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^{1}H NMR Relaxation and Viscosity Measurements on Solutions and Suspensions of Carbohydrates and Starch from Corn: The Investigation of Carbohydrate Hydration and Stereochemical and Aggregation Effects in Relation to ^{17}O and ^{13}C NMR Data for Carbohydrate Solutions

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The ^{1}H NMR relaxation of a series of carbohydrates (glucose, fructose, sucrose, corn syrups) and chemically modified waxy maize starch in solutions or suspensions reveals marked differences in hydration behaviors, determined primarily by compositional and structural differences. Our results suggest that the stereochemistry of the solute plays an important role in determining the extent of hydration. Extensive aggregation of Polar Gel-5 (modified) corn starch and amylopectin in concentrated suspensions is prevented by brief heat treatment at 60 °C, consistent with the involvement of hydrogen bonding in the aggregate formation. Such a preheating treatment results, strikingly, in a linear concentration dependence of ^{1}H NMR relaxation rates of water protons up to concentrations as high as 0.7 g of solids/g of H_2O, in agreement with a two-state, fast-exchange model for relaxation. On the other hand, the concentration dependences of viscosities and apparent viscosities of amylopectin and Polar Gel-5 remain nonlinear above $\simeq$0.2 g of solids/g of H_2O, suggesting the presence of some residual hydrogen bonding between polysaccharides in the preheated samples, at high concentrations. Our ^{1}H NMR study of the molecular "mobility" of water, combined with viscosity measurements, indicates that the macroscopic, flow behavior of starch suspensions is *not* correlated with the molecular mobility of water in such systems. ^{17}O and ^{13}C NMR data are consistent with the results of our ^{1}H NMR relaxation measurements on corn carbohydrates and chemically modified waxy maize starch, concerning compositional and structural differences that determine hydration behavior.

The range of food uses of carbohydrates has greatly increased in the United States since the early 1960s. The use of carbohydrates for the improvement of acceptability and nutritional value of foods is of great interest to food technologists and scientists; the carbohydrate interactions with other food constituents are also potentially important.

The hydration behavior of carbohydrates is of special interest in this context (Tait et al., 1972; Franks et al., 1973; Biswas et al., 1975; Sugget, 1976; Mora-Gutierrez and Baianu, 1985). Understanding the hydration behavior of biopolymers has recently made much progress with the application of high-field nuclear magnetic resonance techniques (Baianu et al., 1982, 1985, 1988; Laszlo, 1983; Pessen and Kumosinski, 1985; Lioutas et al., 1986–1988; Kakalis and Baianu, 1988).

In this report we are primarily concerned with the NMR determination of the molecular "mobility" of water associated with monosaccharides, sucrose, corn syrups, amy-

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lopectin, and chemically modified starch. Related to such NMR investigations are parallel measurements of viscosity of polysaccharide solutions and modified starch suspensions and gels; the combination of viscosity and NMR measurements could, in principle, provide additional information pertinent to both the hydration and hydrogen bonding in solutions of polysaccharide mixtures and suspensions of modified starch. Such materials are now in widespread use in the food industry, and therefore, an improved understanding of their interactions is important for food engineering and product development.

MATERIALS AND METHODS

Samples. Amaizo Polar Gel-5 and Fro-Dex-55L were gifts of the American Maize Products Co. (Hammond, IN). Purified amylopectin from corn starch was obtained from Sigma Chemical Co. (St. Louis, MO). Sucrose was a product of EK Industries, Inc., Addison, IL 60101. Fructose was a gift from General Mills, Inc. Anhydrous dextrose was obtained from Baker Chemical Co. (Phillisburg, NJ 08865).

Moisture contents of Polar Gel-5, Fro-Dex-55L, and amylopectin were determined in triplicate by a vacuum oven method to be, respectively, 11.54%, 2.67%, and 10.72%; the vacuum oven was set at 60 °C (29.8 in.Hg), and samples were dried for 24 h. Moisture contents of fructose, sucrose, and glucose were determined in triplicate and were found to be less than about 0.3%.

Preparation of Samples for NMR Measurements. Solutions or suspensions of these carbohydrate mixtures and starch solids, respectively, were prepared by mixing the appropriate amount of solids with deionized water, followed by warming to 60 °C for 2.5–4 min to achieve complete solubilization. Solutions or suspensions were also shaken in a Fisher minishaker for thorough mixing and were allowed to stand at room temperature at least for 20 min before the measurements. Portions (5 mL) of sample solution were transferred to 10-mm-diameter tubes for NMR measurements.

^{1}H NMR Measurements. ^{1}H NMR measurements were carried out at 10 and 20 MHz with a PC-10 and a PC-20 NMR process analyzer, respectively (Bruker/IBM Instruments, Danbury, CT). Such measurements were carried out in triplicate at 25 ± 1 °C. The inversion recovery sequence, 180° – τ –90°, was used for T_1 measurements, whereas the Carr–Purcell–Meiboom–Gill (CPMG) multipulse sequence was employed for T_2 measurements. The decay of the transverse magnetization (spin-echo maxima) was monitored with a Tektronix storage oscilloscope (Model 5113, dual beam).

Viscosity Measurements. A falling-ball viscometer (Gilmont, Res. Instr. and Control Systems) was employed for determining viscosities of carbohydrate solutions and modified starch suspensions in the range of viscosities 2.0–15 cP. The ball size used was No. 1 (stainless steel), and measurements were carried in the range 25 ± 2 °C, as determined with ±0.1 °C accuracy with a thermocouple (Keithley Instruments, Inc., Cleveland, OH).

RESULTS AND DISCUSSION

Monosaccharides. *A. Glucose.* The ^{1}H NMR longitudinal and transverse magnetization decays (T_1 and T_2, respectively) were characterized by a single time constant, which is consistent with a two-state model with fast exchange, as detailed in eq 1. Parts A and B of Figure 1 present CPMG spin-echo decays for two types of carbohydrate mixtures, Fro-Dex-55L and Polar Gel-5, respectively.

Figure 2A presents ^{1}H NMR relaxation data for D-glucose obtained with the Carr–Purcell–Meiboom–Gill, multipulse sequence (Meiboom and Gill, 1958). To interpret such T_2 NMR relaxation data, we are employing a simple two-state model with fast exchange (Derbyshire, 1982). With increasing concentration of a solute in water, the amount of "free" water decreases, whereas the relative amount of "bound" water increases. If no additional contributions to relaxation were present, a linear relationship between the observed relaxation rate (R_{obs}) and solute concentration is expected

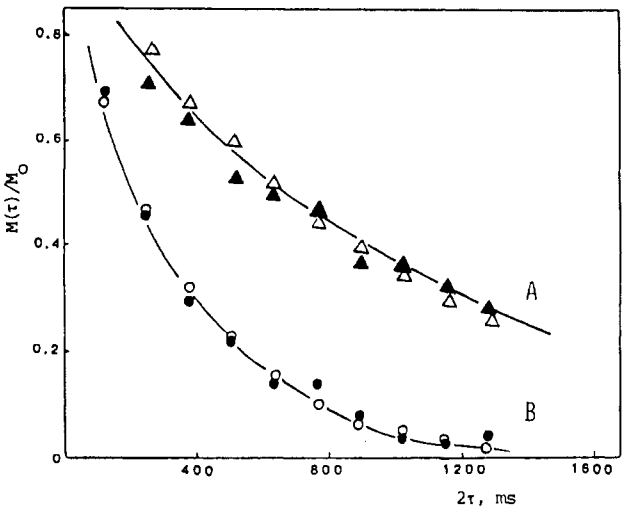


Figure 1. Single exponential decay of the transverse magnetization of water protons in Fro-Dex-55L and Polar Gel-5 suspensions, measured at 10 MHz with a CPMG train of ^{1}H NMR spin-echoes. (A) Fro-Dex-55L: ▲, experimental; △, calculated. (B) Polar Gel-5: ●, experimental; ○, calculated.

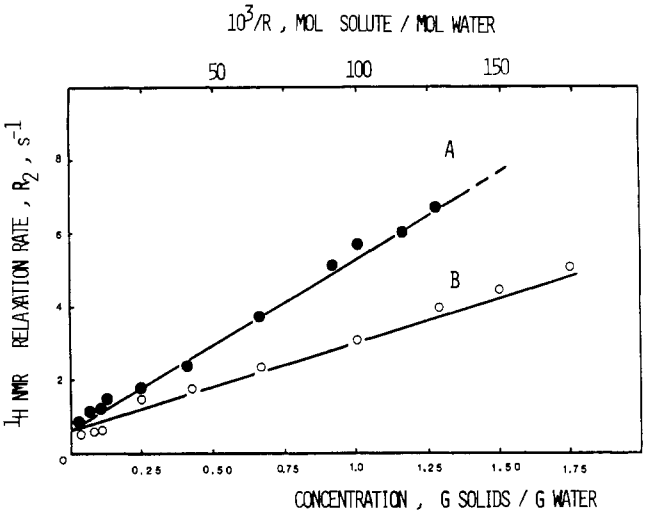


Figure 2. (A) Concentration dependence of 10-MHz ^{1}H NMR relaxation rates, R_2, of glucose solutions. The PC-10 NMR spectrometer settings were CST = 2.0, four scans, receiver attenuation (ATT) = 39, and 14-s recycling delay (RD). (B) Concentration dependence of 10-MHz ^{1}H NMR relaxation rates, R_2, of fructose solutions as a function of concentration. The instrument settings were CST = 1.0, four scans, ATT = 31, and 14-s recycling delay. In both A and B the pulse spacing, 2τ, in the CPMG sequence was 32 ms for the dilute range; for concentrations lower than 0.40 g of solids/g of H_2O a value of CST of 3.0 and a value of 2τ of 16 ms were employed.

$$R_{1,2obs} = P_b R_{1,2b} + P_f R_{1,2f} \quad (1)$$

where P_b and P_f are the percent bound and percent free water in the system and R_b and R_f are, respectively, the relaxation rates of bound and free water. However, departures from linearity are often observed at high concentrations due to either a change in the hydration of the molecule or a change in the relaxation rate of the bound water (Derbyshire, 1982); often this is more likely due to increased interactions between the solute molecules as the concentration increases and the average intermolecular distances become shorter. Furthermore, additional

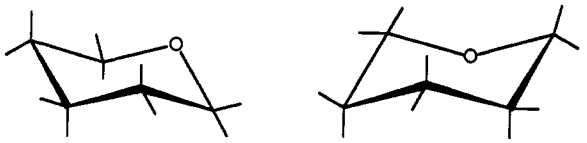
contributions to ^1H NMR relaxation may arise from the chemical exchange of protons or cross-relaxation.

In the case of D-glucose solutions in water, the dependence of R_2 is linear up to the highest concentration measured ($\simeq$1.3 g of glucose/g of water), as predicted by eq 1. Our ^1H NMR data (Figure 2A) are, therefore, in good agreement with previous ^{17}O NMR work on D-glucose at low field (Tait et al., 1972) for a narrower range of concentrations.

B. Fructose. The dependence of the ^1H NMR transverse relaxation data on fructose concentration (Figure 2B) is also linear as predicted by eq 1 up to the highest concentration measured ($\simeq$1.75 g of fructose/g of water). The difference between the T_2 values for glucose and fructose at the same concentration is positive. We are now considering possible explanations for the shorter T_2 values of glucose solutions in comparison with fructose solutions. It is known that the predominant forms of fructose in aqueous solution at room temperature are β-D-fructopyranose (about 67%), β-D-fructofuranose (about 27%), and α-D-fructofuranose (about 6%) (Prince et al., 1982), whereas D-glucose adopts two forms, α-D-glucopyranose and β-D-glucopyranose.

Because of such conformational changes in fructose, the probability of water forming hydrogen bonds with fructose is lower than for glucose. Therefore, the residence time of water on the fructose molecule would be shorter than on the glucose, and this effect would result in longer T_2 values for fructose in solutions. However, the interconversion between the fructose conformations in aqueous solutions is likely to be slower compared with the correlation time of water in these solutions. Therefore, we have to consider an alternative explanation.

For β-D-glucose in the C1 conformation all OH substituents and the CH_2OH group occupy equatorial positions (Shallenberger and Birch, 1975). β-D-Glucopyranose in



C1 1C

the C1 conformation is the favored structure, and the 1C conformation is the least favored structure. The OH groups of glucose in the equatorial configuration are known to fit within the quasi-tetrahedral arrangement of water molecules in the liquid state (Tait et al., 1972). As a result, water binding to glucose is stronger than to fructose. τ_c would be, therefore, longer in glucose solutions than in fructose solutions, causing T_2 values to be shorter in glucose solutions than in fructose solutions at the same molar concentration, as observed (Figure 2A,B).

Disaccharides. The concentration dependence of the proton T_2 values of sucrose solutions in water (Figure 3) is linear only up to 1.5 g of sucrose/g of water (R = 0.08 mol of sucrose/mol of water). We will consider possible explanations for these observations. Our data in Figure 3 could be explained by the formation of a sucrose-associated water structure, or "clusters", over a wide range of concentrations, above 1.5 g of sucrose/g of water. Hydrogen bonding between sucrose and water is likely to be present at sucrose concentrations as low as 1.50 g of dry sucrose/g of water.

Sucrose has eight hydroxyl groups. Three of these groups and one of the ring oxygen atoms are thought to participate in the formation of two intramolecular hydrogen bonds in the solid state. The remaining five hydroxyl groups are involved in intermolecular hydrogen bonding upon formation of the sucrose crystal (Allen and Wood, 1974). In solution there is *no direct* evidence that the sucrose molecule retains its intramolecular hydrogen bonds. If these bonds were retained in solution, the available sites for sucrose–water interactions would be diminished. However, it was suggested that, upon solubilization of sucrose, breaking of the intramolecular hydrogen bonds does occur (Allen and Wood, 1974).

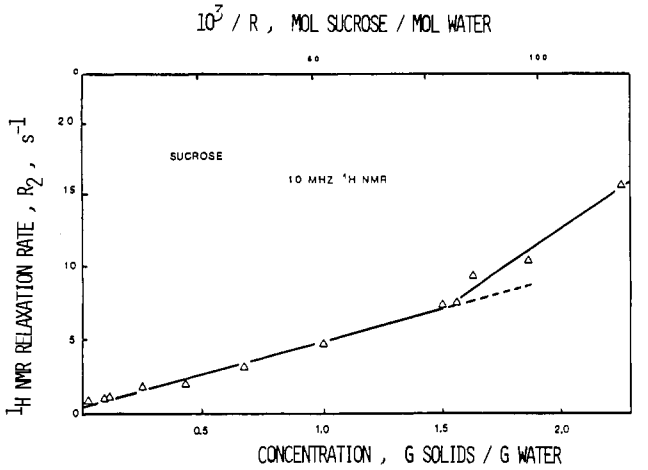


Figure 3. Concentration dependence of 10-MHz ^1H NMR relaxation rates, R_2, of sucrose solutions. The instrument settings were CST = 1.0, four scans, ATT = 35, and 12-s recycling delay. The pulse spacing, 2τ, in the CPMG sequence was 32 ms for the dilute range and 16 ms for the moderate and high concentration ranges, respectively.

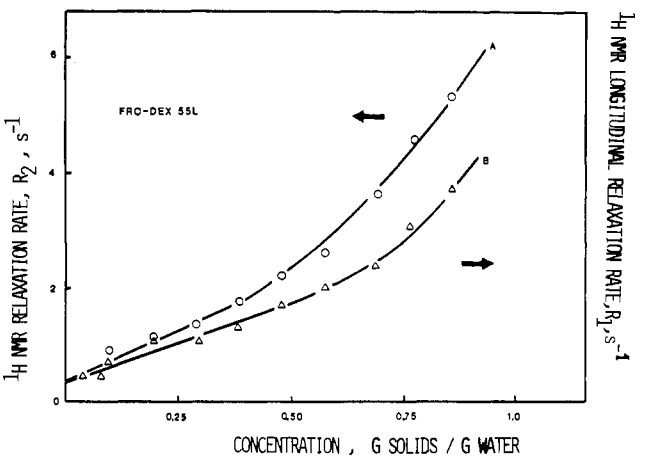


Figure 4. 10- and 20-MHz ^1H NMR relaxation rates of Fro-Dex-55L solutions: (A) concentration dependence of transverse relaxation rates (R_2); (B) concentration dependence of longitudinal relaxation rates (R_1). T_1 values were obtained with the inversion recovery sequence 180° – τ –90°.

At the higher sucrose concentrations, the corresponding OH groups are likely to form intermolecular hydrogen bonds between sucrose molecules and, therefore, form clusters of sucrose molecules in water; such clusters containing bound water molecules would have longer correlation times than the unassociated sucrose molecules. Such longer correlation times would be reflected in shorter T_2 values for the bound water. This would explain the increase in the gradient of the T_2 concentration dependence above 1.5 g of sucrose/g of water (R = 0.08 mol of sucrose/mol of water).

The T_2 values of sucrose solutions are significantly lower than those of glucose and fructose. This is presumably

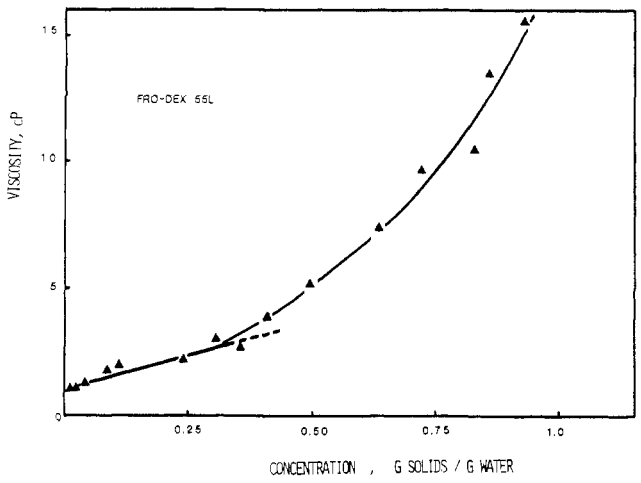


Figure 5. Dependence of viscosity of Fro-Dex-55L solutions on concentration at 25 ± 2 °C.

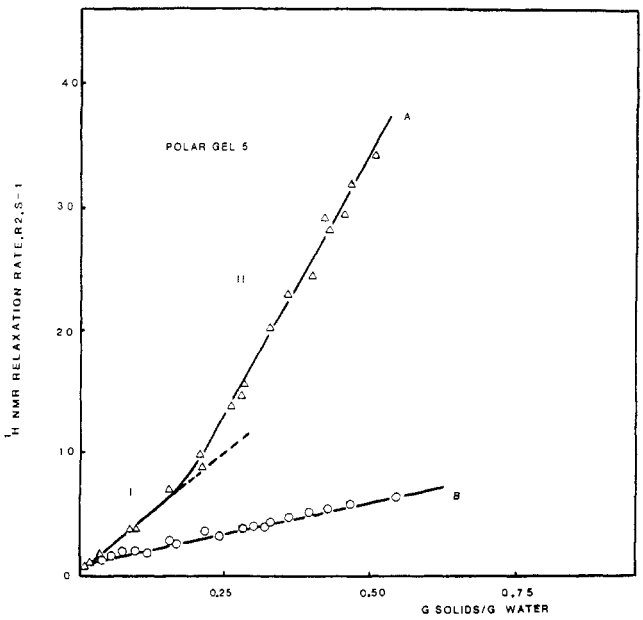


Figure 6. Concentration dependence of 10-MHz ^{1}H NMR relaxation rates, R_2, for Polar Gel-5 suspensions: (A) unheated; (B) preheated at 60 °C for 2.5 min and measured at 25 ± 1 °C.

due not only to the hydrogen-bonding effects discussed above but also to the higher molecular weight of sucrose which would increase the correlation time of bound water, tumbling together with the sucrose molecule.

Corn Syrups. Fro-Dex-55L is a spray-dried blend of corn syrups, high-fructose corn syrups, and oil-free lecithin. Previous ^{13}C NMR work (Mora-Gutierrez and Baianu, 1989a) identified a number of oligosaccharides that are present in high proportions in this polysaccharide mixture.

By comparing the transverse relaxation rate (R_2) of Fro-Dex-55L, fructose, and modified starch (Polar Gel-5), one can see that fructose and Fro-Dex-55L (Figures 2B and 4A, respectively) exhibit quite different NMR relaxation behavior. The presence of large particle sizes in solution, as in the case of Fro-Dex-55L, would favor hydrogen bonding on the particle surface and increased relaxation rates for the bound water. As a result of aggregation of oligosaccharides in the Fro-Dex-55L mix, the tumbling rate will be determined by the particle size rather than by the molecular weight of the component molecules. In the case of fructose solutions, however, the tumbling rate is directly related to the fast reorientation of

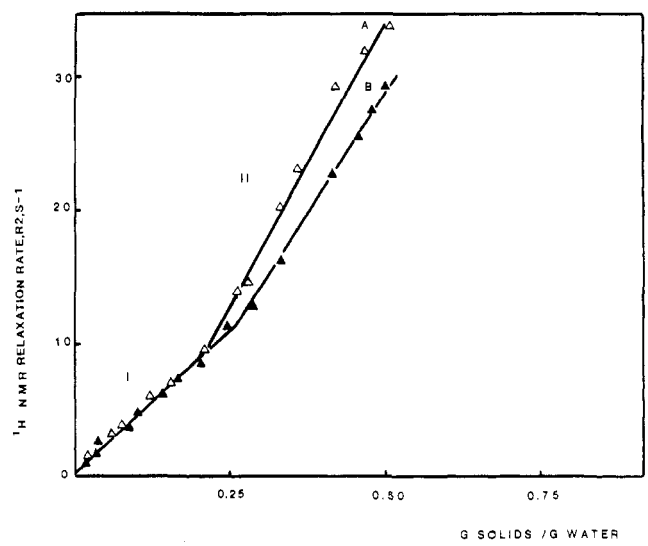


Figure 7. Concentration dependence of 10-MHz ^{1}H NMR relaxation rates, R_2: (A) Polar Gel-5; (B) amylopectin solutions and suspensions.

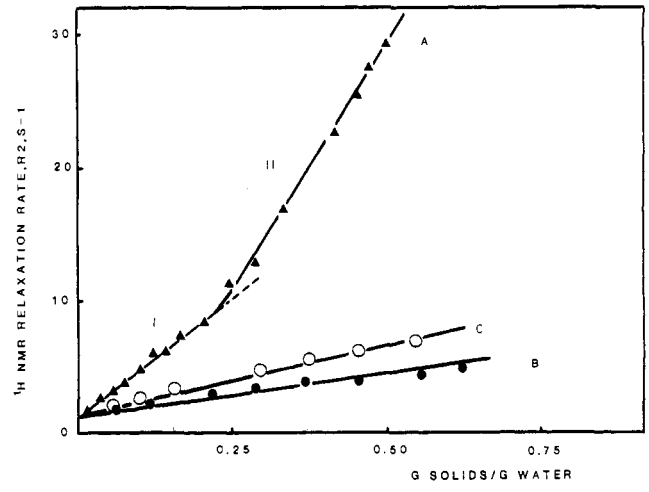


Figure 8. Concentration dependence of 10-MHz ^{1}H NMR relaxation rates, R_2, for amylopectin solutions and Polar Gel-5 suspensions: (A) unheated amylopectin suspensions; (B) preheated amylopectin at 60 °C for 2.5 min and measured at 25 ± 1 °C; (C) preheated Polar Gel-5 at 60 °C for 4 min and measured at 25 ± 1 °C.

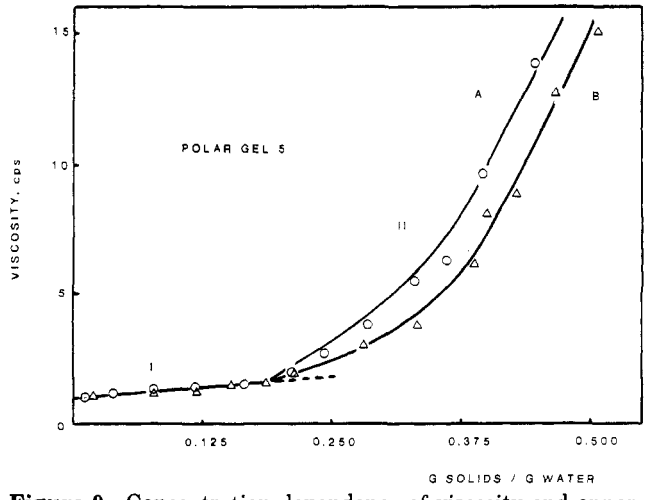


Figure 9. Concentration dependence of viscosity and apparent viscosity for Polar Gel-5 suspensions at 25 ± 2 °C: (A) preheated at 60 °C for 4 min and measured at 25 °C; (B) unheated.

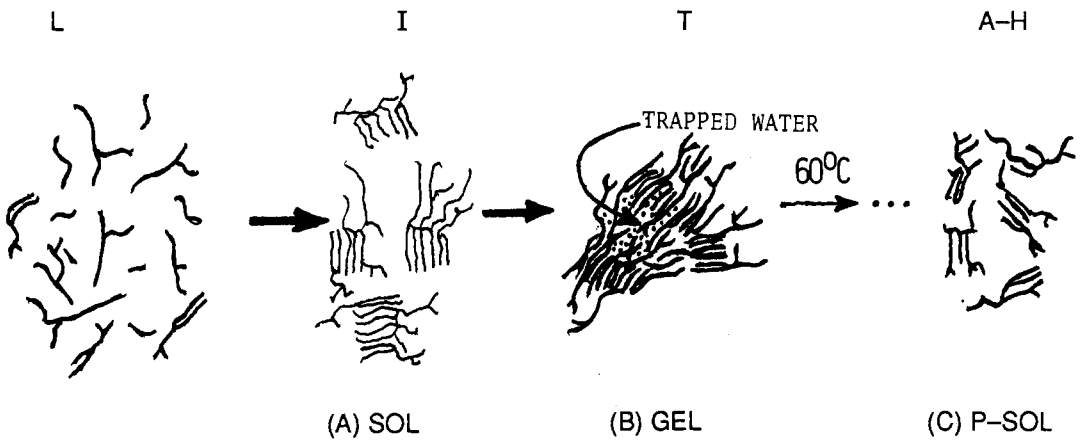


Figure 10. Illustration of a sol-to-gel transition with *two phases* for maltodextrins from corn starch. This model may also be applicable, with some modification, to amylopectin aggregation [modification of Figure 6 of Reuther et al. (1983)]; the presence of trapped water is inferred from our NMR data presented above and is consistent with the previous findings from SAXS studies (Reuther et al., 1983). Key: L = linear region in the NMR concentration dependence (Figures 6A and 8A, region I); I = intermediate region at about 0.23 g of solids/g of H_2O (Figures 6A and 8A); T = trapped water region reflected in a nonlinear concentration dependence of the R_2's of water protons (Figures 6A and 8A, region II); A–H = linear concentration dependence of the water protons R_2's observed after preheating Polar Gel-5 and amylopectins at 60 °C for 2.5 min (Figures 6B and 8B, respectively).

the small fructose molecule. Therefore, the bound water relaxation rates are much lower for fructose than in the case of Fro-Dex-55L solutions.

Although carbohydrate–water interactions are affected by the hydrogen bonding of sugar hydroxyl groups to water, the increased hydration of Fro-Dex-55L (Figure 4A) in comparison with the Polar Gel-5 modified corn starch (Figure 6A) could be due to the participation of lecithin polar groups in the hydration process; such groups could also participate in intermolecular hydrogen bonding with the hydroxyl groups of fructose. This would explain the change in the gradient of the R_2 relaxation rate as a function of concentration above 0.40 g of dry solids/g of water (Figure 4A).

Figure 4B shows that the NMR spin–lattice relaxation rate (R_1) of Fro-Dex-55L exhibits a linear dependence on concentration in the dilute to moderate concentration range. This is similar to the concentration dependence of the NMR transverse relaxation rate (R_2) observed for this system (Figure 4A).

The viscosity of Fro-Dex-55L solutions (Figure 5) shows Newtonian behavior up to 0.3 g of dry solids/g of water, implying that intermolecular interactions between solute molecules are very weak, or negligible, in this concentration range.

Chemically Modified Waxy Maize Starch. Most starches are made of linear and branched polymers (amylose and amylopectin, respectively). Waxy maize starches differ from all other starches since they consist entirely of branched, amylopectin molecules. Polar Gel-5 is a chemically modified waxy corn starch. The type of pretreatment of Polar Gel-5 has a marked effect on its gelling and flow behavior. The concentration dependence of the proton NMR transverse relaxationrates R_2 for Polar Gel-5 (Figure 6A) has two regions of distinct behavior. In the linear region I, up to 0.2 g of dry solids/g of water, water exchanges fast and the two-state model applies. The increase in the gradient of R_2 in region II is ascribed to the presence of a *third* population of water, i.e., water "trapped" between aggregates of amylopectin molecules. For these larger structures, it is likely that cross-relaxation also plays an important role in determining the R_2. Therefore, the relaxation behavior in region II would have a significant contribution from this third population of trapped water. There is a factor of 4 between the ^1H NMR R_2 values for samples of Polar Gel-5 that were not heated and those that were preheated, respectively (Figure 6A,B); presumably, cross-relaxation and trapped-water contributions are considerably reduced upon heating the material. After the material is heated to about 60 °C for 2.5 min, a large amount of swelling occurs and a linear relationship is observed between proton R_2 and the inverse of the moisture content up to high concentrations of Polar Gel-5 (Figure 6B). Heating would facilitate, or increase substantially, the hydration of starch granules; furthermore, it would result in a more open structure of the swollen starch granule that allows fast exchange between the bound and free or (bulk) water. Our observations are consistent with the current view that the beginning of gelatinization is characterized by an increased hydration and fluidity of polymer chains (Lelievre and Mitchell, 1975).

Parts A and B of Figure 7 compare, respectively, the concentration dependences of proton R_2 for Polar Gel-5 starch and amylopectin in water. Both systems show almost identical behavior in the dilute range (region I). A significant difference in the dependence of R_2 on solids to water ratio is observed between Polar Gel-5 starch and amylopectin at higher concentrations (region II). A possible explanation of this observation is that Polar Gel-5 either tends to aggregate faster or retains more water than amylopectin; therefore, a higher slope value is obtained for Polar Gel-5 than for amylopectin.

Figure 8 compares the amylopectin concentration dependence of R_2 (A) with those of preheated (60 °C for 2.5 min) amylopectin (B) and preheated Polar Gel-5 (C). The latter two dependences are linear up to about 0.7 g of dry solids/g of water. R_2 values for preheated amylopectin were slightly lower than for preheated Polar Gel-5 (parts A and C of Figure 8, respectively), this being attributed mainly to polysaccharide chain mobility differences caused by different degrees of close-packing; Polar Gel-5 starch granules are larger and swell more rapidly than amylopectin. In addition to this effect, certain other properties of the starch granule are controlled by chemical modification of the starch. Thus, the amount of granule swelling is determined by the degree of cross-linking between the D-glucose units, which will also affect the ^1H NMR relaxation behavior. Moreover, there is a strong similarity between Figures 8A,B and 6A,B for amylopec-

tin and Polar Gel-5, respectively; this is caused by the fact that the Polar Gel-5 obtained from waxy maize contains mostly amylopectin.

Parts A and B of Figure 9 present viscosity data as a function of concentration in grams of dry solids/grams of water for heated and unheated Polar Gel-5, respectively; as expected, upon heating to 60 °C there is an increase in the viscosity of the suspension (Figure 9A). Two regions are observed for Polar Gel-5, aggregation being the main factor affecting the viscosity (interparticle interactions, or their packing, become important with increasing starch to water ratio).

The marked contrast between the concentration dependence of R_2 and viscosity, respectively, in Figures 6 and 9 suggests a lack of correlation between the molecular mobility of water, as derived from relaxation data (Figure 6) and the macroscopic flow behavior of Polar Gel-5 suspensions (Figure 9). This comparison also suggests the presence of either swollen or retrograded aggregates of amylopectin molecules, which causes higher viscosities in the preheated samples and allows the free movement of water molecules into, and out of, such swollen amylopectin aggregates (note that the plot in Figure 6B is linear); the aggregates would be larger in the heated samples of Polar Gel-5 because the apparent viscosities are higher for such samples than those of the unheated samples at concentrations greater than 0.25 g of solids/g of water.

Viscosity and apparent viscosity measurements for the gelatinized Polar Gel-5 show that a limited amount of aggregation affects the rheology of starch pastes, as expected, but does *not* affect the molecular mobility of water and the proton chemical exchange as measured by ^{1}H NMR relaxation. Our work supports the view that water protons relaxation times in starch pastes do *not* correlate at high concentrations with the rheological properties of the starch pastes and are only governed by exchange between the bulk and bound water, superimposed on two other relaxation mechanisms: proton chemical exchange and cross-relaxation. This happens because ^{1}H NMR relaxation monitors the molecular mobility of water and proton chemical exchange (as well as cross-relaxation) in the starch pastes, whereas the viscosity, or apparent viscosity, is determined by the resistance to flow in the polysaccharide solutions, or starch suspensions, respectively, which is dominated by the size and shape of the matrix components of the starch paste (amylopectin aggregates). High-resolution ^{13}C and ^{17}O NMR at high fields (Mora-Gutierrez and Baianu, 1985, 1989a,b), however, provide additional microrheological information about the motions of specific chemical groups or atoms in the less mobile polysaccharide matrix, which could be then related to the macroscopic, flow behavior of the polysaccharides. The ^{13}C NMR spectrum of Polar Gel-5 in suspension is very similar to that of gelatinized corn starch and exhibits relatively high chain mobility, although lower than the chain mobilities of maltodextrins and corn syrups that are very high (with correlation times of the order of 10 ns or less; Mora-Gutierrez and Baianu, 1989a,b). Maltodextrins, which are partial digestion products from starch, also exhibit an interesting gelling behavior. Thus, our previous ^{1}H, ^{2}H, and ^{17}O NMR results for corn maltodextrin hydration (Mora-Gutierrez and Baianu, 1989a) show a similar behavior to those of Polar Gel-5 and amylopectin, with a nonlinear concentration dependence of R_2 for concentrations higher than 0.23 and 0.4 g of maltodextrins/g of water, respectively, for ^{1}H and ^{2}H/^{17}O NMR.

Previous small-angle X-ray scattering (SAXS) data for maltodextrins suggest the occurrence of a sol-to-gel transition upon cooling of potato maltodextrins, at about 15% w/w, with two phases being present in the gel (Reuther et al., 1983, 1984), as illustrated in Figure 10. A somewhat similar process (Figure 10A,B) with similar aggregates (but of larger sizes) is likely to take place for unheated amylopectin and Polar Gel-5 (and perhaps, also, in Fro-Dex-55L), whereas the preheated amylopectin behavior may be explained as shown in Figure 10B,C. In the gel state, trapped water between hydrogen-bonded, structured domains of amylopectins may be thus responsible for the nonlinear concentration dependence of R_2 for amylopectin above $\simeq$0.2 g of solids/g of H_2O in Figure 8A. However, the higher degree of branching and chain disorder in amylopectins confers to amylopectin gels higher degrees of hydration than those in maltodextrin gels, which have shorter chains, lower degrees of branching, and a tendency toward ordering of parallel chains into disklike structures of about 360-Å width (Reuther et al., 1983).

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Registry No. Glucose, 50-99-7; fructose, 57-48-7; Fro-Dex-55L, 123147-76-2; Polar Gel-5, 123147-77-3; amylopectin, 9037-22-3; water, 7732-18-5; sucrose, 57-50-1.

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Separation, Identification, and Quantification of the Major Carotenoids in Extracts of Apricots, Peaches, Cantaloupe, and Pink Grapefruit by Liquid Chromatography[†]

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The predominant carotenoids and carotenol fatty acid esters in extracts from apricots, peaches, cantaloupe, and a variety of pink grapefruit (Ruby seedless) have been separated and quantitated on C_{18} reversed-phase high-performance liquid chromatography (HPLC) columns with low and high carbon loading. Isocratic and gradient HPLC conditions were developed that separated carotenoids from three classes of xanthophylls, hydrocarbon carotenoids, and carotenol fatty acid esters. The xanthophylls were identified as zeaxanthin and β-cryptoxanthin. The hydrocarbon carotenoids were identified as lycopene, γ-carotene, ζ-carotene, β-carotene, phytofluene, and phytoene, which were accompanied by several of their cis stereoisomers. The carotenol fatty acid esters were identified as saturated straight-chain bis(fatty acid esters) of β-cryptoxanthin, lutein, and zeaxanthin, which were shown to be only present in the extracts from peaches.

Recent laboratory and epidemiological studies have correlated the high consumption of certain foods with reduced incidence of several types of cancers in human beings. These studies have associated several micronutrients as possible active ingredients in certain fruits and vegetables with prevention of cancer (Moon and Micozzi, 1988). Since carotenoids are among one of the most abundant micronutrients found in cancer-preventive foods, the determination of accurate qualitative and quantitative data on these classes of compounds in foods, particularly in fruits and vegetables, has recently become increasingly important. As a result, rigorous analytical techniques have been developed that can separate and quantify carotenoids in several green and yellow/orange fruits and vegetables (Beecher and Khachik, 1984, 1988; Khachik et

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