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Note

Further evidence of the changing nature of biopolymer networks in the presence of sugar

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Abstract—Despite claims made in the literature that polysaccharides maintain a substantially aggregated morphology in the form of 'gel particulates' or 'gel islands' at a high sugar environment, results of differential scanning calorimetry (DSC) discussed now demonstrate that extensive macromolecular order is not thermodynamically stable. Gelatin, on the other hand, appears to demix from the sugar-rich domains, which promote chain association rather than inhibiting it. DSC evidence is supported by previously published transmission electron microscopy (TEM) work and mechanical analysis.

Keywords: Agarose; Deacylated gellan; Carrageenans; Gelatin; Sugar; DSC

High-solid biomaterials increasingly include a number of nonstarchy polysaccharides and proteins that provide a range of properties such as structure, storage stability, processability, delivery control, etc.[1] The market value of these materials is significant, since their low-water content imparts on them a long shelf life, thus making them relatively inexpensive to handle and transport around the world. Commercial products in the food and pharmaceutical industries that fit this category include confections, the unfrozen phase of ice cream, dried fruit leathers, and the hard gelatin capsule as a pharmacotherapeutic.[2,3] By and large, manufacturing of high-solid food products is regarded as being craft based. Thus, there have been no systematic studies on the kinetics of structure development of materials containing sugars, proteins, and/or polysaccharides. The lack of understanding and control of these properties is hampering the ability of the industry to match the kinetics of structure formation to the timescale of manufacturing processes in order to improve the consumer acceptance of many high-solid formulations.

At these levels of solids (70–95%) and under the common conditions of processing, storage, and consumption, biomaterials exhibit properties that relate to 'rubbery' or 'glassy' consistency. However, the importance of the rubber-to-glass transition and the development of the glassy state, as pioneered in the sophisticated 'synthetic-polymer approach', became widely appreciated only recently.[4] In the glassy state, materials exhibit the consistency of an amorphous solid, with molecules remaining in a random orientation during the timescale of practical observation. Cooling from the melt or the rubbery state results in vitrification, and the temperature at which the sample acquires glassy consistency is known as 'T_g'.[5] This is not that well defined as, for example, the melting point (T_m), since the process of vitrification may take place over a wide range of temperatures. The resulting glassy system is thermodynamically unstable, but derives kinetic stability from its high viscosity.

There has been much work in the literature regarding the vitrification of pure compounds (e.g., sugars or

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starch hydrolysates).[6,7] Future research should utilize fundamental knowledge in industrial preparations, which are composites of various ingredients. In the past decade, we have attempted to record and then rationalize the structural properties of mixtures of sugar in the presence of gelatin and/or polysaccharide. These are of great importance for the confectionery industry and have other applications, for example, flavour encapsulation and the preservation of bioactive molecules in glassy carbohydrate matrices. The work has been aiming to

(i) Implement the method of reduced variables in order to introduce a new tool in the analysis of relaxation processes of the mechanical glass transition.[8] Otherwise, there is little advantage in monitoring simple isochronal heating or cooling profiles.
(ii) Report on the observation of two distinct molecular processes in the glass transition region and the glassy state, which were treated with the combined framework of WLF/free volume and the predictions of the reaction rate theory.[9] The approach is clearly differentiated from the flawed use of the WLF equation in macromolecular collapse phenomena, and chemical or enzymatic reactions.
(iii) Use the theoretical advance to define the network glass-transition temperature (T_{gn}), a concept that is distinct from the commonly reported calorimetric T_g of sugars and polyols.[10] The new concept allows for the macromolecular effects observed in the mechanical manifestation of glassy phenomena and can be applied to the curtailed vitrification of dehydrated materials.[11]

The foregoing addressed some of the issues governing the behavior of biopolymers in sugar–low-water systems. These are much less understood than in the high-water environment where biopolymers are also widely employed. In the latter, industrial applications have been undoubtedly benefited from the establishment of relationships between conformational characteristics of biopolymers and their behavior in solutions and gels.[12,13]

Regarding high solids, the network characteristics responsible for the structural properties summarized in the preceding paragraph are under investigation. It has been suggested that systems might demix in polymer-rich regions deemed 'gel particulates' or 'gel islands' in which macromolecular assemblies remain substantially ordered as in the aqueous environment.[14] As shown in Figures 1 and 2, however, our observations using differential scanning calorimetry (DSC) contradict this hypothesis. In aqueous preparations, we dissolved 2% deacylated gellan or 2% ι-carrageenan at 90 °C with stirring for 15 min, followed by addition of 1.5 mM $CaCl_2$ and 100 mM KCl, respectively. Samples containing co-solute were made by lowering the temperature to 80 °C for the addition of sucrose and glucose syrup to the solution. Excess water was evaporated off to bring the total level of solids to the required concentration, comprising in each case 2% polysaccharide and a sugar mixture of 50:50 sucrose to glucose syrup.

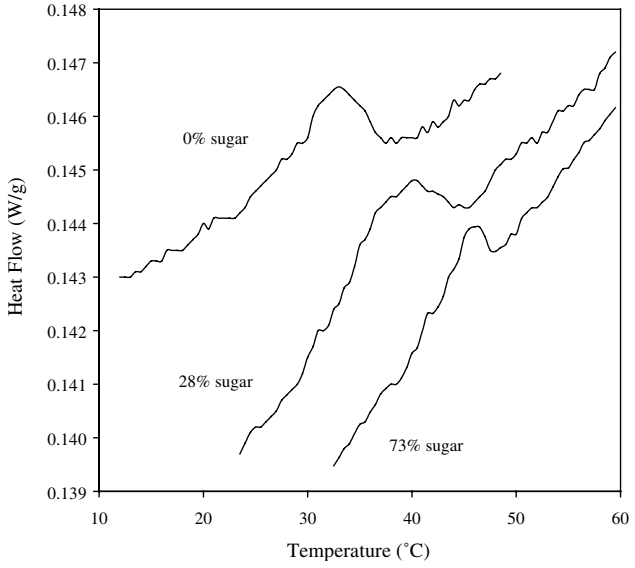

Figure 1. Cooling exotherms of 2% deacylated gellan, ion exchanged in the sodium form, at levels of sugar shown by the individual traces (1.5 mM $CaCl_2$ added; sugar is a 50:50 mixture of sucrose/glucose syrup; scan rate: 3 deg min^{-1}).

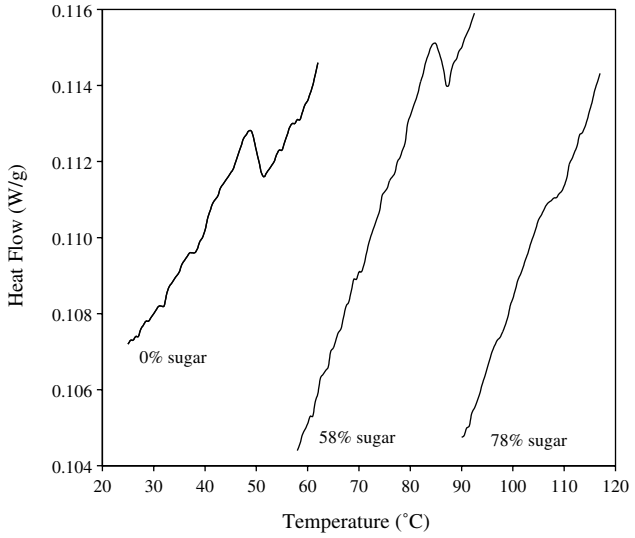

Figure 2. Cooling exotherms of 2% ι-carrageenan at levels of sugar shown by the individual traces (100 mM KCl added; sugar is a 50:50 mixture of sucrose/glucose syrup; scan rate: 3 deg min^{-1}).

Samples were heated to eliminate traces of mechanical history and then cooled at a controlled scan rate to reveal well-defined exothermic events. Three runs were taken, and the average of essentially overlapping traces for each preparation is reported. From the aqueous to

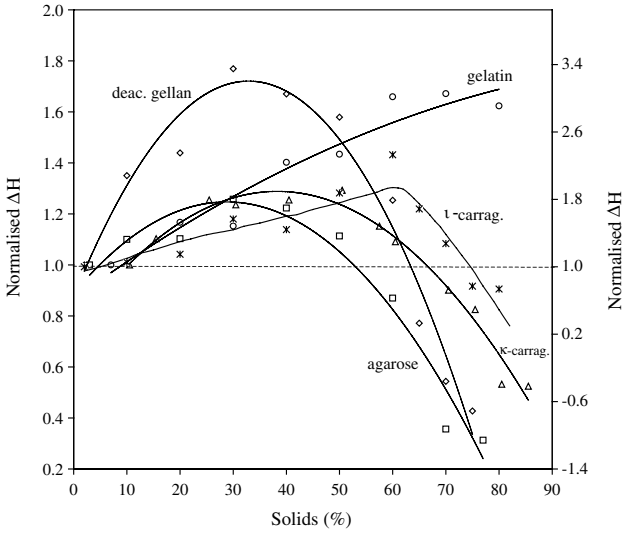

Figure 3. Enthalpy changes as a function of total level of solids for biopolymer–sugar mixtures containing 3% agarose, 0.5% κ-carrageenan (10 mM KCl), 2% ι-carrageenan (100 mM KCl), 2% deacylated gellan (1.5 mM $CaCl_2$), and 7% gelatin (ι-carrageenan data are plotted on the secondary axis; original results and Ref. 19).

the high-solid regime, thermal events possess considerable variation in size, shape, and temperature band. The area underneath the peaks can be used to obtain values of change-in-enthalpy (ΔH), which were determined by numerical integration and interpolating baselines from higher to lower temperatures.

Clearly, there is a positive increase in the values of ΔH up to 30% solids, followed by a dramatic decrease in the ability to form macromolecular associations at higher levels of co-solute in gellan gels (Fig. 3). In spite of the diminishing order at the upper range of sugar, there is no abatement in the shift of exotherms to higher temperatures. Thus the transition midpoint temperatures increase from 33 and 40.5 to 46 °C for the preparations in Figure 1. This result suggests that the tendency to form progressively longer and, hence, more thermally stable helices at high levels of co-solute should be increasingly counterbalanced by the inability to sustain those helices in the form of stable aggregates due to the scarcity of water molecules.

Work was extended to sugar mixtures of linear galactans of ι-carrageenan, (Fig. 2), κ-carrageenan, and agarose having different levels of sulfation. Furthermore, the members of this family show differences in backbone geometry, but they form a thermally reversible double-helix structure. There is evidence that the decrease in degree of sulfation from ι-carrageenan to agarose results in distinct gelation properties.[15] Thus increasingly rigid, brittle and opalescent networks can be formed at low levels of solids, which is due to extensive aggregation of the agarose chains. A clear trend in the enthalpy change of the gelation process of the three galactans is depicted in Figure 3. The transition enthalpy of the agarose gels reaches a maximum at 30% solids and about 70% of the original associations have been lost at 77% solids. By comparison, the threshold of thermodynamic stability of structure formation extends to between 40% and 50% solids in κ-carrageenan, and 60% solids in the case of ι-carrageenan. Evidently, the reduced level of cross-linking in carrageenans is compatible with additional molecular interactions that sustain a rise in ΔH values at levels of sugar higher than those of agarose.

In conclusion, the structural transformation of polysaccharides is due to the 'dissolution' of considerable parts of the network in the saturated sugar environment. This is supported by recent transmission electron microscopy (TEM) work, which documents the absence of dense fibrillar structures of the aqueous polysaccharide network in the high-solid counterparts.[16] Lightly cross-linked biopolymer–sugar mixtures at 80% solids undergo a rubber-to-glass transition that can be treated with the WLF/free volume approach along the lines established in the vitrification of amorphous synthetic rubbers.[17]

Finally, we wish to contrast the aforementioned properties of gelling polysaccharides with those of gelatin. As shown in Figure 3, gelatin appears to respond differently in a high-solid environment, with the co-solute reinforcing chain association rather than preventing it. There is densimetric evidence in the literature that sugars and polyols are excluded from the domain of the protein.[18] This means that reduction of the protein–sugar interface will render the system less unfavorable thermodynamically, thus stabilizing it. The drive to self-association in order to reduce the surface of contact between the gelatin and the co-solute is documented in the increasing ΔH values of the thermal events, which remain up to 80% solids higher than those in the aqueous preparation. Therefore, it is gelatin (not the gelling polysaccharides) that retains a high network strength and coil-to-helix transition enthalpy in the presence of sugars. This is further evidenced by TEM unveiling demixed gelatin and sugar-rich phases of considerable size, as opposed to the homogeneous polysaccharide preparations in the presence of high levels of co-solute.[19]

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