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Abstract: The phase behavior of aqueous mixtures of gelatin and oligosaccharides above their gelation temperature is investigated experimentally, and rationalized according to a simple multicomponent Flory–Huggins model. When the gelatin is only weakly charged, entropic considerations dominate and it is found that the cloud point curve of the mixtures is extremely sensitive to the molecular weight distribution of the oligosaccharide. Even very small quantities of long-chain oligosaccharides present in an otherwise short-chain oligosaccharide population can radically reduce the compatibility. Added salt does not significantly affect the phase diagram, although a strong effect on the kinetics of phase separation is seen. Lowering the pH increases the electrostatic charge on the gelatin and strongly enhances the compatibility. Because the kinetics of gelation and phase separation are different, gelation can freeze in nonequilibrium states. Therefore, all phase diagrams were determined well above the gelation temperature (about 37°C). © 1997 John Wiley & Sons, Inc. Biopoly 41: 607–622, 1997

Keywords: Polymer solution phase behavior; gelatin; oligosaccharides; gelatin and oligosaccharides; compatibility of/dilute solution properties of/light scattering of/size exclusion chromatography of; size exclusion chromatography and light scattering detection; Flory Huggins

INTRODUCTION

Gelatin is obtained from the animal protein collagen by either acid or alkali hydrolysis.1 Gelatin molecules undergo a helix–coil transition at temperature close to 30°C. If the concentration is high enough, its solutions can form thermoreversible gels, which are widely used in the pharmaceutical, photographic, and food industries, among others. By far the largest application of gelatin in the food industry is the manufacture of jelly confectionery. The annual world production of this type of confectionery is about 500,000 tons. A typical composition would be 9% gelatin, 73% carbohydrate (or ‘sugar’), and 18% water. The carbohydrate is a mixture of saccharose and oligosaccharides (~ 80% aqueous
solutions of the latter are sold as ‘‘glucose syrup’’). Despite their economic importance, almost nothing has been published in the scientific literature concerning these mixtures. In recent years, problems of clarity and nongelation of these products have been encountered. These are major problems, since clarity and gel strength are two of the main defining characteristics of this product. These problems were ascribed to ‘‘incompatibility,’’ without giving any precise meaning to the term. This study was carried out in order to better understand the phase behavior of these mixtures above their gelation temperature, which should lead to a better understanding of the problems encountered in practice.

In an early study, Marrs\(^2\) showed that the presence of increasing concentrations of ‘‘sugar’’ in aqueous gelatin gels lead initially to a rise in elastic modulus, followed by a maximum and a fall. The ‘‘sugar’’ concentration at which the maximum occurred was inversely proportional to the number average molecular weight of the ‘‘sugar.’’ He also observed that the maximum coincided with clouding of the clear gel. Finally, he obtained exactly analogous results when the oligosaccharides were replaced by polyethylene oxide, showing that the effect was nonspecific. Microscopic examination of the turbid gels showed the presence of gelatin-rich droplets. This observation shows that the incompatibility was due to segregative phase separation.\(^3\) Marrs’ study was carried out by observing the behavior of mixtures after cooling. The temperature dependence of the phenomena was not studied. This protocol leaves many questions unanswered, since in these mixtures, cooling favors both phase separation and gelation. Competition between these phenomena can lead to a complex range of behavior.\(^4\)

Initial investigations showed that demixing of gelatin/oligosaccharide (G/O) mixtures occurs at temperatures well above the gelation temperature, and that the process often takes several hours or more. Viewed under a phase contrast microscope the initial phase separation involves formation of spherical, gelatin-rich droplets, as observed by Marrs.\(^2\) This leads from a slowly growing cloudiness to a final phase separation in which a yellowish, gelatin-rich layer is superimposed on a colorless, oligosaccharide-rich phase. The initial observations were that gelation essentially ‘‘freezes’’ in nonequilibrium demixing states when the rate of gelation is faster than the demixing rate. Similar observations have been made by other groups.\(^5\)\(^6\)

The immediate consequences of this phenomenon are twofold: First, it is impossible to determine the equilibrium phase behavior of gelled G/O mixtures since these may represent ‘‘frozen,’’ nonequilibrium states. Phase diagrams can only be reliably mapped above the gelation temperature after sufficiently long time for equilibrium to be reached (often several days). Second, from a practical point of view, this difference in demixing and gelation kinetics might be exploited to freeze in one phase nonequilibrium states fast enough that the resulting gel retains its desirable optical and rheological qualities. However, before addressing the complex issue of competition between demixing and gelation, it is important to have a good understanding of the equilibrium phase behavior of these mixtures.

In this article we concentrate first on dilute solution characterization of the gelatinos and oligosaccharides in terms of weight average molecular weight \(M_w\), root mean square radius of gyration \(R_g\), and second virial coefficient \(A_2\), which are relevant to their phase behavior. Then, phase diagrams are determined above the gelation temperature for oligosaccharides of different molecular weights and polydispersities. Observations on kinetics are presented along the way, but no systematic approach to detailed kinetic determinations were made in this work. Tromp et al.\(^4\) have recently used small angle laser light scattering (SALLS) to follow the spinodal decomposition of gelatin/dextran mixtures above and below the gelation temperature.

Finally, a semiquantitative analysis of the behavior in terms of standard Flory–Huggins theory for multicomponent solutions is made, with emphasis on the effects of oligosaccharide degree of polymerization and polydispersity.

**REVIEW OF LITERATURE**

The compatibility of gelatin with high molecular weight polymers has been studied: with dextran by Tromp et al.\(^4\) \((M_w = 160 \text{ kD})\) and Grinberg et al.\(^7\) \((M_w = 83 \text{ kD})\), and with methylcellulose \((M_w = 70 \text{ kDal})\) by Grishcenkova et al.\(^8\) In the latter system it was found that decreasing the pH of the solution below the gelatin isoelectric point (IP) enhanced the compatibility with methylcellulose, whereas an increase in ionic strength at the isoelectric point did not. Grinberg et al.\(^7\) likewise found increased compatibility between gelatin/dextran by decreasing pH below the gelatin IP, and also found a lower critical solution temperature.

From a fundamental point of view, the phase behavior of polymers in aqueous solution has been intensively studied (e.g., Ref. 3). Apart from the classical effects of polymer concentration and mo-
molecular weight,\textsuperscript{9} the most striking additional effect is that of polymer electrostatic charge.\textsuperscript{10,11} The compatibility of a mixture of two neutral polymers is greatly enhanced by the addition of only a few electrostatic charges per chain to one of the two polymers. This enhancement is due to the greater loss of entropy of mixing on phase separation of the polyelectrolyte, since the entity that phase separates is the molecule plus its counterions, as electroneutrality must be maintained. Khoklov and Nyrkova \textsuperscript{11} have modified the Flory–Huggins (FH) approach to include electrostatic interactions between either or both of the polymers in a ternary system. This has been tested, with only rough qualitative agreement, by Wang et al.\textsuperscript{12} for a ternary system of zinc polystyrene sulfonate/poly(ethyl acrylate-4-vinylpyridine)/tetrahydrofuran, in which the two polymers were weakly and oppositely charged to enhance compatibility.

In terms of thermodynamic modeling of the phase separation, surprisingly little has been done for solutions of gelatins with other oligomers and polymers. Durrani et al.\textsuperscript{13} used FH theory to model the phase diagrams of gelatin/amylpectin mixtures. They found reasonable agreement between theory and experiment; however, both the interaction parameters and the gelatin molecular weight were used as fitting parameters, rather than being determined independently. Although the FH theory is a fairly crude model for phase behavior,\textsuperscript{14} we feel that for this study it incorporates the essential thermodynamics of phase separation and that more sophisticated approaches will not necessarily give more insight into the key phenomena involved.

Since the oligosaccharide syrups are polydisperse, it is quite possible, a priori, that the effects of polydispersity on the gelatin/oligosaccharide phase diagram will be important. Because of its practical importance in fractionating polydisperse polymers into narrow fractions, much theoretical and computational work has been done on the detailed effects of polydispersity on phase behavior; e.g. Narasimhan et al.,\textsuperscript{15} Solc and Koningsveld,\textsuperscript{16} Beernaer et al.,\textsuperscript{17} An et al.,\textsuperscript{18} Schichtel and Bindel,\textsuperscript{19} and others.

The usual starting point for computation of cloud point and binodal curves is a model for the Gibbs free energy of mixing $\Delta G$, for several components. From this is calculated the chemical potential for each component $\Delta \mu_i (= \partial \Delta G / \partial n_i)$, where $n_i$ is the number of moles of component $i$. In the final two-phase equilibrium state, $\Delta \mu_i$ for each component $i$ is equal in both phases. This condition provides a series of equations to be solved iteratively, which yield the fraction of each component in each of the two separated phases along the binodal curve for a given set of polymer characteristics and enthalpic interaction parameters between components.

**EXPERIMENTAL**

**Materials**

Gelatins produced by acid processing of pigskins (termed PS), with an IP around 9, were provided by Systems Bio-Industries (France). The molecular weight $M_w$ and the polydispersity index $M_w/M_n$ for the first two successive extractions from a single batch of pigskin, PS1 and PS2, are summarized in Table I. The Bloom value is an industrial standard for the gel strength. Its value is proportional to the elastic modulus of a 66.7 g/L gelatin gel prepared in deionized water and matured for 16 hours at 10°C. The viscosity is that of a solution at the same concentration at 60°C.

Figure 1 shows titration curves for gelatin samples PS1 and PS2. They were determined in deionized water under nitrogen,\textsuperscript{20} using a Mettler DL70ES automatic titrator. Unless otherwise stated, experiments were carried out in water at various ionic strengths with unadjusted pH (about 5.6–5.7). Although polyampholytic, at this pH the gelatin had a net positive charge. The titration data show that under these conditions, each PS1 chain has about 50 (net) positive charges, i.e., 1 charge every 30 amino acids, so the charge density is low. This corresponds to one net elementary charge roughly every 11.2 nm, which gives a charge parameter of $\xi = 0.07$, that is, 0.07 net charges per Bjerrum length (0.72 nm in water at 25°C). At this low $\xi$ the ionic strength $C_s$ is practically equal to the added salt concentration. Sodium chloride was used as electrolyte.

For experiments lasting more than one day, 0.02% sodium azide (equivalent ionic strength = 3 mM) was added to prevent microbial contamination.

Monosaccharides and disaccharides were from Sigma Chemical Co. Oligosaccharides (glucose syrups) were obtained from Cerestar Co. (France). Glucose syrups are obtained by acid and/or enzymatic hydrolysis of starch. They are always polydisperse mixtures of oligo- and monosaccharides. The syrups are characterized and sold according to their dextrose equivalent number (DE), defined as

$$DE = \frac{100}{N_e}$$

where $N_e$ is the number-average degree of polymerization. In this work, four different syrups were used: DE40, DE36, DE21, and DE13. Dextran of $M_w$ around 15,000 and dextran sulfate of the same $M_w$ were obtained from Sigma.

G/O mixtures were prepared as follows: Dry gelatin granules were added to deionized water at $T = 60^\circ C$, and
Table I Characteristics of PS1 and PS2 Gelatin Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_w$ (kD)</th>
<th>$M_w/M_n$</th>
<th>IP</th>
<th>Bloom (g)</th>
<th>Viscosity (mPas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1</td>
<td>162</td>
<td>2.3</td>
<td>8.7</td>
<td>305</td>
<td>3.36</td>
</tr>
<tr>
<td>PS2</td>
<td>131</td>
<td>2.4</td>
<td>7.7</td>
<td>283</td>
<td>4.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Humidity %</th>
<th>Calcium (ppm)</th>
<th>Sulphate %</th>
<th>Chloride %</th>
<th>Phosphate (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1</td>
<td>10</td>
<td>80</td>
<td>&lt; 0.1</td>
<td>0.16</td>
<td>53</td>
</tr>
<tr>
<td>PS2</td>
<td>10</td>
<td>100</td>
<td>0.24</td>
<td>0.13</td>
<td>136</td>
</tr>
</tbody>
</table>

then gently stirred in a jacketed beaker connected to a temperature controlled bath at $T = 60^\circ$C. After the dissolution was complete (ca. 30 min.), oligosaccharide was added to the gelatin/water solution and stirred for 10 min at 65°C. One gram of the sample was then removed, sealed in a glass cell, and kept at $T = 45^\circ$C. Another gram was poured into a cuvette for turbidity measurement and left at $T = 10^\circ$C for 24 h.

Concentrations were measured by weighing the solutions and corrected for the gelatin humidity of 10% by weight, and the amount of water in the glucose syrup. The amount of water content in the syrup was estimated by measuring the difference in weight between a sample before and after complete evaporation of water. About 20% by weight of water was found in the syrup with DE40 and DE36. The others samples—DE21, DE13, saccharose and glucose—were powders and contained less than 1% water.

In all the phase diagrams, concentrations are expressed in volume percent. At 45°C the specific densities of gelatin and carbohydrate are 1.44\(^{21}\) and 1.54 g/mL,\(^{22}\) respectively.

Techniques

Static light scattering measurements were performed with a Wyatt Technology Dawn-F DSP, using instrument control and analysis software written by one of the authors (WFR). The Dawn-F is equipped with a vertically polarized 5 mW He-Ne laser, operating at a wavelength $\lambda = 633$ nm. Light scattering is measured simultaneously at 18 angles from 26° to 145°. Analysis of the dilute solution static light scattering was carried out using the standard Zimm single contact approximation, when $q^2 \langle S^2 \rangle < 1$,

$$\frac{Kc}{I(q)} = \frac{1}{M_w} \left( 1 + \frac{q^2 \langle S^2 \rangle}{3} \right) + 2A_2C_p$$ (2)

Here $C_p$ is polymer concentration in g/cm\(^3\), $M_w$ is the polymer weight average molecular weight, $\langle S^2 \rangle$ is the $z$-averaged mean square radius of gyration, $A_2$ the second virial coefficient in cm\(^2\)mole/g\(^2\), $q$ is the scattering wavevector amplitude $[q = (4\pi n/\lambda)\sin(\theta/2)]$, where $n$ is the solvent index of refraction and $\theta$ is the scattering observation angle, and $I(q)$ is the excess Rayleigh scattering ratio (i.e., solvent background subtracted scattering intensity). $I(q) = 1.408 \times 10^{-5}$ cm\(^3\)/cm for toluene at $T = 25^\circ$C, independent of angle, for vertically polarized incident light of $\lambda = 633$ nm. $K$ is an optical constant given for vertically polarized incident light by

$$K = \frac{4\pi^2 n^2 (dn/dc)^2}{N_A\lambda^4}$$ (3)

Here $N_A$ is Avogadro’s number, $n$ is the solvent refractive index, and $dn/dc$ is the differential refractive index for the polymer/solvent. The value of $dn/dc = 0.18$ mL g\(^{-1}\) has been used for gelatin,\(^{21}\) and $dn/dc = 0.15$ was used for the oligosaccharides.

As discussed below, the pore size for membrane filtration was important for eliminating aggregates in the dilute solution light scattering studies on gelatin. Unless otherwise noted, 0.1 μm Millipore Durapore membrane filter were used throughout.

Dynamic light scattering was carried out using a Brookhaven BI 2030 autocorrelator, with a vertically polarized argon ion source ($\lambda = 488$ nm).

Size exclusion chromatography (SEC) measurements of oligosaccharides were made using a system consisting of an ISCO 2350 pump and injector, Shodex OH-pak 805 and OH-pak 806 columns in series, and a separate Wyatt Dawn-F for multi-angle light scattering detection, and

![FIGURE 1 Titration curves for gelatin samples (a) PS1 and (b) PS2.](https://example.com/figure1.png)
an ANSPEC ERC 7522 differential refractometer. Data
collection and analysis software was written by one of
the authors (WFR), and the basis of the procedures has
recently been detailed.23

Turbidity measurements for gelled samples were made
on a Spectronic 21 spectrophotometer at 500 nm. Thirty
minutes before starting the measurements samples were
left at room temperature. Deionized water was used as a
blank.

RESULTS

Gelatin Characterization in Dilute
Solution

Because gelatin tends to aggregate, light scattering
measurements must be made carefully on dilute,
transparent solutions, in order to obtain reliable esti-
mates of molecular parameters. The authors are
acutely aware of the problem of aggregates, and at
the expense of brevity, have included the following
details of measurements and observations on the
pure gelatin/water solutions.

Dynamic light scattering (DLS) measurements
were made at \( T = 25^\circ \text{C} \) on salt-free solutions of
gelatin PS1 at concentrations between 0.1 to 1.0
mg/mL after filtering through 0.22 \( \mu \text{m} \) Millipore
Milllex GS filters. The autocorrelation function
showed a bimodal population, with a \( z \)-average ap-
parent hydrodynamic radius \( R_{H, ap} \approx 38 \text{ nm} \). When
the same stock solutions were filtered through a 0.1
\( \mu \text{m} \) filter, however, only a single fast relaxation
mode was observed with DLS, with \( R_{H, ap} \) of about
6 nm. When salt was added, the scattering intensity
increased and the single fast mode yielded a larger
\( R_{H, ap} \) of about 12 nm. Most simple models lead to
an expression for \( R_{H, ap} \) as

\[
R_{H, ap} = R_{H,0} \left( 1 + \gamma \frac{C_p}{C_e} \right)
\]

where \( R_{H,0} \) is the true hydrodynamic radius obtained
at \( C_p = 0 \), and \( \gamma \) is a constant involving \( A_2 \) and
hydrodynamic interactions (it is positive for \( A_2 \) posi-
tive).

These results are reminiscent of the ‘‘fast’’ and
‘‘slow’’ modes frequently observed in low ionic
strength polyelectrolyte solutions. The increase in
intensity and drop in \( R_g \) with increasing ionic
strength associated with the single fast relaxation
mode is well known and understood for polyelectro-
lytes. Recently, it was shown that the slow mode in
many polyelectrolyte solutions can be permanently
removed by filtering the solutions through suffi-
ciently small pore size membranes.24±27 Although
this removability of the slow mode is also seen here
for the gelatin, it is important to note that, whereas
the aggregates never reappeared for such polyelec-
tralytics as poly(\( L \)-lysine), poly(styrene sulfonate),
heparin, chondroitin sulfate, poly(\( \text{methyl acrylate} \)
copolymers, and others, aggregates do reappear in
the gelatin solutions. These aggregates grow with
time, are irreversible, and are due to the inherent
‘‘stickiness’’ of the gelatin molecules toward each
other. In contrast, it has been conjectured that the
permanently removable aggregates responsible for
the slow mode in other polyelectrolyte solutions are
due to incompletely dissolved material, the type and
amount of which is critically dependent on the precip-
itation and other steps used in preparing the dry
polyelectrolyte previous to reconstitution in solu-
tion.

Consistent with the results of Boedtker and Doty,28
aggregation at low ionic strength was more pro-
nounced than at high ionic strength. A series of Zim-
plots at \( T = 25^\circ \text{C} \) for PS1 were made at low and high
ionic strength at different times. Solutions of fixed
concentration were prepared at the same time and
allowed to age, with Zimm plot determinations being
made at intervals. Figures 2a±c show the time evolu-
tion of \( M_w, R_g, \) and \( A_2 \), respectively. It is seen that
both the mass and \( R_g \) increase in time, and \( A_2 \) becomes
increasingly negative. Aggregates reached masses of
over \( 10^3 \) after roughly a week and eventually precipi-
tated at low ionic strength. At the relatively high ionic
strength of 150 mM \( \text{NaCl} \) a small amount of aggrega-
tion occurred over two to three days, after which the
solution remained stable for weeks. In this case, the
aggregates had molecular weights of the order of
300,000, 50\% higher than in the initial solutions.
\( R_g^2/M_w \) (not shown) remains approximately constant
in time and suggests that the aggregates maintain
the same essential random coil structure (for which
\( R_g^2 \propto M \)), and do not involve increasingly
dense aggregates, where \( R_g^2/M_w \) would decrease in
time.

Instantaneous dilution of the large aggregates
yielded a slightly positive \( A_2 \); i.e., the aggregates
are stable against dilution, and their size is not ap-
preciably concentration dependent. The kinetics of
formation, however, are concentration dependent,
since the higher concentration solutions precipitated
before the lower concentration ones; i.e., the nega-
tive \( A_2 \) values found for measurements on the same
aggregating samples on succeeding days is actually
a nonequilibrium effect, reflecting the different ki-
the gelatin has net charge at the pH studied, it is still a polyampholyte, containing both positive and negative charge groups. Whether the aggregation is due to purely electrostatic forces or other forces or interactions, such as secondary structural changes, which might be sensitive to ionic strength, but are not directly ionic, is not pursued here.

The gelatins show interesting polyelectrolyte properties. Figure 3 shows typical $Kc_p/I(q)$ vs $q^2$ for PS2. At no added salt for 1 mg/mL gelatin, there is a negative slope for $Kc_p/I$ vs $q^2$, which becomes positive with addition of even a small amount of salt (e.g., 1.2 mM NaCl). Such negative slopes of $Kc_p/I$ vs $q$ are typical of polyelectrolytes at no added salt,24,29–31 and have been interpreted in terms of liquid-like correlations due to electrostatically enhanced excluded volumes between particles.24,31–33

As salt is added, $A_2$, which measures the effective excluded volume between the gelatin molecules, decreases. This is manifested as a decreasing intercept in the $Kc_p/I$ vs $q^2$ curves at fixed $C_p$. Approximate $A_2$ vs $C_p$ for PS2 is shown in Figure 4.

**Oligosaccharide Characterization**

Oligosaccharide solutions were always transparent up to the highest concentrations used for light scattering measurements. There was no evidence of aggregates or time dependent aggregation.

Because the nature of the interaction of the oligosaccharides with the solvent is an important factor in determining the G/O phase behavior, it was necessary to estimate $A_2$ for the syrups and saccharides. Although traditionally the molecular mass and $A_2$ of low molecular weight polymers has been determined by osmometry, there is no reason, a priori, that the same determinations cannot be made by static light scattering. For the oligosaccharide syrups and for solutions of glucose and saccharose, there was no angular dependence to the intensity of scattered light, as expected. Figure 5 shows $Kc_p/I$ vs $q^2$ curves at $\theta = 90^\circ$. Figure 6 shows the corresponding $A_2$ vs $M_w$, which follows the empirical fit.

$$A_2 (\text{cm}^3 - \text{mole/g}^2) = \frac{0.22}{M^{0.71}} \quad (5)$$

This mass dependence of $A_2$ is between that for an ideal random coil ($A_2 \propto M^{-0.5}$), and a sphere ($A_2 \propto M^{-1}$), whereas for a random coil with excluded volume $A_2 \propto M^{-0.2}$. Also shown in the figure is the Flory–Huggins parameter $\chi$, computed according to
Gelatin / Oligosaccharide Mixtures

**FIGURE 3** Typical $\frac{KCP}{I}$ vs $q^2$ for PS2 gelatin solutions with and without salt (NaCl), showing negative slope at no salt, turning positive with added salt.

$$\chi = \frac{1}{2} \left(1 - 2\rho_o^2 v_o A_2\right)$$

where $\rho_o$ is the oligosaccharide density, taken as 1.542 g/cm$^3$, and $v_o$ is the solvent specific volume, which for water is 18 cm$^3$/mole; i.e. $\chi = 0.5 - 43A_2$.

Surprisingly, the batch light scattering revealed that $M_w$ was much larger than the manufacturer’s specified $M_n$ of the syrups, suggesting extremely high polydispersity. This led to SEC characterization on all the oligosaccharides used during the experiments. Figures 7a–c show data from the raw chromatograms for DE36, DE21, and glucose, respectively. The raw data show the voltages from the refractive index detector (RI) and light scattering at $\theta = 90^\circ$ vs elution volume. The RI data are essentially monomodal with a small shoulder at low elution volume. For each of the DE36 and DE21 light scattering spectra, however, there are two peaks; a dramatically large light scattering peak precedes a narrower light scattering peak at high elution volumes. Since the RI signal measures concentration and the light scattering measures the product of molecular mass and concentration, these results indicate that all three syrups contain small fractions of high molecular weight starch fragments, in addition to the majority population of small oligosaccharides, which form the bulk of the RI peak. The existence of these high molecular weight fractions in glucose syrups does not seem to have been observed previously. This is probably due to the fact that the traditional method of measuring molecular weight distributions for oligosaccharides does not separate and detect oligosaccharides above decamers. Using SEC coupled with a light scattering detector, however, allows both sterically based separation of the larger masses and highly sensitive detection of small populations of them.

Although one does not usually expect a highly

**FIGURE 4** Estimates of $A_2$ vs $C_s$ (Molar ionic strength) for PS2.

**FIGURE 5** $KC_p/I(\theta = 90^\circ)$ vs $C_p$ for different ‘sugars’ and syrups.

**FIGURE 6** $A_2$ and $\chi$ values vs. $M$ for the different ‘sugars’ and syrups (mono-, di-, and oligosaccharides), from batch SLS, determined from Figure 5 and Eqs. (2) and (4).
polydispersity $M_w/M_n$ in each of the samples is striking.

**Phase Behavior Data**

Phase diagrams for monosaccharide, disaccharide, and syrups with DE40, DE36, DE21, and DE13 were determined by leaving sealed samples for several days at $T = 45^\circ C$. The stock solution was initially prepared at $T = 60^\circ C$ and several samples were obtained by successive dilution of the stock, before being left in an oven at $45^\circ C$. Thus, each stock solution gave a line of samples with the same G/O ratio. The boundary between the single phase and two phase samples along each dilution line permitted the determination of the binodal line. A typical phase diagram for PS2/DE36/water at $T = 45^\circ C$ is shown in Figure 9. The open points represent one-phase samples and the solid points are two-phase samples. The experimental resolution of the phase boundary is approximately the size of the symbols in this and the other phase diagrams.

The region above the binodal curve (dashed line) corresponds to the two-phase systems while the region under the line represents one phase systems. The more compatible the G/O mixture, the higher the binodal and the larger the region under it. For mono- or disaccharide systems, there was no phase separation in the concentration range studied (up to a volume fraction of 47%). These samples are represented in Figure 9 by open star symbols for glucose and open triangle symbols for saccharose.

For all the syrups, phase separation was observed under certain conditions. Binodal curves obtained for different syrup and for $G/dextran/W$ are shown in Figures 10a,b for PS1 and PS2, respectively.

There is some difference in the phase diagram accurate molecular weight from light scattering from low molecular weight materials, Figure 8 shows the approximate cumulative mass fraction of the samples, obtained from the full light scattering treatment of the data. From these figures it can be seen that the two syrups have long high mass tails, which are wholly absent for glucose and saccharose. This is predicted to have a profound effect on phase behavior of the G/O system, as discussed below.

Table II shows the $A_2$ and $M_w$ determined by static light scattering and the $M_w$ and $M_n$ by SEC. Again, although the absolute values of $M_w$ and $M_n$ may contain some systematic error, the very large

![FIGURE 7 SEC results for syrups: Raw data consisting of RI and $\theta = 90^\circ$ light scattering voltages for (a) glucose, (b) DE21, (c) DE36, and (d) DE40.](image1)

![FIGURE 8 Cumulative mass fractions for DE20 and DE36 from the SEC data.](image2)
between PS1 and PS2, but the most significant difference is due to the syrup; different syrups give quite different binodals. On increasing the syrup molecular weight (i.e., decreasing the DE), the coexistence lines move to lower syrup and gel concentrations.

Figures 11a,b show the lack of effect of salt concentration on the binodal. We investigated the effect of 1 mM NaCl on PS2/DE40/water (Figure 11a) and the effect of 150 mM NaCl on PS2/DE36/water (Figure 11b). The dotted line and the crosses were determined in pure water, and the triangles show the phase boundary for salt solutions. The phase separation occurs at the same points as with pure water; i.e., the presence of salt does not affect the compatibility of these systems at $T = 45^\circ$C. Increasing ionic strength, however, did increase phase separation kinetics in the metastable zone (see below).

Figures 12a and 12b show the effect of pH on the compatibility of PS2/DE13/water mixtures. At pH 2 all compositions of syrup and gelatin are compatible, while at pH 5.6 and pH 8 there is a phase separation. As the pH decreases the net charge on the gelatin increases (see Fig. 1), and the compatibility of the system increases. This is consistent with the known fact that increasing the charge density of a polyelectrolyte greatly increases its compatibility with other polymers at low to moderate ionic strength, since there is both a high entropic penalty in separating polyelectrolytes and their small counterions into a phase of reduced volume, and an electrostatic enthalpic penalty due to confining the charged polymers in the reduced volume. The three highest concentration points in Figure 12a were repeated with 150 mM NaCl at pH 2.0, and were also found to be compatible, so the enhanced compatibility due to electrostatic charge persists even when such interactions are well screened.

**Effect of Polydispersity by Mixing Syrups and/or Other Carbohydrates.** In order to investigate the effect of oligosaccharide polydispersity, syrups of different size were mixed with gelatin and water. For example, all the samples in a dilution series starting from a 8.75% gelatin and 41.5% saccharose were compatible. When 20% of the saccharose was replaced by DE13 syrup, the samples with more than 29% volume percent of oligosaccharide were incompatible.

The simple Flory–Huggins model considered below suggests that the weight average mass $M_w$ of the syrups is far more important in determining demixing behavior than $M_n$. To test this idea we made saccharose/DE13 mixtures having the same $M_n$ as the DE40 and DE21 syrups. The first mixture contained 23.8% DE13/76.2% saccharose, yielding $M_w = 6400$ and $M_n = 420$, and the second mixture contained 50.43% DE13/49.57% saccharose, yielding $M_w = 13,200$ and $M_n = 550$.

The computations of these above $M_n$ and $M_w$ were made according to the following equations, which
can be simply derived for a mixture of \( N \) different polydisperse populations, each with a concentration fraction \( f_i \), and weight and number average molecular weights of \( M_{w,i} \) and \( M_{n,i} \), respectively:

\[
M_w = \sum_{i=1}^{N} f_i M_{w,i} \tag{7a}
\]

\[
\frac{1}{M_n} = \sum_{i=1}^{N} \frac{f_i}{M_{n,i}} \tag{7b}
\]

where the \( f_i \) obey the normalization condition \( \sum_{i=1}^{N} f_i = 1 \). For the \( N = 2 \) system of the above mixtures, for saccharose \( M_{n,1} = M_{w,1} = 342 \), and

for DE13 \( M_{w,2} = 26,000 \), was taken as an average value from Table II, and the manufacturer’s value of \( M_{n,2} = 1400 \) (from \( N_n = 100 / 13 \) ) was used.

These mixtures were used to prepare dilution series starting from composition of 5% gelatin and 29.13% syrup. Point 1 on Figure 10b is the phase boundary for the first mixture (which simulates the \( M_w \) of DE40) and point 2 is the phase boundary for the second mixture (which simulates the \( M_w \) of DE20). The phase boundaries for the mixed syrups are identical to those of the respective syrups whose \( M_w \) values they equal.

**Experimental Attempt to Increase Compatibility of Longer Chain Polysaccharides.** The critical importance of the degree of polymerization on the phase behavior has been clearly demonstrated in the preceding section by showing how compatibility sharply decreases with increasing oligosaccharide \( M_n \). This is a direct consequence of the decreasing entropy penalty for phase separation for longer chains. The question then arises as to whether the least compatible systems, i.e., those with the longest oligosaccharides, might be made compatible by increasing the enthalpic penalty of demixing. To test this a negatively charged, long-chain polysaccharide, dextran sul-
Observations on Demixing Kinetics

For the phase behavior at a given temperature there is a zone of metastable states on the phase diagram. This region is bounded by the spinodal curve at the top, for which "instantaneous" instability (as observed by immediate cloudiness in the solutions) and subsequent phase separation occurs, and by the binodal curve on the bottom, along which there is no free energy difference between the mixed and separated states. Between these two curves, there is a zone of metastable compositions, for which the demixing, defined as time before noticeable cloudiness occurs takes a finite time. This can be up to several days in the case of the G/O system. In Figure 9 the solid circles represent samples that were cloudy within 12 h, which we define operationally as within the spinodal. The shaded circles show compositions that were still transparent after 12 h and became cloudy subsequently. We define these as within the metastable region. The open symbols show compositions that did not become cloudy even after several months. For experimental convenience we have represented the phase diagrams in terms of the binodal.

The phase separation kinetics in the metastable zone depended on ionic strength; higher ionic strength promoted faster phase separation kinetics, without appreciably changing the phase diagram. For this reason also, clouding was much faster for FIGURE 12 Increased compatibility of G/O by lowering pH: Demixing line for PS2 gelatin and DE13 syrup with no salt at pH 5.6 (+). The open squares show the same system, but at pH 2, with and without 150 mM NaCl, for which no demixing occurred anywhere along the points shown. (b) Demixing line for PS2 gelatin and DE36 syrup with and without 150 mM salt at pH 5.6 (+). The solid triangle shows the demixing point for the same system at pH 8; there is no discernible difference compared to pH 5.6.

fate (DS) of $M_w = 15,000$ was used. It was surmised that the electrostatic attraction between the negatively charged DS and positively charged PS gelatin would enhance compatibility. In fact, there was no demixing with dextran sulfate and PS2 in pure water at least up to point `2` in Figure 10b, clearly showing the compatibility enhancement. Interestingly, this compatibility enhancement was observed even when the experiment was repeated with 150 mM NaCl, despite the greatly reduced magnitude of electrostatic interactions at high electrolyte concentration.

When samples are cooled, or "quenched," two separate effects occur: (a) Below the gelation temperature, if gelation proceeds more quickly than demixing, the gelation can actually "freeze-in" transparent metastable states. This is illustrated in Figure 13a, where a well-mixed sample of PS1 with no added salt was cooled from 60 to 10°C by placing the sample in the refrigerator. For this system quenching moves the "apparent binodal" to higher concentrations, i.e., it increases the apparent compatibility. (b) The equilibrium phase behavior changes as a function of the temperature. Hence, for example, samples which are in the one phase or metastable regions at high temperature can cross the spinodal on cooling, causing instantaneous clouding. This is illustrated in Figure 13b, where a similar quench from 60 to 10°C for PS2 with no salt (but
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with a few mM of intrinsic ionic strength, as mentioned) moved the ‘‘apparent binodal” to lower concentrations. The opposite can also occur—that is, samples that are cloudy at high temperature (i.e., in the process of phase separation) can become clear on quenching. This was observed for several samples, such as PS1/DE40/no salt from 1.68% gel/39.5% syrup to 2.21% gel/45.1% syrup, which were cloudy at 60°C but became clear at 10°C.

**DISCUSSION**

Faced with the problem of phase separation in the G/O systems, the first question is to identify the controlling factors. A priori the list includes volume fractions of G, O, and water, temperature, ionic strength and chemical identity of added salts, pH, average molecular masses of G and O, the polydispersity of the G and O populations, the type of gelatin used, and possibly the purity of the G and O starting materials.

The most salient feature of the experimental demixing data is that the chain length of the syrup has the most profound effect. The effect of ionic strength at unadjusted pH is secondary compared to syrup chain length and polydispersity, and has more effect on the kinetics of demixing than on the phase diagram. Data indicate that if the pH is lowered to the point where the gelatin becomes more highly charged, however, the compatibility increases markedly. A systematic study of the effects of charge state on the phase diagram is deferred to future work. The object at this point is to qualitatively rationalize the trends observed in phase behavior, but not to construct a quantitatively rigorous model.

Thermodynamically, the free energy of mixing $\Delta G_{\text{mix}}$, and hence the phase behavior, is controlled by the entropy of mixing of the solution components, and the energy of interaction between them. In general, for a given level of interaction energy between components, the higher the degree of polymerization of the components, the more incompatible the system, since the entropy penalty for phase separation decreases. The extent to which this occurs will depend on the enthalpy/entropy ratio for a given degree of polymerization and interaction energy.

**Flory–Huggins Model**

For the purposes of rationalizing the data, the standard Flory–Huggins lattice model for free energy of a system with several components is used. The early works of Tompa$^{34}$ and Scott$^{35}$ provide a good background on how the FH model was originally adapted to three component and higher mixtures involving polymers.

We have not dealt explicitly with the electrostatic interaction here, so in fact it is incorporated into the enthalpic interaction parameters. For the future, low pH work this may no longer be adequate. Let $\phi_i$ and $N_i$ be the volume fraction and degree of polymerization, respectively, of component $i$, and $\chi_{ij}$ is the enthalpic interaction parameter between components $i$ and $j$. The excess free energy of mixing per mole of lattice sites of the multicomponent solution containing $m$ components is

$$\frac{\Delta G}{RT} = \sum_{i=1}^{m} \frac{\phi_i}{N_i} \ln \phi_i + \sum_{i \neq j}^{m} \sum_{j=1}^{m} \chi_{ij} \phi_i \phi_j$$  \hspace{1cm} (8)

$\Delta G$ must be negative for mixing (compatibility) of
the system to be favored, but the stability of the system is determined by the second derivatives of \( \Delta G \). For the multicomponent system, this stability criterion is given by the spinodal curve, which is found from the condition that \( \det Q = 0 \), where \( Q \) is an \((m - 1) \times (m - 1)\) matrix, whose components \( Q_{ij} \) are given by

\[
Q_{ij} = \frac{\partial^2 \Delta G}{\partial \phi_i \partial \phi_j} \quad (9)
\]

We note that although we are carrying out this analysis in terms of the spinodal, and the data have been represented in terms of the binodal (Figures 9–13), we assume, with some experimental justification (e.g., Figure 9), that the difference between the two lines is quite small compared to the effects due to differences in oligosaccharide chain length and polydispersity. This avoids the computationally intensive procedures associated with computing the binodal curve. For the three-component system gelatin/oligosaccharide/water \((m = 3)\), the spinodal line is given by

\[
\left( \frac{1}{N_1 \phi_1} + \frac{1}{\phi_3} - 2\chi_{13} \right) \left( \frac{1}{N_2 \phi_2} + \frac{1}{\phi_3} - 2\chi_{23} \right) - \left( \frac{1}{\phi_3} - \chi_{13} - \chi_{23} + \chi_{12} \right)^2 = 0 \quad (10)
\]

where \( \phi_1 = \phi_{\text{gelatin}}, \phi_2 = \phi_{\text{oligosaccharide}}, \) and \( \phi_3 = \phi_{\text{solvent}} = \phi_s \), where \( \phi_s = 1 - \phi_1 - \phi_2 \). This equation is equivalent to the standard quadratic form

\[
a\phi_1^2 + b\phi_1 + c = 0 \quad (11)
\]

where \( N_1 \) and \( N_2 \) represent the gelatin and oligosaccharide degree of polymerization, respectively. To save the interested reader, the algebraic labor involved in converting Eq. (9) to the quadratic form, the constants \( a, b, \) and \( c \) are given:

\[
a = 2\chi_{13}N_1(1 - 2\chi_{23}N_2\phi_2) + N_1N_2\phi_2(\chi_{12} - \chi_{13} - \chi_{23})^2 \quad (12a)
\]

\[
b = (N_1 - 1) + 2\chi_{23}N_2\phi_2(1 - N_1)
\]

\[
+ 2\chi_{13}N_1(\phi_2 - 1 - N_2\phi_2)
\]

\[
+ 4\chi_{13}\chi_{23}N_2N_2\phi_2(1 - \phi_2)
\]

\[
+ 2(\chi_{13} + \chi_{23} - \chi_{12})N_1N_2\phi_2
\]

\[
- N_1N_2\phi_2(1 - \phi_2)(\chi_{12} - \chi_{13} - \chi_{23})^2 \quad (12b)
\]

\[c = 1 + (N_2 - 1)\phi_2 - 2\chi_{23}N_2\phi_2(1 - \phi_2) \quad (12c)
\]

The \( \chi_{ij} \) parameters in the above equations group together the interactions of the components \( i, j \), and \( ij \), that is,

\[
\chi_{ij} = \chi_{ij}^{\text{dir}} - \frac{\chi_{ii}^{\text{dir}} - \chi_{ij}^{\text{dir}}}{2} \quad (13)
\]

where \( \chi_{ii} \) and \( \chi_{ij}^{\text{dir}} \) represent the interactions of \( i \) with \( i \), and \( j \) with \( j \), respectively, and \( \chi_{ij}^{\text{dir}} \) is the ‘‘direct’’ interaction between components \( i \) and \( j \). These direct interactions are related to \( A_2 \) via Eq. (6). \( A_2 \) measures the interactions between any pair of components via the two body excluded volume integral \( B \),

\[
A_{2,ij} = \frac{N_A}{2M_iM_j} \beta_{ij} \quad (14)
\]

where

\[
\beta_{ij} = \int [1 - e^{-U(r_{ij})/kT}]d^2r_{ij} \quad (15)
\]

and \( U(r_{ij}) \) is the potential energy between particles \( i \) and \( j \) at a distance of \( r_{ij} \) from each other.

Using Eq. (6) and the \( A_2 \) for the gelatins at finite salt, \( \chi_{13} = 0.49 \). The values of the oligosaccharide/solvent interaction \((=\chi_{23})\) are shown in Figure 6. In what follows, \( 1 = \) gelatin, \( 2 = \) oligosaccharide, and \( 3 = \) water. Using these values, a gelatin polymerization number of \( N_1 = 400 \), and an assumed gelatin/oligosaccharide interaction value of \( \chi_{12} = 0.25 \), leads to the spinodal lines shown in Figure 14, for different values of the oligosaccharide polymerization number \( N_2 \). For \( N_2 = 1 \) and 2 there is no incompatibility, but above this, increasing \( N_2 \) rapidly and dramatically decreases compatibility, as seen by the spinodal line shifting lower in the diagram.

The \( \chi_{23} \), the oligosaccharide interaction parameter with the solvent, is important. For a theta relationship between oligosaccharide and water \((\chi_{23} = 0.5)\), the relationship between gelatin and water, \( \chi_{13} \), is relatively unimportant; i.e., from \( \chi_{13} = 0 \) to 0.5, there is little effect on the demixing curve. For an athermal relation between oligosaccharide and water \((\chi_{23} = 0)\), however, the effect of varying \( \chi_{13} \) is quite pronounced.

The effect of the interaction between gelatin and oligosaccharide, as measured by \( \chi_{12} \), is obviously important, since it is the positive enthalpy of interaction of these two species that drives the phase sepa-
compatibility of the mixture as the chain length of the oligosaccharides increases. \( N_1 \) = 400, \( \chi_{12} \) = 0.25, \( \chi_{13} \) = 0.49. From top to bottom the curves have oligosaccharide polymerization number \( N_2 \) and \( \chi_{23} \) as follows: (3, 0.391), (4, 0.410), (5, 0.424), (7, 0.438), (10, 0.452), (50, 0.485).

For the case of PS1, for example, which has an enormous effect on the G/O phase behavior, it is reasonable that varying degrees of polydispersity among different lots of syrup with nominally identical DE (\( N_a \)) values should produce significant differences in phase behavior.

The simplest approach to the polydispersity problem is to continue using the Flory-Huggins model and to work in the discrete representation for the free energy of a multicomponent system, and consider oligosaccharides of two different sizes. While this is an oversimplification of a polydisperse system, it still provides insight into the effect of different size polymers of the same species on phase behavior. The Flory-Huggins model now involves a quaternary system, and \( Q \) is a \( 3 \times 3 \) determinant, for which the solution is

\[
a(bc - f^2) - d(df - ef) + e(df - eb) = 0
\]

where

\[
a = \frac{\partial^2 \Delta G}{\partial \phi_1^2} = \frac{1}{N_1 \phi_1} + \frac{1}{\varphi_4} - 2\chi_{14} \quad (17a)
\]

\[
b = \frac{\partial^2 \Delta G}{\partial \phi_2^2} = \frac{1}{N_2 \phi_2} + \frac{1}{\varphi_4} - 2\chi_{24} \quad (17b)
\]

\[
c = \frac{\partial^2 \Delta G}{\partial \phi_3^2} = \frac{1}{N_3 \phi_3} + \frac{1}{\varphi_4} - 2\chi_{34} \quad (17c)
\]

\[
d = \frac{\partial^2 \Delta G}{\partial \phi_1 \partial \phi_2} = \frac{1}{\varphi_4} + \chi_{12} - \chi_{24} - \chi_{14} \quad (17d)
\]

\[
e = \frac{\partial^2 \Delta G}{\partial \phi_1 \partial \phi_3} = \frac{1}{\varphi_4} + \chi_{13} - \chi_{34} - \chi_{14} \quad (17e)
\]

\[
f = \frac{\partial^2 \Delta G}{\partial \phi_2 \partial \phi_3} = \frac{1}{\varphi_4} + \chi_{23} - \chi_{34} - \chi_{24} \quad (17f)
\]

Figure 15 shows some of the salient features of various bimodal oligosaccharide distributions. In referring to the parameters below and in Figure 15, 1 = gelatin, 2 = short oligosaccharide, 3 = long oligosaccharide, and 4 = water. Table III gives the list of parameters used for the curves, as identified from top to bottom. The interaction between the two oligosaccharides was taken as \( \chi_{23} = 0.00 \), which corresponds to athermal solutions conditions; i.e., it is assumed the oligosaccharides interact with each other the same way they interact with themselves. The gelatin polymerization number was taken as \( N_1 = 400 \), in accord with the molecular weight determinations, the gelatin/water interaction was taken as \( \chi_{14} = 0.49 \), following the \( A_s \) determination, and the...
Gelatin/oligosaccharide interaction parameters were taken as $\chi_{12} = \chi_{13} = 0.25$.

Several important trends are immediately apparent in Figure 15: The solutions become increasingly incompatible as $N_w$ (and hence $M_w$) increases, regardless of $N_n$ (and hence $M_n$). The fourth curve from the top, in fact, has a lower $N_n$ (=3.15) than the first curve ($N_n = 5$), and yet is less compatible since its $N_w$ (=26.7) is larger than the top curve’s ($N_w = 5$). Such cases were seen experimentally when an even wider range of syrups than presented here were tested for compatibility (SBI, Alan Parker, unpublished results).

Although syrups are commercially specified in terms of DE (i.e., $N_n$), this parameter is actually unrelated to the compatibility. Furthermore, the top two curves are virtually indistinguishable, and in fact have the same $N_n$ (=5), whereas their $N_w$ values differ (3.2 vs 5). In fact, for the athermal oligosaccharide conditions assumed, the spinodal line is virtually independent of the details of the bimodal distribution of oligosaccharide, and only depends on $M_w$. This conclusion was first stated by Koningsveld and Staverman. This conclusion does not continue to apply, however, if the oligosaccharides do not interact athermally (i.e., when $\chi_{23} \neq 0$). Also, recent numerical work has shown this conclusion does not apply if both polymer species are significantly polydisperse with different shape distributions. Finally, the fourth line from the top illustrates a striking effect. Namely, the compatibility of a highly compatible syrup, with $N = 3$, say, can be virtually destroyed by the addition of a small percentage of long chain polysaccharides. On that line a mere 5% weight fraction of 175 unit polysaccharide has dramatically reduced the compatibility of the $N = 3$ syrup (see top curve in Figure 15, for unimodal $N = 3$ curve).

**Summary**

The factors controlling the phase separation of G/O systems, when the gelatin has a small or zero net charge, have been defined and investigated experimentally. Both experiment and calculations based on Flory–Huggins theory show that $M_w$ is an excellent indicator of the compatibility of polydisperse syrups, virtually independently of the detailed mass distribution and polydispersity, whereas $M_n$ is virtually unrelated to the phase behavior. The kinetics of the phase separation, however, does depend on the gelatin/gelatin interaction, so that highly screened gelatins (in moderate ionic strength solutions) may more quickly undergo phase separation. Since the demixing and gelation have their own kinetic time scales, whereby gelation can “freeze in” nonequilibrium states when it proceeds more rapidly than demixing, highly charged gelatin (away from the IP) in very low ionic strength solutions may get frozen in by gelation before demixing, giving a transparent gel with good mechanical properties.

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**Table III Spinodal Line Parameters for Fig. 15**

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<tr>
<th>$N_2$</th>
<th>$N_3$</th>
<th>$\chi_{24}$</th>
<th>$\chi_{34}$</th>
<th>$\phi_2/\phi_3$</th>
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<th>$N_w$</th>
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FIGURE 15 Effect of polydispersity on phase separation: All the spinodal lines have the following parameters constant: $\chi_{12} = \chi_{13} = 0.25, \chi_{14} = 0.49, \chi_{33} = 0.00, N_i = 400$. The spinodal lines from top to bottom have the parameters found in Table III. Note that incompatibility increases strictly in accordance with $M_w$ and is independent of $M_n$. 

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