Rheological interaction of sage seed gum with xanthan in dilute solution

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Abstract
Polysaccharide-polysaccharide interactions are attractive in the food, polymer and pharmaceutical systems because they may impart novel or improved functional properties to products and reduce costs. In this work, the rheological interaction of sage seed gum (SSG) as a novel hydrocolloid with xanthan gum (XG) was studied in the dilute region. The intrinsic viscosity of the XG-SSG mixture at five blending ratios (100:0, 75:25, 50:50, 25:75, 0:100) and three temperatures (25, 40 & 55°C) was determined. Antagonistic interaction between sage seed gum and xanthan gum was occurred at 25°C and 40°C, while synergistic interaction was observed for all XG-SSG blends at 55°C.

Introduction
Polysaccharides extracted from plants are considered as new sources of hydrocolloids in the food/pharmaceutical area and they are more desirable for many applications according to their functionality, safety, availability and low processing costs. It is well known that there is still a significant search for new natural hydrocolloids with excellent functional properties and good price, which could be a potential alternative to some commercial gums. Some plants, growing in different regions of Iran, have valuable polysaccharides, whose seeds extract can be used as novel hydrocolloid sources (Razavi et al., 2007).

The genus salvia (Labiatae) contains more than 700 species, which about 200 out of them exist in Iran and is also found in neighboring countries. Plants belonging to this genus are pharmacologically active and have been used in folk medicine all around the world. Wild sage seed (Salvia macrosiphon) is a small rounded seed, which quickly produces a transparent mucilaginous gum as it is wetted by water. It has been traditionally used in Iran for pharmaceutical and food applications (Razavi et al., 2010). The conditions of sage seed gum (SSG) extraction have been previously optimized by Bostan et al. (2010). Steady shear flow properties and viscoelastic behavior of SSG have been also investigated (Razavi et al., 2011; Razavi et al., 2013). It was reported that SSG has high viscosity, yield stress, strong shear thinning and gel like characteristics, which were comparable with commercial gums. It was found that the hydrocolloid extract from SSG can be used as a novel hydrocolloid and it has excellent functional properties as a stabilizer, thickener, binder and gelling agent in food, cosmetics and pharmaceutical.

Razavi et al. (2012) recently studied the effect of salt type (sodium and calcium chlorides), salt concentration (0, 0.5, 20 and 50 mM) and temperature (20, 30 and 40°C) on the properties of dilute sage seed gum (SSG) solutions. The increase in ionic strength of the NaCl and CaCl$_2$ from 0 to 0.5 mM caused to increase in intrinsic viscosity, but increasing the temperature from 20 to 40°C and salts concentrations from 0.5 to 50 mM decreased the intrinsic viscosity. Divalent ions from CaCl$_2$ showed a more pronounced effect on the intrinsic viscosity compared with monovalent ions from NaCl. The weight-average molecular weight of sage seed gum was obtained as 1.5x10$^6$ Da.

Synergistic polysaccharide–polysaccharide interactions are attractive in the food industry because they impart novel and improved texture and rheological characteristics to food products and reduce polymers costs (Williams and Phillips, 2000). One of the easiest ways to assess and identify the interactions between gums is measurement of intrinsic viscosity. Intrinsic viscosity is an ability of polymer to increase the viscosity of solution. The viscosity measurement of binary blends has been a very useful approach to understand how molecules behave and interact in solution (Wang, 2001). In recent years, aqueous polysaccharide mixtures have attracted great interest and many studies have characterized the polysaccharide-polysaccharide behavior in dilute solution. For example, Richardson et al. (1998),

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Khouryieh et al. (2006) and Khouryieh et al. (2007) determined the intrinsic viscosity of guar-locust bean gum, xanthan-guar gum, and deacetylated xanthan-guar gum, respectively. Characterization of xanthan gum and sage seed gum behavior in dilute solution is important in understanding their performance and application where they are used jointly. Therefore, the objective of this paper was to investigate the effect of mixing temperature (25, 40 and 55 ºC) and blending proportions (100:0, 75:25, 50:50, 25:75 and 0:100) on the rheological behavior of xanthan-sage seed gum mixture dilute solution.

**Materials and Methods**

**Preparation of samples**

The sage seeds were procured from a herb plants market, Mashhad, Iran. The seeds were manually cleaned to remove all foreign matter such as dust, dirt, stones, chaff, immature and broken seeds. Xanthan gum was purchased from Sigma Com, Spain. Sage seed gum was extracted, at optimized conditions, using the same procedure as described by Bostan et al. (2010). The dispersions were dried overnight in a forced convection oven (Model 4567, Kimya Pars Com., Iran) at 70°C, then milled and sieved using a mesh 18 sifter.

To study the interaction between the selected gums in dilute domain, they were blended at the following ratios: 100% XG: 0% SSG; 75% XG: 25% SSG; 50% XG: 50% SSG; 25% XG: 75% SSG and 0% XG: 100% SSG. Sample solutions (w/w) were prepared by dispersing the hydrocolloid powder in de-ionized water, and stirring at room temperature overnight for complete hydration. To study the effect of temperature, the intrinsic viscosity of XG and SSG and their blends were also determined at 25°C, 40°C and 55°C.

**Solution viscosity measurement**

The solution viscosity of XG, SSG and their blends were measured a Cannon-Ubbelohde viscometer (No. 75, Cannon Instruments, USA; viscometer constant, k = 0.007690 mm² s⁻²) immersed in a paraffin bath to maintain the appropriate temperatures (25, 40 and 55°C). The ratio of the solution viscosity (selected gums) to solvent viscosity (de-ionized water) is called as relative viscosity (Cui, 2005):

\[
\eta_{rel} = \frac{\eta_{solution}}{\eta_{solvent}}
\]  

The specific viscosity (\(\eta_\text{sp}\)) i.e. the fractional increase in viscosity upon addition of polysaccharide was calculated as (Cui, 2005):

\[
\eta_{sp} = \frac{\eta_{solution} - \eta_{solvent}}{\eta_{solvent}} - \eta_{rel} - 1
\]  

**Intrinsic viscosity determination**

The intrinsic viscosity is determined experimentally from measurements of the solution viscosity of very low concentrations. Drawing the specific (or relative) viscosity data against the concentration and extrapolating the data to zero concentration will result in obtaining intrinsic viscosity value (Rao, 1999). The extrapolations are usually done for relative viscosity values between 1.2 and 2.0, the corresponding specific viscosities being between 0.2 and 1.0 (Da Silva and Rao, 1992). In this paper, the intrinsic viscosity ([\(\eta\)]) was estimated through extrapolation to infinite dilution according to the Huggins and Kraemer empirical expressions below:

1. Huggins’ equation (Huggins, 1942):

\[
\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2C
\]  

Where, \(k'\) is the Huggins constant and \(C\) is gum concentration.

2. Kraemer’s equation (Kraemer, 1938):

\[
\ln \frac{\eta_{rel}}{C} = [\eta] + k''[\eta]^2C
\]  

Where, \(k''\) is the Kraemer constant. For very dilute solutions, however, Eq. (4) can be shortened by retaining only the first-order term, and \([\eta]\) can be determined from the slope of a plot of ln\(\eta_{rel}\) against \(C\) (Sornsrivichai, 1986).

The intrinsic viscosity can also be calculated by measuring the slope of relative viscosity or specific viscosity vs. concentration. For this case, there are some developed equations (Eqs. 5, 6, and 7) to determine the intrinsic viscosity as follows:


\[
\eta_{rel} = 1 + [\eta]C
\]  

4. Higiro’s equations (Higiro et al., 2006):

\[
\eta_{rel} = e^{[\eta]C}
\]  

According to that, the intrinsic viscosity is the slope obtained by plotting ln\(\eta_{rel}\) vs. \(C\).

\[
\eta_{rel} = \frac{1}{1 - [\eta]C}
\]  

According to that, the intrinsic viscosity is the slope
obtained by plotting $1 - \frac{1}{\eta_s}$ vs. C.

Result and Discussion

Coil overlap parameter

The transition from dilute solutions to concentrated solutions is usually accompanied by a pronounced change in the concentration dependence of solution viscosity (Morris et al., 1981; Morris, 1995). The corresponding concentration is called critical or coil-overlap concentration (C'). Estimating the coil overlap for SSG, XG and their blends are necessary for study of dilute domain. Double logarithmic plots of $\eta_s$ against C[η] is shown in Figure 1 for different temperatures. It can be seen that no change in the slope of selected gums was observed for all blends and temperatures, indicating that no molecular entanglement were occurred.

For random-coil polysaccharide solutions except for galactomannans, like xanthan, Morris et al. (1981) reported that the slope of double logarithmic plots of $\eta_s$ against C[η] was close to 1.4 in a dilute regime, whereas, in the concentrated regime, the slope increased to 3.3. Khouryi et al. (2006) reported the amount of this parameter 1.38. As shown in Table 1, the slope values were in the range of 1.017–1.195 means that sage seed gum, xanthan, and their blends were in the dilute domain at the studied temperatures. The higher slope of master curve in 40°C than 25°C indicates more rigidity of selected gums and their blends in this temperature. In addition, the slopes of the double logarithmic plots of $\eta_s$ against C[η] of the blends were slightly lower than the slopes of either XG or SSG alone.

Intrinsic viscosity

Intrinsic viscosity, denoted as [η], is a useful experimental parameter in the study of dilute solutions. The intrinsic viscosity is a measure of the hydrodynamic volume occupied by a macromolecule, which is closely related to the size and conformation of the macromolecular chains in a particular solvent (Lai and Chiang, 2002). In dilute solutions, the polymer chains are separate and the [η] of a polymer in solution depends only on the dimensions of the polymer chain. Because [η] indicates the hydrodynamic volume of the polymer molecule and is related to the molecular weight, it provides deep insights into the molecular characteristics of a biopolymer (Flory, 1953; Rao, 1999).

The rheological interaction and intermolecular binding between xanthan and sage seed gum can be determined by the intrinsic viscosity of XG-SSG mixtures. In this paper, the intrinsic viscosity was calculated by using five models that two models were based on extrapolation method (intercept value) and three models were based on slope determination.

Tables 2-4 show the predicted value of intrinsic viscosity by Kraemer (Eq. 3), Huggins (Eq. 4), Tanglerpaibul and Rao (Eq. 5) and Higiro (Eqs. 6 and 7) models at 25, 40 and 55°C, respectively. It can be found that intrinsic viscosity values calculated by using Tanglerpaibul and Rao model were larger considerably from those determined by using others. It was also showed a better fit, with higher correlation (R²) value obtained by Tanglerpaibul and Rao model.
McMillan (1974) reported that methods of determination of intrinsic viscosity based on slope of plot had larger correlation coefficient and smaller standard error than those based on intercept of plot. As shown in Table 2, intrinsic viscosity values of SSG at 25°C determined by different models were between 10.25 and 16.37 dl/g that were comparable to the values reported by Razavi et al. (2012). The [η] of XG in water at 25°C was ranged from 39.34 to 67.38 dl/g (Table 2), whereas Khouryieh et al. (2006) obtained 154 dl/g and were reported elsewhere ranging from 44.93 to 168 dl/g (Launay et al., 1997; Wang, 2001). Xanthan gum had a much higher [η] than SSG, which can be attributed to the significant difference in their chain stiffness. Xanthan had a stronger chain stiffness, which increased its chain dimensions and hydrodynamic volume (Khouryieh et al., 2006).

At 25°C temperature, the intrinsic viscosity of XG and SSG blends were lower than the intrinsic viscosity calculated from weight averages of the two individually (Figure 2a), indicating no synergy was present between SSG and XG in all proportions. Even though that SSG and XG were attracted to each other in solution, the attraction was not strong enough to yield the synergistic phenomenon. Razavi et al. (2012) reported that as the concentration of salts (NaCl and CaCl$_2$) increased from 20 to 50 mM, intrinsic viscosity of SSG decreased and its structure was more compact. It has been shown that the sage seed gum is negative charged (Mohammadzadeh, 2012) and as regards the xanthan gum is probably an anionic hetero-polysaccharide. Therefore, the reason of antagonistic reaction could be the same charged of two selected gums.

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As shown in Table 4, the intrinsic viscosity of SSG and XG determined by selected models were increased considerably as the temperature increased from 40 to 55°C and they were ranged from 37.26 to 98.05 dl/g and 8.3 to 21.68 dl/g, respectively. At 55°C temperature, the intrinsic viscosity of different proportions of xanthan and sage seed gum were higher than those calculated from weight averages of the two individually, indicating that molecular binding occurred between XG and SSG (Figure 2c). The synergy effect observed in this study may be resulted from the increase in xanthan side chains flexibility due to removal of counter-ions. Among gum blends, 75% xanthan–25% SSG blend showed the largest intrinsic viscosity (Figure 2c). In addition, sharp increase in the intrinsic viscosity of SSG and XG were observed at 55°C temperature that may be related to the gelatinization temperature range or conformational change of these gums. Therefore, synergistic interaction between SSG and XG can be attributed to the neutralization of SSG and XG charged due to gelatinization or conformational change of SSG and XG at 55°C. At high temperature and low ionic strength, xanthan exists in solutions as a disordered structure, but reduction in temperature and/or addition of salts induces an ordered structure (Norton et al., 1984). Rochefort and Middelmann (1987) reported that the backbone of xanthan is disordered (or partly ordered in the form of a randomly broken helix) in distilled water at 25°C, but highly extended at temperature higher than 50-60°C and low salt concentrations due to the electrostatic repulsions from the charged groups on the side chains. They also showed that, as the temperature increased, a transition to coil-like configuration occurred, which caused a dissociation of the molecules and a subsequent change in the rheological properties. Milas and Rinaudo (1986) also demonstrated that intrinsic viscosity of xanthan decreased as the temperature increased except 40-55°C temperature that intrinsic viscosity increased.

In dilute solution, the polymer conformation depends mainly on the interactions between the polymer chain segments and the solvent molecules (Sperling, 2001). Polymer coils can expand or contract from their unperturbed dimensions depending on the quality of the solvent they are dissolved into. A solvent can be good (the number of solvent–segment contacts is maximized and the polymer coil expands), bad (the polymer coil contracts in order to minimize the segment–solvent interactions), or theta (unperturbed coil dimensions) (Marcus, 2002; Hammouda and Worcester, 2006). Therefore, it can be concluded that coil volume of SSG and XG were increased when the temperature increased to 55°C, representing the loss of deionized water power. In contrast to these biopolymers, temperature dependence studies on water and polar solvents indicated that the structure of these solvents is not affected significantly by temperature.

It is rather the hydrogen bonding stability that is influenced (i.e., it decreases as temperature increases). The expansion of the SSG coils that we observed upon heating can be attributed to a decrease of the stability of the hydrogen bonds between SSG and solvent molecules (Antoniou et al., 2010 and Güner, 1999) and a relative increase of the stability of intramolecular interactions among the polymer segments of SSG (Antoniou et al., 2010).

Conclusion

Double logarithmic plots of $\eta_p$ against $C[\eta]$ showed that sage seed gum, xanthan, and their blends were in the dilute domain at the measured temperatures (25°, 40°, 55°C) and there were no molecular entanglements. At 25°C and 40°C temperatures, the intrinsic viscosity of XG and SSG blends were lower than the intrinsic viscosity calculated from weight averages of the two gums individually, indicating no synergy was present between SSG and XG. At 55°C, the strong attraction observed that may have resulted from the increase in xanthan side chains flexibility due to removal of counter-ions or gelatinization and conformational change of SSG and XG at 55°C. Among blends, 75% XG–25% SSG blend showed the largest intrinsic viscosity and synergy.

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