

A comparison of various models for obtaining the intrinsic viscosity of salep gum and sweeteners mixture in dilute solutions

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Abstract: Rheological methods were applied to study the effect of sweeteners on the intrinsic viscosity of salep in dilute solutions. The concentration of these sweeteners were 0.1, 0.2% w/v for Aspartame, Acesulfame-K and Cyclamate, 0.001, 0.002% w/v for Neotame. Gum was evaluated for intrinsic viscosity by various models i.e. Huggins, Kraemer, Tanglertpaibul and Rao equations. The results showed that the values obtained for intrinsic viscosity were different upon various equations used. The plot of relative viscosity versus gum concentration obtained from Tanglertpaibul and Rao model was found as best model. Synthetic sweeteners in these ranges of concentration had no significant effect on intrinsic viscosity of salep gum in solutions.

Keywords: Salep gum, intrinsic viscosity, sweetener, rheology

Introduction

Food additives are chemical materials used in food formulations for various purposes, for example, to enhance flavor, to add color, to increase shelf life by preserving or to modify rheological and physical properties. Hydrocolloids are polymers interacting strongly with water and are widely used as food additives. In the food industry, they are primarily used for thickening, gelling, film forming and stabilizing purposes. Many food products such as sauces, syrups, ice cream, instant foods, beverages and confectionaries, marshmallows and candies contain hydrocolloids in their formulations (Aliste *et al.*, 2000; Dogan and Kayacier, 2004; Kayacier and Dogan, 2006). The rheological properties of hydrocolloids are particularly important when they are used in the formulation of any food for their effects on the textural attributes (Patmore *et al.*, 2003). The rheological properties of fluid food should be carefully taken into account for designing and modeling purposes. Many factors including the concentration of hydrocolloids, temperature, dissolution, electrical charge, previous thermal and mechanical treatments and the presence of electrolytes may affect the rheology of the fluid food containing hydrocolloids (Marcotte *et al.*, 2001; Rao and Anantheswaran, 1982). One of the best known hydrocolloids is Gluco- mannans.

Gluco- mannans found in the hemicellulose as well as in the roots, bulbs and tubers of many plants are generally considered linear random block copolymers even though species with sidechains of α -(1-6) linked D-galactose units are known. A polysaccharide of this type the salep glucomannan, produced by orchid

tubers (*Orchis morio*, *Tubera salep*) as reserve material and used as low-calorie dietary product, is regarded as a neutral linear molecule with ca. 1:3.6 glucose/mannose ratio in which the sporadic lateral chains (ca. seven per molecule) do not seem to be long enough to affect molecular conformation. The most important constituent of salep is glucomannose amounting to 16±55%, which acts as a stabilizer (Tekinsen, 1996). Salep also contains starch (2.7% wt), moisture (12%) and ash (2.4%) (Tekinsen and Karacabey, 1984) and plays an important role in the favor of the final product.

One of the key parameters to measure salep gum rheological behavior is to study Intrinsic Viscosity (IV). The intrinsic viscosity, $[\eta]$, is not a very specific parameter and depends upon several factors (Bohdanecky and Kovar, 1982). It is dependent upon the hydrodynamic volume occupied per unit mass of the macromolecule, which consists of the intrinsic volume occupied by the polymer chain and its excluded free volume. It is also influenced by hydrodynamic properties which include a measure of the permeability of the polymer coil to solvent (if it is free draining, then $[\eta]$ is higher) and chain anisotropy. Deviations from spherical geometry add a frictional component to viscosity. The solution properties of galactomannans in water have been characterized in different research reports (Launay *et al.*, 1986).

More recently, Goycoolea *et al.* (1995) removed specific chain/chain association by dissolving guar and locust bean gum neutral polysaccharides to weak polyelectrolyte. This resulted in a significant reduction in the intrinsic viscosity, for LBG, 12.1 to 5.2 dl/g

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and to a lesser extent for guar, 12.5 to 11.9 dl/g. These intrinsic viscosities were then in accordance with the relative actual molecular weights for these two galactomannans. Upon neutralization, the intrinsic viscosities returned to their original values clearly showing that reversible aggregation does occur. Thus, not only does the specific association of galactomannan chains possibly contribute to higher viscosity dependencies with concentration, but it also increases the intrinsic viscosity of the macromolecule (a measure of the hydrodynamic volume) (Richardson *et al.*, 1998).

In another work, effect of some salts (NaCl, KCl, and CaCl₂) on intrinsic viscosity of xanthan and locust bean gum interaction in dilute solution were investigated (Higiro *et al.*, 2006). Their results showed that, addition of any of the three salts reduced significantly the intrinsic viscosity, with a pronounced effect from divalent ions, compared with monovalent ions. Sweeteners are widely used as low molecular weight additives. However, limited work has been published on the influence of sweeteners on the solution properties of hydrocolloids. In the case of guar and locust bean gum, Elfak *et al.* (1977) found that the intrinsic viscosity decreased upon the addition of sucrose and other low molecular weight additives. Recently, in a similar but more extensive study by Launay *et al.* (1997), no change in intrinsic viscosity was measured for 0, 10 and 40% sucrose concentrations. The objective of this study was to explore the effect of some Synthetic sweeteners on the intrinsic viscosity of salep gum in dilute solutions and comparison of various models for obtaining the intrinsic viscosity of salep gum and sweeteners mixture in dilute solutions.

Materials and methods

Solutions preparation

Salep gum was obtained from Arman Shayan Co., Iran. Acesulfame-K and Cyclamate were purchased from PT Golden Sari (Jakarta Utara 14440, Indonesia). Aspartame and Neotame were obtained from Nutrasweet Company, USA. Sweeteners/gum solutions were prepared by first dry mixing the appropriate amounts of each sweeteners and salep gum. Cold de-ionised water was then added and the solutions were heated at 60°C for 15 min whilst stirring with a magnetic agitator. Molecular weight analyses (Russell, 1997) showed that the polymer did not degrade at these temperatures unless excessive mechanical stirring was used. Dilute salep gum solutions were prepared at 0.5% w/v for physical study, and 0.005, 0.010, 0.020, 0.030, 0.040 gr/dl

for rheological study, in the ranges of sweeteners concentrations (0.1, 0.2% w/v for Aspartame, Acesulfame-K and Cyclamate, and 0.001, 0.002% w/v for Neotame). Gum solution without any sweetener added was used as the control sample.

Viscosity measurements

The viscosity was determined at a frequency of 2 Hz (Thurston, 1996) within a shear-rate range of 0.8–20 s⁻¹. Morris and Taylor (1982) reported that oral perception of solution viscosity correlated well with viscosity measurements at 10 s⁻¹. Thus, all viscosity measurements were statistically assessed at 10 s⁻¹. Rheological measurements were carried out at 20°C by using a temperature-controlled circulating water bath (Haake DC5, Gebr. Haake GmbH, Karlsruhe, Germany). The analyzer was calibrated with deionized distilled water at 20°C, followed by the verification procedure to further ensure that the rheometer was operating at optimum conditions. Microsoft Excel 2000 (Microsoft Corporation, Seattle, WA) was used to plot viscosities against concentrations, as well as to obtain linear regression lines with the corresponding equations and correlation coefficients (R²) in order to assess the best model.

Intrinsic viscosity determination

The intrinsic viscosity $[\eta]$ was measured according to the method used by Higiro *et al.* (2006). In their work, they proposed five ways to determine the intrinsic viscosity following:

$$\text{- Huggins equation: } \frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \quad (1)$$

$$\text{- Kraemer equation: } \frac{\ln \eta_{rel}}{C} = [\eta] + k''[\eta]^2 \quad (2)$$

$$\text{- Tanglertpaibul and Rao equation: } \eta_{rel} = 1 + [\eta]c \quad (3)$$

Higiro *et al.* (2006) have proposed two other Ways to obtain intrinsic viscosity from the equation (3):

$$\text{- } \eta_{rel} = e^{[\eta]c} \quad (4)$$

According to that, the intrinsic viscosity is the slope obtained by plotting Ln η_{rel} vs. C.

$$\text{- } \eta_{rel} = \frac{1}{1 - [\eta]c} \quad (5)$$

According to that, the intrinsic viscosity is the slope obtained by plotting vs. C.

$$\text{- } 1 - \frac{1}{\eta_{rel}} \quad (6)$$

Statistical analysis

A two-way factorial design was used to generate the best-fitting intrinsic viscosity model. The sweeteners (Aspartame, Acesulfame-K, Cyclamate and Neotame) at two concentrations (0.1 and 0.2% w/v for Aspartame, acesulfame-K and cyclamate, 0.001 and 0.002% w/v for neotame) were compared for intrinsic viscosity of gum, in a factorial design. In each instance, three replications were made. The analysis of variance and means comparison were conducted by the general linear models procedure (Proc GLM), with Statistical Analysis System software (version 9.1, SAS Institute, Inc., Cary, NC). Comparisons among the treatments were analyzed by using Fisher's least significant.

Results and Discussion

Intrinsic viscosity (IV)

In general, an increase in specific viscosity/con. was observed as the concentration of gum increased (Figure 1). The same results have been reported by Lapasin and Pricl (1995), indicating that neutral polysaccharides (i.e., salep gum) exhibited linear plots of lower slope, whereas ionic polysaccharides displayed a sharp increase of the slope, possibly due to the expanded coil dimensions and electrostatic repulsion between the chain segments. The similar results were observed for salep gum in this study. Figure 1 displays linear increase of specific viscosity/concentration as concentration increased. Lai *et al.* (2002) reported similar results when determining the intrinsic viscosity of hsian-tso leaf gum in different salt solutions. Other works have also shown the same results for the mixture of gum and salts (Pals and Hermans 1952; Higiro *et al.*, 2006).

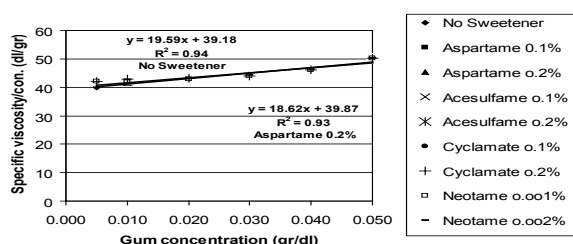


Figure 1. Huggins plot (specific viscosity/concentration vs. concentration) for salep gum with the addition of sweeteners

The data from Huggins plot showed that at applied sweetener concentrations there was no significant effect on IV. The non-linear relationship between $\ln \eta_{rel}/C$ and gum concentration (kraemer model) was observed, thus making impossible the determination of the intrinsic viscosity by extrapolation of experimental data with this equation to zero concentrations; this prompted the use of slope models (Chou and Kokini, 1987; Tangeltrpaibul and

Rao, 1987) and Huggins plot (Kraemer, 1938).

From Eq. (1), the Huggins constant k' theoretically should lie between 0.3 and 0.8, and values larger than 1 represents polymer-polymer aggregation (Morris, 1995), increasing as the solvent quality decreases, resulting in polymer coil contraction and larger values possibly indicate a poorer solvent (Bohdanecky and Kovar, 1982). It was found that the k' values lied in the range (data not shown), indicating molecular association was absent and solvent quality was good. This was in accordance with sum of the Huggins constant k' and Kraemer constant k'' which equals $0.5 \pm 10\%$, and larger or smaller values are attributed to molecule association (Morris *et al.*, 1981; Higiro *et al.*, 2006).

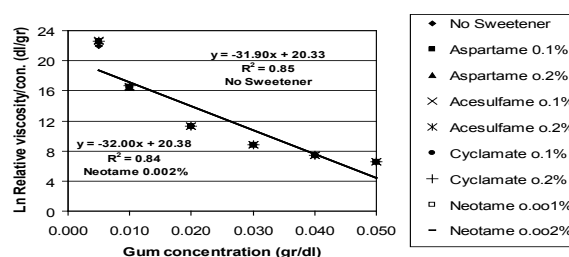


Figure 2. Kraemer plot (Ln relative viscosity/concentration vs. concentration) for salep gum with the addition of sweeteners

To determine the intrinsic viscosity by plotting relative viscosity vs. C (Eq. 1) and $\ln \eta_{rel}$ vs. C (Eq.4), Straight-line relationship with large linear regression coefficients were obtained (Figures. 3, 4). In the case of $1-1/\eta_{rel}$ vs. C (Eq. 5). Straight-line relationship with low linear regression coefficients was obtained (Figure 5). McMillan (1974) reported that methods of determination of intrinsic viscosity based on slopes of plots had larger correlation coefficients and smaller standard errors than did those based on intercepts of plots. The representative comparison of different models used to estimate the intrinsic viscosity is shown for each gum (Table 1).

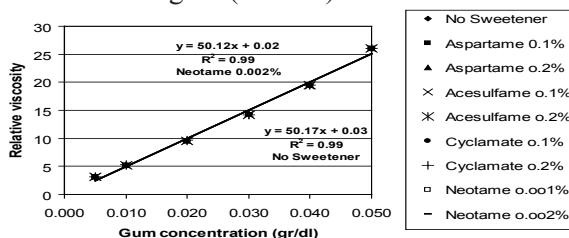


Figure 3. Relative viscosity for salep gum as a function of gum concentration with the addition of sweeteners

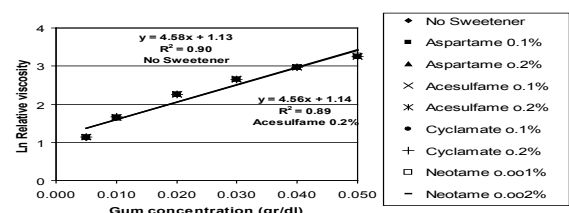


Figure 4. Ln Relative viscosity for salep gum as a function of gum concentration with the addition of sweeteners

Table 1. Intrinsic viscosity values (dl/gr) obtained for salep gum using different models

sweetener	concentration (%)	1	2	3	4	5
Aspartame	0	39.18±1.7 ^B _a	20.33±1.5 ^C _a	50.17±3.1 ^A _a	4.58±0.3a ^D	0.57±0.05a ^E
	0.1	40.01±1.6 ^B _a	20.68±1.6 ^C _a	50.22±3.2 ^A _a	4.57±0.2a ^D	0.55±0.04a ^E
	0.2	39.87±1.5 ^B _a	20.62±1.4 ^C _a	50.15±2.9 ^A _a	4.55±0.2a ^D	0.54±0.03a ^E
Acesulfame-k	0	39.18±1.7 ^B _a	20.33±1.5 ^C _a	50.17±3.1 ^A _a	4.58±0.3a ^D	0.57±0.05a ^E
	0.1	40.12±1.5 ^B _a	20.68±1.4 ^C _a	50.11±3.1 ^A _a	4.54±0.2a ^D	0.55±0.04a ^E
	0.2	40.26±1.8 ^B _a	20.70±1.5 ^C _a	49.95±2.9a ^A	4.56±0.2a ^D	0.54±0.04a ^E
Cyclamate	0	39.18±1.7 ^B _a	20.33±1.5 ^C _a	50.17±3.1 ^A _a	4.58±0.3a ^D	0.57±0.05a ^E
	0.1	40.18±1.6 ^B _a	20.61±1.4 ^C _a	50.06±2.9a ^A	4.53±0.3a ^D	0.55±0.03a ^E
	0.2	40.15±1.8 ^B _a	20.74±1.6 ^C _a	50.05±3.2a ^A	4.52±0.2a ^D	0.55±0.04a ^E
Neotame	0	39.18±1.7 ^B _a	20.33±1.5 ^C _a	50.17±2.9 ^A _a	4.58±0.3a ^D	0.57±0.05a ^E
	0.001	40.19±1.5 ^B _a	20.69±1.6 ^C _a	50.12±3.0 ^A _a	4.53±0.2a ^D	0.55±0.04a ^E
	0.002	39.37±1.5 ^B _a	20.38±1.3 ^C _a	50.12±3.3 ^A _a	4.57±0.3a ^D	0.56±0.03a ^E

Results are expressed as means ± SD for three replications.

A-E: Means followed by the same letters in the same row are not significantly different ($P < 0.05$).

a-b : Means followed by the same letters in the same column are not significantly different ($P < 0.05$).

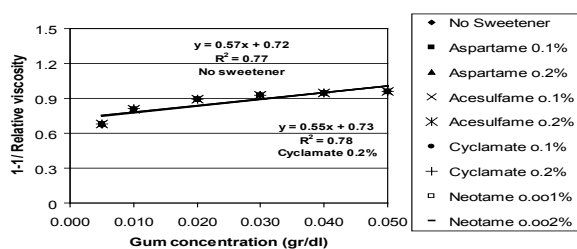


Figure 5. Plot of $(1-1/\eta_{rel})$ as a function of gum concentration for salep gum with the addition of sweeteners

Intrinsic viscosity values calculated by using Eq. (3) were larger and significantly different from those obtained by using Eqs. (4) and (5) for all sweeteners and sweetener concentrations. Tanglertpaibul and Rao (1987) successfully implemented the model from Eq. (3) in the intrinsic viscosity determination of tomato serum. The authors reported higher correlation coefficients and fewer errors. Significant differences between salep gums were detected with Eq. (3), whereas these differences were few with Eqs. (4) and (5). We used Eq. (3) as the best model to determine intrinsic viscosity as it best fitted with higher correlation (R^2) for gum, sweeteners, and sweetener concentrations. Furthermore, the results were similar to other findings (Tanglertpaibul and Rao, 1987). Richardson *et al.* (1998) studied the effect of sucrose on dilution solution properties of guar gum. Their results showed that addition of sucrose at low concentrations (0-10% w/w) decrease the intrinsic viscosity (because of decreasing the solvent quality), and these results complies with Huggins model. Sucrose affects the intrinsic viscosity by changing structure of the hydrocolloid (coil expansion) hence

its rheological behavior.

Our results showed that the values obtained for intrinsic viscosity were different by the equation used for estimation the intrinsic viscosity. We found kraemer equation and $1-1/\eta_{rel}$ versus concentration plot did not provide a better fit for salep gum. Addition of these sweeteners in these ranges of concentration had no significant effect on intrinsic viscosity of salep gum solutions, indicated these synthetic sweeteners had any affects on solvent quality and hydrodynamic volume of salep gum.

Conclusion

The kraemer and $1/1-\eta_{rel}$ plots do not provide a better fit the solutions of gum containing artificial sweeteners in regular concentrations. To determine the intrinsic viscosity by plotting specific viscosity/concentration versus C (Huggins equation, (Eq. 1), relative viscosity vs. C (Eq. 3), $\ln\eta_{rel}$ vs. C (Eq. 4), Straight-line relationships with large linear regression coefficients obtained for salep gum but among them relative viscosity versus gum concentration plot (Eq. 3) was found as the best model because of its higher linear regression coefficient. By using these appropriate equations, there are not any significant different among these sweeteners, indicated these synthetic sweeteners had any affects on solvent quality and hydrodynamic volume of salep gum may be due to their low concentrations. It seems possible that at higher concentration of sweeteners the gum behavior would be affected more significantly.

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