IJFAAS International Journal of Food and Allied Sciences
ISSN: 2415-0290 (Print)
ISSN: 2413-2543 (Online)
DOI:10.21620/ijfaas.2017249-55

Research Article

Salt effects on the dilute solution properties of bototo gum (*Cochlospermum vitifolium*)

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History

Submitted: Sep 18, 2017
Revision: March 03, 2018
Accepted: March 15, 2018

Keywords
Cochlospermum vitifolium, gum exudates, intrinsic viscosity, conformational studies, salt effects.

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Abstract

Salt effects on the dilute solution properties of bototo gum (Cochlospermum vitifolium) were investigated. There were measured the intrinsic viscosities of the polymer under study in water, in NaCl (at different concentrations) and in NaCl, $CaCl_2$ and $AlCl_3$, at the same ionic strength (I = 1), using an Ubbelohde viscometer at 25 ° C. The Huggins intersection model (R^2) is more appropriate to determine the intrinsic viscosity than the Kraemer's equation. This parameter tends to reduce with the increase in the salt concentration and the cation charge. The molecules of C. vitifolium gum exudate in water (solvent Φ) tend to adopt a "random coil" conformation, of great volume and radius, which exist in two regimes: diluted and semi-diluted; in the presence of salts, the coils exist only in a diluted regime where they are separate each other. The macromolecules in water have probably an extended ellipsoid shape but in presence of salts, they change to another more compact, probably spherical, with radii and smaller volumes. The knowledge of the behavior of C. vitifolium gum molecules in dilute solutions is an important criterion for its rational use in various industries

1. Introduction

C. vitifolium (bototo), a species that grows in Venezuela in arid and semi-arid zones, exudates gum which contains galactose (37%), glucose (0.30%), arabinose (traces), and rhamnose (62%). Its high intrinsic viscosity, in correspondence with its high molar mass, confers to the bototo gum a potentiality as a thickener and emulsion stabilizer in different industries (Martínez et al., 2016).

Intrinsic viscosity supplies details about fundamental properties of the polymer and its interaction with the solvent, stiffness, conformation and chains overlapping, volume and shape of the rigid particles Therefore, determination of intrinsic viscosity can provide deep understanding of molecular properties of macromolecules in solution (Irani et al., 2016).

These properties of a polymer in dilute solutions, based on its intrinsic viscosity, are important in determining its processability and applications (as a stabilizer, thickener, or gelling agent) (Eddy et al., 2014), i.e can provide basic information about the behavior in diluted solutions of a polymer with additives like salts, sugars and other hydrocolloids in food systems. Furthermore, determination of critical concentration (C*) is important in the process of gelation to indicate when the network is formed (Irani et al., 2016).

According to the above expressed, the present study would allow to predict the usefulness of the bototo gum in various industries that require saline medium: in the food industry, as a stabilizer and thickener (in salad dressings and frozen foods); in pharmacy, as a suspending agent in oral liquid formulae for children and in intravenous shots and in cosmetics, as an emulsion stabilizer in lotions and protective creams, and as a foam stabilizer in liquid soaps (Nussinnovitch, 2010). They could also suggest its application in biotechnology, as a coagulant in the water potabilization process (Rodiño et al.,2015) and in the synthesis of nanofibers (Colby & Rubinstein, 2009).

Studies were carried out on the properties in dilute solutions of *Enterolobium contortisilliquum* (Oliveira et al., 2001), *Albizia zygia* (Eddy et al., 2013a), *Ficus platyphylla* (Eddy et al., 2013b), *Eucalyptus citriodora* (Eddy et al., 2014), *Khaya ivorensis* (Isreal, Innocent & Ammeh, 2013) and *Acacia senegal* (Masuelli, 2013) gum exudates, which demonstrated its potential industrial use.

The objective of this research is to evaluate the salt effects on the dilute properties of bototo gum exudate (*C. vitifolium*).

1. Materials and methods.

2.1 Origin and purification of gum

The gum was collected from a specimen duly identified by M.Sc. Antonio Vera, terrestrial ecologist from the Universidad del Zulia, as *Cochlospermum vitifolium* (bototo), located at Wildlife Reserve "Ciénaga de La Palmita e Isla de Pájaros", Santa Rita Municipality, Zulia State, Venezuela (N: 10° 35 '44.8 ", W: 71° 30' 09.9", elevation: 2 mosl).

Gum collection was performed after the practice of wounds at trunk level and the periodic removal of the gum exudate, every 7 days, during the dry season (December 2015 - March 2016 period). The raw gum was manually cleaned and stored in dry containers.

The gum was purified according to the methodology previously described (Martínez et al., 2016), without modifications. The raw gum (2g) was dissolved in water (100 mL), filtered through Watman 41, dialized against tap water for 48 h, and was lyophilized to obtain the pure gum. This process guarantees the elimination of remains of other plant parts and impurities of low molecular weight.

1.2. Determination of intrinsic viscosities:

Intrinsic viscosities of the polymer, in water, were measured using a size 75 Cannon-Ubbelohde capillary viscometer (Cannon Instruments Co., Germany; viscometer constant, k = 0.01875 mm^2/s^2) immersed in a thermostatic water bath under precise temperature control (± 0.1 °C) at 25 °C. Measurements were made at different concentrations (0.006 to 0.15 g /dL) and extrapolated to infinite dilution using the Huggins model Eq. (1) (Oliveira et al., 2001) where K_H, is the Huggins constant.

$$\eta_{red} = K_H[\eta]^2C + [\eta] \quad (1)$$

where:

$\eta_{red} = \eta_{sp}/C \longrightarrow \eta_{sp} = \eta_{rel} - 1 \longrightarrow \eta_{sp} = t - t_o/t_o$

$[\eta]$ = intrinsic viscosity

C = Concentration

Reported flow times were at least three replicates.

The effect of the concentration of a salt, NaCl, on the $[\eta]$ of the bototo gum, was studied by measuring the latter parameter in solutions 0.1, 0.2, 0.5, and 1 M.

On the other hand, the intrinsic viscosities in the presence of salts of different charges, were determined while maintaining the same ionic strength (I=1): 1.0 M NaCl, 0.335 $CaCl_2$ and 0.170 $AlCl_3$, as was reported (Oliveira et al., 2001).

Also, the intrinsic viscosity in water, and its respective constant was calculated using the Kraemer model according to Eq.(2) for comparison purposes:

$$1/C \; Ln \; \eta_{sp} = [\eta] + K_K [\eta]^2 C \quad (2)$$

2.3 Determination of the stiffness (S) and relative stiffness parameters (ß)

The $[\eta]$ of the gum exudate under study, in NaCl at different ionic strengths, I = 0.1, 0.2, 0.5, 1.0 M, were determined and plotted $[\eta]$ vs. $I^{-0.5}$

The relative stiffness parameter (β), related to the flexibility of the molecule, was calculated diectly using the Eq. (3) of SmidsrΦd, in which the exponent υ reported for 0.1M NaCl (1.3) was used and the parameter stiffness, S, slope of the curve $[\eta]$ vs. $I^{-0.5}$ (Smidsrod & Haug, 1971). The value of β was compared with those reported for other polyelectrolytes to predict the possible conformation of the *C. vitifolium* gum molecules in the electrolyte solution as flexible, semi-flexible or rigid (Oliveira et al., 2001).

$$S = ß[\eta]^{U}_{0,1} \quad (3)$$

2.4. Molecular conformation parameter (b)

The molecular conformation was predicted based on the slope of the double log plot η_{sp} vs C (power law graphic) (Lai, Tung & Lin, 2000). A value of b> 1 is associated with a "random coil" conformation while b <1 suggests a more rigid (rod-like) conformation (Lapasin & Priel, 1995).

2.5. Coil-overlap parameter (C*)

The plot of logη_{sp} against log C[η], known as the master curve, was used to estimate the coil overlap parameter C*, in the case of a random coil conformation. If the slope value of the curve is below or equal to 1.4, the "coils" are considered to be in a diluted regime (separate coils) whereas if this value increased to 3.3, they are described in a concentrated regime, with overlapping of the coils. The limiting concentration between both regimes is called the critical concentration C* (Morris et al., 1981).

2.6. The Berry number (Be)

The Berry number, so called the space occupancy, was calculated by the Eq. (5)

$$Be = C \times [\eta] \quad (5)$$

Values of Be that approximately equal to 1, confirm the existence of molecules in a diluted regime whereas if Be> 1 indicate that the molecules of the polymer are rather in a semi-liquid regime. In this last case, if the values are between 1-10, they suggest that the molecules do not overlap enough to form a lattice or network (Irani et al., 2016).

2.7. Coil radius and volume

Variation of the coil radius, Rcoil, either known as hydration radius R_H, in water, at different concentration of one cosolute and cosolutes of different charges, at a same ionic force, was determined according to previously reported (Antoniu et al., 2010), through following Eq. (7), where Mw and N_{AV} are the average molecular weight (Martínez et al, 2016) and molecular weight Avogadro's number (6,022 × 1023 mol-1), respectively.

$$R_{coil} = [3\,[\eta]\,Mw \setminus 10\pi\,Nav]^{1\setminus 3} \quad (6)$$

Supposing that the shape of coil is spherelike, the coil volume was determined by the following equation:

$$V_{coil} = 4/3\ \pi(Rcoi)l^3 \quad (7)$$

2.8. Voluminosity (Us) and shape factors (U) of the macromolecules

Plotting the parameter Y (equation 8) against C leads to the calculation of a factor, known as voluminosity (Us) or swollen specific volume, from the intercept at C = 0, in water and electrolytes solutions. Us is a measure of the volume of the solvated polymer molecule (Antoniu et al., 2010).

$$Y = \eta_{rel}^{0.5}\text{-}1 \,/\, C\ (1.35.\ \eta_{rel}^{0.5}\text{-}0.1) \quad (8)$$

The intrinsic viscosity and Us are related through Eq.(9):

$$U = [\eta] \,/\, Us \quad (9)$$

where U is the shape factor or the universal shape function, also called the Simha number, which unlike [η] is directly related to the shape of the particle independent of its volume.

3. Results and Discussion

3.1 Intrinsic viscosities of bototo gum in water.

The Huggin′s model reveals a higher degree of linearity (R^2 = 0.9713) than the Kraemer′s equation (R^2 = 0.77), Fig 1, which indicates that the intrinsic viscosity is better fit to the first one in contrast to *A. zigya* gum exudate (Eddy et al., 2013a).

The [η] calculated from Kramer model (9.61 dL/g) is lower than that obtained from Huggins plot and so $K_K < K_H$, Table 1.

The $K_K + K_H$ = 1.06, above the range (0.5 ± 10%), indicates that the polymer molecules are associated in solution [3,7]. However, the bototo gum Huggins constant in water K_H <1, Table 1, which lies between 0.3-0.8, characteristic range of a Φ solvent, suggests that the polymer-solvent and polymer-polymer interactions are balanced (Oliveira et al., 2001).

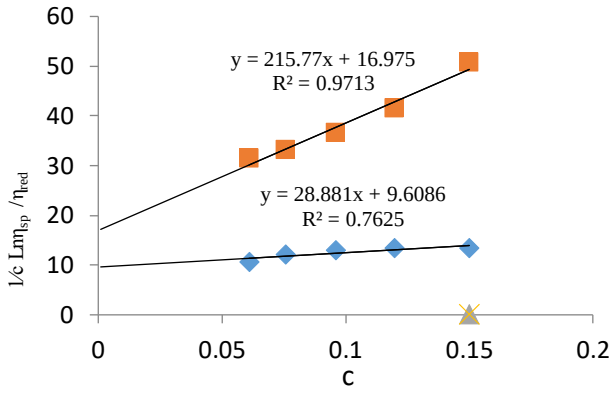

Figure 1 Huggins and Kramer's models

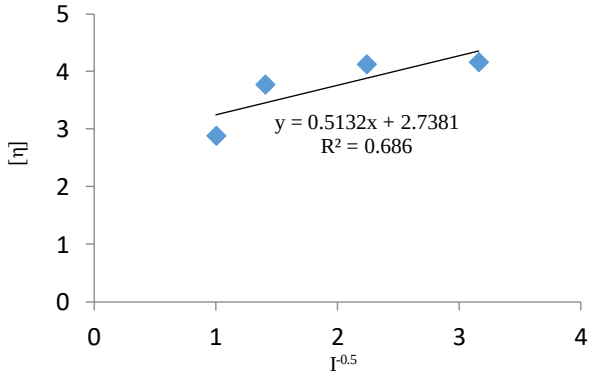

Figure 2 [η] vs. $I^{-0.5}$ plot of bototo gum

3.2. Intrinsic viscosities of the bototo gum in the presence of salts.

On the other hand, the viscosity of the polymer under study decreased markedly with the increase in the concentration of a salt, in particular, NaCl, Table 1. The viscosity decrease corresponds to the Huggins constants as indicative of a molecular aggregation, Table 1. This behavior has been reported for seed gums (Irani et al., 20169 and may be important for pharmaceutical purposes (Vieria et al., 2009).

The data shown in Table 2, reveal that the increase in the Z/R ratio decreases the intrinsic viscosity according to the following order: $Al^{+3} < Ca^{+2} < Na^{+1}$, as reported for similar gums (Oliveira et al., 2001; Eddy et al., 2014). The affinity of the gum for metal ions depends on this Z/R because the charge enhances the polymer-polymer interaction (Eddy et al., 2014).

In the same way, the K_H calculated for the gum in the presence of NaCl, $CaCl_2$ and $AlCl_3$, Table 1, confirms a strong interaction between the macromolecular chains, especially in the case of Al^{+3}. The order of interaction may be ascribed to the rather intermolecular crosslinking effect of $Al^{+3} > Ca^{+2} > Na^{+1}$ although Na^{+1} is not a crosslinking agent (Oliveira et al., 2001).

3.3. Stiffness (S) and relative stiffness parameters (ß) in the presence of NaCl (I=1).

The value of the stiffness constant, S, 0.51, the slope of the graph [η] vs. $I^{-0.5}$, Fig. 2, is greater than those described for Balangu seed and tragantin gums (Yousefi, Razavi & Khodabakhsh, 2014), whereas the relative stiffness, ß, 0.08, calculated from Eq.(3) describes the bototo polymer as semi-flexible in NaCl, analogous to that suggested for *Enterolobium contorsilliquom* gum exudate (Oliveira et al., 2001).

3.4. Molecular conformation parameter (b).

The slope of the power-law model (b value), in water, Table 1, is greater than 1, which suggests that the molecules of the polymer adopt a "random coil" rather than a rod-like conformation as was reported for other gum exudates (Eddy et al., 2013b; Masuelli, 2013).

The random coil conformation is a typical property of linear polysaccharides in solution. It was described that most of the polysaccharides such as k-carrageenan, alginates and locust bean gum, even with certain structural regularities, will assume a random coil conformation in dilute solutions. Furthermore, many random coil hydrocolloids such as agar impart ability to form a physically crosslinked gel-network, given appropriate solvent conditions or temperature (Naji-Tabasi et al., 2015). The gelling capacity of *C. vitifolium* gum (Martínez et al., 2016) may be related with the formation of this gel-network starting from these random coil macromolecules.

The slopes of power-law model (b values), at different concentrations of NaCl, still correspond to a "random coils" molecules and decrease to 1.21, Table 1.

Table 1. Conformational and overlapping parameters related to the [η] of bototo gum

Salt	[ ]	Parameters [η], dL/g	K_H K_K	b	m_{morris}	B_e
a		16.97[b]	0.75	2.18	2.09	1.03-2.03
		9.61[c]	0.31			
NaCl	0,1	4.16	1.11	1.31	1.31	0.25-0.62
	0,2	4.13	1.38	1.35	1.38	0.25-0.62
	0,5	3.79	0.99	1.21	1.22	0.19-0.47

	1	2.89	2.38	1.31	1.31	0.15-0.37
$CaCl_2$	0,335	1.97	3.77	1.34	1.34	0.10-0.25

[a] In water [b] Huggins model [c]Kraemer's model b=power law slope [ ]=molar m_{morris}=Morris slope B_e= Berry number

Table 2. Salt effects[a] on the intrinsic viscosity of pototo gum

Cation	Parameters				
	Z	RI (pm)	Z/R(pm^{-1})	[η] dL/g	K_H
Na^{+1}	+1	102	0.010	2.90	2.38
Ca^{+2}	+2	100	0.020	1.97	3.77
Al^{+3}	+3	53	0.056	b	b

[a]at the same ionic force [b] Below t_o

The values of "b" obtained for the gum of bototo in cations of different charge, at the same ionic strength, are comparable Table 1, although in the case of calcium the inter-chain interactions must be predominant as expressed in previous paragraphs

3.6. Coil-overlap parameter (C*)

The graph of double log ηsp vs log c [η], Fig. 3, in water shows two regimes: the first, slope 1.2, below the described for a dilute regime (Morris et al., 1981) and the second , with a higher slope of 1.63, which suggests the existence of molecules in a rather semi-dilute regime (Colby & Rubinstein, 1990). This semi-dilute regimen slope is comparable to that reported for the medium-concentration region in a levan polysaccharide from *Bacillus* sp. (Arvidson, Rinehart & Gadala-María, 2006).

The product C* [η], corresponding to the limit between the two regimes, is 1.29 and in consequence the C* is 0.076. It has been described that the critical concentrations of the polyanions are low in comparison with the neutral polymers (Harding, 1997).

Knowledge of the critical concentration of the gum is important in the synthesis of nanofibers (Colby & Rubinstein, 1990).

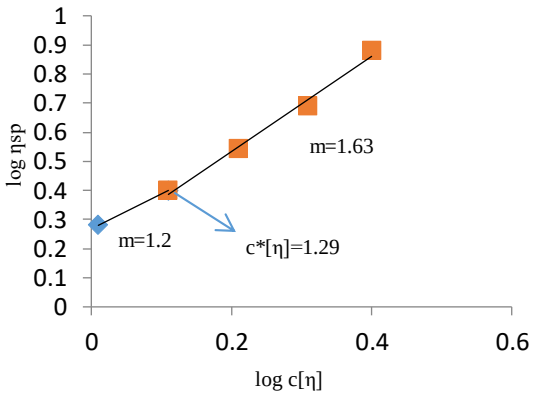

Figure 3: Master curve for bototo gum in water

In contrast, the graphs of double log ηsp vs log c [η], in the presence of salts, at different concentrations, show single slopes (b), whose values oscillate between 1.22-1.38, lower than those described for a dilute regime (Morris et al., 1981), Table 1. The macromolecules do not exist in two regimes as in the case of water.

3.7. The Berry number (Be)

The Berry numbers in water, greater than 1, and between 1.03-2.03, Table 1, confirm, first of all, that there are molecules present in a semi-dilute regime and second, that they do not overlap enough to form a lattice or network (Irani et al., 2016). A particular situation was described, within the semi-dilute domain, characterized by overlapping coils but not enough to form a lattice or network (Colby & Rubinstein, 1990). Values of the ratio c / c * between 1.00 <c / c *> 1.97 confirm the possibility of existence of this semi-diluted regime without forming a lattice (Graessley, 1974).

These concentration values could be used as references in the food and pharmaceutical industries, in order to achieve the desired effect of the bototo gum, as an emulsion stabilizer, thickener, or gelling agent when water is used as a solvent (Nussinovtich, 2010).

Table 3. Size and shape factors related with the [η] of bototo gum

Salt	[ ]	Parameters				
		[η] (dL/g)	Us (dL/g)	U	R_{coil} (nm)	V_{coil} x10^8 (nm^3)
a		16.97[b]	5.29	3.21	612	9.6

NaCl	0.2	4.13	1.78	2.32	81.9	0.013
	0.5	3.79	1.62	2.34	81.7	0.012
	1	2.89	-	-	79.4	0.008
$CaCl_2$	0.335	1.97	0.99	1.99	63.92	0.006

[a] In water. [b]Huggins model. Us =volumen factor U=shape factor [ ]=molar

On the other hand, the values of Be, close to 1, Table 1, confirm the existence of the molecules in a dilute domain, in the presence of salts (Graessley, 1974) in correspondence with the slopes of their master curves.

3.9 Coil radius and volume

The values of R_{coil} and V_{coil} confirm the tendency towards compactness, with the increase in the concentration of a salt and the charge of the cation, Table 3.

The R_{coil} of the random coil decreases 88% when passing from the water to the solution more concentrated of the electrolyte (1M NaCl); the volume also experienced a decrease of three orders of magnitude. It has been reported that the molecules of the polymer experience their greatest extension in water due to the repulsion forces; by adding salts, the cations screen the charged sites, and the molecules tend to be compacted to minimize the solvent-polymer interaction (Dobrynin, 2012).

The R_{coil} and the volume of the coils, both in water and in different salt concentrations and charges, Table 3, are higher than those reported for seed gums and linear polymers (Yousefi, Razavi & Khodabakhsh, 2014) and they correspond with its high molar mass (Martínez et al., 2016)., Table 1.

3.8. Voluminosity (Us) and shape factors (U) of the macromolecules

The factor U for the macromolecules of bototo gum, in water, 3.21, Table 3, suggests that these probably have an ellipsoidal shape (of an oblate or prolate) (Yousefi, Razavi & Khodabakhsh, 2014), rather than an spheroidal one, characterized by an axial ratio a / b. It has been reported that the fraction F1 of the arabic gum corresponds to an ellipsoid oblate form with an internal network (Sánchez et al., 2008).

Flory proposes an ellipsoid model for solution polyelectrolytes in which the size of the chain depends on an internal fraction of charged monomers (Dobrynin, 2012). In bototo gum this fraction may be represented by an important number of carboxylate groups (Martínez et al., 2016).

On the other hand, the values of Us and of U for the macromolecules decrease, when increasing the concentration of a salt or the charge of the cation, Table 3, indicating less interaction with the molecules of the solvent, a tendency to the compactness and towards the sphericity. It has been described that in poor solvents, like the salt solution studied, where K_H are> 1. Table 1, the spheroids have lesser flexibility to minimize contact with the solvent and hence the viscosity decreases. (Yousefi, Razavi & Khodabakhsh, 2014)

4. CONCLUSIONS

The bototo gum shows a higher viscosity in water which could be related with the existence of semi-flexible and extended rod-like chains, which overlap in a semi-dilute regime, and ellipsoidal particles.

In the presence of salts, the viscosities become smaller because there is a tendency to a compactness and the macromolecules tend to adopt a spheroidal shape, to minimize the interaction with the solvent.

The different behavior of the bototo gum, in water or in the presence of salts, at a different concentration and charge of the cations, can be used as a rational criterion for its application in different industries.

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