



HYDRODYNAMIC PROPERTIES OF TRAGACANTHIN. STUDY OF TEMPERATURE INFLUENCE

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Abstract

The molecular weights and hydrodynamic properties are important parameters affecting the performance of tragacanthin as thickening and gelling agents in foods, pharmaceutical, biochemical and mining industry. The most common and economical of the hydrodynamic properties is the determination of viscosity, intrinsic viscosity and diffusion coefficient. They indirectly measure the molecular weight (M); hydrodynamic radius (R_H); and hydrodynamic parameters at different

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temperatures. This work studies tragacanthin dependence with temperature, finding that this biopolymer in aqueous solution behaves rigid random coil (stiff chain) with $\nu_{(p)} > 2.5$, when temperature increases R_H decreases (tends to compact).

Mark-Houwink (MH) equation constants, a and k , for tragacanthin in water solvent-temperature systems have been already reported; and two equations are proposed to determine, a and k at different temperatures from viscometric data. The molecular weight determined for tragacanthin is 17×10^4 g/mol.

Key words. tragacanthin, hydrodynamic, Mark-Houwink parameters, temperature

Resumen

El peso molecular y las propiedades hidrodinámicas son parámetros importantes que afectan el rendimiento de tragacantina como agente espesante y gelificante en los productos alimenticios, farmacéuticos, bioquímicos y de la industria minera. La más común y económica de las propiedades hidrodinámicas es la determinación de la viscosidad, densidad, viscosidad intrínseca y el coeficiente de difusión. La medida de la viscosidad intrínseca nos permite, indirectamente, estimar el peso molecular (M); radio hidrodinámico (RH), y a su vez parámetros hidrodinámicos a diferentes temperaturas. En este trabajo se estudia la dependencia con la temperatura del biopolímero tragacantina en solución acuosa, encontrando que adquiere una conformación de un ovillo aleatorio rígido (cadena rígida) con $\nu(p) > 2,5$, donde un incremento de la temperatura de la solución provoca una disminución en el radio hidrodinámico (tiende a compactarse).

Las constantes de la ecuación de Mark-Houwink (MH), a y k , son informadas para tragacantina en solución acuosa a diferentes temperaturas. Además, se proponen dos ecuaciones para determinar, a y k a diferentes temperaturas a partir de los datos viscométricos, cuyo peso molecular determinado para tragacantina es de 17×10^4 g/mol.

Palabras clave: tragacantina, hidrodinámica, parámetros de Mark-Houwink, temperatura

INTRODUCTION

Gum tragacanth is a dried exudation obtained from the stems and branches of Asiatic species of *Astragalus*. It has been used as a stabilizer, emulsifier and thickener in food, pharmaceutical, cosmetic industries. Tragacanth is one of the most acid resistance gums and most efficient natural emulsifier for acidic oil-in-water emulsions. Gum tragacanth is a branched, heterogeneous, and anionic carbohydrate which consists of two major fractions: tragacanthin (water-soluble) and bassorin (water-swelling). It is not understood yet if the two polysaccharides are in a physical mixture or chemically bonded to each other, although easy separation procedures favor the former hypothesis. Bassorin and tragacanthin composition differ particularly in terms of their uronic acid and methoxyl content; it has been suggested that bassorin is a complex structure of polymethoxylated acids and on demethoxylation, probably yields tragacanthin. The capacity of gum tragacanth to extensively modify the rheology of aqueous media even at fairly low concentration is the most important factor in evaluating tragacanth and is regarded as a measure of its quality and also a guide to its behavior as a suspending agent, stabilizer, and emulsifier. A few studies have been devoted to functional properties of gum tragacanth, and most of the works on structural and functional properties of the gum as well as its application in various fields have been done on samples with unknown botanical sources, and often, experimental conditions were not clearly explained, then the results are diverse, nonconforming, and misleading [1].

Tragacanthic acid was found to contain residues of D-galacturonic acid, D-xylose, L-fucose, D-galactose and trace amounts of D-glucuronic acid. Tragacanthin is based on essentially linear chains of 1,4-linked α -D-galacturonic acid residues. The majority of these residues carry xylose-

containing side-chains through C-3 a study of the structure of arabinogalactan established that the polymer is composed of interior chains of D-galactopyranose residues, in which the majority of units are joined by 1,6-linkages and to lesser degree by 1,3-linkages. These basal chains carry highly branched exterior chains of l-arabinofuranose. It has been reported that the polymer has a high content of arabinose and galactose residues [2].

Guner group 2000-2001 [3-4], studied theta temperatures evaluated via interpolation methods employing interaction parameter and second virial coefficient from intrinsic viscosity measurements achieved between the temperature intervals of 25°C and 43°C for the same system.

Kar & Arslan 1999 [5], reported about the effects of temperature and concentration on the viscosity of orange peel pectin solutions were examined at five different temperatures between 20 and 60°C and five concentration levels between 2.5-20 kg/m³. They found that the effect of concentration was described by two types of equation, power-law and exponential.

Chen & Tsaih 1998 [6], studied the effect of temperature on the conformation of chitosan. Herein, intrinsic viscosities of chitosans with different molecular weights were determined at temperatures between 10 and 50°C. They obtained from the slope of the plot of $\ln[\eta]$ versus $1/T$, was the $d\ln[\eta]/d(1/T)$, a stiffness index of chitosan molecules. The effects of temperature on the intrinsic viscosity and conformation of chitosan were elucidated calculating the MH parameters.

Morris et al. 2009 [7], investigated the solution conformation of chitosan at 4, 25 and 40°C from: (a) the MH power law relationship and (b) the persistence length, L_p .

A work done on tragacanthin and other polysaccharides at 35°C using high performance size exclusion chromatography (HPSEC) with concentration-viscosity detection was carried out by Hoagland et al. 1993 [8].

Mohammadar et al. 2006 [9], evaluated the solution properties of tragacanthin by gel permeation chromatography (GPC) combined with multi-angle light scattering, photon correlation spectroscopy, and viscometry at 25°C. These authors founded the dependence of intrinsic viscosity $[\eta]$ on weight average molecular mass M for this biopolymer, whose relationship was of $[\eta] = 9.077 \times 10^{-5} M^{0.87}$ (dL g⁻¹) in the range of M from 1.8×10^5 to 1.6×10^6 (g mol⁻¹).

Another most current work is done by Balaghi et al. 2010 [10], which evaluated six species of Iranian gum tragacanth dispersions was investigated at different temperatures and ionic strengths, within a concentration range (0.05-1.5% w/w) using a rheometer.

In this paper, the tragacanthin was characterized by viscometry in bidistilled water solution, in order to determine the conformational characteristic of tragacanthin. Then the data of intrinsic viscosity ($[\eta]$) and molecular weight (M) were analyzed on the basis to obtain molecular parameters.

MATERIALS AND METHODS

Tragacanth gum dispersion was prepared by weight (1% w/v). Five gram of biopolymer powder was dispersed in pure deionized water and then gentle stirring at room temperature for 2h. The dispersions were left at 3°C for 24h to enable biopolymer hydration. Centrifugation at 9g for 60 minutes allowed the separation of soluble from insoluble part. Both fractions were freeze-dried and sealed in zip plastic bags and then kept in desiccators. Finally, 1g of tragacanthin was dissolved in 100ml of distilled water. Density of solution and solvent were measurement with Anton Paar densimeter DMA5N. Solutions and solvents were analyzed using an Ubbelohde viscometer (IVA 1), under precise temperature control using thermostatic bath (Haake 1C).

THEORY

The capillary viscosity η is,

$$\eta = A\rho t \quad (1)$$

Where η (poise) is the viscosity, A (cm^2/s^2) is the viscometer constant, ρ is density (g/cm^3), and t is the draining time of liquid (s).

The relative, η_{rel} , was calculated from

$$\eta_r = \frac{t\rho}{t_0\rho_0} \quad (2)$$

Where t is the drainage time of polysaccharide sample, and t_0 is the water drainage time [10]. Where

$$\eta_{sp} = \eta_r - 1 \quad (3)$$

$$\eta_{red} = \frac{\eta_{sp}}{c} \quad (4)$$

Where c (g/cm^3) is the concentration of the polysaccharide sample.

A plot η_{red} vs. concentration yields the intrinsic viscosity, $[\eta]$ at the intercept and the slope is related to the concentration dependence, k_H [11].

$$\eta_{red} = [\eta] + k_H [\eta]^2 c \quad (5)$$

This way of calculating the intrinsic viscosity requires several concentrations in order to determine it. The intrinsic viscosity may be easily calculated by the Solomon-Ciuta single-point equation [12],

$$[\eta] = \frac{1}{c} \sqrt{2\eta_{sp} - 2 \ln \eta_r} \quad (6)$$

The relation between molecular weight (M , g/mol) and the intrinsic viscosity is given by MH equation,

$$[\eta] = k_{MH} (M)^a \quad (7)$$

The calculation of MH parameters is carried out by the graphic representation of the following equation:

$$\ln[\eta] = \ln k_{MH} + a \ln M \quad (8)$$

Where k_{MH} and a are MH constants, these constant depend of the type of polymer, solvent, and temperature of viscosity determinations. These constants can be determined experimentally by measuring the intrinsic viscosity of several polymer samples for which the molecular weight has been determined by an independent method (i.e. osmotic pressure or light scattering). Using the polymer standards, a plot of the $\ln [\eta]$ vs $\ln M$ usually gives a straight line. The slope is a value and intercept is equal to $\ln k_{MH}$ value. The MH exponent bears the signature of a three-dimensional configuration of a polymer chain in the solvent environment. For a values are from 0-0.5 rigid sphere in ideal solvent, from 0.5-0.8 random coil in good solvent, and from 0.8-2 rigid or rod like (stiff chain). A long-chain polymer molecule in solution takes on a somewhat kinked or curled shape, intermediate between a tightly curled mass (coil) and a rigid linear configuration. All possible degrees of curling may be displayed by any molecule, but there will be an average configuration which will depend on the solvent. The polymer molecule assumes a tighter configuration, and the solution has a lower intrinsic viscosity. The MH “ a ” constant is close to 0.5 in “poor” solvents. For a rigid or rod like polymer molecule that is greatly extended in solution, the Mark-Houwink “ a ” constant approaches a value of 2.0 [13-14].

The hydrodynamic radius (R_H), for a sphere ($\nu_{(p)} = 2.5$) is given by the Einstein relation,

$$[\eta]M = \nu_{(p)}N_A \frac{3}{4}\pi(R_H)^3 \quad (9)$$

The viscosity of liquids is highly dependent on temperature and presents complex relationships. The change of viscosity at different temperatures is commonly calculated with an equation of the Arrhenius form:

$$\eta = A_{vf} \exp\left(\frac{E_{avf}}{RT}\right) \quad (10)$$

Where η is the viscosity (poise), E_{avf} is the energy of the viscous flow activation (cal/mol); R is the gas constant (1.98 cal/mol K) and T the temperature (K).

Stokes-Einstein equation, which assumes diffusion of a solute (rigid sphere) in a solvent continuum at constant temperature (typically 25°C). The correlation of this equation with the molecular weight and the viscosity is as follows:

$$D(cm^2 / s) = 8.34 \times 10^{-8} \left(\frac{T}{\eta M^{1/3}} \right) \quad (11)$$

Diffusion coefficients are related to hydrodynamic properties which can provide information about the size and the shape of macromolecules.

Hydrodynamic properties, such as the η and D , the intrinsic viscosity, $[\eta]$, and equilibrium solution properties such as the hydrodynamic radius R_H can be combined to construct dimensionless quantities that are universal in the sense of being independent of the size of the macromolecular particle, while they depend more or less sensitively on its shape or conformation.

Typical examples are the Scheraga-Mandelkern parameter, β given by

$$\beta = \frac{\eta_0}{f} \left(\frac{M[\eta]}{100} \right)^{1/3} \quad (12)$$

The friction coefficient f is measured from the diffusion coefficient, from equation of $f = k_B T/D$ where k_B is the Boltzmann constant and T the absolute temperature. In equation 11, where M is the molecular weight of the macromolecule, and η_0 the solvent viscosity. Other classical size-independent combinations are the Flory parameters that combine the intrinsic viscosity, $[\eta]$, and the radius of gyration, R_g :

$$\phi_0 = \frac{[\eta]M}{6^{3/2} R_g^3} \quad (13)$$

and another combining the friction coefficient with the radius of gyration:

$$P_0 = \frac{f}{6\eta R_g} \quad (14)$$

β takes the values of 2.112×10^6 and about 2.3×10^6 for a sphere and a random coil, respectively, while the values for these two structures in the case of the ϕ_0 are 9.23×10^{23} and 2.60×10^{23} . Thus, it is accepted that, for every flexible-chain polymer in a Θ (ideal) solvent, there is a universal value of $\phi_0 = 2.50 \times 10^{23}$ [15].

For rigid macromolecules, it is also conventional, particularly for rigid macromolecules, to combine a solution property with the volume of the particle itself, or with a quantity directly derived from it. Thus, it is common practice to express the frictional coefficient of rigid structures as

$$P \equiv \frac{f}{f_0} = \frac{f}{6\pi\eta_0(3V/4\pi)^{1/3}} \quad (15)$$

Where (in our notation) f_0 is the frictional coefficient of a sphere having the same hydrodynamic (hydrated or solvated) volume V as the particle. The term f/f_0 is sometimes denoted as P , Perrin number. A similar combination involves the intrinsic viscosity and specific volume:

$$v_{(p)} = \frac{[\eta]}{V_s} \quad (16)$$

$v_{(p)}$ is called Einstein viscosity increment or Simha number, and V_s is specific volume (cm^3/g).

The corresponding value of the 'hydration' δ of the molecule, defined by

$$\delta = (V_s - \bar{v})\rho_0 \quad (17)$$

to be $\sim 50\text{g}$ solvent bound per g of solute. Although, because of the approximations we have made, the actual numerical value must be treated with very great caution, this treatment does however suggest that polysaccharide is highly expanded, but perhaps not to the same extent as found for coil-like polysaccharide structures.

RESULTS AND DISCUSSION

Figure 1-a shows the linear relation between viscosity and temperature, where the value obtained from E_{avf} 5529.4 cal/mol, and A_{vf} 7.21×10^6 g/cm s, with σ^2 0.9963. This occurs due to the higher resistance to flow of bigger biomacromolecules requiring, therefore, more energy. Figure 1-b shows that the intrinsic viscosity is influenced by temperature for tragacanthin.

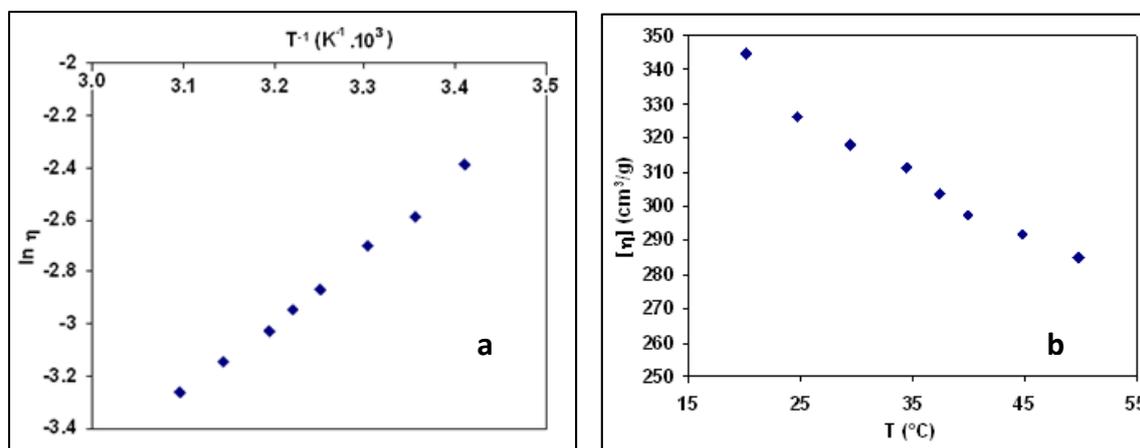


Figure 1. a- Logarithmic plot of viscosity in function of $1/T$ of tragacanthin. b- Influence of temperature on intrinsic viscosity.

Within a limited temperature range, the viscosity of a polymer solution varies generally following a relation similar to that of usual liquids (equation 9), provided that polymer concentration is not too high and that temperature remains far enough from glass transition state [16-17].

According to Stokes-Einstein equation, the diffusion coefficient is inversely proportional to the solution viscosity which increases with temperature. Hence, a lower diffusion coefficient corresponds to a lower size molecule [18] (see Table 2).

Mohammadar et al. 2006 [9], reported M for tragacanth from 180000 to 1600000 g/mol, and MH parameters of $0.009077 \text{ cm}^3/\text{g}$ and “ a ” of 0.87 at 25°C , data similar to those obtained in this work. Balaghi et al. 2010 [10], evaluated six species of Iranian gum tragacanth, but not reported intrinsic viscosity and molecular weight data.

Table 1 shows the classical values for MH parameters, a and k for temperature. These studies on MH parameters are usually carried out at a given temperature, obtaining a consistent result but in a very limited range of temperature [19]. This value shows a clear functionality between these parameters and temperature, as expected in equations 18 and 19 are exponential type.

$$k = 0.0117 \exp(-0.0098T) \quad \sigma^2 = 0.9804 \quad (18)$$

$$a = 0.8624 \exp(0.0003T) \quad \sigma^2 = 0.9776 \quad (19)$$

Table 1: Dates of intrinsic viscosity and Mark-Houwink parameters on temperature function.

T ($^\circ\text{C}$)	$[\eta]$ (cm^3/g)	k (cm^3/g)	a
20.1	344.9891	0.009780	0.8681
24.8	326.2436	0.009077	0.8700
29.5	318.1233	0.008701	0.8715
34.5	311.2440	0.008490	0.8721
37.4	303.5430	0.008055	0.8739
39.9	297.3088	0.007788	0.8748
44.8	291.8366	0.007512	0.8764
49.8	285.2091	0.007298	0.8768

The value of a given at different temperatures shows that this polysaccharide in aqueous solution behaves in a conformation predominantly confined to the form of rigid random coil, as observed by other authors [8-9].

Furthermore, the influence of temperature on the intrinsic viscosity is given by the parameter of chain flexibility ($d\ln[\eta]/dT$), which gives information about the conformation of the macromolecule chain in solution.

The parameter of chain flexibility ($d\ln[\eta]/dT = 586.95 \text{ K}^{-1}$), indicates that the chain flexibility of this molecular weight tragacanthin is less flexible. Analysis of the relative stiffness parameter indicates that at 172300 g/mol molecular weight of tragacanthin is less flexible, as this molecule is of low molecular weight rigid behaves [6].

Analyzing the values of the hydrodynamic properties of tragacanthin in aqueous solution can be seen in Figure 2 a-b and table 2, all vary with the temperature. β values increases from 2.30 to 3.01×10^6 to increase with temperature, indicating the changes file from a flexible structure to a rigid. Values of ϕ_0 and P_0 decreases from 2.54 to 2.10×10^{23} and 5.92 to 4.25 demonstrating a high compaction of particles. The value of P is of 7.92, and $v_{(P)}$ is of 5.57 which confirms that tragacanthin is a rigid random coil in aqueous solution with a tendency to compaction with increasing temperature. The value of δ as expected decreases from 58.08 to 52.59 g/g this phenomenon is due to loss of water due to compression of tragacanthin colloidal particle by the effect of increasing temperature, similar phenomenon is the drying gels to form films, or compaction of a sponge [20-21].

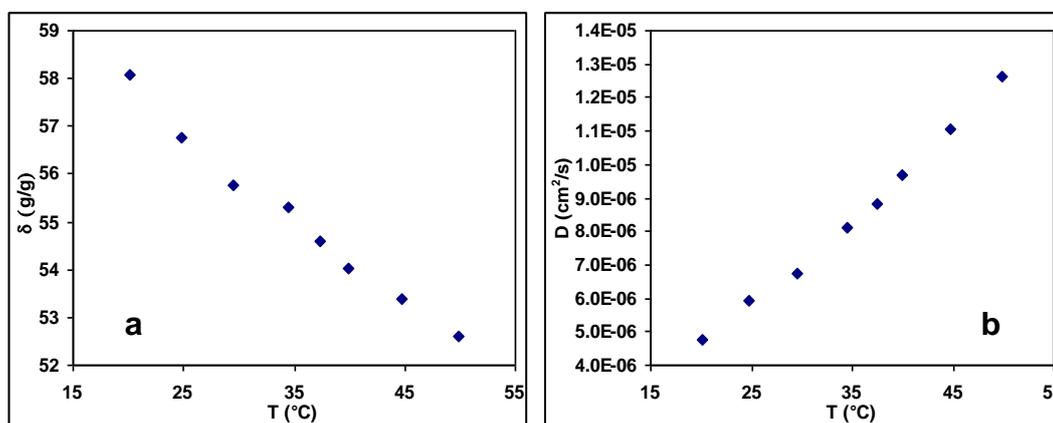


Figure 2. Hydrodynamic properties of tragacanthin. **a.** Diffusion coefficient. **b.** δ Hydration value.

The Mark-Houwink parameters for polymers and biopolymers may vary with the type of solvent used, and with temperature. This is because the macromolecule hydrodynamic radius changes with type solvent and temperature via change in its chain flexibility. In a good solvent, a temperature increase results in an intrinsic viscosity decrease and in a less-extended conformation ($D >$ and $R_H <$), because the entropy value increases with an increase in temperature and it is unfavorable for an extended conformation ($E_{avf} >$). In the case of a poor solvent, a temperature increase causes an increase in entropy and intrinsic viscosity and is favorable for the not extended conformation [21].

M-H values confirm that for these conditions tragacanthin behave as rigid random coil. To facilitate the calculation of these parameters in an acceptable way exponential function given in equations 18-19 can be used. Such empirical equations relating the parameters of Mark-Houwink with T , which ultimately describe this type of thermodynamic parameters are relations between properties the solute with the solvent and temperature dependence.

Table 2. Hydrodynamic properties of tragacanthin.

T (°C)	R_H (cm) x 10 ⁶	D (cm ² /s) x 10 ⁶	δ (g/g)	β x 10 ⁻⁶	ϕ_0 x 10 ⁻²³	v_{ϕ}	P	P_0
20.1	2.11	4.78	58.08	2.30	2.54	5.92	9.13	5.92
24.8	2.07	5.94	56.77	2.40	2.40	5.87	8.68	5.54
29.5	2.05	6.74	55.50	2.50	2.34	5.67	8.42	5.31
34.5	2.04	8.11	55.31	2.67	2.29	5.55	7.88	4.93
37.4	2.02	8.82	54.58	2.76	2.23	5.47	7.61	4.72
39.9	2.01	9.69	54.02	2.84	2.19	5.40	7.40	4.56
44.8	1.99	11.0	53.37	2.92	2.14	5.35	7.21	4.42
49.8	1.98	12.6	52.59	3.01	2.10	5.27	6.99	4.25

CONCLUSIONS

The MH parameters have an exponential functionality with temperature. The numerical value of a indicates that tragacanthin acquire the shape of a rigid random coil in aqueous solution; and k demonstrates that water solution their value decreases with temperature [6, 22].

Due to the lack of data on the uniformity of intrinsic viscosity measurements in the water system / tragacanthin clearly shows an increase in " a " with temperature. The equations 18-19 were empirical equations relating the parameters of Mark-Houwink with T , and facilitate the calculation of these parameters in an acceptable temperature range and this M for tragacanthin.

Tragacanthin behavior in this system indicates that it behaves rod like that tends to contract with increasing temperature. This conclusion is supported by the observed data from the hydrodynamic properties analyzed.

An increase in temperature causes the tragacanthin/water system to show a trend of the macromolecule to compaction (decrease in R_H and $[\eta]$), which requires an increase of energy consumption due to a greater difficulty in flowing (increase in D and E_{avf}). This phenomenon is observed in the case of ideal solvents, evidencing an increase of a with temperature.

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