

Review of the characterization of sodium alginate by intrinsic viscosity measurements. Comparative analysis between conventional and single point methods

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Abstract

In the pharmaceutical and food industry, alginate is used in aqueous solutions and excipients as thickener, gelling agent, coagulant and encapsulant. This biopolymer is very important, with many key industrial applications, especially if the intrinsic viscosity $[\eta]$ and the viscometric molecular weight (M_v) are known. Alginate is obtained from the cell walls of algae such as *Macrocystis pyrifera*, *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Sargassum vulgare*, etc.; bacteria such as *Pseudomonas aeruginosa* and *Azotobacter vinelandii* can also synthesize it. This work studied alginate in aqueous solution from 0.25 to 1% wt., measuring its intrinsic viscosity in order to obtain its M_v . The intrinsic viscosity is calculated by the classical methods of Huggins, Kremer, Schulz-Blashke and Martin. We compared several single point methods to determine which of them would be more appropriate for an alginate-water system. We also obtained Mark-Houwink parameters and the corresponding viscometric molecular weight.

Keywords

Alginate, Intrinsic Viscosity, Single Point, Hydrodynamic

1. Introduction

Alginates are unbranched copolymers of (1→4)-linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues. If the uronic acid groups are in the acid form (–COOH), the polysaccharide, called alginic acid, is water insoluble. The sodium salts of alginic acid (–COONa), or sodium alginates, are water-soluble. The sequence of mannuronic and guluronic residues significantly affects the physicochemical properties of alginates. The ratio between the residues of β -D-mannuronic acid and those of α -L-guluronic acid (MG) is usually 2:1, although it may vary with the algal species, the age of the plant and the type of tissue from which the alginates are extracted. The main advantage of alginate as a gel former [1-4] is its ability to

form heat-stable gels that can set at room temperatures. In food applications is of interest the gels formation, ie calcium ions.

Alginate is located in the cell wall and in the matrix of the algae, cementing the cells together and giving certain mechanical properties to the algae. In its native state, alginate exists as an insoluble mixed salt of all the cations that are found in seawater, the main ones being sodium, magnesium, and calcium. It maintains a fast ion-exchange equilibrium with seawater. The alginate extraction process can be performed in two steps: transformation of insoluble alginate into a soluble form, namely sodium alginate, followed by diffusion of the soluble glycuronan into solution.

The three types of blocks in alginate have been characterized by partial hydrolysis with HCl; ie mannuronic-guluronic (MG-block), mannuronic (M-Block) and guluronic (G-Block). The material thus solubilized corresponds to the MG-block. The resistant part was then fractionated at pH 2.9. The soluble fraction corresponds to the M-block, the insoluble to the G-block. For commercial and scientific purposes, the most important property of alginates is their ability to form viscous solutions in water, and thus alginate samples can be characterized by intrinsic viscosity. The ability of alginates to form gels in the presence of calcium ions is due to glycuronan as it has one of its main biofunctional properties, and therefore is of great industrial interest. The formation of gels depends mainly upon autocoooperatively-formed functions between chain regions enriched in GG-sequences [2]. These polysaccharides show interesting rheological properties: that improve the viscosity of aqueous solutions to low concentrations, and to gels form or thin films. They are widely used in various industrial fields, such as in the textile, food, paper, cosmetics and pharmaceutical industries. The extraction protocol used by the alginate industry is divided into five steps: acidification, alkaline extraction, solid/liquid separation, precipitation and drying. Acidification consists in immersing seaweeds in a sulphuric acid bath (about 0.5 N) for several hours to convert the insoluble alginate salts present in the cell walls into alginic acid. Alkaline extraction is the central step, as it corresponds to the extraction phase itself. Acidified seaweeds are soaked in a sodium carbonate solution: insoluble alginic acid is converted into soluble sodium alginate, which passes into the aqueous phase. It requires several hours to reach the optimum extraction yield, depending on the seaweed species considered. Seaweed residues are then separated from the sodium alginate solution using floatation/flocculation and filtration. Sulphuric acid or calcium chloride is then added to precipitate alginates in their acid or calcium salt form, respectively, the latter being easier to dewater. The product is afterwards pressed and dried by heating. The different alginate salts are finally produced by making alginic acid react with the appropriate base. A previous work highlighted a decrease of dynamic viscosity and average molecular weight during alkaline extraction of alginates from *Laminaria digitata* [5]. This depolymerization phenomenon seems to be mainly due to pH and temperature effects. Bacterial development and endogenous alginate lyases activities are also mentioned. The most evident solution to avoid these depolymerization phenomena, would be, from the industrial point of view, to reduce the extraction time [5-6]. Soluble sodium alginate can be cross-linked with divalent or polyvalent cations to form an insoluble alginate. Due to this property, alginate can be employed as a controlled release device for some drugs. Calcium cations are commonly used to cross-link sodium alginate. They have been reported to bind preferentially to the polyguluronic acid units of alginate in a planar two-dimensional manner, producing the so-called

“egg-box” structure [7-11]. Acetylation dramatically affects both the solution properties and the metal-induced precipitation of alginates. The presence of acetyl groups in both bacterial and seaweed alginate polymers marginally increased the weight average molecular weight (M_w) of each polymer by 7% and 11 %, respectively. Acetylated bacterial alginate showed a significant increase in solution viscosity compared to its deacetylated counterpart. However, microbial acetylation of seaweed alginate did not change its solution viscosity. Acetylation altered the calcium-induced precipitation of both alginates. The presence of acetyl groups decreased the ability of each polymer to bind with calcium but increased their ability to bind with ferric ions (Fe^{3+}). By controlling the degree of acetylation on the alginate chains, it was possible to modify the solution viscosity and the cation-induced precipitation of these polymers [12].

Alginate microspheres can be produced by the external or internal gelation method using calcium salts. The addition of calcium chloride solution by external gelation method, using an emulsification technique, in the final phase of the production of microspheres, causes the disruption of the equilibrium of the system being stirred, resulting in a significant degree of clumping of the microspheres [13-15].

Alginate is a linear, anionic polysaccharide with an abundance of free hydroxyl and carboxyl groups distributed along the polymer chain backbone; it, therefore, unlike neutral polysaccharides, has two types of functional groups that can be modified to alter its characteristics compared to the parent compounds [16-17]. The viscosities of aqueous solutions of dilute sodium alginate have been measured at various temperatures. The upward bending phenomenon of the reduced viscosity of the solution of sodium alginate in the dilute concentration region resulted from the adsorption of polymer on the glass capillary wall. The polyelectrolyte behavior can be described by the effects arising from the intra-chain electrostatic forces between the charges present on the chain backbone. For example, the famous phenomenon of upward bending of reduced viscosity versus the concentration plot of the polyelectrolyte solution in the dilute concentration region is ascribed to the intra-chain electrostatic repulsion of charges on the same backbone. This leads to chain extension and an increase of reduced viscosity upon dilution. The upward bending phenomenon of viscosity has therefore been regarded as typical of the nature of polyelectrolytes; it is defined as the “polyelectrolyte effect” [18]. Some of the first authors to work with sodium alginate in water solution were Donnan & Rose, who determined Mark-Houwink parameters through intrinsic viscosity measurements [19]. Harkness & Wassermann [20] determined the nominal molecular weight ($M_n = 120000\text{g/mol}$) of alginate with intrinsic viscosity data of $620\text{-}690\text{ cm}^3/\text{g}$, in 0.1M of NaCl solutions. In the 1960s, Haug & Smidsrod worked especially hard on the physicochemical and structural characterization of alginate [22]. Table 1 contains the data of the intrinsic viscosity measurements, M_w and Mark-Houwink parameters

calculated by different authors in different periods.

Table 1. Previous antecedents of the values of intrinsic viscosity and Mark-Houwink parameters for aqueous solutions of alginate.

Supplier and producer organisms of alginate	Solution	T (°C)	[η] (cm ³ /g)	M _w (g/mol)	k (cm ³ /g)	a	References
<i>Laminaria claustroni</i>	Water	25	420-1430	48000-186000	0.0123	0.960	Donnan & Rose 1950 [19]
Alginate –(Non specified)	0.1M NaCl	20	620-690	120000 Mn	0.0320	1.000	Harkness & Wasserm-ann 1952 [20]
<i>Laminaria, Fucus</i> and <i>Ascophyllum</i> species	0.1M NaCl	25	360-1920	42000-2202000	-	-	Vincent et al. 1955 [21]
Alginate (Non specified)	Water	-	31-170	30000-370000	-	-	Cook & Smith 1955 [22]
<i>Laminaria</i> species	0.1M NaCl	20	2600-3000	-	-	-	Haug & Smidsrod 1962 [23]
Alginate (Non specified)	0.01M NaF	20	1390	790000	0.0020	1.000	Smidsrod & Haug 1968 [24]
	0.01M		305-1200		0.00048	1.150	
	0.1M		225-5500		0.0020	1.000	
<i>Laminaria digitata</i>	1 M NaCl	20	195-3600	112000-2700000	0.0091	0.870	Smidsrod 1970 [25]
	∞		185-2450		0.0120	0.840	
<i>Laminaria hyperborea</i>			350-2500	170000-940000	0.0120	1.110	
<i>Fucus vesicularis</i>	0.1 M NaCl	25	500-2850	280000-1640000	0.0178	1.000	Mackie et al. 1980 [26]
<i>Azotobacter vinelandii</i>			810-1700	270000-610000	0.0501	0.910	
Alginates	-	-	-	-	0.0020	1.000	Launey et al. 1986 [27]
<i>Macrocystis pirifera</i>	0.1M NaCl	25	548	197300	0.00730	0.920	Martinsen et al. 1991 [28]
<i>Laminaria hyperborea</i>			807	234500	0.00069	1.130	
<i>Ascophyllum nodosum</i> and <i>Macrocystis pyrifera</i>	0.1M NaCl	25	-	-	-	0.910-1.130	Moe et al. 1995 [29]
Alginate (Janssens)	0.1M NaCl	20	142.01	526000	-	-	Velings & Mestdagh. 1995 [30]
<i>Macrocystis pirifera</i>	0.1M NaCl	5-35	618-1213	76312-153710 Mn	0.01228	0.963	Mancini et al. 1996 [31]
<i>Fucus vasicularis</i>	0.1M NaCl	20	250	12500 Mn 750000	0.00200	1.000	Fourest & Volesky 1997 [32]
<i>Laminaria japonica</i>			1540	Mn 750000			
<i>Azotobacter vinalandii</i>	0.1M NaCl	25	328-1510	154600-730000	0.0230	0.984	Clementi et al. 1998 [33]
			725				
<i>Laminaria hyperborea</i>	0.1M NaCl	20	910	-	-	-	Ouwerx et al. 1998 [34]
<i>Macrocystis pyrifera</i>			610				
			290				
<i>Macrocystis pyrifera</i>	-	-	920	250000	-	-	Pelletier et al. 2000 [35]
<i>Laminaria hyperborea</i>	0.1M NaCl	20	170-1440	5100-46500	0.00485	0.970	Stokke et al. 2003 [36]
Alginate (Adrich)	0.1M NaCl	25	1074	410000	0.00730	0.920	Gomez-Diaz & Navaza 2003 [37]
<i>A. nodosum</i>	0.1M NaCl	20	500-1440	155000-465000	0.00484	0.970	Draguet et al. 2003 [38]
<i>Laminaria Hyperborea</i>							
<i>Cystoseira trinode</i>			860				
<i>Cystoseira myrica</i>			860				
<i>Sargassum dentifolium</i>	0.1M NaCl	20	1260	-	-	-	Larsen et al. 2003 [39]
<i>Sargassum asperifolium</i>			1520				
<i>Sargassum latifolium</i>			870				
<i>Laminaria hyperborea</i>	0.1M NaCl	20	93.5-572	35000-280000	-	-	Larsen et al. 2003 [40]
Oxidized Alginates	0.1M	20	-	20000-100000	0.0051	1.000	Vold et al.

Supplier and producer organisms of alginate	Solution	T (°C)	[η] (cm ³ /g)	M _w (g/mol)	k (cm ³ /g)	a	References
<i>(Laminaria Hyperborea)</i>	NaCl			100000-300000	0.0349	0.830	2006/2007 [41-42]
				300000-1000000			
					0.0305	0.660	
Oxidized Alginates	Water	25	325	100000	-	-	Gomez et al. 2007 [43]
<i>Sargassum vulgare</i>	0.1M NaCl	25	410-690	194000-330000	0.0300	0.984	Torres et al. 2007 [44]
<i>Alginate (Kimica)</i>	-	-	-	59000-255000	0.002	1.000	Kakita & Kamishima 2008 [45]
Alginate (Non specified)	0.1M NaCl	25	2021.21	-	0.011	0.930	Holme et al. 2008 [46]
<i>Laminaria hyperborea</i>			590				
leaf <i>Laminaria hyperborea</i>			1200		0.0054	1.000	Morch et al. 2008 [47]
<i>DurVillea potatorum</i>			1110				
Alginate (Sinopharm)	water	25	760	430000	-	-	Yang et al. 2009 [48]
Alginate (Kibun Food Chem.)	10mM NaCl	25	1300	104000	-	-	Minami et al. 2010 [49]
Alginate (FMC Biopolymer)	0.1M NaCl	20	287.65-364.8	8330-106000	0.00485	0.970	Davidovic-Pinhas et al. 2010 [50]
Alginate (FMC Biopolymer)	0.1M NaCl Ca<0.51%	25	553-1126	263000-541000	0.0230	0.984	Fu et al. 2010/2011 [51-52]
Alginate				100000-1000000			Andersen et al. 2012 [53]
<i>Macrocystis pyrifera</i>	0.01-0.4 M NaCl	30	490-657	178000-199000	0.47-1.13	-	Díaz Baños et al. 2014 [54]

Cook & Smith [22] measured the molecular weights obtained from sedimentation–diffusion and sedimentation; the intrinsic viscosity ranged from 46000 to 370000 g/mol, demonstrating that the results indicate that sodium alginate has a relatively high extension ratio.

Smidsrod [25] and Launey et al. [27] measured the intrinsic viscosity of several alginate samples ranging in weight-average molecular weight from about 100000 to 2700000 g/mol; the measurements were carried out in aqueous salt solutions of various strengths. They found that the exponent a in the Mark-Houwink equation decreased with increasing salt concentration, but even at infinite ionic strength the value was very high ($a = 0.84$), suggesting that the discharged alginate molecule is very extended in water, but it is argued that the main cause of the extension is a high degree of chain rigidity.

Martinsen et al. [28] analyzed different molecular weights of alginates by different methods. They found the data of [η], the Mark-Houwink parameters and the viscosity molecular weight of *Macrocystis pyrifera* and *Laminaria hyperborea*, by GPC measurements using a universal calibration curve.

Mancini et al. [30] analyzed both high-Mannuronic and high-Glucuronic alginates in aqueous dispersions, where they exhibited non-Newtonian shear-thinning behaviour when the alginate concentration and temperature ranged from 0.125 to 1.5% w/v and from 278.16 to 308.16 K, respectively. Thus, intrinsic viscosity is correlated to the average molecular weight via the well-known Mark-Houwink equation, using Donnan and Rose's experimental results [19], listed in Table 1.

Gomez-Diaz & Navaza [37] conducted studies on the

rheological properties of carboxymethyl cellulose and alginate sodium salts. The molecular weights of these polymers were calculated using Huggins and Kramer equations, and intrinsic viscosity in aqueous solutions was determined.

Vold et al. [41-42] found that, due to the curvature, the M-H equation was split into three regions describing different molecular weight regions of oxidized alginates.

In the pharmaceutical and food industry, the use of biopolymers in aqueous solution as thickeners and additives is very important, and their molecular weight (M_w) and size (RH) are key to their application. The technologies used for the determination of M_w and R_H are: gel permeation chromatography (GPC, SEC [55-59]), ultracentrifugation [60], osmometry [20], NMR [38, 58], Light Scattering [55, 61], intrinsic viscosity ([η]) (see table 1), etc. In this work, [η] was used to study the conformational aspects of alginate in aqueous solution at a concentration of 0.25 to 1% by weight, while intrinsic viscosity was calculated by different methods in order to assess which is the most appropriate.

The Hagen-Poiseuille law describes the flow through the capillaries, starting from the rate of flow (for a given volume) proportional to the fluid density (ρ g/cm³) and inversely proportional to viscosity η [62],

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

Where η (poise) is the viscosity, A (cm²/s²) is the instrumental constant of the viscometer, and t is the liquid draining time (s).

The relationship of density and drainage time between solute and solvent is given by the relative viscosity:

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

Where ρ is the density in g/cm^3 .

The inherent viscosity is:

$$\eta_{inh} = \eta_r - 1$$

The $[\eta]$ is defined according to Huggins [63]:

$$[\eta] \cong \lim_{c \rightarrow 0} \frac{\eta_{inh}}{c} \quad 3$$

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

$$\eta_{red} = [\eta] + k_H [\eta]^2 c \quad 4$$

This way of calculating the intrinsic viscosity requires several concentrations in order to determine it.

Kraemer (1938) [64],

$$\frac{1}{c} \ln \eta_r = [\eta] + k_K [\eta]^2 c \quad 5$$

Schulz-Blaschke (1941) [65],

$$\frac{\eta_{sp}}{c} = [\eta] + k_{S-B} [\eta] \eta_{sp} \quad 6$$

and Martin (1942) [66],

$$\frac{\eta_{sp}}{c} = [\eta] e^{k_M [\eta] c} \quad 7$$

For molecules of high intrinsic viscosity, a correction must be made for the effect of the rate of shear strain. For relatively low intrinsic viscosity, the rate of shear strain does not have any appreciable effect.

The intrinsic viscosity data calculated with the methods of Huggins, Kraemer, Schulz-Blaschke and Martin are shown in Table 2; how each of these methods performed is shown in Figure 2.

A graphical method was used for the estimation of the constant “ k_{B-P} ” in the Baker-Philippoff equation [67] for intrinsic viscosity measurement, for this method makes use of data obtained from the different concentrations required to determine intrinsic viscosity by extrapolation to zero concentration.

$$[\eta] = \frac{k_{B-P}}{c} \left\{ \left(10^{\frac{\log \eta_r}{k_{B-P}}} \right) - 1 \right\} \quad 8$$

Where “ k_{B-P} ” is the constant being investigated. Instead of using “ k_{B-P} ” is setting a value, this constant is adjusted to fit each separate case. The big problem with this equation is that the value of “ k_{B-P} ”, an integer, must be calculated for each system under study, which complicates its application.

All values were calculated for intrinsic viscosity measurements of sodium alginate solutions at 25°C and were compared against the value obtained by Huggins method, normally used as standard.

It frequently occurs that extrapolations do not have a common value at their origin ordinates. These deviations may be caused by inadequate lineal extrapolations. The above-mentioned method is the one routinely used for $[\eta]$ determination. The procedure is laborious and consumes a considerable amount of time and reactive; because of this, several equations were developed that estimate intrinsic viscosity at one single concentration and do not require a graphic. They are known as “single-point” methods. Single-point equations assume that k_H , k_K and k_{SB} are constants and that $k_H + k_K = 0.5$, as is indicated by the combination of equations Huggins and Kraemer. They all include the values for relative viscosity, increment of viscosity and concentration. For example, Solomon-Ciuta [68] proposes:

$$[\eta] \cong \frac{1}{c} \sqrt{2\eta_{sinh} - 2 \ln \eta_r} \quad 9$$

By studying the molecular weights of various polymer solutions, Solomon-Ciuta arrived at the formula that allowed the calculation of the intrinsic viscosity of polymer solutions by a single viscosity determination. The formula was verified for different polymer-solvent systems and the values are in accord with those obtained by extrapolation.

Deb and Chatterjee [68-69] suggested that:

$$[\eta] \cong \frac{1}{c} \sqrt[3]{3 \ln \eta_r + 1.5 \eta_{inh}^2 - 3 \eta_{inh}} \quad 10$$

More recently, Rao & Yansen [70] provided a simplified expression:

$$[\eta] \cong \frac{1}{2c} \{ \eta_{inh} + \ln \eta_r \} \quad 11$$

Kuwahara [71] uses the expression:

$$[\eta] \cong \frac{1}{4c} \{ \eta_{inh} + 3 \ln \eta_r \} \quad 12$$

While Palit & Kar [72] suggest:

$$[\eta] \cong \frac{1}{c} \sqrt[4]{4\eta_{inh} - 4 \ln \eta_r + 1.33 \eta_{inh}^3 - 2\eta_{inh}^2} \quad 13$$

and the following equation, due to Maron [73], makes use of the previously calculated parameters:

$$[\eta] \cong \frac{\eta_{sp} + \gamma \ln \eta_r}{c \{ 1 + \gamma \}}, \quad 14$$

where $\gamma = \frac{k_H}{k_K}$

Rao & Yaseen [70] and Chee [74] have examined the applicability of the single-point method and have found that some equations are inadequate or applicable only to some specific macromolecule-solvent systems.

Curvale & Cesco [75] suggest a double point equation

$$[\eta] = \frac{0.5c_2}{c_2 - c_1} \left\{ \frac{\eta_{inh,1}}{c_1} + \frac{\ln \eta_{r,1}}{c_1} \right\} - 0.5 \frac{c_1}{c_2 - c_1} \left\{ \frac{\eta_{inh,2}}{c_2} + \frac{\ln \eta_{r,2}}{c_2} \right\} \quad 15$$

where subscript 1 and 2 refer to the concentrations measured. The application of different single point methods and their comparison against the Huggins method are shown in Table 2. Single point methods fit with minor errors of 3.54%, but the Solomon-Ciuta method is only valid for concentrations below 0.25% wt. of alginate. The error of double point methods is low, of 2.00%. While all single and double point methods are used to characterize a polymer solution, it is always advisable to increase the statistical weight (to reduce errors) with at least four different concentrations of polymer in a given solvent.

A simple way to implement a single point method for such systems (alginate-water) is to adjust it according to the following equation:

$$[\eta] \cong \frac{\eta_{inh} \rho}{c \rho_0} \quad 16$$

The relation between M_w and the intrinsic viscosity is given by the Mark-Houwink-Sakurada equation,

$$[\eta] = k (M_w)^a \quad 17$$

The calculation of Mark-Houwink (M-H) [76-77] parameters is carried out by the graphic representation of the following equation:

$$\ln[\eta] = \ln k + a \ln M_w \quad 18$$

Where k and a are M-H constants, which depend on the type of polymer, solvent, and temperature of viscosity determinations [78]. The exponent a is a function of polymer geometry, and varies from 0.5 to 2. A rigid sphere in ideal solvent gives a values from 0 to 0.5, a random coil in good solvent from 0.5 to 0.8, and a rigid or rod-like (stiff chain) from 0.8 to 2 [79-80].

2. Materials and Methods

The technique consists of preparing four solutions at concentrations of sodium alginate (Sigma-Aldrich N° 180947) from 0.25 to 1% wt in NaCl 0.1M. Density is measured by a DMA35N densimeter provided by ANTON-PAAR. The measurement of drainage time was performed for triplicate, and carried out in a HAAKE C

thermostatic bath at 25°C. The viscometer used was Ubbelodhe 1C (IVA) with a water drainage time of 30s, and constant A of 2.69 cm²/s².

3. Results and Discussion

Figure 1 shows the density data as a function of concentration, which shows the strong dependence of density with respect to concentration, i.e., as the concentration increases, the density increases. Table 2 shows the viscosity, density and diffusion coefficient data for different alginate concentrations, where it can be reasonably seen that as concentration increases, viscosity and density increase while D decreases. The data of $[\eta]$, determined for an alginate-water system, was 1269.11 cm³/g, using the Huggins method as standard; with this data, an M_v of 490000g/mol and an R_H of 46.36 nm were calculated, shown in Table 3-5.

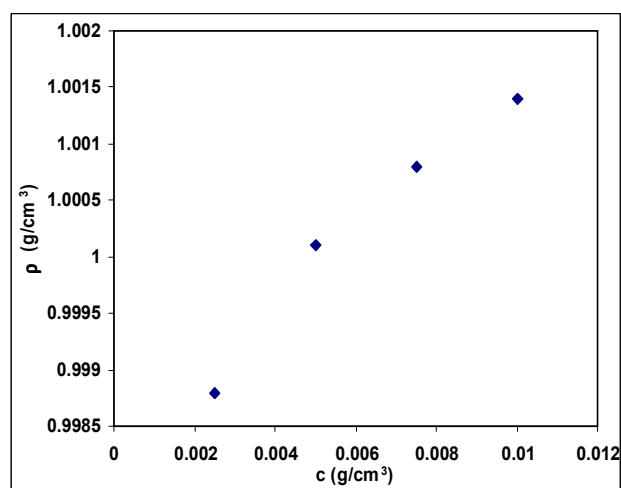


Figure 1. Plot of density as a function of concentration.

Table 2. Data of c , η y D .

c (g/cm ³)	η (poise)	ρ (g/cm ³)	D (cm ² /s) x 10 ⁶
0.0025	0.0382	0.9988	8.08
0.0050	0.0684	1.0001	4.51
0.0075	0.0989	1.0008	3.12
0.0100	0.1274	1.0014	2.42

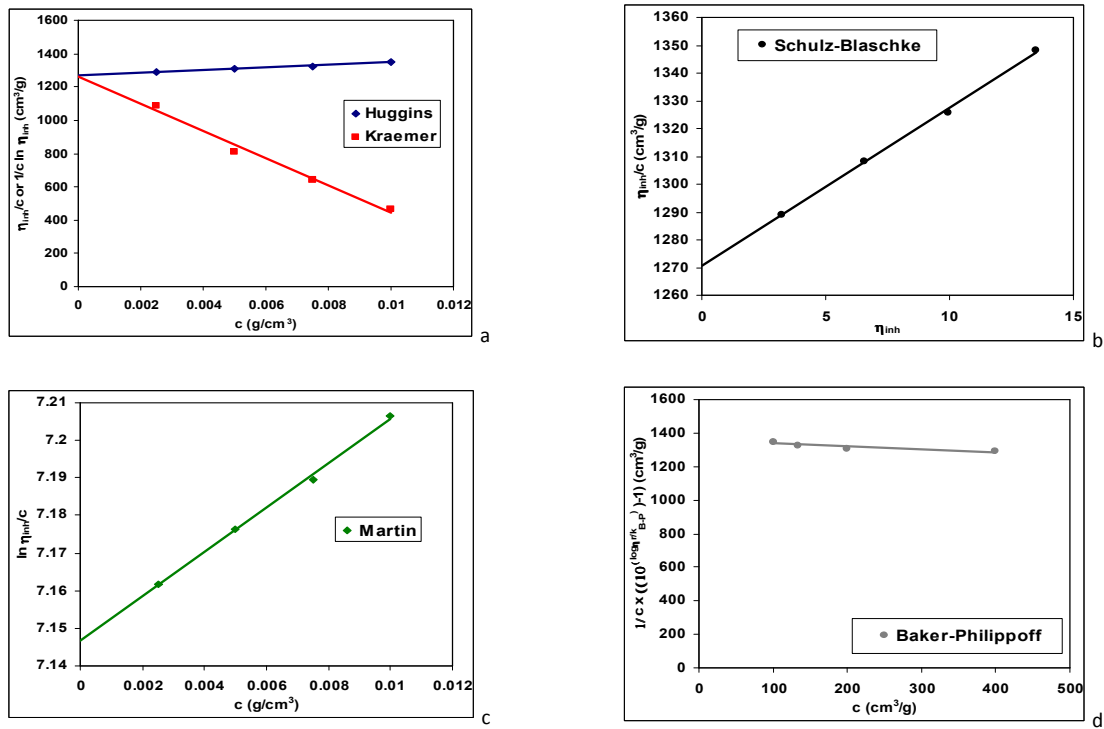


Figure 2. Classic methods for determination of intrinsic viscosity. a- Huggins and Kraemer methods. b- Schulz-Blaschke method. c- Martin method. d- Baker-Philippoff method.

Table 3 shows data obtained by applying the most common procedures to calculate intrinsic viscosity, namely, those based upon Equations 4, 5, 6 and 7, to the sodium alginate water solution, and their respective percentage relative error (ER %) with the Huggins method [81-82]. Table 4 includes the experimental data and errors resulting from single point Equations 9 to 15. The most commonly used equation is the Maron equation, which gives values

with acceptable errors ranging from 1.18 to 3.31%. The Deb & Chaterjee method responds quite well to alginate-water system in very dilute solutions with an ER of 2.63%; Palit & Car has an ER of 0.13%. The equation proposed in this work achieves an ER from 1.69 to 6.63% over the whole range of concentrations, much better than the rest of the authors using single point equations for more concentrated solutions.

Table 3. Classic methods for intrinsic viscosity determination.

	Huggins	Kraemer	Schulz-Blaschke	Martin	Baker-Philippoff
[η]	1269.11	1261.42	1270.51	1269.78	1353.5
σ ²	0.9972	0.9828	0.9983	0.9977	0.9712
Constant (k)	0.004834	0.050750	0.004489	0.004653	1.0000
E _R %	-	0.6059	0.7201	0.0573	6.5931

Table 4. Intrinsic viscosity determination by single and double point methods.

Methods →	Solomon-Ciuta	Deb & Chaterjee	Rao & Yanseen	Kuwahara	Palit & Kar	Maron	Curvale-Cesco	Eq. 16
C (g/cm³)				[η] (cm³/g)				
0.0025	988.32	1302.46	932.62	754.40	1267.51	1227.08	1089.42	1290.61
0.0050	673.06	1109.54	856.10	630.08	979.49	1229.53	1009.14	1311.41
0.0075	554.42	1001.47	822.29	570.65	862.70	1238.06	923.71	1329.82
0.0100	487.44	932.24	807.70	537.49	798.00	1254.15	866.06	1353.25
C (g/cm³)				%E_R				
0.0025	22.12	2.630	26.51	40.56	0.130	3.31	14.16	1.69
0.0050	46.97	12.57	32.54	50.35	22.82	3.12	20.48	3.33
0.0075	56.31	21.09	35.21	55.04	32.02	2.45	27.22	4.78
0.0100	61.59	26.54	36.36	57.65	37.12	1.18	31.76	6.63

Table 5. Hydrodynamic properties calculated.

R_H (nm)	\bar{V} (cm ³ /g)	$v_{a/b}$	V_s (cm ³ /g)
46.35	0.66159	10.52	122
δ (g/g)	$\beta \times 10^{-6}$	$\Phi_0 \times 10^{-23}$ (1/mol)	P
120.6708	2.32	4.2763	4.22

Across the entire set of equations, the determinations for alginate water system present errors higher than expected and do not adjust properly. From Table 4, it appears that none of the ‘single-point’ equations used provides an overall good estimation for $[\eta]$. Relative errors always increase when the concentration values become bigger. Figure 2 provides a graphical explanation for that observed behavior. The crosses represent the values of different methods for equations 3, 5, 6 and 7 at the different concentrations levels for the alginate-water system with a pH of 6.7, the common intersection point of which at $c \rightarrow 0$ provides an extrapolation estimation for $[\eta]$. Each pair of full lines passing through the points at concentrations of 0.25, 0.5, 0.75 and 1% wt. has a common intercept at $c \rightarrow 0$, which is the Solomon-Ciuta ‘single-point’ estimation for the corresponding concentration value obtained through equation 9. This is one of the most widely used ‘single-point’ estimations, and for the systems considered in this work, it provides relative errors ranging from 22.12 to 61.59 %. Moreover, it is also clear now why the Solomon-Ciuta gives the worst estimations; a concentration increases the ER% increases, as in almost all the methods previously reported, as shown in Tables 3 and 4. It is worth noting that ‘single-point’ estimators would provide good estimations for $[\eta]$ whenever the rheological behavior of the solute-solvent system under study was well represented by a set of Huggins and Kraemer equations with graphic representations like those shown in Figure 2, where $k_H + k_K = 0.056204$. Based on the intrinsic viscosity data obtained by the Huggins method, we also calculated the hydrodynamic properties shown in Table 5, according to references [62, 79-81, 84].

However, when a system does not present such an appropriate behavior, ‘single point’ methods will always provide estimations for $[\eta]$ with poor relative errors, which increase with concentrations. Therefore, if a system has not been studied previously in order to determine what its behavior is going to be, ‘single-point’ methods involve the risk of providing unacceptable errors when estimating $[\eta]$. As an alternative strategy, Curvale & Cesco propose a ‘double-point’ method of estimation. In Curvale & Cesco work, the intrinsic viscosity of BSA-water solutions changes with different pH because BSA size and shape are modified; in this work the ER% ranged from 14.16 to 31.76%.

The highest errors are observed in the run at high concentration, with the data showing a linear regression of 0.9828, but with a negative and very high k_K . In addition, the sum of the k_H and k_K constants deviates more from the 0.5 value. In the other runs, $k_H + k_K$ deviates approximately 10.24%. In general, it can be stated that these Maron, Deb &

Chatterjee equations can be used for approximate determinations of intrinsic viscosity in the alginate-water system when concentrations are similar or lower to 0.25%. For higher concentrations, all ‘single-point’ estimators provide poor estimations. Therefore, this work proposes a ‘single point’ method to estimate $[\eta]$ that has given errors smaller than 6.59 % in the alginate-water system. This method does not require a graphical adjustment and is economic, simultaneously adjusting series of Huggins and Kraemer’s values with two lines with a common intercept at $c \rightarrow 0$, providing good estimates for $[\eta]$, but requiring at least four determinations. Methods based upon ‘single point’ equations are strongly dependent on the system under consideration, its concentration and the verification of the condition $k_H + k_K = 0.5$. Regarding the alginate-water system, the ‘single point’ method of estimation performs better, needs only the density of the solution and of the solvent exhibits less dependence on the concentration and does not require previous knowledge about the values of k_H and k_K .

4. Conclusions

The cost of a viscometer is low, and the measurement took two hours to complete. Therefore, the quality analysis of water-soluble biopolymers using capillary viscometry is a viable alternative when equipment that is more sophisticated and is not available. The intrinsic viscosity and M_v calculated are approximate, and comparing them with literature data shows (table 1) they are validated with references [19, 21, 4-26, 31-33, 36-39, 47, 49, 51-52, 54]. The best method to calculate the intrinsic viscosity for the alginate-water system is Huggins (taken as standard), followed by Martin, Kraemer, Schulz-Blashke and Baker-Philippoff. Among the single point methods, Maron, Deb & Chatterjee and Palit & Kar stand out for their low ER% in dilute concentrations (0.25% wt.). It is noteworthy that the method proposed in this work has ER% lower than that of the single point methods mentioned above, and is useful for concentrations of 0.25 to 1% wt. [85].

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