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Viscometric study of pectin. Effect of temperature on the hydrodynamic properties

Martin Alberto Masuelli*

Instituto de Física Aplicada, CONICET, FONCyT, Cátedra de Química Física II, Área de Química Física, Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco, 917 (5700) San Luis, Argentina

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ABSTRACT

Hydrodynamic properties are important parameters affecting the performance of pectin. This polysaccharide is used as a thickening and gelling agent in food and pharmaceutical industries. The most common and economical of the hydrodynamic properties is the determination of viscosity, in which are determined the intrinsic viscosity and the diffusion coefficient. They indirectly measure the molecular weight (M_w); hydrodynamic radius (R_H); number of Simha, ($\nu_{(a/b)}$); Perrin parameter (P); Scheraga–Mandelkern parameter (β); and Flory parameters (ϕ_0 and P_0). All the hydrodynamic parameters are dependent on temperature. Normally these parameters are reported at a temperature of 25 °C, which limits their application to different temperatures. This work studies pectin dependence on temperature, finding that this biopolymer in aqueous solution presents a conformation of rod-like with $\nu_{(a/b)} = 10.5$, and a value from 0.8232 to 0.8129. Pectin behavior in this system indicates that it behaves like a colloidal particle that tends to compact with increasing temperature (R_H decrease). The molecular weight calculated for pectin is 180,000 g/mol. Mark–Houwink–Sakurada (M–H–S) equation constants, a and k, for pectin in water solvent-temperature systems have been already reported.

1. Introduction

Pectin is a major component of primary cell walls of all land plants and encompasses a range of galacturonic acid-rich polysaccharides. Three major pectic polysaccharides (homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II) are thought to occur in all primary cell walls. Biochemical definition of pectin is that it is a group of polysaccharides that are rich in galacturonic acid (GalA). GalA occurs in two major structural features that form the backbone of three polysaccharide domains that are thought to be found in all pectin species: homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). HGA is a linear homopolymer of (1 → 4)-α-linked-D-galacturonic acid and is thought to contain some 100–200 GalA residues [1–3].

Extracted pectin is widely used as a functional food ingredient and it is listed among the ingredients of innumerable food products. Worldwide annual consumption is estimated to be around 45 million kilograms. The gelling properties of pectin are well known to home jam makers and industrial producers alike [4,5].

The AFM images of pectin show conformation of rods, segmented rods, linked rods; rings, branched molecules, and dense circular areas [6].

Viscosity of water solution polysaccharides depends on intrinsic biopolymer characteristics (such as molecular mass, volume, size, shape, surface charge and deformation facility) and on ambient factors (such as pH, temperature, ionic strength, solvent, esterification degree, and galacturonic content). The method of choice has been capillary viscosimetry because it is a simple and useful method that requires low cost equipment and yields useful information on soluble macromolecules. Although in the literature there is much information on hydrodynamic measurements from determinations of viscosity, very few of them evaluate the situation at different temperatures. The importance of this type of study lies in analyzing the behavior of the polysaccharide in industrial processes so as to reduce energy requirements and avoid flow problems and product quality control.

Pectins were obtained by acid, base and enzymatic hydrolysis of citrus and apple peel; where M–H–S relation was $[\eta]$ (cm^3/g) = $1.4 \times 10^{-4} M_w^{1.34}$ (25 °C, pH 6, 0.155 M NaCl), equation corresponding to a rod-like model [7].

Citrus pectin was studied with a degree of esterification of 70% and galacturonan content of 70%, where $[\eta]$ values were from 106.7 to 809.3 cm^3/g at 25 °C for M_w range 20,000–200,000 g/mol taking essentially rod-like characteristics in solution [8].

Pectins were researched from citrus, apple and sunflower with degrees of esterification between 30 and 95%. They observed that majority of the samples that had M_w below 100,000 g/mol at 25 °C obeyed the M–H–S relation of $[\eta] = 0.0955 M_w^{0.73}$ equation corre-

* Tel.: +54 2652 424689 (116); fax: +54 2652 430224.
E-mail address: masuelli@unsl.edu.ar

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sponding to a random-coil model; however, the intrinsic viscosity of high molecular weight fractions (M_w 10^5–10^7) did not correlate with molecular weight [9].

Intrinsic viscosity and molecular weight of pectins that were obtained by extraction with HCl, ammonium oxalate and EDTA were 262, 281, and 309 cm^3/g and 84,500, 91,400, and 102,800 g/mol, respectively, where M–H–S equation to 25 °C was $[\eta] = 0.0234 M_w^{0.8224}$ (limit between random-coil and rod-like model) [10].

Acid-extracted pectin was reported from low quality 'Golden Delicious' apple fruit and presented a galacturonic acid content of 65% (w/w), an esterification degree of 57%, and an $[\eta]$ of 307 cm^3/g with M_w of 112,000 g/mol [11].

Measures of $[\eta]$ were based on the rod like model with values of 41–527 cm^3/g for degrees of methylesterification (DM) in the range of 35–73.9%, respectively [12]. In a similar work [13] performed measurements for five commercial pectins were employed to compare LiAc/HAc buffer against $NaNO_3$ solution. These samples were studied at 25 °C, M_w from 41,000 to 307,000, with $[\eta]$ from 86 to 976 cm^3/g, and using M–H–S constants from 0.62 to 0.94 (transition between random-coil and rod-like conformation).

Five citrus pectins were reported with average degree of esterification of 77.8, 65.0, 53.9, 37.8 and 27.9%, respectively, and were studied with M_w range 190,000 ± 30,000 g/mol. They estimated $[\eta]$ from 315 to 417 cm^3/g, $\nu_{(a/b)}$ from 6.9 to 37, and δ from 33 to 117 g/g, for the conformation dependent Wales–van Holde $k_s/[\eta]$ values between 0.34 and 0.85, and f/f_0 values between 7.8 and 9.6 ratios from the hydrodynamic data clearly indicating increasing chain stiffness with decreasing degree of esterification [14].

Hydrodynamics was conducted for high-methoxy (HM) and low-methoxy (LM) pectin solutions, and was examined by capillary viscometric analysis; where M_w for HM-pectin was 138,000 g/mol and for LM-pectin was 226,000 g/mol, with $[\eta]$ ranging from 249 to 330 cm^3/g [15].

The flexibility/rigidity of four pectins of low degree of esterification of 17–27% and one of high degree of esterification (70%) that were characterized in aqueous solution (0.1 M NaCl to 25 °C) in terms of intrinsic viscosity $[\eta]$, sedimentation coefficient ($s_{20,w}$) and M_w was studied. They showed an extended coil conformation for M_w measured in a range between 145,000 and 180,000 g/mol, $[\eta]$ between 325 and 600 cm^3/g, f/f_0 from 7.1 to 8.6, and ϕ of 2.86×10^{-23} mol^{-1} [16].

Although intrinsic viscosity is a molecular parameter that can be interpreted in terms of molecular conformation, it does not offer as high resolution on molecular structure as other methods do such as light scattering, circular dichroism, sedimentation velocity, sedimentation equilibrium, size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS), high-performance size exclusion chromatography (HPSEC), gel permeation chromatography (GPC), NMR, X-rays, etc. But intrinsic viscosity measurement is a very economical alternative and is easy to determine with a few experiences.

Different authors conducted studies on the hydrodynamic properties of polysaccharides such as dextran [17,18] and chitosan [19,20] in aqueous solutions at different temperatures, in whose work highlights the default values for the parameters of Mark-Houwink-Sakurada.

In this work, an experimental study was conducted on pectin in semi-dilute region. Effects of temperature on hydrodynamic properties of the pectin were characterized by viscometry in water solution, in order to determine the conformational characteristic. Then the data of intrinsic viscosity, $[\eta]$, and molecular weight, M_w, were analyzed on the basis to obtain hydrodynamic parameters of pectin in solution (hydrodynamic radius, R_H; number of Simha, $\nu_{(a/b)}$; Perrin parameter, P; hydration value, δ; Scheraga–Mandelkern parameter β; and Flory parameters, ϕ_0 and P_0).

2. Materials and methods

2.1. Sample preparation

Pectin from citrus peel was supplied by Sigma (Galacturonic acid ≥74.0%, methoxy groups 6.7%). Pectin dispersion was prepared 2% w/v. Five grams of biopolymer powder was dispersed in 250 mL of pure deionized water and under gentle stirring at room temperature for 2 h at 40 °C. The dispersions were left at 3–1 °C for 24 h to enable biopolymer hydration. Insoluble part was separated from the soluble part by centrifugation. Both the fractions were dried and sealed in zip plastic bags and then kept in desiccators. Finally, pectin was dissolved in distilled water preparing a solution of 1, 0.75, 0.5, 0.25 and 0.15% (w/v).

2.2. Density measurement

The densities of solution and solvent were measured with an Anton Paar densimeter DMA5N.

2.3. Capillary viscometry

Solutions and reference solvents were analyzed using an Ubbelohde 1B viscometer (IVA), under precise temperature control using thermostatic bath (Haake 1C).

3. Theory

The Hagen–Poiseuille law describes the flow through capillaries starting from the rate of flow (for a given volume) proportional to the fluid density (ρ g/cm^3) and inversely proportional to the viscosity η,

$$\eta = A\rho t \tag{1}$$

where η (poise) is the viscosity, A (cm^2/s^2) is the instrumental constant of the viscometer, and t is the time of draining of liquid (s) [21].

The relative, η_{rel}, specific, η_{sp}, and reduced, η_{red}, viscosities [22] were calculated from

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

where t is the flow time of polysaccharide sample, and t_0 is the flow time of solvent 34:95 s. Where,

$$\eta_{sp} = \eta_r - 1 \tag{3}$$

$$\eta_{red} = \frac{\eta_{sp}}{c} \tag{4}$$

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.

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

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 [23],

$$[\eta] = \frac{1}{c}\sqrt{2\eta_i - 2\ln\eta_r} \tag{6}$$

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

obtained by extrapolation. Another possible method is the double point of Curvale and Cesco [24].

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

$$[\eta] = k(M_w)^a \tag{7}$$

The calculation of Mark–Houwink–Sakurada (M–H–S) parameters is carried out by the graphical representation of the following equation:

$$\ln[\eta] = \ln k + a \ln M_w \tag{8}$$

where k and a are M–H–S constants, these constants depend on the type of polymer, solvent, and temperature of viscosity determinations. The exponent a is a function of polymer geometry, and varies from 0.5 to 2. 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, [8]. Using the polymer standards, a plot of the $\ln[\eta]$ vs. $\ln M_w$ usually gives a straight line. The slope is a value and intercept is equal to $\ln k$ value [25]. The M–H–S exponent bears the signature of a three-dimensional configuration of a polymer chain in the solvent environment. For a values are from 0 to 0.5 rigid sphere in ideal solvent, from 0.5 to 0.8 random coil in good solvent, and from 0.8 to 2 rigid or rod like (stiff chain). The fact that the intrinsic viscosity of a given polymer sample is different according to the solvent used gives an insight into the general shape of polymer molecules in solution. 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. In a good solvent which shows a zero or negative heat of mixing with the polymer, the molecule is fairly loosely extended, and the intrinsic viscosity is high. The Mark–Houwink "a" constant is close to 0.75 or higher for these "good" solvents. In a "poor" solvent which shows a positive heat of mixing, segments of a polymer molecule attract each other in solution more strongly than the surrounding solvent molecules. The polymer molecule assumes a tighter configuration, and the solution has a lower intrinsic viscosity. The M–H–S "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 M–H–S "a" constant approaches a value of 2.0 [26]. The hydrodynamic radius (R_H), for a sphere ($\nu_{(a/b)} = 2.5$) is given by the Einstein relation [27],

$$[\eta]M_w = \nu_{(a/b)} N_A \frac{3}{4}\pi (R_H)^3 \tag{9}$$

The viscosity of liquids is highly dependent on temperature and its complex relations [28]. The change of viscosity at different temperatures is commonly calculated with an equation of the Arrhenius form:

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

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 is the temperature (K). The pre-exponential factor A_{vf} is considered independent or approximately independent of the temperature. The Eq. (10) is convenient for calculating E_{avf} in a discrete range of temperatures.

For simplicity reasons, proteins and macromolecules may be treated as rigid molecules for a hydrodynamic study. It is worth noting that the size of proteins is much bigger than that of solvent (water) molecules [29]. Thus, D from spherical proteins in dilute aqueous solutions can be approximately described by the Stokes–Einstein equation, which assumes a solute rigid sphere diffusing in a continuum of solvent. The correlation of this equation with the molecular weight and the viscosity is as follows:

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

The diffusion coefficient is an important physical chemistry property of biological molecules. In several biological and industrial processes, the diffusivity datum is required for the design of the process and its analysis. For example, the protein diffusion coefficients are crucial for analysis, extraction, and transport in porous media and drying processes. Also, diffusion coefficients are related to hydrodynamic properties which can provide information about the size and the shape of macromolecules and proteins.

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 example is the Scheraga–Mandelkern parameter, β given by

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

The friction coefficient f is obteined from the measured of the diffusion coefficient as $f = k_B T/D$ where k_B is the Boltzmann constant and T is the absolute temperature. In the Eq. (12), M is the molecular weight of the macromolecule, and η_0 is 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:

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

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

$$P_0 = \frac{f}{6\eta R_g} \tag{14}$$

These quantities have been proposed along the years, at different times and by different eminent scientists, after whom they are named. As a consequence of the diversity in their origin, the set of classical universal size independent quantities suffers some inconveniences. Two of them, unimportant but also somehow cumbersome, are related to the diversity not only in the symbols employed to represent them, but mainly in the disparity of their numerical values and the order of magnitude for typical cases at 25 °C; thus, 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 $9.23 \times 10^{23}\ \mathrm{mol^{-1}}$ and $2.60 \times 10^{23}\ \mathrm{mol^{-1}}$. Thus, it is accepted that, for every flexible-chain polymer in a Θ (ideal) solvent, there is a universal value of $\phi_0 = 2.50 \times 10^{23}\ \mathrm{mol^{-1}}$ [30].

The application of universal shape functions, either the classical ones or the new ratios of radii, requires the consideration of an unclear problem: hydration. In the definition of shape functions, either the classical ones or the ratios of radii, it is implicitly assumed that the particle "seen" by the various solution properties is the same. Then, for compatibility with hydrodynamic properties, the particle volume used for the calculation of V must be the hydrated volume, i.e., V_{hyd}. From the molecular weight and partial specific volume, M_w and $\bar{v}$, we can readily calculate the anhydrous volume, $V_{anh} = \bar{v}M_w/N_A$. For large particles, the thickness of the hydration layer will be small in comparison with the size of the

Table 1
Data of intrinsic viscosity by Huggins and Salomón–Ciuta methods.

c (g/cm^3)	T (°C)							
	20.2	26.6	29.6	34.9	37.0	39.9	44.8	49.8
η_{red}/c (cm^3/g)								
0.0100	1632.23	1520.64	1398.17	1340.30	1323.35	1299.89	1186.38	1048.29
0.0075	1344.45	1218.11	1234.91	1151.56	1148.13	1072.54	955.87	906.56
0.0050	1046.46	948.25	890.51	859.25	837.83	785.13	759.48	697.60
0.0025	808.03	753.28	720.62	695.75	687.94	658.45	623.37	571.45
0.0015	668.09	620.15	587.76	575.66	558.87	549.34	516.96	483.68
Method	T (°C)							
	20.2	26.6	29.6	34.9	37.0	39.9	44.8	49.8
Huggins								
$[\eta]$ (cm^3/g)	507.06	466.32	453.61	447.65	433.43	412.88	406.22	389.54
σ^2	0.9985	0.9943	0.9861	0.9927	0.9884	0.9866	0.9923	0.9943
Solomon–Ciuta								
$[\eta]$ (cm^3/g)	513.89	489.37	469.59	458.63	451.01	431.85	422.31	398.66
$\%E_R$	1.35	4.94	3.52	2.45	4.05	4.59	3.96	2.34

macromolecule and both volumes will be approximately equal. However, for other macromolecules, particularly small or medium sized proteins, this approximation is not valid. It is commonly assumed that the hydration effect causes a uniform expansion of the macromolecule (in terms of the ellipsoidal models employed below, P is the same for the hydrated and the "dry" particle) [30]. We must take

$$V = V_{hyd} = h^3 V_{anh} \tag{15}$$

where h is the hydration expansion factor. The quantity usually employed to express hydration of biopolymer is the ratio δ, of the grams of water per gram of biomacromolecule. Then the expansion factor is

$$h = \left(1 + \frac{\delta}{\bar{\nu}\rho}\right)^{1/3} \tag{16}$$

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 a common practice to express the frictional coefficient of rigid structures as

$$P \equiv \frac{f}{f_0} = \frac{f}{6\pi\eta_0(3V/4\pi)^{1/3}} \tag{17}$$

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 constant. A similar combination involves the intrinsic viscosity and specific volume:

$$\nu_{(a/b)} = \frac{[\eta]}{V_s} \tag{18}$$

$\nu_{(p)}$ is called Einstein viscosity increment, and V_s is specific volume (cm^3/g). For ellipsoids, as studied by Simha, $\nu_{(a/b)}$ is a function of axial ratio [31].

Combination of the Perrin function, P often referred as the 'frictional ratio due to shape' with the frictional ratio (f/f_0) enables the degree of expansion of the molecule ($V_{sw}/\bar{\nu}$) to be estimated, where V_{sw} (cm^3/g) is the volume of the swollen molecule (polysaccharide + associated solvent) per unit mass of polysaccharide and $\bar{\nu}$ is the partial specific volume (essentially the anhydrous molecule):

$$\frac{f}{f_0} = P\left(\frac{V_{sw}}{\bar{\nu}}\right)^{1/3} \tag{19}$$

When the polysaccharide is contracted, term of expansion is negligible.

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

$$\delta = (V_s - \bar{\nu})\rho_0 \tag{20}$$

to be ~50 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 [32].

4. Results and discussion

Solomon–Ciuta single point [22] method or Curvale–Cesco [23] double point is generally applied to polymers synthetic and linear, but when there is thin room for error and reduced viscosity measurements by the method of Huggins can be used without an indication misuse. Table 1 is performed the comparative study of Huggins method and the single point method, and the single point, note that the percentage relative error ($\%E_R$) is between 1.35 and 4.95%. So with some precaution the single point method can be used. In this work was used 0.5% of pectin solution for single point determination.

Fig. 1 shows the decrease of the relative viscosity as temperature increases. Fig. 2a shows the linear relation between logarithmic of viscosity and reverse temperature, where the pectin value was

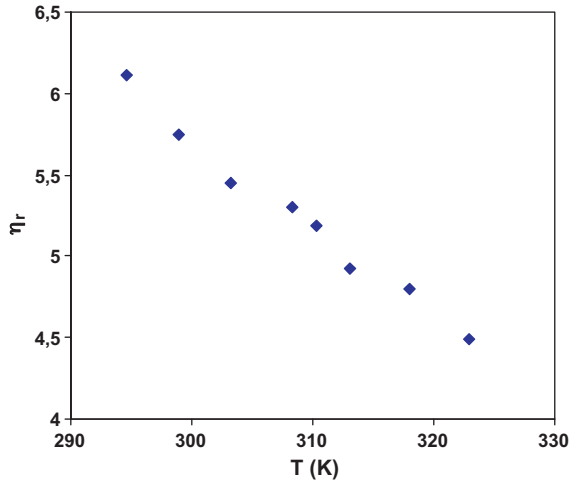

Fig. 1. Influence of temperature on the relative viscosity of pectin aqueous solution.

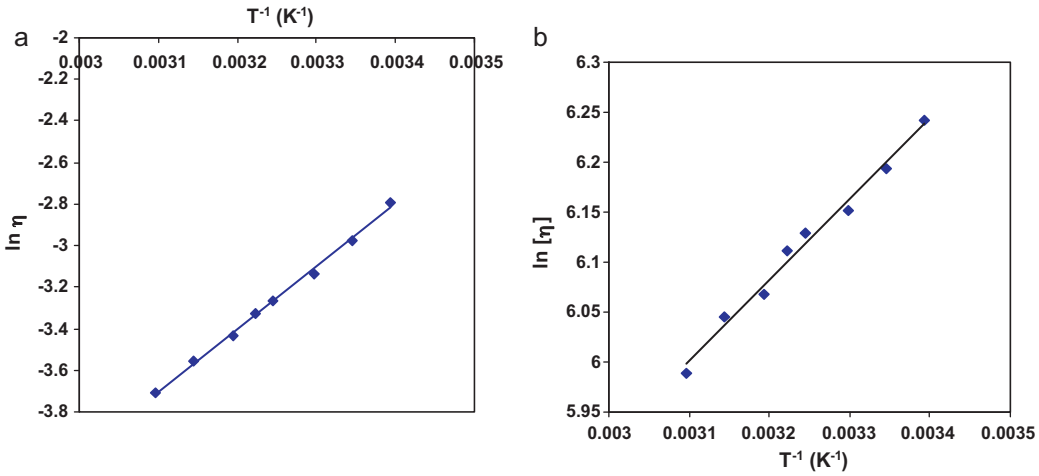

Fig. 2. (a) Plot of logarithm of viscosity in function of $1/T$, for pectin solution. (b) Influence of temperature on intrinsic viscosity.

obtained from E_{avf} 6012.39 cal/mol, and A_{vf} 2.21×10^{-6} g/cm s, with σ^2 0.9965; and water values from experimental data are E_{avf} 4039.40 cal/mol and A_{vf} 1.04×10^{-5} g/cm s, with σ^2 0.9963. These experimental values of solvent viscosity are used for calculated η_r. The increment of activation energy of viscous flow occurs due to the higher resistance to flow of biopolymer with respect to solvent. The increase of E_{avf} is 1974.55 cal/mol for pectin in water solution. Fig. 2b shows that the intrinsic viscosity is influenced by temperature for pectin solution.

Noting 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 [20]. The chain flexibility parameter ($d \ln[\eta]/dT$) whose value is 810.56 K^{-1}, this chain flexibility value is low for the molecular weight of pectin. Analysis of the relative stiffness parameter indicates that 180,000 g/mol molecular weight of pectin is lesser flexible [19].

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 (see Fig. 3a). Phillies and Quinlan [33], showed a mathematical relationship between D and T and in turn between D and M_w for dextran.

The hydrodynamic radius and intrinsic viscosity for polysaccharides and proteins are higher at high molecular weights and decrease with increasing temperature [34] (Fig. 3b). Table 2 shows the classical values calculating for Mark–Houwink–Sakurada parameters, a and k, and for temperature. These studies on M–H–S parameters are usually carried out at a given temperature, obtaining a consistent result but in a very limited range of temperature [7,10,16]. This value shows a clear functionality between these parameters and temperature, as expected in Table 1.

Table 2
Data of Mark–Houwink–Sakurada parameters on temperature function.

T (K)	k (cm^3/g)	a
294.66	0.0242	0.8232
298.86	0.0234	0.8221
303.26	0.0226	0.8215
308.26	0.0222	0.8213
310.26	0.0219	0.8208
313.06	0.0217	0.8180
318.06	0.0215	0.8169
322.96	0.0213	0.8129

The molecular weight calculated for pectin is 180,000 g/mol. The value of a given at different temperatures shows that this polysaccharide in aqueous solution behaves in a conformation predominantly confined to the rod-like conformation, as observed by other authors [7,10,16].

The values of the hydrodynamic properties of pectin in aqueous solution can be seen in Table 3, all vary with the temperature. β values increases from 3.29 to 3.91×10^6 with temperature increase, indicating the changes in a rod structure. The values of ϕ_0 and P_0 decreases from 8.05 to 5.37×10^{23} mol^{-1} and from 10.42 to 2.57 demonstrating a low flexibility of particles. The value of P decreases from 6.09 to 2.52, and $\nu_{(a/b)}$ with 10.5 which confirms

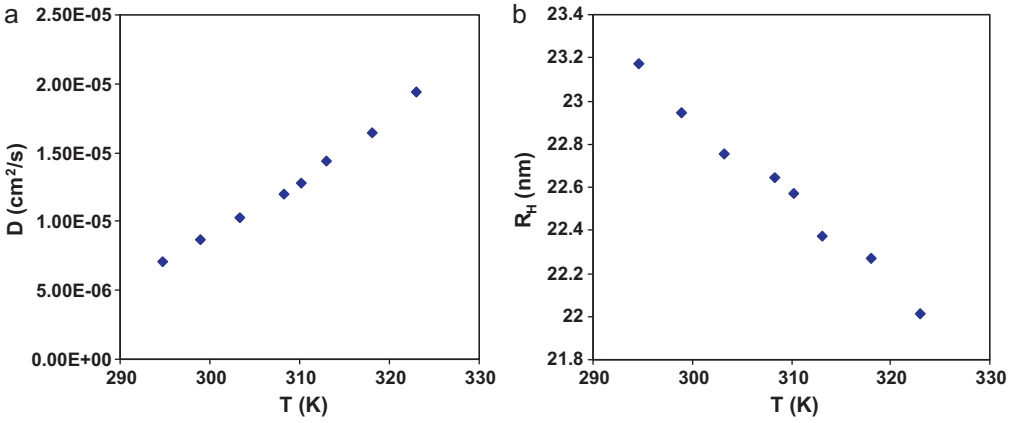

Fig. 3. Pectin data on temperature function: (a) diffusion coefficient, (b) hydrodynamic radius.

Table 3
Hydrodynamic properties of pectin in water solution at different temperatures.

T (K)	$p = f/f_{hyd}$	$\beta \times 10^{-6}$	$\phi_0 \times 10^{-23}$ (mol^{-1})	δ (g/g)	P_0
294.66	6.1	3.29	8.05	205.1	10.42
298.86	5.7	3.38	7.30	195.0	7.91
303.26	5.4	3.49	6.85	186.5	6.21
308.26	5.2	3.55	6.61	182.0	5.16
310.26	5.2	3.59	6.45	178.8	4.70
313.06	4.9	3.70	6.04	171.0	3.91
318.06	4.8	3.76	5.84	166.9	3.31
322.96	4.5	3.91	5.36	157.2	2.57

that pectin in aqueous solution is a biopolymer with a rod-like conformation, and tendency to compaction with increasing temperature (R_H decreases). The value of δ as expected decreases from 205.1 to 157.2 g/g with increases of temperature, this phenomenon is due to loss of water due to compression of pectin by the effect of increasing temperature [32].

The parameters of Mark–Houwink–Sakurada for biopolymers may be varied with polymer solution and temperature [35]. This is because the macromolecule changes hydrodynamic radius with type solution and temperature via change in their 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} solute > E_{avf} solvent) [36].

Mark–Houwink–Sakurada values confirm that for these conditions pectin behaves as a rod-like biopolymer. Such empirical equations relating the parameters of Mark–Houwink–Sakurada with T, which ultimately describe this type of thermodynamic parameters are relations between properties of the solute with the solvent and temperature dependence.

5. Conclusions

The Mark–Houwink–Sakurada parameters have functionality with temperature. The numerical value of a indicates that pectin acquire a shape of a rod-like in aqueous solution; and k demonstrates that under water their value decreases with temperature [19,37]. The values of M–H–S parameters may be universalized with certain precautions, being an indication for the calculation of molecular weight in a temperature range of 25–50 °C.

Due to the lack of data on the uniformity of intrinsic viscosity measurements in the pectin/water system, clearly shows a decrease in "a" with temperature, and this M_w is 180,000 g/mol for pectin. Molecular weight and Simha number do not change in this temperature range, so it changes the hydrodynamic properties of the biopolymer in aqueous solution as η, D, $[\eta]$, R_H, β, ϕ_0, P_0, δ, and P.

Pectin behavior in this system indicates that it behaves like rod 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 pectin/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 difficulty in flowing (increase in D and high increment E_{avf} respect solvent). This phenomenon is observed in the case of ideal solvents, evidencing a decrease of a with temperature.

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References

[1] W.G.T. Willats, L. McCartney, W. Mackie, J.P. Knox, Plant Molecular Biology 47 (2001) 9–27.
[2] D. Mohnen, Current Opinion in Plant Biology 11 (2008) 266–277.
[3] B.L. Ridley, M.A. O'Neill, D. Mohnen, Phytochemistry 57 (2001) 929–967.
[4] M.L. Fishman, P.H. Cooke, H.K. Chau, D.R. Coffin, A.T. Hotchkiss Jr., Biomacromolecules 8 (2007) 573–578.
[5] W.G.T. Willats, J.P. Knox, J.D. Mikkelsen, Trends in Food Science and Technology 17 (2006) 97–104.
[6] I.A. Brownlee, Food Hydrocolloids 25 (2011) 238–250.
[7] H.S. Owens, H. Lotzkatr, H. Schultz, W.D. Maclay, Journal of American Chemical Society 68 (1946) 1628–1632.
[8] S.E. Harding, G. Berth, A. Ball, J.R. Mitchell, J. Garcia de la Torre, Carbohydrate Polymers 6 (1991) 1–15.
[9] H. Anger, G. Berth, Carbohydrate Polymers 6 (1986) 193–202.
[10] F. Kar, N. Arslan, Carbohydrate Polymers 40 (1999) 277–284.
[11] A. Rascón-Chu, A.L. Martínez-López, E. Carvajal-Millán, N.E. Ponce de León-Renova, J.A. Márquez-Escalante, A. Romo-Chacón, Food Chemistry 116 (2009) 101–103.
[12] M.L. Fishman, D.T. Gillespie, S.M. Sondey, R.A. Barford, Journal of Agricultural and Food Chemistry 37 (1989) 584–591.
[13] M.L. Fishman, H.K. Chau, F. Kolpak, J. Brady, Journal of Agricultural and Food Chemistry 49 (2001) 4494–4501.
[14] G.A. Morris, T.J. Foster, S.E. Harding, Food Hydrocolloids 14 (2000) 227–235.
[15] S.-H. Yoo, M.L. Fishman, A.T. Hotchkiss Jr., H.G. Lee, Food Hydrocolloids 20 (2006) 62–67.
[16] G.A. Morris, J. Garcia de la Torre, A. Ortega, J. Castile, A. Smith, S.E. Harding, Food Hydrocolloids 22 (2008) 1435–1442.
[17] A. Guner, Journal of Applied Polymer Science 72 (1999) 871–876.
[18] A. Guner, G. Kibarer, European Polymer Journal 37 (2001) 619–622.
[19] R.H. Chen, M.L. Tsaih, International Journal of Biological Macromolecules 23 (1998) 135–141.
[20] M.R. Kasaai, Carbohydrate Polymers 68 (2007) 477–488.
[21] M.C. López Martínez, F.G. Díaz Baños, A. Ortega Retuerta, J. García de la Torre, Journal of Chemical Education 80 (9) (2003) 1036–1038.
[22] S. Matsuoka, M.K. Cowman, Polymer 43 (2002) 3447–3453.
[23] O.F. Solomon, I.Z. Ciuta, Journal of Applied Polymer Science 6 (1962) 683–686.
[24] R.A. Curvale, J.C. Cesco, Applied Rheology 19 (5) (2009) 53347.
[25] S.E. Harding, K. Day, R. Dham, P.M. Lowe, Carbohydrate Polymers 32 (1997) 81–87.
[26] S.E. Harding, K. Varum, B. Stoke, O. Smidsrod, Advances in Carbohydrate Analysis 1 (1991) 63–144.
[27] D.R. Smith, J.W. Raymonda, Journal of Chemical Education 49 (8) (1972) 577–579.
[28] R. Curvale, M. Masuelli, A. Perez Padilla, International Journal of Biological Macromolecules 42 (2008) 133–137.
[29] A. Ortega, J. García de la Torre, Biomacromolecules 8 (2007) 2464–2475.
[30] G.A. Morris, T.R. Patel, D.R. Picout, S.B. Ross-Murphy, A. Ortega, J. Garcia de la Torre, S.E. Harding, Carbohydrate Polymers 72 (2008) 356–360.
[31] J. Garcia de la Torre, B. Carrasco, Progress in Colloid and Polymer Science 113 (1999) 81–86.
[32] S.E. Harding, Progress in Biophysical Molecules Biological 68 (1997) 207–262.
[33] G.D.J. Phillies, C.A. Quinlan, Macromolecules 25 (1992) 3110–3116.
[34] K. Monkos, Biochimica et Biophysica Acta 1700 (2004) 27–34.
[35] A. Durand, European Polymer Journal 43 (2007) 1744–1753.
[36] G.A. Morris, J. Castile, A. Smith, G.G. Adams, S.E. Harding, Carbohydrate Polymers 76 (2009) 616–621.
[37] R.H. Chen, W.Y. Chen, S.T. Wang, C.H. Hsu, M.L. Tsaih, Carbohydrate Polymers 78 (2009) 902–907.