5

Hydrodynamic Properties of Gelatin – Studies from Intrinsic Viscosity Measurements

Martin Alberto Masuelli[1] and Maria Gabriela Sansone[2]
[1]Instituto de Física Aplicada, CONICET, FONCyT
Cátedra de Química Física II, Área de Química Física
[2]Área de Tecnología Química y Biotecnología, Departamento de Química
Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis
Argentina

1. Introduction

Gelatin is a natural polymer widely used in pharmaceutical, cosmetic, photographic, and food industries. It is obtained by denaturation and partial hydrolysis of fibrous collagen. Collagen is the most abundant structural protein of animals and by far the main organic component of skin and bone of vertebrates. However, collagen is standing for a family of proteins with 21 different types of aminoacids described to date. Skin and bones, the raw materials for gelatin manufacture, mainly consist of type I collagen and a small fraction of type III collagen. Each type I collagen molecule consists of polypeptide chains are twisted into left-handed helices. The rod like triple-helical collagen molecules is arranged in a parallel but staggered orientation to form fibrils (Meyer & Morgenstern 2003).

To convert insoluble native collagen into gelatin requires pre-treatment to breakdown the non-covalent bonds and disorganize the protein structure, allowing swelling and cleavage of intra and intermolecular bonds to solubilize the collagen. Subsequent heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple helix, resulting in helix-to-coil transition and conversion into soluble gelatin (Harrington & von Hippel 1962). The degree of conversion of the collagen into gelatin is related to the severity of both the pre-treatment and the extraction process, depending on the pH, temperature, and extraction time. Two types of gelatin are obtainable, depending on the pre-treatment. These are known commercially as type-A gelatin (isoelectric point from 7.5-9.2), obtained in acid pre-treatment conditions, and type-B gelatin (IP from 4.5-5.1), obtained in alkaline pre-treatment conditions. Industrial applications call for one or the other gelatin type, depending on the degree of collagen cross-linking in the raw material, in turn depending on a number of factors, such as collagen type, tissue type, species, animal age, etc. (Karim & Bhat 2009). The most important property of gelatin is its ability to form stiff gels at about 30°C, when cooling a hot gelatin solution with sufficient high concentration (>1%). In contrast to soluble collagen, gelatins show a broad molar mass distribution on solution. The gelatins fractions can be prepared by coacervation, precipitation, or fractionated dissolution

Gelatin has long been used as a food ingredient (e.g., gelling and foaming agent), in the preparation of pharmaceutical products (e.g., soft and hard capsule, microspheres), in the

biomedical field (wound dressing and three-dimensional tissue regeneration) and in numerous non-food applications (e.g., photography) (Chatterjee & Bohidar 2008).

Gels are commonly classified into two groups: chemical gels and physical gels, which are different essentially through the nature of the bonds linking the polymer molecules together. If a gel is formed by chemical reaction, the bonds created are covalent bonds and the gel formed is irreversible. It is possible to treat the viscoelastic properties of chemical gels through percolation theory because of the very nature of the bonding in chemical gelation. However, the physical gels achieve solution stability through an array of possible secondary forces, like hydrogen bonds, van der Waals' forces, dipolar interactions and hydrophobic interactions, etc. Since these are weaker bonds the physical gels are reversible when thermodynamic parameters such as pH, ionic strength, and/or temperature are modified. Unlike chemical gels, physical gels are fragile and the viscoelastic properties cannot be described through models like percolation theory. Regardless, all the gels have a characteristic temperature, called gelation temperature T_{gel} below which gelation occurs and, a threshold polymer concentration. Gelatin gels are made of an ensemble of physically interconnected triple helices, which are held together by intermolecular hydrogen bonding. The gelation transition in gelatin has been modeled through a variety of theoretical models to name a few; percolation model, kinetic aggregation model and Smoluchowski aggregation model, etc. In these gels, the continuous phase, which is usually water, stays in equilibrium with the dispersed gelatin network in a state of dynamic equilibrium and exhibits two distinct physical states, namely; water bound to the gelatin chains and interstitial water trapped inside the gel network. The hydrodynamic environment of the network characterizes the possible relaxation modes that the gel can execute at temperature, $T < T_{gel}$. The structure of bound and interstitial water undergoes significant change as the network structure is altered affecting the hydrodynamic environment of the network and thus modulating its relaxation features, which can be easily captured in experiments (Karim, & Bhat 2008).

Biodegradable films made from edible biopolymers from renewable sources could become an important factor in reducing the environmental impact of plastic waste. Proteins, lipids, and polysaccharides are the main biopolymers employed to make edible films and coatings. Which of these components are present in different proportions and determine the properties of the material, as a barrier to water vapor, oxygen, carbon dioxide, and lipid transfer in food systems (Gomez-Guillen et al. 2002 and 2009).

Influence of molecular weight heterogeneity and drug solubility, drug loading and hydrodynamic conditions on drug release kinetics from gelatin nanoparticles as a potential intravascular probe for diagnostic purposes and in improving the biodelivery of drug (Saxena et al. 2005).

The thermodynamic properties of the gelatin-water system have been studied using methods of static light scattering (LS), osmosis, mixing calorimetry, differential scanning calorimetry, ultracentrifugation, swelling and swelling pressure. At temperatures below 35°C the system undergoes a coil-helix transition, which is clearly demonstrated by temperature-dependent measurements of LS and optical rotation. If the concentration of gelatin is above its critical value for network formation, the solutions (sols) undergo a phase transition to the gel state (Kaur et al. 2002).

One of the techniques to determine the molecular weight of macromolecules is size exclusion chromatography (SEC) with size exclusion chromatography-multi-angle laser light scattering (SEC-MALLS) and sedimentation equilibrium (Borchard 2002). The determination of the molar mass distribution of this acid soluble collagen (ASC or gelatin) using the technique SEC-MALS by direct measurement of M_w (from 19,000-61,000g/mol). According to the next scaling law $\langle s^2 \rangle^{1/2} = KM^{\alpha}$, $\alpha = 0.78$ was determined for the gelatin. This α could reflect a structure in solution, which is more similar to an ellipsoid than to a random coil (Meyer & Morgenstern 2003).

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, but intrinsic viscosity measurement is a very economical alternative and easy determination with a few experiences. Viscosity of protein water solution depends on intrinsic biopolymer characteristics (such as molecular mass, volume, size, shape, surface charge, deformation facility, estherification degree in polysaccharides, and aminoacids content) and on ambient factors (such as pH, temperature, ionic strength, solvent, etc.). The method of choice has been capillary viscometry because it is a simple and useful method that requires low cost equipment and yields useful information on soluble macromolecules. Although in 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 protein in industrial processes so as to reduce energy requirements and avoid flow problems and product quality control. (Masuelli 2011)

Pouradier & Venet (1952), showed that an equation of the type $[\eta] = 0.166M^{0.885}$ exists between the intrinsic viscosity and molecular weight of gelatin. Whereas *k* and *a* were each the same for two alkali-processed gelatins, they were different for an acid-processed gelatin (Zhao 1999).

Boedker and Doty (1954), worked with gelatin B and $[\eta]$ in water solution at 40.2°C was 38.1cm^3/g for an M_w of 90,000g/mol and R_g of 17nm.

Nishihara and Doty (1958), prepared soluble calf-skin collagen by extraction in citrate buffer at pH 3.7, and was fractionated by different time of sonication from 10 at 440 minutes. They obtained $[\eta]$ (measured at 24.8°C) varied from 1075 to 220 cm^3/g with M_w calculated from 336,000 to 137,000g/mol. They proposed Mark-Houwink equation as following $[\eta] = 1.23 \times 10^{-7} M^{1.80}$. This value of the *a* exponent is that of the upper limit for ellipsoids having a constant minor axis or for cylinders of constant diameter, and confirms assumption concerning the homologous, rod-like nature of the collagen sonicates.

Olivares et al. (2006), study dilute gelatin (M_n 133,000 g/mol) prepared to pH 9.8, ionic strength of 0.110M (NaCl) and mature 20hs, where intrinsic viscosity with an range temperature of 5-35°C was of 116.2 to 56.8 cm^3/g.

Haug et al. (2004), worked with fish gelatin at 30°C and 0.1M NaCl, M_w 199,000 to 140,000 g/mol and $[\eta]$ from 42 to 47 cm^3/g.

Domenek et al. (2008), released measurement of the intrinsic viscosity of gelatin type B in water solution at 42°C, where given values were of 27 cm^3/g and 70 cm^3/g.

Works where study the hydrodynamic properties of a biopolymers in aqueous solution at different temperatures are made by Guner (1999), and Guner & Kibarer (2001) for dextran; Chen & Tsaih (1998) and Kasaii (2008) for chitosan, Bohidar for gelatin (1998), and Monkos for serum proteins (1996, 1997, 1999, 2000, 2004 and 2005).

Bohidar (1998), study the aggregation properties of Gelatin chains in neutral aqueous solutions, were reported in the temperature range of 35-60°C. Data measured to 35°C were: intrinsic viscosity $[\eta]$ = 384cm^3/g, diffusion coefficient D = 1.12x10^{-7}cm^2/s, molecular weight M_w = 410,000 g/mol, and hydrodynamic radius of R_H = 28nm. The Mark-Houwink equation of $[\eta] = 0.328\ n^{0.69}$, with monomer molecular weight of 110 g/mol to the repeating unit, and n values of 3,727. Bohidar concluded that the intermolecular interaction was found to be repulsive which showed significant decrease as the temperature was reduced; and random coil shape was confirmed with date obtained.

In this work, an experimental study was conducted on gelatin in semi-dilute region in water solution; and research the effect of temperature, pH, zeta potential, and ionic strength on hydrodynamic properties by viscometry, in order to determine the conformational characteristic, and phase transition (T_{gel}).

2. Materials and methods

2.1 Sample preparation

Gelatin B from cow bone was supplied by Britannia Lab Argentine with isoelectric point of 5.10. Gelatin was dried and sealed in zip plastic bags and then kept in desiccators. Finally, gelatin was dissolved in distilled water preparing a solution of 0.25, 0.5, 0.75 and 1% (wt.).

2.2 Density measurement

Density of solution and solvent were measurement with Anton Paar densimeter DMA5N. For determining the hydration value is used the following concentrations of gelatin in aqueous solution 0.2, 0.4, 0.5 and 0.6%.

2.3 Electrokinetic measurement

Electrokinetic measurements consisted of measuring the viscosity with and without NaCl (Carlo Erba, Argentine) (Figures 3-a and 3-b), while the isoelectric point (figure 2) and zeta potential (figure 3-c) were measured at different pH (HCl Ciccarelli and NaOH Tetrahedrom, Argentine).

3. Results and discussion

3.1 Intrinsic viscosity

If a solution tends to be independent of shear, then the measurement of viscosity (η) is based on Poiseuille's law can be made easy by grouping all those terms related to a specific viscometer as a calibration constant A.

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

where ρ is the density of solution. If we divide both sides by ρ, we have

$$\upsilon = \frac{\eta}{\rho} = A\,t \tag{2}$$

where υ is the cinematic viscosity of solution.

In macromolecular chemistry, the relative viscosity η_r is often measured. The relative viscosity is the ratio of the viscosity of the solution to that of the solvent:

$$\eta_r = \frac{\rho\,t}{\rho_0\,t_0} \tag{3}$$

The specific viscosity η_{sp} is obtained from the relative viscosity by

$$\eta_{sp} = \eta_r - 1 \tag{4}$$

3.1.1 Methods for determining the intrinsic viscosity

The intrinsic viscosity, denoted by [η], is defined as

$$[\eta] = \lim_{c\to 0} \frac{\eta_{sp}}{c} = \lim_{c\to 0} \frac{1}{c} \ln \eta_r \tag{5}$$

or as

$$[\eta] = \lim_{\eta_{sp}\to 0} \frac{\eta_{sp}}{c} \tag{6}$$

where c is the concentration of the polymer in grams per 100 cm^3 or grams per milliliter of the solution. The quantity η_{sp}/c is called the reduced viscosity. The unit of intrinsic viscosity is deciliters per gram (dL/g), milliliters per gram (mL/g) or (cm^3/g) depending on the concentration unit of the solution. The intrinsic viscosity is also called the limiting viscosity number. The plot of η_{sp}/c versus c or 1/c ln η_r versus c often gives a straight line, the intercept of which is [η].

3.1.2 Huggins, Kraemer and Schulz-Blaschke methods

Huggins (1942) showed that the slope is

$$\frac{d\eta_{sp}/c}{dc} = k_H [\eta]^2 \tag{7}$$

Rearranging and integrating the resulting equation is

$$\frac{\eta_{sp}}{c} = [\eta] + k_H [\eta]^2 c \tag{8}$$

where k_H is a dimensionless constant, called the Huggins constant. The value of k_H is related to the structures of polymers or biopolymers. Some other equations for the determination of [η] are:

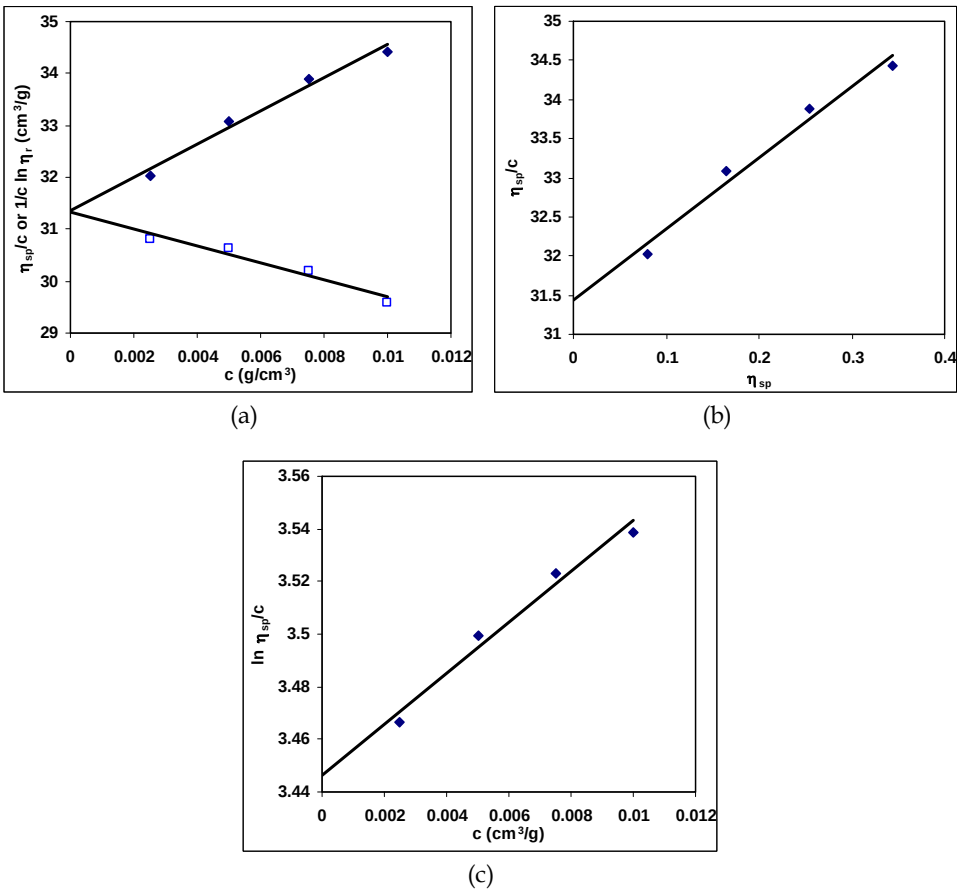


Fig. 1. Methods of Huggins (positive slope), Kraemer (negative slope) (a), Schulz-Blaschke (b), and Martin (c). Data obtained from experimental measures for gelatin B at 37.4ºC.

Kraemer (1938)
$$\frac{1}{c}\ln\eta_r = [\eta] + k_K[\eta]^2 c\,, \tag{9}$$

Schulz-Blaschke (1941)
$$\frac{\eta_{sp}}{c} = [\eta] + k_{S-B}[\eta]\eta_{sp} \tag{10}$$

and Martin (1942)
$$\frac{\eta_{sp}}{c} = [\eta]e^{k_M[\eta]c} \tag{11}$$

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.

Temperature	37.4ºC			
Method	Huggins	Kramer	Schulz-Blaschke	Martin
C (g/cm^3)	η_{sp}/c	$1/c \ln \eta r$	η_{sp}	$\ln \eta_{sp}/c$
0.0025	32.02	30.80	0.0800	3.46
0.0050	33.09	30.62	0.1654	3.50
0.0075	33.89	30.20	0.2542	3.52
0.0100	34.43	29.59	0.3443	3.54
[η] (cm^3/g)	31.35	31.32	31.44	31.39
Constant	k_H	k_K	k_{S-B}	k_M
	0.34853	0.1660	0.3139	0.3078
σ^2	0.9787	0.9712	0.9751	0.9757
$\%E_R$	-	0.0957	0.2552	0.1286

Table 1. Data obtained applying the classic methods from experimental measurement.

The intrinsic viscosity data calculated from the methods of Huggins, Kramer, Schultz-Blaschke and Martin are shown in Table 1, and how to perform these steps are shown in Figure 1.

All values were calculated for intrinsic viscosity of gelatin B solutions to 37.4ºC and compared against the value of Huggins, normally used as standard. It is noteworthy that each method has a relative error percentage and low for methods of more than four pair's values ($E_R\% > 0.30$).

3.1.3 Single point methods

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 is the routine method used for [η] determination. The procedure is laborious and consumes a considerable amount of time and reactive; because of this, several equations were developed which estimate intrinsic viscosity at one single concentration and do not require a graphic. They are known as "single-point" methods.

Single-point equations suppose 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 (1962) proposes:

$$[\eta] \cong \frac{1}{c}\sqrt{2\eta_{sp} - 2\ln\eta_r} \tag{12}$$

In 1968, Deb and Chatterjee (1968 and 1969) suggested that:

$$[\eta] \cong \frac{1}{c}\sqrt[3]{3\ln\eta_r + 1.5\eta_{sp}^2 - 3\eta_{sp}} \tag{13}$$

More recently, Rao and Yanseen (1986) gave a simplified expression:

$$[\eta] \cong \frac{1}{2c}\left\{\eta_{sp} + \ln\eta_r\right\} \tag{14}$$

Kuwahara (1963) uses the expression:

$$[\eta] \cong \frac{1}{4c}\left\{\eta_{sp} + 3\ln\eta_r\right\} \tag{15}$$

While Palit and Kar (1967) suggest:

$$[\eta] \cong \frac{1}{c}\sqrt[4]{4\eta_{sp} - 4\ln\eta_r + 1.33\eta_{sp}^3 - 2\eta_{sp}^2} \tag{16}$$

and the following equation due to Maron (1961) makes use of the previously calculated parameters:

$$[\eta] \cong \frac{\eta_{sp} + \gamma\ln\eta_r}{c\{1+\gamma\}} \tag{17}$$

$$\gamma = \frac{k_H}{k_k} \tag{18}$$

Chee (1987) and Rao & Yaseen (1986) 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 and Cesco suggest a double point equation

$$[\eta] = \frac{0.5c_2}{c_2 - c_1}\left\{\frac{\eta_{sp,1}}{c_1} + \frac{\ln\eta_{r,1}}{c_1}\right\} - 0.5\frac{c_1}{c_2 - c_1}\left\{\frac{\eta_{sp,2}}{c_2} + \frac{\ln\eta_{r,2}}{c_2}\right\} \tag{19}$$

where subscript 1 and 2 referrer to concentrations measured. The application of different methods of single point are shown and compared against the method of Huggins (Table 2).

For single point methods fit with minor errors of 3.54%, but the method of Solomon-Ciuta is only valid for concentrations below 0.25% wt of gelatin. Double point error is low of 2.00%.

While all methods of single and double point are used to characterize a polymer solution is always advisable to increase the statistical weight (to reduce errors) with at least four different concentrations of polymer in a given solvent.

Methods						
c (g/cm^3)	Solomon-Ciuta	Deb-Chaterjee	Ram, Mohan, Rao, Yanseen	Kuwahara	Palit & Kar	Maron
0.0025	31.97	31.41	31.41	31.10	31.17	31.19
0.0050	16.23	31.83	31.85	31.24	31.90	31.42
0.0075	10.94	31.99	32.04	31.12	32.24	31.39
0.0100	8.26	31.93	32.01	30.79	32.31	31.15
			$\%E_R$			
0,0025	2.44	0.65	0.64	0.33	0.12	0.05
0,0050	47.99	2.01	2.07	0.09	2.20	0.67
0,0075	64.94	2.52	2.67	0.28	3.30	0.57
0,0100	73.52	2.30	2.56	1.32	3.54	0.19

Table 2. Data obtained applying the methods of single point from experimental measurement.

3.2 Hydrodynamic properties

In physics, fluid dynamics is a sub-discipline of fluid mechanics that deals with fluid flow—the natural science of fluids (liquids and gases) in motion. It has several subdisciplines itself, including aerodynamics (the study of air and other gases in motion) and hydrodynamics (the study of liquids in motion). Fluid dynamics offers a systematic structure that underlies these practical disciplines, that embraces empirical and semi-empirical laws derived from flow measurement and used to solve practical problems. The solution to a fluid dynamics problem typically involves calculating various properties of the fluid, such as velocity, pressure, density, viscosity and temperature, as functions of space and time.

3.2.1 Mark-Houwink parameters

Staudinger (1932) suggested that the molecular weight M of polymers is proportional to the reduced viscosity:

$$[\eta] = k_0 M \tag{20}$$

where k_0 is proportionality constant. Mark (1938) and Houwink (1940) independently correlated the intrinsic viscosity with molecular weight:

$$[\eta] = k\ M^a \tag{21}$$

where k and *a* both are constants. The Mark-Houwink equation is applicable to many polymers and is extensively used to determine molecular weight. The constants k and *a* both vary with polymers and solvents.

Equation (21) describes the relationship between viscosity and molecular weight. Since molecular weight is related to the size of the polymer chain. Generally, for proteins using the following equation:

$$[\eta] = k\left(\frac{M}{M_0}\right)^a \tag{22}$$

Where M_0 is the molecular weight of the repeating unit, gelatin is 540g/mol, according Pouradier-Venet (1954), and Bohidar is 110g/mol. The calculation of Mark-Houwink (M-H) parameters is carried out by the graphic representation of the following equation:

$$\ln[\eta] = \ln k + a \ln M_w \tag{23}$$

Where *k* and *a* are M-H constants, these constant depend of the type of polymer, solvent, and temperature of viscometric 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). Using the polymer standards, a plot of the ln [η] vs ln M_w usually gives a straight line. The slope is *a* value and intercept is equal to ln *k* value. The M-H 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). The fact that the intrinsic viscosity of a given polymer sample is different according to the solvent used gives and 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 attract the surrounding solvent molecules. The polymer molecule assumes a tighter configuration, and the solution has a lower intrinsic viscosity. The M-H "*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 "*a*" constant approaches a value of 2.0 (Masuelli 2011).

Table 3 shows the classical values calculating for Mark-Houwink parameters, *a* and *k* for temperature. These studies on M-H parameters are usually carried out at a given temperature, obtaining a consistent result but in a very limited range of temperature (for gelatin: Pouradier & Venet 1954 and Bohidar 1998; Monkos for serum proteins 1996, 1997, 1999, 2000, 2004 and 2005). This value shows a clear functionality between these parameters and temperature.

T(°C)	[η] (cm^3/g)	k (cm^3/g)	*a*
20.00	62.12	0.1681	0.9119
25.00	48.65	0.1660	0.8850
26.60	44.46	0.1631	0.8737
28.30	41.15	0.1626	0.8621
31.00	39.28	0.1621	0.8554
34.00	35.53	0.1618	0.8382
37.40	31.35	0.1614	0.8198

Table 3. Data obtained of intrinsic viscosity and Marck-Houwink parameters of gelatin B at different temperatures.

The molecular weight calculated for gelatin is 333,000g/mol. The value of *a* given at different temperatures shows that this biopolymer in aqueous solution behaves in a conformation predominantly confined to the rod-like, different as observed by Bohidar 1998.

3.2.2 Hydration values

It is well known that biopolymers adsorbed water during dry storage and its quality depends on water content. For example the length of keratin depends on water content and therefore it is used as a hygrometer. The amount of adsorbed water depends on temperature and pressure of water vapor.

On the other hand, biopolymers in solution exhibit the phenomenon of hydration due to the polar properties of water molecules. The electronic formula of water shows that the center of charge of the negatively charged electrons is nearer to the oxygen atom to the positively charged hydrogen nuclei. The center of the positive charges is nearer the two hydrogen atoms. Assuming a molecule, in which the centers of the positive and negative charges do not coincide, a polar molecule or dipole. Always, dipoles are attracted by ions. In the fits phase, the ion attracts the opposite pole and repels the pole of same sign; in a second phase, attraction is stronger than repulsion because the attracted pole is nearer the ion than the repelled pole. For similar reasons attraction takes place between two dipoles.

Hydration consists of the binding of water dipoles to ions or ionic groups, to dipoles, or polar groups. Hydration takes place in solid substances as well as in solution.

Since the components of a compound are linked to each other in such a way that they have lost same of their free translational mobility, the volume of hydrated molecule is always smaller than the sum of the volumes of its components, the hydration is accompanied by a decrease of the total volume.

The amount of water bound to the proteins and polysaccharides depends primarily on the ratio of water to the biopolymer in the investigated system. The two extreme cases are the dry biopolymer (water content tend to zero) and highly diluted aqueous solutions of the biopolymers. The dry biopolymer undergoes hydration if is exposed to the water vapor of increased vapor pressure. The extent of hydration can be determined y measuring the

increment in weight. It is much more difficult to determine the extent of hydration in aqueous solutions of biopolymers. Although hydration is accompanied by a volume contraction of the solute and the solvent, this change in volume is very small and difficult to measure directly. It is customary to measure the density of biopolymer solution.

The amount of hydrated biopolymer and of free water in the biopolymer-water system, the thermodynamic notion of partial specific volume has been introduced and is frequently determined. The relation to v_{sp}, the specific volume, is shown by the equation:

$$v_{sp} = g_p \bar{v} + g_0 \bar{v}_0 \tag{24}$$

where g_p and g_0 are the amount of biopolymer and water, respectively, in 1g of the mixture. The magnitude of $\bar{v}$ can be determined by varying the biopolymer-water ratio (g_P/g_0) and plotting v_{sp} agains g_p, the slope is $\bar{v}$.

However is not to measure solution density at each concentration since the correction of Tanford (1955) can be applied:

$$[\eta] = [\eta]_0 + \left\{ \frac{1 - \rho_0 \bar{v}}{\rho_0} \right\} \tag{25}$$

$$\text{or } \left(\frac{\eta_{sp}}{c} \right) = \left(\frac{\eta_{sp}}{c} \right)_0 + \left\{ \frac{1 - \rho_0 \bar{v}}{\rho_0} \right\} \tag{26}$$

Of course if this latter is not known for the solvent conditions being used, or cannot be calculated from the chemical composition of the macromolecule then solution density measurements are required:

$$\bar{v} = \left\{ \frac{1 - \partial\rho / \partial c}{\rho_0} \right\} \tag{27}$$

ρ_0 and ρ are density of solvent and solution, respectively, and can be measured using densimeter or picnometer.

The swollen specific volume v_{sp} (cm^3/g) is defined when an anhydrous biomacromolecules essentially expand in suspended or dissolved in solution because of solvent association, and

$$v_{sp} = \frac{v_H M}{N_A} \tag{28}$$

where v_H is swollen or hydrodynamic volume (cm^3), M the molecular weight (Da or g/mol), and N_A is Avogadro's number. This associated solvent which we consider in more detail below can be regarded as which is either chemically attached or physically entrained by the biomacromolecules. v_{sp} can be related to a popular term called the hydration value δ, by the relation

$$v_{sp} = \bar{v} + \frac{\delta}{\rho_0} \tag{29}$$

The corresponding value of the 'hydration' δ of the molecule (see table 4), defined by

$$\delta = (v_{sp} - \bar{v})\rho_0 \tag{30}$$

where v_{sp} is specific volume (cm^3/g). 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 to is ~50g/g solvent bound per g of solute. For globular proteins a δ value of 0.3-0.4 has been inferred by RMN, IR, and simulation computer. Hydration value from sedimentation or diffusion data varied from 0.1 to 1.

3.2.3 Perrin number

Most biological polymers, such as proteins and nucleic acids and some synthetic polymers, have relatively inflexible chains. For rigid particles, the size is no longer of predominant importance, because the polymer chain is no longer in the form of a flexible random coil; instead, shape becomes an important parameter. Following are some theoretical proposals for the estimation of the shape factor p from the viscosity measurement (table 4). The term f/f_0 is sometimes denoted as p, Perrin constant.

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 ($v_H/\bar{v}$) to be estimated, where v_H, (cm^3/g) is the volume of the swollen molecule (Polysaccharide or protein + associated solvent) per unit mass of polysaccharide and $\bar{v}$ is the partial specific volume (essentially the anhydrous molecule):

$$\frac{f}{f_0} = P\left(\frac{v_H}{\bar{v}}\right)^{1/3} \tag{31}$$

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

3.2.4 Einstein viscosity increment (Simha number)

There are two molecular contributions to the intrinsic viscosity: one from shape, the other from size or volume, as summarized by the relation

$$[\eta] = \upsilon_{a-b} v_{sp} \tag{32}$$

Where $\upsilon_{a\text{-}b}$ is a molecular shape (Simha 1940) parameter known as viscosity increment and v_{sp} defined in equation 28.

The viscosity increment $\upsilon_{a\text{-}b}$ is referred to as a universal shape function or Simha number (table 4); it can be directly related to the shape of a particle independent of volume. For its experimental measurement it does however require measurement of v_{sp}, $\bar{v}$,δ, ρ_0, as well as of course [η].

In a study of the viscosity of a solution of suspension of spherical particles (colloids), suggested that the specific viscosity η_{sp} is related to a shape factor $\upsilon_{a\text{-}b}$ in the following way:

$$\eta_{sp} = \upsilon_{a-b}\phi \tag{33}$$

where ϕ is the volume fraction;

$$\phi = \frac{n\, v\, \upsilon_{a-b}}{V} \tag{34}$$

where n is the number of no interacting identical particles, v is the volume of each particle, and V is the volume of the solution or suspension. Assume that the molecules are of a spherical shape, rigid and large relative to the size of the solvent molecules, and that the particles are small enough to exhibit Brownian motion but large enough to obey the laws of macroscopic hydrodynamics (Teraoka 2002). Then $\upsilon_{a\text{-}b} = 2.5$ is for spherical particle.

The Einstein equation is now used as a reference to estimate the shape of macromolecules. Any deviation can be interpreted as the fact that the molecules are not a sphere.

3.2.5 Scheraga-Mandelkern parameter

The first attempt to the problem of the hydration for ellipsoids of revolution, suggests a combination graphic $\upsilon_{a\text{-}b}$ with the contribution of the form of the Perrin function "p" (ratio of friction). This was followed in for Flory and Scheraga-Mandelkern describing and analytical combination of $\upsilon_{a\text{-}b}$ with p to yield a function β, which, with [η] in cm^3/g is given by:

$$\beta \equiv \frac{[\eta]^{1/3}\eta_0}{M^{2/3}\left(1-\rho_0\bar{v}\right)100^{1/3}} = \frac{N_A^{1/3}}{\left(16200\pi^2\right)^{1/3}}\frac{\upsilon_{a-b}^{1/3}}{p} \tag{35}$$

Unfortunately the β-function proved very insensitive to shape change. Fortunately further combination of $\upsilon_{a\text{-}b}$ with other universal shape parameters have proved more successful.

Scheraga–Mandelkern equations (1953), for effective hydrodynamic ellipsoid factor β (Sun 2004), suggested that [η] is the function of two independent variables: p, the axial ratio, which is a measure of shape, and V_e, the effective volume. To relate [η] to p and V_e, introduced *f*, the frictional coefficient, which is known to be a direct function of p and V_e. Thus, for a sphere we have

$$\frac{\eta_{sp}}{c} \equiv [\eta] = \upsilon_{a-b}\frac{N_A}{100}\frac{V_e}{M} \tag{36}$$

$$f_0 = 6\pi\eta_0\left(\frac{3V_e}{4\pi}\right)^{1/3} \tag{37}$$

Using the Stokes-Einstein equation of diffusion coefficient

$$D = \frac{k_B T}{f} \tag{38}$$

where k_B is Boltzmann constant and T is the temperature.

And Svedberg equations of sedimentation coefficient

$$S = \frac{M\left(1-\rho\bar{v}\right)}{N_A f} \tag{39}$$

obtain

$$\beta \equiv \frac{D[\eta]^{1/3} M^{1/3}\eta_0}{k_B T} \tag{40}$$

or

$$\beta \equiv \frac{N_A S[\eta]^{1/3}\eta_0}{M^{2/3}\left(1-\rho\bar{v}\right)} \tag{41}$$

The value of β is a measure of the effective hydrodynamic ellipsoid (table 4).

3.2.6 Flory parameters

The classical size-independent combinations are the Flory parameters that combine the intrinsic viscosity, $[\eta]$, and the radius of gyration, R_g:

$$\phi_0 = \frac{[\eta]M_w}{6^{3/2}R_g^3} \tag{42}$$

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

$$P_0 = \frac{f}{6\eta R_g} \tag{43}$$

These quantities have been proposed along the years, at different times by Flory. 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 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; thus, while the values for these two structures in the case of the ϕ_0 are $9.23 \times 10^{23} mol^{-1}$ and $2.60 \times 10^{23} mol^{-1}$. Thus, it is accepted that, for every flexible-chain polymer in a Θ (ideal) solvent, there is a universal value of $\phi_0 = 2.50 \times 10^{23} mol^{-1}$ (Ortega & Garcia de la Torre 2007). The values obtained for gelatin B are show in table 4.

3.3 pH effect and Isoelectric point

The IEP for aminoacid can be calculated from the ionization constant according to the equation:

$$IEP = \frac{pK_1 + pK_2}{2} \tag{44}$$

If the acid and basic groups of aminoacid were ionized to the same extent, its salt-free water solution would have the same pH value as pure water. Since the ionization of carboxyl groups is higher than of amino groups the IEP are neighborhood of pH 6. An aqueous solution of aminoacid contains small amounts of hydrogen ions and of anions $H_2N{\cdot}R{\cdot}COO^-$ in addition to large amounts of dipolar ion $H_3N^+{\cdot}R{\cdot}COO^-$. Since the aminoacid are weak acids and weak bases at the same time, their mixtures with strong acids and bases are used as buffer solutions.

Since the binding of extraneous ions considerably alters the value of IEP of an aminoacid or protein, this point is not a constant. The term isoionic point (IP) is used to designate the pH value of a pure protein in salt-free water. The direct determination of this constant is difficult and because many proteins are insoluble in the absence of salts. The isoionic point is usually determined indirectly, that is, by measuring the IEP at different concentrations of the neutral salts and extrapolating to zero concentration. The value of the isoionic point may differ from IEP by more than a pH unit (Haurowitz 1963).

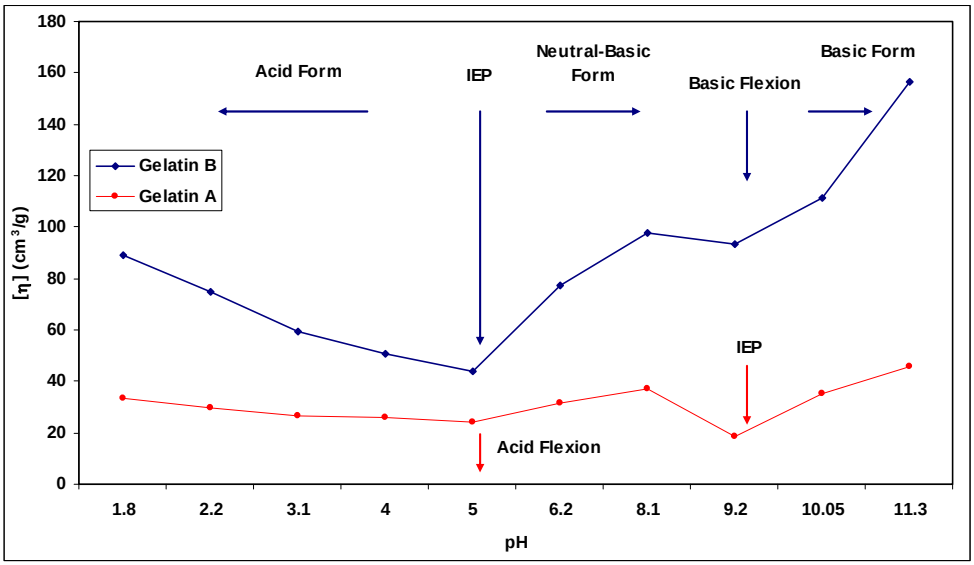


Fig. 2. Influence of pH on the intrinsic viscosity of gelatin A and B.

On point of interest at the pH at which a protein or order polyampholite has zero charge (isoelectric point) the mobility should be zero. At lower pH values a protein will be positively charged and move toward the negative electrode, and at higher pH transport will be in the opposite direction. Thus the measure of isoelectric point (IEP) can be made simply and unambiguously by means electrophoresis (van Holde 1971).

Gelatins are classified according to whether an acid or an alkali is used in the final preextraction step. If an acid solution is used as the final solvent, type-A gelatin (acid process) is obtained. In case of alkali as the final solvent, type-B gelatin (alkali process) is obtained. Type-A gelatin's isoelectric point is higher compared to that of type-B gelatin, as a

milder acid process does not remove the amide nitrogen of glutamine and aspargine, therefore, the resulting gelatin's isoelectric point might be as high as 9.4. If a more severe acid treatment is required, then some of the amide groups are hydrolyzed and the isoelectric point would be similar to that of the original collagen molecule, which generally lies between 6 and 8. Type-B gelatin's isoelectric point might be as low as 5.2 or 4.8, as the alkali process results in the loss of the amide groups. Benson 1963, prepared protein solutions at various pH and determining by plott the viscosity relative or intrinsic viscosity as a function of pH is a minimum corresponding to the isoelectric point.

Kenchington and Ward 1954, conducted studies of gelatin obtained by titration of acid and basic process. They claim the IEP gelatin A to the amide groups in addition to carboxyl groups. In the case of gelatin B, awarded the IEP conversion of arginine to ornithine, where the conversion of the guanidine groups in the amino acid occurs during treatment extent slight alkaline of the collagen.

The figure clearly shows two minimum for a gelatin B corresponds to the isoelectric point, pH 5.1, and the other corresponds to a flexion in the basic medium at pH 9.1; in the case of gelatin A the isoelectric point corresponds to pH 9.2 and at pH 5 an flexion. The isoelectric point is presented as the more compact form of gelatin, i.e. less drainage time, evidenced with smaller hydrodynamic radius. Both the basic or acidic flexion is due to compact forms of gelatin but not so small as to be isoelectric point. No less are the forms they take in acidic or basic, identified as linear gain widespread forms of gelatin A or B. In the neutral pH from the isoelectric points bending is the neutral form. A basic pH after the isoelectric point 9.2 shall register a new or extended basic shape.

3.4 Solvent effect

Solubility of protein in water varies within wide limits. While some proteins dissolve easily in salt-free water, others dissolve only in the presence of certain concentrations of salts, a third group is soluble in mixtures of water and ethanol, and insoluble in any solvent.

The solubility protein in water is determined by chemical structure, number of aminoacids that forming the molecule, and folding of the peptide chains in the molecule. Peptide structure and charged ionic groups increase the affinity of the protein for water and its solubility. The solubility of proteins depends to a great extent on pH and on the concentration of salts present on solution. The minimum solubility is found at the isoelectric point. Neutral salts have a two-fold effect on protein solutions. Low concentration of the salts increase the solubility of proteins phenomena denominated as salting-in, while high concentrations of neutral salts reduce the solubility and give rise to the formation of precipitates (salting-out). Salting-out effect is evidently due to a competition between the salt and protein for molecules of the solvent.

Bohidar et al. 1998, realized studies of Sol and Gel state properties of aqueous gelatin solutions of concentrations 4%, 6%, 8% and 10% (w/v) were investigated through dielectric relaxation studies done at various temperatures in the range from 20 to 60°C carried out over a frequency range 20Hz-10MHz and no relaxation of any nature was observed.

Quite generally, high dielectric constants are found in polar molecules, low constant in non-polar molecules. The proteins are highly polar substances; one would expect zwitterions to

have high dielectric constants. The dielectric constant of protein aqueous solutions is increase and proportional to the dissolved substance.

3.5 Ionic strength and electroviscous effect

The ionic strength dependence of intrinsic viscosity is function of molecular structure and protein folding. It is well known that the conformational and rheological properties of charged biopolymer solutions are dependent not only upon electrostatic interactions between macromolecules but also upon interactions between biopolymer chains and mobile ions. Due electrostatic interactions the specific viscosity of extremely dilute solutions seems to increase infinitely with decreasing ionic concentration. Variations of the intrinsic viscosity of a charged polyampholite with ionic strength have problems of characterization.

It was found earlier by experiment and theory that the viscosity intrinsic of polyelectrolyte solutions is nearly linear with the reciprocal square root of the ionic strength over a certain range, such as

$$[\eta] = [\eta]_{\infty} + \varepsilon\, I^{-1/2} \tag{45}$$

Where the slope ε determined by the plot is the extension coefficient. The $I^{-1/2}$ dependence viewed in terms of an increased Debye length can be explained as the electrostatic excluded volume contribution.

To study the electroviscous effect should address the theory of the electrical double layer developed by Smolowchoski, Helmholtz, Guy and Stern (Lyklema 1995). According to the Stern double layer consists of two parts: one, which is about the thickness of anion remains almost fixed to the solid surface. In this layer there is a definite fall of potential. The second part extends some distance within the liquid phase and is diffuse in this region, thermal motion allows the free movement of particles, but the distribution of positive and negative ions is not uniform, because the electrostatic field in the surface would cause preferential attraction of the opposite charge. The result is a fall of potential within the liquid where the charge distribution is uniform. The approximate boundary between the bulk liquid and bulk solution is the electrokinetic potential or zeta potential. The magnitude of the zeta potential could be alter, or even sign, the presence of ions in solution. The higher the valence of the ion of opposite sign is greater effect to reduce the potential electrokinetic. Increasing the concentration of electrolyte on a surface of negative charge, cations tend to accumulate on the fixed layer, reducing the thickness of the double layer and consequently charge density and zeta potential. To study the stability of colloids are essential trace amounts of electrolytes, at least in water, but larger quantities cause aggregation of particles and precipitate formation. The phenomenon of precipitation, flocculation, coagulation, and conservation depends on what kind of effect on the type of colloid electrolyte concentration exercises. Given the concentration of salts and the concentration of colloid is possible to determine whether precipitation occurs or not. As a result of these studies it is clear that the ion is effective for the coagulation is the opposite to that of the colloidal particle, and that the clot can grow considerably increases the valence of the ion (Schulze-Hardy rule).

The stability of a colloid such as gelatin in water is determined by the electric charge and hydration. The addition of large amounts of electrolytes to colloids (biopolymers) causes

precipitation dispersed substance, a phenomenon known as salting. That is, high concentrations of salts dehydrate the solvated biopolymer and reduce the zeta potential. The salificantion effect depends on the nature of the ion, and salts of a given cation. Can be arranged according to decreasing ability to remove the lyophilic substances on colloidal solution, these series have been called Hofmeister or lyotropic series.

Electroviscous effect occurs when a small addition of electrolyte a colloid produces a notable decrease in viscosity. Experiments with different salts have shown that the effective ion is opposite to that of the colloid particles and the influence is much greater with increasing oxidation state of the ion. That is, the decrease in viscosity is associated with decreased potential electrokinetic double layer. The small amount of added electrolyte can not appreciably affect on the solvation of the particles, and thus it is possible that one of the determinants of viscosity than the actual volume of the dispersed phase is the zeta potential.

The electroviscous effect present with solid particles suspended in ionic liquids, to increase the viscosity over that of the bulk liquid. The primary effect caused by the shear field distorting the electrical double layer surrounding the solid particles in suspension. The secondary effect results from the overlap of the electrical double layers of neighboring particles. The tertiary effect arises from changes in size and shape of the particles caused by the shear field. The primary electroviscous effect has been the subject of much study and has been shown to depend on (a) the size of the Debye length of the electrical double layer compared to the size of the suspended particle; (b) the potential at the slipping plane between the particle and the bulk fluid; (c) the Peclet number, i.e., diffusive to hydrodynamic forces; (d) the Hartmann number, i.e. electrical to hydrodynamic forces and (e) variations in the Stern layer around the particle (Garcia-Salinas et al. 2000).

The primary electroviscous effect occurs, for a dilute system, when the complex fluid is sheared and the electrical double layers around the particles are distorted by the shear field. The viscosity increases as a result of an extra dissipation of energy, which is taken into account as a correction factor ``p_i'' to the Einstein equation:

$$\eta_r = 1 + \nu_{a-b}(1 + p_i)\phi \tag{46}$$

The effective viscosity of a suspension of particles in a fluid medium is greater than that of the pure fluid, owing to the energy dissipation within the electrical double layers.

Finally, p_i is the primary electroviscous coefficient which is a function of the charge on the particle or, more conventionally, the electrostatic potential, ζ , on the "slip-ping plane" which defines the hydrodynamic radius of the particle, and properties (charge, bulk density number, and limiting conductance) of the electrolyte ions (Rubio–Hernandez et al. 2000).

Equation 46 suggests that, maintaining p_i constant, η_r must depend linearly on ϕ if only a first-order electroviscous effect exists, and an increase in the electrolyte concentration implies a decrease in the thickness, $1/\kappa$, of the electrical double layer,

$$\frac{1}{\kappa} = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_B T}{4\pi e^2 \sum_i c_i z_i^2}} \tag{47}$$

where ε_r is the dielectric constant of the liquid medium, ε_0 is the vacuum permittivity, e is the electron charge, k_BT is the Boltzmann energy, and c_i and z_i are the concentrations and

the valencies, respectively, of the various ionic species in the solution, far away from any particle (Oshima 2008 and Overveek 1976).

The slopes of the different curves correspond to the full electrohydrodynamic effect, $\phi + \phi\, p_i$, where the first term expresses the hydrodynamic effect, and the second is the consequence of the distortion of the electrical double layer that surrounds the particles. To determine this second term and, more exactly, the primary electroviscous coefficient, p_i.

The calculation of zeta potential from electoviscous effect measures (Rubio-Hernandez et al. 1998 and 2004), is given by the equation

$$\eta_{sp} = \nu_{a-b}\phi\left[1 + \frac{1}{\eta_o \kappa R_H^2}\left(\frac{\varepsilon_r \varepsilon_0 \zeta}{2\pi}\right)^2\right] \tag{48}$$

Proteins and polysaccharides are biopolymers carrying charges due to dissociation of their ionizables groups in an aqueous solution and have been widely used in paints, cosmetics, and film industries. Different from neutral polymers, polyelectrolytes have a comparatively extended conformation, owing to the repulsive intrachain electrostatic interaction screened by the surrounding small ions in the solution. As a result, their rheological properties in a dilute solution due to an applied flow will change accordingly. This is called a tertiary electroviscous effect. In addition, the imposed flow distorts the ionic cloud around each polyelectrolyte from its equilibrium state, leading to two effects. First, it results in additional energy dissipation associated with the electrical interaction between each polyelectrolyte and small ions. This direct effect, depending on the chain configuration, is analogous to the primary electroviscous effect of charged rigid spheres, rods, or polyions of arbitrary shape but has sometimes been ignored in the field of flexible polyelectrolyte rheology. Second, an indirect effect changes the polyelectrolyte conformation through the modification of the intrachain electrostatic interaction, compared to that with equilibrium double layer. Therefore, the primary and tertiary effects are indeed coupled for polyelectrolytes (Hunter 1981).

The increasing dilution of flexible polyelectrolytes at low ionic strength, the reduced viscosity may increase first, reach a maximum, and then decrease. Since a similar behavior can also be observed even for solutions of polyelectrolyte lattices at low salt concentration, the primary electroviscous effect was thought as a possible explanation for the maximum, as opposed to conformation change.

It is very difficult and scarce to find literature to study the electrokinetic phenomena of proteins or macromolecules in solution therefore limit us to the basic concepts of electrokinetic changes observed, they are conformational change because of the presence of salts and the zeta potential change in pH.

For polyelectrolyte solutions with added salt, prior experimental studies found that the intrinsic viscosity decreases with increasing salt concentration. This can be explained by the tertiary electroviscous effect. As more salts are added, the intrachain electrostatic repulsion is weakened by the stronger screening effect of small ions. As a result, the polyelectrolytes are more compact and flexible, leading to a smaller resistance to fluid flow and thus a lower viscosity. For a wormlike-chain model by incorporating the tertiary effect on the chain

conformation to predict the intrinsic viscosity in zero-shear limit. The effect of the intrachain electrostatic repulsion on the chain conformation is obtained based on the equilibrium interaction, which depends on the Debye screening length (Li Jiang et al. 2001).

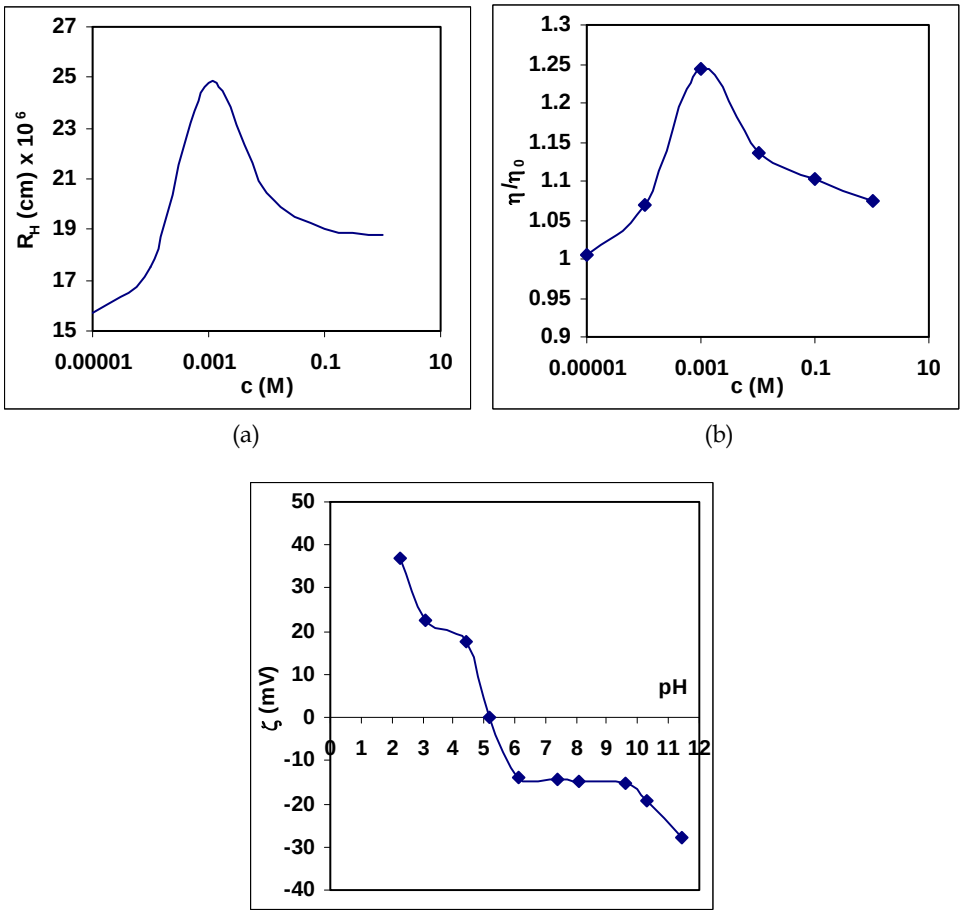


Fig. 3. Study of the electroviscous effect of NaCl on gelatin B. a-Hydrodynamic radius, b-η/η_0. c- Zeta potential at different pH (0.001M NaCl).

Note that when the concentration of added salt is very low, Debye length needs to be modified by including the charge contribution of the dissociating counterions from the polyelectrolytes. Because the equilibrium interaction is used, their theory predicts that the intrinsic viscosity is independent of ion species at constant ionic strength. At very high ionic strength, the intrachain electrostatic interaction is nearly screened out, and the chains behave as neutral polymers. Aside from the tertiary effect, the intrinsic viscosity will indeed be affected by the ionic cloud distortion and thus cannot be accurately predicted by their theory.

The effects of ion valence and polyelectrolyte charge density showed that at very low ionic strength found that when the counterion valence of added salt changes from monovalent (NaCl) to divalent ($MgSO_4$), the reduced viscosity decreases by a factor of about 4.5. If $La(NO_3)_3$ is used, the reduced viscosity will be further decreased although not drastically. As for polyelectrolyte charge density, the intrinsic viscosity was found to increase with it because of an enhanced intrachain electrostatic repulsion (Antonietti et al. 1997).

The data on the electroviscous effect of NaCl in gelatin B can be seen in Figure 3. Figure 3 b shows that in 0.001M is a maximum, this maximum defines the most expanded form of gelatin B, at higher concentrations this value is constant from 0.1M, a phenomenon known as salting-out. At lower concentrations found in the salting-in. Figure 3 c, we find the zeta potential and pH where it is zero corresponds to the isoelectric point to a concentration of 0.001M NaCl.

3.6 Temperature effect

Theta temperature (Flory temperature or ideal temperature) is the temperature at which, for a given polymer-solvent pair, the polymer exists in its unperturbed dimensions. The theta temperature, Θ, can be determined by colligative property measurements, by determining the second virial coefficient. At theta temperature the second virial coefficient becomes zero. More rapid methods use turbidity and cloud point temperature measurements. In this method, the linearity of the reciprocal cloud point temperature ($1/T_{cp}$) against the logarithm of the polymer volume fraction (ϕ) is observed. Extrapolation to $\log \phi = 0$ gives the reciprocal theta temperature (Guner and Kara 1998).

Theta temperature is one of the most important thermodynamic parameters of polymer solutions. At theta temperature, the long-range interactions vanish, segmental interactions become more effective and the polymer chains assume their unperturbed dimensions. It can be determined by light scattering and osmotic pressure measurements. These techniques are based on the fact that the second virial coefficient, A_2, becomes zero at the theta conditions.

Another method is known as the cloud point/turbidity measurements yielding more rapid and accurate determination of the theta temperature. This technique is mainly based on the observed linearity of the reciprocal of the cloud point temperature when plotted versus the logarithm of the polymer volume fraction and the extrapolation to ϕ. The information on unperturbed dimensions has been accessed via extrapolation methods by viscometry yielding intrinsic viscosity values (Gouinlock et al. 1955). In these methods, it is also possible to determine the long-range interactions, and evaluate the theta temperature by the temperature dependence of long-range interaction parameter. Simply, the intrinsic viscosity may be given in the form:

$$[\eta]_T = [\eta]_\Theta \alpha_\eta^3 \tag{49}$$

where $[\eta]_T$ and $[\eta]_\Theta$ are the intrinsic viscosities determined at the studied temperature interval (20-60°C) and the theta temperature. α_η is the expansion of the polymer for the employed solvent system. The relation between hydrodynamic linear expansion factor, α, and α_η is given by $\alpha_\eta^3=\alpha^{5/2}$.

The thermodynamic linear expansion factor has been related to Flory or thermodynamic interaction parameter, χ, and the entropy of dilution parameter, χ_S, through the Flory-Fox [10] equations,

$$\alpha^5 - \alpha^3 = 2C_M\left(0.5 - \chi_s\right)\left(1 - \Theta / T\right)M^{1/2} \tag{50}$$

where Θ is the theta temperature. The coefficient C_M is given by

$$C_M = \frac{27\nu_{sp}^2 M^{3/2}}{2N_A v_1\left(2\pi < r^2 >_0\right)^{3/2}} \tag{51}$$

where v_{sp} is the specific volume of the polymer, N_A is the Avogadro's number, v_1 is the molar volume of the solvent (for water, 18 cm^3/mol) and $<r^2>_0^{1/2}$ denotes the unperturbed root-mean-square end-to-end distance.

α depends on the factor $(0.5-\chi_s)(1-\Theta/T)$, measuring the intensity of the thermodynamic interactions and also representing the power of the solvent, i.e. the higher is this factor, the better is the solvent. The coefficient C_M seems to be less dependent on temperature, however, the parameter C_M involves the unperturbed dimension end-to-end distance, $<r^2>_0^{1/2}$, which depends on temperature through the effective bond character of the chain. Consequently, C_M is indirectly governed by temperature through $<r^2>_0^{1/2}$ term present in Eq. (51). So, the temperature dependence of α is much more governed by this factor, whereas temperature dependence of C_M is not negligible. The decrease of alpha with increasing temperature is for different molecular weights of sample. Considering the structure of gelatin, it is strongly expected that hydrogen bonding will form between polymer segments, and obviously these bonds will break at increased temperature and hydrophobic interactions between polymer segments will be more dominant and can be related to hydrodynamic systems. Hydration, hydrogen bonding/molecular association between polymer segments and water molecules are destroyed with an increment of temperature for water-soluble polymers. It is observed that the strong interaction between polymer segments and solvent molecules through hydrogen bonding will form for gelatin/water system. Therefore, χ_H must be negative. Experimental χ values seem to be higher than 0.5 and χ_S values are the only dominant driving force in setting χ numerically to 0.5 which is believed to be the ideal condition of polymer solutions. The relation between χ_H, χ_S and the interaction parameter χ is defined as $\chi - 0.5 = \chi_H - (0.5 - \chi_s)$.

This stipulation of the interaction parameter to be equal to 0.5 at the theta temperature is found to hold with values of χ_H and χ_S equal to $0.5 - \chi < 2.7 \times 10^{-5}$, and this value tends to decrease with increasing temperature. The values of Θ = 308.6 K were found from the temperature dependence of the interaction parameter for gelatin B. Naturally, determination of the correct theta temperature of a chosen polymer/solvent system has a great physic-chemical importance for polymer solutions thermodynamically. It is quite well known that the second virial coefficient can also be evaluated from osmometry and light scattering measurements which consequently exhibits temperature dependence, finally yielding the theta temperature for the system under study. However, the evaluation of second virial

coefficient from Zimm plot is really a time consuming and difficult task although quite reliable (Guner and Kibarer 2001).

Gelatin is easily dissolved in water by heating the solutions at about 40 to 50ºC. Then the gelatin chains are believed to be in the coil conformation. When solutions are cooled below 30ºC a reverse coil→helix transition takes place which can be detected by important modifications of the optical rotation, mainly due to the left-handed helix conformation. The helices have to be stabilized by hydrogen bonds which are perpendicular to their axes. Thus, at very low concentration 10^{-4} to 10^{-3} w/w intramolecular hydrogen bonds are formed preferentially by a back refolding of single chains. A higher concentrations > 1% w/w, can be proved the helix growth indices chain association, and three-dimensional network formation. Models of a chain have been proposed: 1- a conformation coil→helix transition by local association of three different chains (intermolecular bonds) along short helical sequences; 2- a crystallization mechanism leading to fiber growth, similar to fringe micelle model of synthetic polymer crystallization. The diameter of fiber would depend on temperature and concentration.

Eysturskard et al. (2009), found that the gelation temperature and helix content are very affected (increase) by increasing molecular weight above 250,000 g/mol. This phenomenon is found in the present work, which accounts for a sol-gel transition different from those found by the team of Djabourov (1988a, 1988b, 1991, 1995, 2007). In this work this phenomenon is awarded to the high molecular weight gelatin B studies; also this biopolymer has high numbers Simha and Perrin, which realizes that this macromolecule is related to the rod-like shape than the random-coil shape.

Olivares et al. (2006), studies performed viscometers very dilute gelatin solutions with concentrations between 10^{-5} and 10^{-3} g/cm^3, where either intermolecular aggregation or intramolecular folding are possible, respectively, and the sol–gel transition is not observed.

Djabourov (1988a, 1988b, 1991, 1995, 2007) proposes a T_g of 26-30°C or below 30ºC in this study we found a T_g of about 30°C.

In this work, the linear relation between viscosity and temperature, where the values obtained in the temperature range of 20-29ºC is E_{avf} 8,556.62 cal/mol, and A_{vf} $8.59x10^{-9}$ g/cm s, with σ^2 0.9983, and range of 31-37.4ºC is E_{avf} 5,649.81 cal/mol, and A_{vf} $7.55x10^{-7}$ g/cm s, with σ^2 0.9971. This occurs due to the higher resistance to flow of biopolymers requiring, therefore, more energy in gel state. The increment of activation energy of viscous flow ($\Delta E_{avf} = E_{avf} - E_{avf0}$) occurs due to the higher resistance to flow of biopolymer respect to solvent; where ΔE_{avf} is 4,454.2 and 1,547.4cal/mol, respectively.

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 (Kasaii 2007, Chen and Tsaih 1998). The chain flexibility parameter in the temperature range of 20-29ºC is $d\ln[\eta]/dT$ = 4,404.11K^{-1}, σ^2 0.9993; and in the range of 31-37.4ºC is 2,987.89, σ^2 0.9845. This phenomenon indicates that the chain flexibility, for this gelatin molecular weight (333,000 g/mol) is rigid for temperature range of 20-29ºC and flexible for range of 31-37.4ºC (figure 4).

Figure 4 shows that the intrinsic viscosity is influenced by temperature for gelatin. Where the influence of temperature is manifested in a phase transition at 30°C, presented as a

change of slopes given between 20-29ºC and another between 31-37.4ºC, with gelation temperature, T_{gel} of 30°C. This phenomenon is repeated by looking at other hydrodynamic properties as seen in the change in hydrodynamic radius as in Figure 4. The hydrodynamic radius and intrinsic viscosity for proteins decrease with increasing temperature (Bohidar for gelatin 1998, and Monkos for serum proteins 1996, 1997, 1999, 2000, 2004 and 2005).

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.

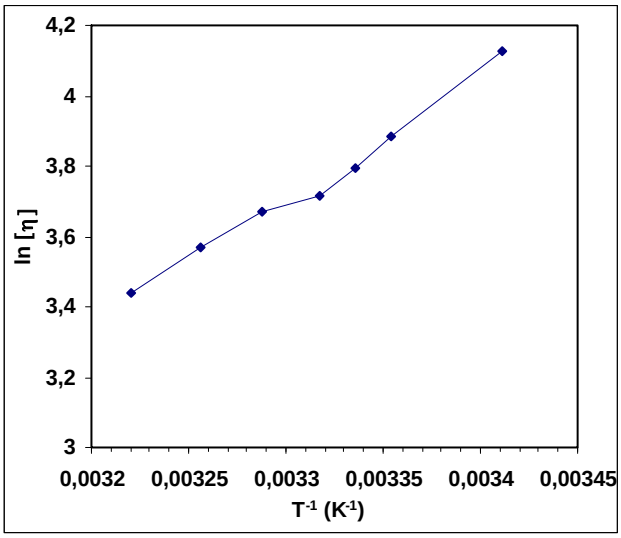


Fig. 4. Ln [η] i function of 1/T.

Analyzing the values of the hydrodynamic properties of gelatin in aqueous solution shows that all values vary with the temperature (table 4). β values increases from 2.24 to 2.83×10^6 with temperature increase, indicating a coil structure. The values of ϕ_0 and P_0 decreases from 12.70 to $6.37 \times 10^{21} mol^{-1}$ and 8.88 to 5.56 demonstrating a low flexibility of the colloid. The value of p decreases from 6.70 to 5.28, and $\nu_{(a/b)}$ with 14.6 which confirms that gelatin in aqueous solution is a biopolymer with a rod-like conformation with tendency to compaction with increasing temperature (R_H decreases). The value of δ as expected decreases from 4.07 to 1.50g/g with increases of temperature, this phenomenon is due to loss of water due to compression of gelatin by the effect of increasing temperature (Durand 2007 and Morris et al. 2009).

The parameters of Mark-Houwink for biopolymers may be varied with solvent and temperature (Chen et al. 2009, Chen & Tsai 1998). This is because the macromolecule changes hydrodynamic radius with type solution and temperature via change in their chain

flexibility. In a good solvent and sol phase, 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 ($\Delta E_{avf}>$).

T (°C)	[η] (cm^3/g)	R_H (cm)	δ (g/g)	β	ϕ_0 (mol^{-1})	$p = f/f_{hyd}$	P_0
20.00	62.12	3.25E-06	4.07	3.40E+06	1.27E+22	6.70	8.88
25.00	48.65	3.00E-06	3.04	3.56E+06	9.95E+21	6.39	7.81
26.60	44.46	2.91E-06	2.72	3.82E+06	9.09E+21	5.95	7.05
28.30	41.15	2.83E-06	2.45	3.94E+06	8.42E+21	5.78	6.68
31.00	39.28	2.79E-06	2.25	4.00E+06	8.03E+21	5.68	6.47
34.00	35.53	2.70E-06	1.91	4.21E+06	7.27E+21	5.41	5.95
37.40	31.12	2.58E-06	1.50	4.31E+06	6.37E+21	5.28	5.56

Table 4. Gelatin B hydrodynamic parameters, measurement to different temperatures.

Mark-Houwink values confirm that for these conditions gelatin is behaves rod-like conformation. Such of empirical equations can be 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.

4. Conclusions

The temperature influence is manifested in a phase transition about at 30°C (gelation temperature) presented as a change of slopes given between 20-29°C and another between 31-37.4°C. The ΔE_{avf} and chain flexibility parameter values obtained in the gel range temperature of 20-29°C is slightly less than twice the sol range of 31-37.4°C. This phenomenon indicates that the chain flexibility is rigid and low flows for temperature range of 20-29°C, and is flexible and more fluid for range of 31-37.4°C.

The Mark-Houwink parameters are influenced by temperature. The numerical value of a indicates that gelatin acquire a shape of a rod-like in aqueous solution with temperature increases; and k demonstrates that under water their value increases with temperature.

Due to the lack of data on the uniformity of intrinsic viscosity measurements in the gelatin/water system, clearly shows a decrease in "a" with temperature, and this M_w is 333,000g/mol.

Gelatin 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 gelatin/water system to show that the biopolymer tends to compaction (decreasing in R_H and $[\eta]$), which requires an increase of energy consumption due to a difficulty in flowing (increase in D and high ΔE_{avf}). This phenomenon is observed in the case of ideal solvents, evidencing a decrease of a with temperature.

The effect of pH on the intrinsic viscosity testing gives a minimum at the isoelectric point at pH 5.1 for gelatin B to pH 9.1 for gelatin A. from electroviscous effect analysis shows that 0.001 M ionic strength the hydrodynamic radius is at its maximum.

5. Acknowledgment

The author thanks Universidad Nacional de San Luis, FONCyT, and CONICET for their contribution. And thanks Dr. R. Curvale, Dr. J. Marchese (CONICET) and Dr. Ariel Ochoa (CONICET) for their help.

6. References

Antonietti M., Briel A., Fosrster S. Quantitative Description of the Intrinsic Viscosity of Branched. Polyelectrolytes. Macromolecules 1997, 30, 2700-2704.

Benson J.E., Viscometric detrmination of isoelectric point of a protein. J. Chem. Educ. 40 (1969) 468-469).

Boedtker H., Doty P. 1954. A study of gelatin molecules aggregates and gels. Journal of American Chemical Society 58, 668-983.

Bohidar H.B. 1998. Hydrodynamic properties of gelatin in dilute solutions. International Journal of Biological Macromolecules 23, 1-6.

Bohidar H. B., Maity S., Saxena A., Jena S. Dielectric behaviour of Gelatin solutions and Gels. Journal Colloid & Polymer Science 276, 1 (1998) 81-86.

Borchard W., Colfen H., Kisters D., Straatmann A. 2002. Evidence for phase transitions of aqueous gelatin gels in a centrifugal field. Progress in Colloid Polymers Science 119, 101-112.

Chatterjee S., Bohidar H.B. 2005. Effect of cationic size on gelation temperature and properties of gelatin hydrogels. International Journal of Biological Macromolecules 35, 81-88.

Chee K.K. A critical evaluation of the single-point determination of intrinsic viscosity. Journal of Applied Polymer Science 34, 3 (1987) 891-899.

Chen R.H., Tsaih M.L. 1998. Effect of temperature on the intrinsic viscosity and conformation of chitosans in dilute HCl solution. International Journal of Biological Macromolecules 23, 135-141.

Chen R.H., Chen W.Y., Wang S.T., Hsu C.H., Tsai M.L. 2009. Changes in the Mark-Houwink hydrodynamic volume of chitosan molecules in solutions of different organic acids, at different temperatures and ionic strengths. Carbohydrate Polymers 78, 902-907.

Curvale, R.A., Cesco, J.C. 2009. Intrinsic viscosity determination by "single-point" and "double-point" equations. Applied Rheology 19, 5, 53347.

Curvale, R.; Masuelli, M.; Perez Padilla, A. 2008. Intrinsic viscosity of bovine serum albumin conformers. International Journal of Biological Macromolecules 42, 133-137.

Deb P.C., Chatterjee S.R. On polynomial expansion of log relative viscosity. Die Makromolekulare Chemie 125, 1 (1969) 283-285.

Deb P.C., Chatterjee S.R. Unperturbed dimension of polymer molecules from viscosity measurements. Die Makromolekulare Chemie 120, 1 (1968) 49-57.

Djabourov M., Leblond J., Papon P. Gelation of aqueos gelatin solutions. I. Structural investigation. J. Phys. France 49 (1988a) 319-332.

Djabourov M., Leblond J., Papon P. Gelation of aqueos gelatin solutions. II. Rheology of the sol-gel transition. J. Phys. France 49 (1988b) 333-343.

Djabourov M. Gelation- A review. Polymer International 25 (1991) 135-143.

Djabourov M., Grillon Y., Leblond J. The sol-gel Transition in gelatin viewed by Diffusing colloidal probes. Polymer Gels and Networks 3 (1995) 407-428.

Domenek S., Petit E., Ducept F., Mezdoura S., Brambati N., Ridoux C., Guedj S., Michon C. 2008. Influence of concentration and ionic strength on the adsorption kinetics of gelatin at the air/water interface. Colloids and Surfaces A: Physicochem. Eng. Aspects 331, 48-55.

Durand, A. 2007. Aqueous solutions of amphiphilic polysaccharides: Concentration and temperature effect on viscosity. European Polymer Journal 43, 1744-1753.

Elharfaoui N., Djabourov M., Babel W.. Molecular weight influence on gelatin gels : structure, enthalpy and rheology. Macromolecular Symposia 256 (2007) 149-157.

Eysturskiard J., Haug I., Elharfaoui N., Djabourov M., Draget K. Structural and mechanical properties of fish gelatin as function of extraction conditions. Food Hydrocolloids 23 (2009) 1702-1711.

García de la Torre J., Carrasco B. 1999. Universal size-independent quantities for the conformational characterization of rigid and flexible macromolecules. Progress in Colloid Polymers Science 113, 81-86.

García-Salinas M. J., Romero-Cano M. S., de las Nieves F. J.. Zeta potential study of a polystyrene latex with variable surface charge: influence on the electroviscous coefficient. Progr Colloid Polym Sci (2000) 115 : 112-116.

Gomez-Guillen M.C., Perez-Mateos M., Gomez-Estaca J., Lopez-Caballero E., Gimenez B., Montero P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. Trends in Food Science & Technology 20, 3-16.

Gomez-Guillen M.C., Turnay J., Fernandez-Diaz M.D., Ulmo N., Lizarde M.A., Montero P. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloids 16, 25-34.

Gouinlock E. V., Flory P.J., Scheraga H.A.. Molecular configuration of gelatin. Journal of Polymer Science 16 (1955) 383-395.

Guner A. 1999. Unperturbed dimensions and theta temperature of dextran in aqueous solutions. Journal of Applied Polymer Science 72, 871-876.

Guner, A., Kibarer, G. 2001. The important role of thermodynamic interaction parameter in the determination of theta temperature, dextran/water system. European Polymer Journal, 37, 619-622.

Guner A. and Kara M. Cloud points and Θ temperatures of aqueous poly(N-vinyl-2-pyrrolidone) solutions in the presence of denaturing agents. Polymer 39, 8 9, 1569-1572, 1998.

Hunter R. Zeta potential in colloid science. Academic Press 1981.

Harding, S.E.; Day, K.; Dham, R.; Lowe, P.M. 1997a. Further observations on the size, shape and hydration of kappa-carrageenan in dilute solution. Carbohydrate Polymers 32, 81-87.

Harding, Stephen E. 1997b. The Viscosity Intrinsic of Biological Macromolecules. Progress in Measurement, Interpretation and Application to Structure in Dilute Solution. Progress in Biophysical Molecules Biological 68, 207-262.

Harrington, W.F. & von Hippel P.H. The stricture of collagen and gelatin. Advances in Protein Chemistry, 16 (1962) 1-138.

Haug I.J., Draget K.I., Smidsrød O. 2004. Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocolloids 18, 203-213.

Haurowitz F., The Chemistry and Functions of Proteins, Academic Press, 1963.

Houwink, R., Zusammenhang zwischen viscosimetrisch und osmotisch bestimm- ten polymerisationsgraden bei hochpolymeren. J. Prakt. Chem., 157 (1940) 15.

Huggins M.L. The Viscosity of Dilute Solutions of Long-Chain Molecules. IV. Dependence on Concentration. J. Am. Chem. Soc., 64, 11 (1942) 2716–2718.

Kasaai M.R. 2007. Calculation of Mark-Houwink-Sakurada (MHS) equation viscometric constants for chitosan in any solvent-temperature system using experimental reported viscometric constants data. Carbohydrate Polymers 68, 477-488.

Karim A.A., Bhat R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids 23, 563-576.

Karim A.A., Bhat R. 2008. Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends in Food Science & Technology 19, 644-656.

Kaur M., Jumel K., Hardie K.R., Hardman A., Meadows J., Melia C.D. 2002. Determining the molar mass of a plasma substitute succinylated gelatin by size exclusion chromatography-multi-angle laser light scattering, sedimentation equilibrium and conventional size exclusion chromatography. Journal of Chromatography A 957, 139-148.

Kenchington A. W. & Ward A.G. 1954, The titration curve of gelatin. J. Biochemistry 58 (1954) 202-207.

Kraemer, E. O., Molecular Weights of Celluloses and Cellulose Derivates. Ind. Eng. Chem. 30, (1938) 1200-1204.

Kuwahara N. On the polymer–solvent interaction in polymer solutions. Journal of Polymer Science Part A.1, 7, (1963) 2395-2406.

Li Jiang, Dahong Yang, and Shing Bor Chen. Electroviscous Effects of Dilute Sodium Poly(styrenesulfonate) Solutions in Simple Shear Flow. Macromolecules 2001, 34, 3730-3735.

López Martínez, M.C.; Díaz Baños, F.G.; Ortega Retuerta, A.; García de la Torre, J. 2003. Multiple Linear Least-Squares Fits with a Common Intercept: Determination of the Intrinsic Viscosity of Macromolecules in Solution. Journal of Chemical Education 80(9), 1036-1038.

Lyklema J. Fuedamentals of inteface and colloid science II, Academic Press 1995.

Mark, H. in Der feste Körper (ed. Sänger, R.), 65–104 (Hirzel, Leipzig, 1938).

Maron, Samuel H.. Determination of intrinsic viscosity from one-point measurements. Journal of Applied Polymer Science 5, 15 (1961) 282-284.

Martin, A. F. Abstr. 103rd Am. Chem. Soc. Meeting, p. 1-c ACS (1942).

Masuelli, Martin Alberto. Viscometric study of pectin. Effect of temperature on the hydrodynamic properties., International Journal of Biological Macromolecules 48 (2011) 286-291.

Matsuoka S., Cowman M.K. 2002. Equation of state for polymer solution. Polymer 43, 3447-3453.

Meyer M., Morgenstern B. 2003. Characterization of Gelatine and Acid Soluble Collagen by Size Exclusion Chromatography Coupled with Multi Angle Light Scattering (SEC-MALS). Biomacromolecules 4, 1727-1732.

Monkos K. 1996. Viscosity of bovine serum albumin aqueous solutions as a function of temperature and concentration. International Journal of Biological Macromolecules 18, 61-68.

Monkos, Karol 1997. Concentration and temperature dependence of viscosity in lysozyme aqueous solutions. Biochimica et Biophysica Acta 1339, 304-310.

Monkos K., Turczynski B. 1999. A comparative study on viscosity of human, bovine and pig IgG immunoglobulins in aqueous solutions. International Journal of Biological Macromolecules 26, 155-159.

Monkos, Karol 2000. Viscosity analysis of the temperature dependence of the solution conformation of ovalbumin. Biophysical Chemistry 85, 7-16.

Monkos, Karol 2004. On the hydrodynamics and temperature dependence of the solution conformation of human serum albumin from viscometry approach. Biochimica et Biophysica Acta 1700, 27-34.

Monkos, Karol 2005. A comparison of solution conformation and hydrodynamic properties of equine, porcine and rabbit serum albumin using viscometric measurements. Biochimica et Biophysica Acta 1748, 100-109.

Morris G.A., Patel T.R., Picout D.R., Ross-Murphy S.B., Ortega A., Garcia de la Torre J., Harding S.E. 2008. Global hydrodynamic analysis of the molecular flexibility of galactomannans. Carbohydrate Polymers 72, 356-360.

Morris, G. A.; Castile, J.; Smith, A.; Adams, G.G.; Harding, S.E. 2009. Macromolecular conformation of chitosan in dilute solution: A new global hydrodynamic approach. Carbohydrate Polymers 76, 616-621.

Nishihara T., Doty P. 1958. The Sonic Fragmentation of Collagen Macromolecules. Proceeding National Academic Sciences 44, 411-417.

Olivares M.L., Peirotti M.B., Deiber J.A. 2006. Analysis of gelatin chain aggregation in dilute aqueous solutions through viscosity data. Food Hydrocolloids 20, 1039-1049.

Ortega, A.; García de la Torre, J. 2007. Equivalent Radii and Ratios of Radii from Solution Properties as Indicators of Macromolecular Conformation, Shape, and Flexibility. Biomacromolecules 8, 2464-2475.

Ohshima H. Primary Electroviscous Effect in a Dilute Suspension of Soft Particles. Langmuir 2008, 24, 6453-6461.

Overbeek J. Th. G. Polyelectrolytes, Past, Present and Future. Pure & Appi Chem., 46 (1976) 91-101.

Palit S.R., Kar I. Polynomial expansion of log relative viscosity and its application to polymer solutions. Journal of Polymer Science Part A-1, 5, 10 (1967) 2629-2636.

Pouradier, J. & Venet, M. (1952). Contribution a l'etude de la structure des gélatines V. – Dégradation de la gélatine en solution isoélectrique, Journal de Chimie Physique et de Physico-Chimie Biologique 49, 85-91.

Ram Mohan Rao M. V., Yaseen M. Determination of intrinsic viscosity by single specific viscosity measurement. Journal of Applied Polymer Science 31, 8 (1986) 2501-2508.

Rubio-Hernandez F.J., Carrique F., Ruiz-Reina E. The primary electroviscous effect in colloidal suspensions. Advances in Colloid and Interface Science 107 (2004) 51–60.

Rubio-Hernandez F. J., Ruiz-Reina E., and Gomez-Merino A. I. Primary Electroviscous Effect with a Dynamic Stern Layer: Low κa Results. Journal of Colloid and Interface Science 226, 180–184 (2000).

Rubio-Hernandez F.J., Gomez-Merino A.I., Ruiz-Reina E., Carnero-Ruiz C. The primary electroviscous effect of polystyrene latexes. Colloids and Surfaces A: Physicochemical and Engineering Aspects 140 (1998) 295–298.

Saxena A., Sachin K., Bohidar H.B., Verma A.K. 2005. Effect of molecular weight heterogeneity on drug encapsulation efficiency of gelatin nano-particles. Colloids and Surfaces B: Biointerfaces 45, 42-48.

Scheraga, H. A., and Mandelkern L. Consideration of the Hydrodynamic Properties of Proteins. J. Am. Chem. Soc. 75 (1953) 179-184.

Schulz, G. V. and Blaschke, F. (1941) Eine Gleichung zur Berechnung der Viskositatszahl fur sehr kleine Konzentrationen. J. Prakt. Chem., 158 (1941) 130-135.

Simha, R. The Influence of Brownian Movement on the Viscosity of Solutions. J. Phys. Chem. 44, (1940) 25-34.

Solomon, O. F.; Ciută, I.Z. 1962. Détermination de la viscosité intrinsèque de solutions de polymères par une simple détermination de la viscosité. Journal of Applied Polymer Science 6, 683-686.

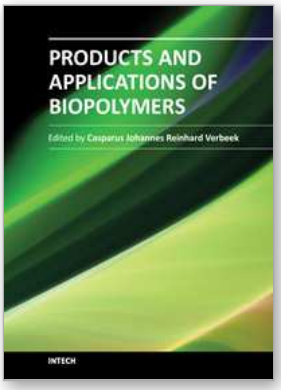
Staudinger, H., Die hochmolekularen organischen Verbindungen. Berlin: Julius Springer, 1932.

Sun S.F. Physical chemistry of Macromolecules. John Wiley & Sons, 2004.

Tanford, Charles. The electrostatic free energy of globular protein ions in aqueous salt solution. Journal of Physical Chemistry 59 (1955) 788-793.

Teraoka, Iwao. Polymer Solutions: An Introduction to Physical Properties. John Wiley & Sons, 2002.

van Holde K.E. Physical Biochemistry, Foundations of Modern Biochemitry Series, Prentice-Hall, 1971.
Zhao W. B. Gelatin. Polymer Data Handbook. Oxford University Press, 1999.



Products and Applications of Biopolymers

Edited by Dr. Johan Verbeek

ISBN 978-953-51-0226-7
Hard cover, 220 pages
Publisher InTech
Published online 07, March, 2012
Published in print edition March, 2012

It is interesting to consider that biopolymers are by no means new to this world. It is only because of our fascination with petrochemical products that these wonderful materials have been neglected for so long. Today we face a different challenge. Environmental pressure is pushing away from synthetic or petro-chemically derived products, while economic factors are pulling back from often more expensive "green" options. This book presents two aspects of biopolymers; potential products and some applications of biopolymers covering the current relevance of biopolymers.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Martin Alberto Masuelli and Maria Gabriela Sansone (2012). Hydrodynamic Properties of Gelatin - Studies from Intrinsic Viscosity Measurements, Products and Applications of Biopolymers, Dr. Johan Verbeek (Ed.), ISBN: 978-953-51-0226-7, InTech, Available from: http://www.intechopen.com/books/products-and-applications-of-biopolymers/hydrodynamic-properties-of-gelatin-studies-from-intrinsic-viscosity-measurements

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.