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Intrinsic viscosity calculated out of single point measurements for chondroitin-4-sulfate and chondroitin-6-sulfate solutions

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Abstract

The differences in the structure of polymer chain between the chondroitin-4-sulfate (C4-S) and the chondroitin-6-sulfate (C6-S) are reflected in the intrinsic viscosity values calculated starting from the traditional methods of Huggins, Kraemer, Mead and Fouss, and Martin, and by the method for a single-point determination of intrinsic viscosity $[\eta]$. For the range between 0.4 and 0.5% (w/v) concentrations, we get an agreement in the intrinsic viscosity values. The chains of both polymers present a flexible structure and are not ramified between 0.28 and 1.00% (w/v) concentrations.

Keywords: Chondroitin-4-sulfate; Chondroitin-6-sulfate; Intrinsic viscosity; Characteristic concentration

1. Introduction

Chondroitin-4-sulfate (C4-S) and Chondroitin-6-sulfate (C6-S), together with the hyaluronic acid, are the only glycosaminoglycans that present steric arrangements among donor and acceptor groups that they allow to establish intramolecular hydrogen bonds between the backbone amide nitrogens and carbony oxygens [1].

In both, the primary structure is a polysaccharide of linear chain, whose repetitive unit is formed by the glucuronic acid and the 2-acetamido-2-deoxygalactosamine with the ester group for each residual of disaccharide in the positions four or six of the ring of galactose. The chondroitin-4-sulfate present this group in axial position and the chondroitin-6-sulfate in equatorial position [1–5]. The secondary structure is established by three hydrogen bonding in those that involve the acetamido group: the residuals of sugar, the uronate and the hexosamine ring oxygen atom [5].

As a result of these intramolecular bonds, the chains of chondroitin sulfate present a certain rigidity that is related with the physiological properties of these substances, as the lubricant function of the sheaths of the collagen fibrils and the length of these fibrils [6].

The structure of the macromolecule conditions and the properties in aqueous solution can be characterized by the versatile conformation in the function of pH, ionic strength and the presence of cations

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[7,8]. The behaviour of all the glycosaminoglycans is the same in relation to the aggregation, hydratation and stability. Although the literature about chondroitin sulfate is very scarce, there are certain differences: for example, the different capacity of bonding to ions and proteins [2]. So, the interaction with the collagen fibrils will have the following order: chondroitin-6-sulfate > dermatan sulfate > hyaluronic acid > keratan sulfate > chondroitin-4-sulfate [9].

The difference of structure in the polymer chain between the chondroitin-4-sulfate and chondroitin-6-sulfate should be reflected in the values of intrinsic viscosity of solutions, since this parameter depends on the dimensions of the polymer chain and polymer–solvent interactions. For determination of intrinsic viscosity, one could utilize the traditional Huggins' equation [10], Kraemer's equation [11], Mead and Fouss' equation [12] and Martin's equation [13]. Another more practical method is a single-point determination of intrinsic viscosity [14,15]. The limitation of this method is in that these expressions are valid for specific viscosity values lower than the unit ($\eta_{sp} < 1$). The most remarkable feature of equations for a single point determination is that they contain no adjustable parameter and give directly the value of $[\eta]$ from a single determination of relative viscosity.

The objective of this work is to evaluate the intrinsic viscosity $[\eta]$ of chondroitin-4-sulfate and chondroitin-6-sulfate solutions in relation to the dimension of polymer chain and the interactions of the same with the medium starting from a comparative study of several traditional procedures of intrinsic viscosity $[\eta]$ calculation with the method of a single-point measurements.

2. Materials and methods

Chondroitin-4-sulfate, sodium salt, from whale cartilage (lot 104 F-0549) was obtained from Sigma. Chondroitin-6-sulfate, sodium salt, from shark cartilage (lot 102 F-0418) was obtained from Sigma.

The solutions to concentrations between 0.28% (w/v) and 1% (w/v), were prepared with phosphate buffer (pH = 7.3) and ionic strength of 0.15, utilizing reagents of Merck.

Density was measured using a PAAR DMA 60 densitometer, with constants $A = 28.52$ and $B = 28.36$ at 25°C and 37°C.

The viscosity measurements were determined in a Fica MS viscomatic with an Ubbelhod viscometer of constants $B = 1.7$ and $C = 0.01$ at 25°C and 37°C.

From these kinematic viscosity and density measurements for the different solutions, the relative viscosity measurements were obtained.

The calculation of the intrinsic viscosity $[\eta]$ was made by the expressions ofHuggins [10]

$$\frac{\eta_{sp}}{c} = [\eta] + K'[\eta]^2 c + \dots \quad (1)$$

where η_{sp} is the specific viscosity and the constant K' depends on the interactions solute–solvent and it can indicate us the degree of crosslinking of the polymer;Kraemer, Mead and Fours [11,12]:

$$\frac{1}{c} \ln \eta_{rel} = [\eta] - K''[\eta]^2 c + \dots \quad (2)$$

where η_{rel} is the relative viscosity and K'' is related with the constant of Huggins through equation $K' = 0.5 + K'$;Martin [13]:

$$\ln \frac{\eta_{sp}}{c} = \ln [\eta] + K[\eta]c \quad (3)$$

this equation gives values of $[\eta]$ higher than Eqs. (1) and (2).

Solomon and Ciuta [14] have proposed an equation for a single-point determination of intrinsic viscosity $[\eta]$:

$$[\eta]c = \sqrt{2\eta_{sp} - 2\ln \eta_{rel}} \quad (4)$$

Deb and Chatterje [14] have derived another equation:

$$[\eta]c = \sqrt[3]{3\ln \eta_{rel} - 3\eta_{sp} + \frac{3}{2}\eta_{sp}^2} \quad (5)$$

and Palit and Kar [14] leading to the equation:

$$[\eta]c = \sqrt[4]{4\eta_{sp} - 2\eta_{sp}^2 + \frac{4}{3}\eta_{sp}^3 - 4\ln \eta_{rel}} \quad (6)$$

These equations are deriving Huggins' relation (Eq. (1)), in which only the first term involving K' of the quadratic, cubic, or biquadratic expansion was accepted and the others were ignored. This is permis-

Table 1
Values of density (ρ) (g ml^{-1}), kinematic viscosity (μ) (mm^2/s) and relative viscosity (η_{rel}) calculated for chondroitin-4-sulfate and chondroitin-6-sulfate solutions at two temperatures

c% (w/v)	T (°C)	Chondroitin-4-sulfate			Chondroitin-6-sulfate		
		ρ	μ	η_{rel}	ρ	μ	η_{rel}
1	25	1.009	12.720	1.497	1.008	18.830	2.205
	37	1.002	9.602	1.434	1.002	14.050	2.100
0.66	25	1.008	11.690	1.326	1.008	15.080	1.764
	37	1.000	8.623	1.286	1.001	11.360	1.690
0.5	25	1.007	11.340	1.244	1.006	13.340	1.559
	37	0.999	8.162	1.216	0.999	10.000	1.491
0.4	25	1.007	10.288	1.195	1.006	12.560	1.467
	37	0.999	7.880	1.174	0.999	9.250	1.379
0.33	25	1.007	9.930	1.160	1.006	11.680	1.364
	37	0.999	7.720	1.150	0.999	8.820	1.315
0.285	25	1.006	9.704	1.138	1.006	11.220	1.310
	37	0.999	7.550	1.123	0.999	8.506	1.311

sible only if they are negligible compared to the first term. It appears that they can include more and more terms to get better and better agreement with experiment.

The characteristic concentration values or concentration of 'coil overlap' has been calculated starting from the representations $\log \eta_{rel} = f(\log c)$ and $\eta_{sp}/c^2 = f(c)$.

3. Results and discussion

The values of density ρ, kinematic viscosity μ and relative viscosity η_{rel} are given in Table 1.

The intrinsic viscosity values were obtained by linear extrapolation at zero concentration of Huggins' Eq. (1), Kraemer's Eq. (2) and Martin's Eq. (3). The three equations are linear functions and the slope of these straight line corresponds to the value of the respective interaction constants (Table 2).

The values of $[\eta]$ calculated by the traditional procedures are practically identical with the three methods, and they are similar to those obtained by Mathews [16] who calculated for several fractions of chondroitin sulfate, values of intrinsic viscosity between 0.37 and 0.72.

The values of $[\eta]$ for chondroitin-4-sulfate solutions are lower than those of the isomeric chondroitin-6-sulfate, which could indicate a greater ease in order to form aggregates in this molecule. It may be due to the fact that the sulfate group in position 6 of the galactose ring presents a less steric impediment than the sulfate group in the position 4 of the same ring. Some authors [12] relate this fact with the difference of solubility that presents both molecules, corresponding to the lower solubility for the molecule of greater viscosity. The constants of Huggins and of Kraemer are small values, less than 0.3, which would indicate that the macromolecule presents a flexible and extended conformation as it corresponds to a good solvent.

The formation of intermolecular and intramolecular bonds is facilitated for the chondroitin-6-sulfate with increased values of the constants of Huggins and Kraemer because this molecule has a less steric impediment, which would also explain that this has a greater intensity of interaction with the collagen fibrils and other substances [9].

Concerning the temperature, we expected to find a greater influence of this parameter. According to Scott and Tigwell [3], at 40°C, a structure type melting that produced a conformational change that affects the dimensions of the chain is produced; therefore, the variation of $[\eta]$ in the temperature function would show this conformational change. However, we did not observe conformational changes

Table 2
Values of the intrinsic viscosity $[\eta]$ (dl g^{-1}) and of constants (K, K' and K'') determined by the methods of Huggins, Kraemer and Martin

	T (°C)	Huggins			Kraemer			Martin		
		$[\eta]$	K'	r	$[\eta]$	K''	r	$[\eta]$	K	r
C4-S	25	0.479	0.019	0.970	0.473	−0.069	0.999	0.479	0.018	0.969
	37	0.431	0.004	0.959	0.426	−0.066	0.997	0.430	0.035	0.959
C6-S	25	1.044	0.162	0.993	1.008	−0.221	0.996	1.048	0.146	0.992
	37	0.867	0.239	0.976	0.861	−0.116	0.961	0.877	0.205	0.974

Table 3
Comparative values of intrinsic viscosity $[\eta]$ (dl g^{-1}) at two temperatures determined by the method of a single-point measurements for chondroitin-4-sulfate (C4-S) and chondroitin-6-sulfate (C6-S)

	$[\eta]$ Solomon and Ciutta			$[\eta]$ Deb and Chatterjée		$[\eta]$ Palit and Kar	
	C% (w/v)	25°C	37°C	25°C	37°C	25°C	37°C
C4-S	0.285	0.769	–	0.778	–	0.782	–
	0.330	0.611	0.861	0.619	0.872	0.623	0.877
	0.400	0.513	0.495	0.468	0.502	0.472	0.506
	0.500	0.432	0.405	0.462	0.412	0.467	0.415
	0.660	0.421	0.394	0.457	0.403	0.463	0.408
	1.000	0.410	0.384	0.448	0.396	0.458	0.403
C6-S	0.285	1.706	1.480	1.706	1.050	1.770	1.529
	0.330	1.312	1.148	1.384	1.176	1.369	1.192
	0.400	1.229	1.021	1.271	1.050	1.297	1.068
	0.500	0.959	0.857	0.997	0.887	1.020	0.906
	0.660	0.941	0.869	0.990	0.911	1.020	0.936
	1.000	0.910	0.846	0.977	0.905	1.020	0.9419

pH = 7.3, $\mu = 0.15$.

at 37°C, and a decrease in $[\eta]$ is only obtained at 25°C.

The values of the intrinsic viscosity calculated by the method of a single-point determination (Eqs. (4)–(6)), are given in Table 3. In relation to these results, in those that the existent literature is practically null, we could detect a dependence of $[\eta]$ with the concentration. In solutions of concentration lower than 0.4% (w/v), the values of intrinsic viscosity calculated do not belong with their habitual form; however, we could consider that the method is adequate to the solutions of concentrations between 0.4 and 0.5% (w/v) for the two chondroitin sulfates utilized.

The determination of the characteristic concentration as a concentration limit of 'coil overlap' has been carried out for the methods suggested by Baloch [17] for flexible molecules (Fig. 1) and for rigid or semirigid chains (Fig. 2). The results indicate that our molecules are adjusted better to the first representation because the hyperbolic form does not present the minimum point that was waiting in the representation of $\eta_{sp}/c^2 = f(c)$.

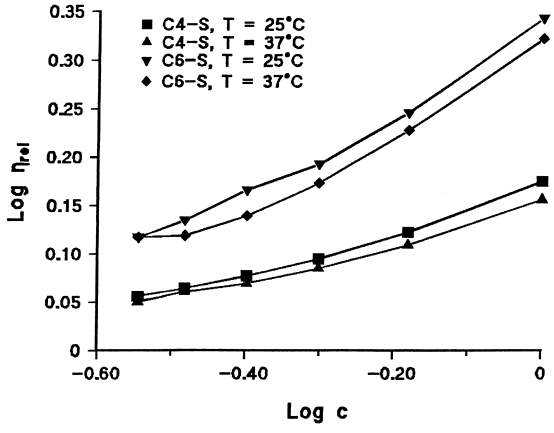

Fig. 1. Log η_{rel} vs. log c to chondroitin-4-sulfate (C4-S) and chondroitin-6-sulfate (C6-S) at 25°C and 37°C.

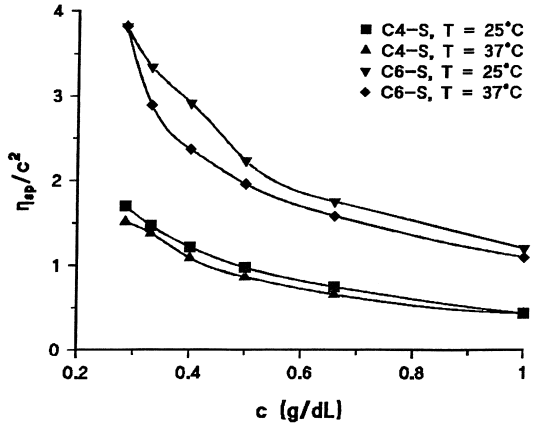

Fig. 2. η_{sp}/c^2 vs. concentration to chondroitin-4-sulfate (C4-S) and chondroitin-6-sulfate (C6-S) at 25°C and 37°C.

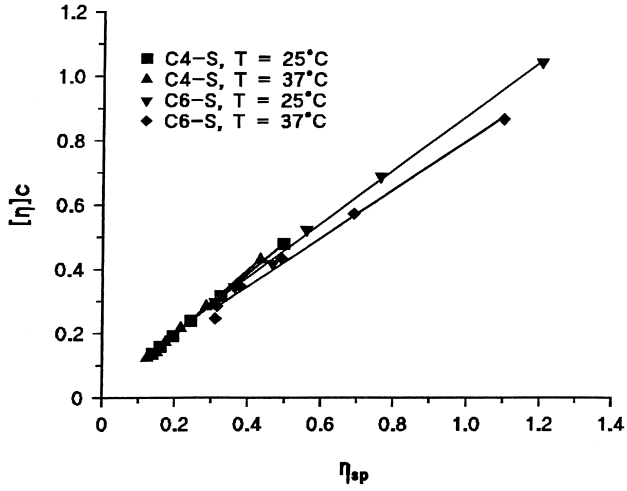

Fig. 3. Specific viscosity dependence of $c[\eta]$ for chondroitin-4-sulfate (C4-S) and chondroitin-6-sulfate (C6-S) at 25°C and 37°C.

Fig. 3 shows the evolution of $[\eta]c$ as a function of the η_{sp}, for the two utilized temperatures so much for the C4-S as for the C6-S. The variation is linear, for the one which the chains in both chondroitin sulfate do not present entanglements between the chains in the range of concentrations utilized.

4. Conclusion

The conformations for the two molecules of chondroitin sulfate correspond to flexible and extended linear chains without evident conformational change by temperature effect. A good agreement exists among the value of the constant of Huggins obtained for these macromolecules and the one obtained by the pattern of flexible molecules of Baloch [17].

Reference to difference of intrinsic viscosity between chondroitin-6-sulfate and chondroitin-4-sulfate is a consequence of aggregate formation in the molecule of chondroitin-6-sulfate.

The difference between the intrinsic viscosity values obtained by the method of a single-point determination and the traditional, are a consequence of a merely algebraic treatment. In our case, the values obtained by Eqs. (4)–(6) are lower than the others given by Eqs. (1)–(3).

The intrinsic viscosity values calculated by the method of single-point determination (Eqs. (4)–(6)), present differences that could be due to the increased sensitivity of Eqs. (5) and (6) as they contain cubic and biquadratic terms; thus, there is a slight increase of results. Our experimental data adjust better to Palit and Kar's equation (Eq. (6)).

We considered that, in spite of the fact that the values of $[\eta]$ starting from Eqs. (4)–(6) are slightly different with concerning those derived by means of the traditional equations. The use of single-point determination is valid for polymers of few ramified flexible chain, and it is advantageous that their experimental determination is very simple.

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