

## On the stiffness of the welan chain

S. P. Campana Filho\*, A. Regiani, E. Frollini

Instituto de Química de São Carlos, Universidade de São Paulo,  
Caixa Postal 780-São Carlos, SP-13560-970-Brasil

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### Summary

Welan, purified as its sodium salt from unpasteurised culture broth, was analyzed by dilute aqueous viscometry. Measurements in water and at low ionic strength were carried out to evaluate the charge effect on intrinsic viscosity. The value of intrinsic viscosity at low ionic strength, determined by the isoionic dilution method, is close to that obtained at higher salt concentrations. A comparison is made among the stiffness parameters determined by different treatment of experimental data. The results agree with previous reports on the weak polyelectrolyte character and high stiffness of the welan chain.

### Introduction

The gellan family of bacterial polysaccharides includes four members, namely, welan, rhamsan, S-657, and S-198 (1-7). The primary structure of the parent polysaccharide is a linear chain composed of tetrasaccharide repeat units (1,2). The same repeat unit is present in the primary structure of the other members also but gellan is the only one which has no side chains. Welan contains a monosaccharide side chain at O-3 of the 4-linked glucopyranosyl residue which can be either  $\alpha$ -L-rhamnose or  $\alpha$ -L-mannose, and the terminal repeat units of welan is reported to be a  $\alpha$ -L-rhamnopyranosyl or  $\alpha$ -L-mannopyranosyl group in a 3:1 proportion (3,4). In the native state gellan contains one acetyl substituent per repeat unit (5,8) and also some glyceryl groups located on O-2 of the 3-linked glucopyranosyl residues (8,9). In welan the acetyl group is present in 80-90% of the repeating units and it is located at the O-3 position of the 3-linked glucopyranosyl residues (10). The strength and stability of gellan gels depend on its degree of acetylation and on the type and concentration of cationic species present in solution (11-14).

Welan is a non-gelling polysaccharide in the native state or in the deacetylated form but its aqueous solutions exhibit high viscosity at low shear rates and good thermal stability (11,15). Recent X-ray studies attribute a threefold double helical structure for welan (16,17), as found for gellan, but in spite of being similar, these ordered conformations exhibit very different stabilities. Native or deacetylated welan adopts a double helix conformation which is much more stable than that of gellan (18-21). No experimental condition has been found to produce the conformational transition to a disordered coil by changing the temperature, pH, and ionic strength of aqueous solutions of welan (19). However, welan undergoes a rapid temperature-induced conformational transition when dissolved in dimethyl sulphoxide (22). The unusual stability of the welan double helix has been attributed to the occurrence of side chain-main chain interactions which are disturbed when dimethyl sulphoxide is the solvent. These intramolecular forces,

\* Corresponding author

van der Waals' interactions or hydrogen bonding involving the side chain and the D-glucuronosyl residue of the main chain of welan, have also been suggested to be responsible for the non-gelling behavior (23,24), the weak polyelectrolyte character (11,18,19) and the unusual low value of  $pK_a$  of this polysaccharide (19). The treatment of Smidsrod (25) has been applied to the intrinsic viscosity data determined at various ionic strengths and the stiffness of the welan chain has been compared to that of DNA (19). A recent study has shown that chain extension as a result of electrostatic repulsions, and the rheological behavior of welan solutions depend on the source and on the purification procedure of the polysaccharide (26). In this paper aqueous solutions of sodium salts of welan samples purified from the unpasteurised culture broth are studied by viscometry, and the observed behavior is discussed from the point of view of the stiffness of the welan chain and of the occurrence of aggregation.

### **Experimental**

Welan was kindly given by Kelco, Division of Merck and Co. (San Diego, CA, USA) as a concentrated (3%) unpasteurised broth. Treatment of diluted broth (1 g/L) with ultrasound radiation and purification as sodium salt, as usual, of the bacterial polysaccharide (27) resulted in the sample studied in this work. Its molecular weight is  $0,37 \times 10^5$  g/mol as determined in a Chromatix KMX-6 low angle laser light scattering apparatus with aqueous solutions of welan at 0,1M NaCl concentration. Viscosity measurements were carried out in Low Shear 30 from Contraves ( $10^{-2} < \dot{\gamma} < 128$  s $^{-1}$ ) or in a Brookfield ( $\dot{\gamma} < 70$  s $^{-1}$ ) always in the Newtonian domain. The welan dissolution in deionized and redistilled water ( $\Omega \cong 1,0$   $\mu$ S / cm) was carried out through stirring for 12 hours, heating at 80°C for 3 minutes, and addition of aqueous salt solution to adjust the ionic strength. In order to avoid the presence of ionic impurities in pure water and low ionic strength solutions, polyethylene bottles were used for dissolution and storage of solutions. Solutions were bubbled with dry N $_2$  to prevent contamination by carbon dioxide. Before viscosity measurements all solutions were filtered through 0,2  $\mu$ m membranes. The isoionic dilution of the welan solutions at initial  $C_{pi}$  concentration was carried out through the addition of aqueous NaCl solutions of concentration  $C_s$ , such that  $X C_{pi} = C_s$ . To carry out the viscosity measurements at higher ionic strengths, it was assumed that the contribution of Na $^+$  ions from the ionization of the polyelectrolyte to the total ionic strength was negligible since  $(C_s)_{ext.} \gg \Phi C_p$  in these cases.

### **Results and Discussion**

Polyelectrolytes show a peculiar behavior as the concentration of their aqueous solutions is decreased. The reduced viscosity of solutions prepared by dissolution of the polyelectrolyte in pure water increases with progressive dilution, reaches a maximum and then it decreases. In solutions containing high salt concentrations the polyelectrolytes recover, at least partially, the behavior of neutral polymers, i.e., the reduced viscosity decreases linearly with decreasing concentration. Due to the presence of D-glucuronosyl residue in its primary structure, welan presents polyelectrolyte properties. A curve showing the behavior of the reduced viscosity of welan in pure water as a function of polymer concentration is displayed in Figure 1. As expected from the above mentioned structural feature, welan displays typical polyelectrolyte behavior with the  $\eta_{red}$  versus polymer concentration plot passing through a maximum at  $C_p \cong 2.5 \times 10^{-4}$  g/mL. To evaluate the charge effect on the viscosity of welan solutions, measurements were carried out by employing the isoionic dilution procedure. In these experiments the initial polymer concentration in pure water was  $5.0 \times 10^{-4}$  g/mL and X was an empirical parameter related to the salt concentration necessary for screening of the electrostatic (Figure 2).

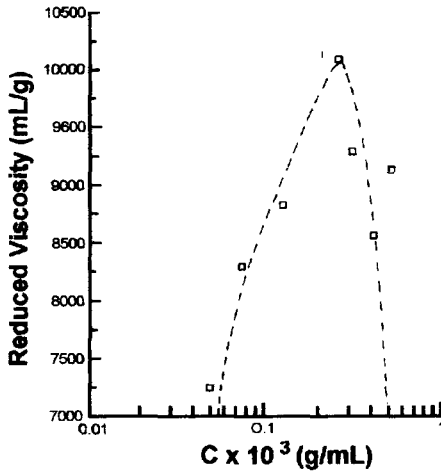


Figure 1: Reduced viscosity of welan solutions in pure water.

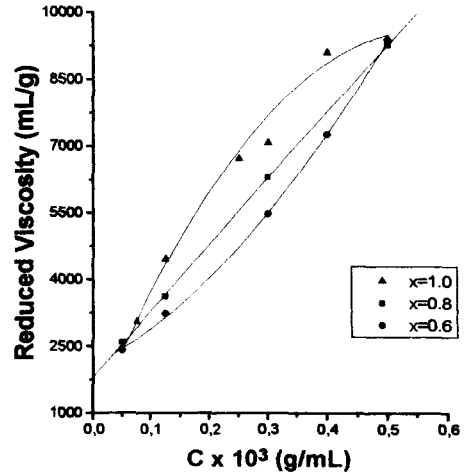


Figure 2: Reduced viscosity of welan in low ionic strength.

As can be seen in Figure 2 a linear correlation of  $\eta_{\text{red}}$  versus  $C_p$  corresponds to  $X = 0.8$ . Convex or concave curvatures are obtained when  $X$  is too low or too high, respectively. The linear dependence corresponds to Huggins' equation and extrapolation of the straight line at  $X = 0.8$  to  $C_p \rightarrow 0$  yields  $|\eta| \cong 1826 \text{ mL/g}$  and  $k_H \cong 4,4$ . The dependence of intrinsic viscosity and  $k_H$  on the ionic strength determined by dilution at constant added salt concentration is shown in Table 1.

Table 1: Intrinsic viscosities and  $k_H$  values of welan as a function of ionic strength.

$\mu$ (mol/L)	$ \eta $ (mL/g)	$k_H$
1,0	1416	0,72
0,3	1464	0,57
0,1	1492	0,66
0,03	1560	0,50
0,01	1572	0,50

The data in Table 1 show that the change in the intrinsic viscosity as a function of the ionic strength is unusually small for a polyelectrolyte. Also the value of  $|\eta|$ , determined by using the isoionic dilution method, is lower than that expected for a polyelectrolyte. For instance, the aqueous solution of the anionic derivative of guar gum changes its intrinsic viscosity from 1275 mL/g to 6000 mL/g when the isoionic dilution is applied (28). A similar behavior, namely a weak polyelectrolyte effect, has been reported by Urbani et al.(29). This has been attributed to the stiffness of the welan chain which is almost as high as that of the DNA chain as estimated by Smidsrod's stiffness parameter(19). This conclusion is supported by Yamakawa-Fuji's approach (30) relative to the stiffness of worm-like chains, which gave an average value of 750nm for the persistence length of the welan chain (31). However, when the treatment of Odjik (32) is applied, the best agreement among the theoretical and experimental values of intrinsic viscosity is found for an intrinsic persistence length of 300nm. Aggregation has been claimed to be the cause of this disagreement. As pointed out recently (26), the presence and the shape of aggregates result in changes in the chain expansion due to electrostatic repulsions. Also, the rheological properties of welan solutions change according to the nature of the sample and to the method of purification. The high values of  $k_H$ , found for samples corresponding to

higher molecular weights, as well as their increase with increasing ionic strength have been taken as further evidences for aggregation in welan solutions (31). On the other hand, the much higher value of  $k_H$  determined by using the isoionic dilution of the welan solution as compared to those shown in Table 1, may correspond to an increase of the effective collision diameter of the swollen polymer (32). In view of two-body collisions, decreasing ionic strength results in swelling of the polymer and in expansion of the electrical double layer. As a consequence more collisions will occur and greater amounts of energy will be dissipated, causing the remarkable increase in  $k_H$ , reported here. In this sense, the parameter  $k_H$  can be taken as a quantitative manifestation of the secondary electroviscous effect (33). Moreover, according to literature reports the Huggins coefficients are not independent of the method of dilution, and their values are usually greater when the isoionic dilution is used (34), as reported in this work.

### Conclusions

Only a small charge effect has been observed in the intrinsic viscosity of welan solutions as its ionic strength increased. Although aggregation has caused a measure of imprecision to the experimental data obtained at high ionic strengths, the three different treatments agreed quite well, in respect to the rigid character of the welan chain. Thus the stiffness of the welan chain was the cause of this unusual behavior for a polyelectrolyte.

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### References

- O'Neill MA, Selvendran RR, Morris JV (1983) *Carbohydr Res* 124: 123.
- Jansson PE, Lindberg B, Sandford PA (1983) *Carbohydr Res* 124: 135.
- Jansson PE, Lindberg B, Widmalm G, Sandford PA (1985) *Carbohydr Res* 139: 217.
- O'Neill MA, Selvendran RR, Morris VJ, Eagles J (1986) *Carbohydr Res* 147: 295.
- Jansson PE, Lindberg B, Lindberg J., Maekawa E, Sandford PA (1986) *Carbohydr Res* 156: 157.
- Jansson PE, Kumar NS, Lindberg B (1985) 156: 165.
- Chowdhury TA, Lindberg B, Lindquist U, Baird J (1987) *Carbohydr Res* 164: 117.
- Kuo MS, Mort AJ (1986) *Carbohydr Res* 156: 173.
- Chandrasekaran R, Radha A, Thailambal VG (1992) *Carbohydr Res* 224: 1.
- Stankowski JD, Zeller SG (1992) *Carbohydr Res* 224: 337.
- Crescenzi V, Dentini M, Dea IMC (1987) *Carbohydr Res* 160: 283.
- Grasdalen H, Smidsrod O (1987) *Carbohydr Polym* 7: 371.
- Tako M, Sakae A, Nakamura S (1989) *Agric Biol Chem* 53(3): 771.
- Shi X (1990) Thesis, Université Joseph Fourier / Grenoble-France.
- Sandford PA, Cotrell IW, Pettitt DJ (1984) *Pure Appl Chem* 56: 879.
- Cairns P, Miles MJ, Morris VJ (1991) *Carbohydr Polym* 14: 367.
- Lee EJ, Chandrasekaran R (1991) *Carbohydr Res* 214: 11.
- Urbani R, Brant DA (1989) *Carbohydr Polym* 11: 169.
- Campana S, Andrade C, Milas M, Rinaudo M (1990) *Int J Biol Macromol* 12: 379.
- Campana S, Ganter J, Milas M, Rinaudo M (1991) *Carbohydr Res* 231: 31.
- Chandrasekaran R, Radha A, Lee EJ (1994) *Carbohydr Res* 252: 183.
- Hember MWN, Richardson RK, Morris ER (1994) *Carbohydr Res* 252: 209.
- Talashak T, Brant DA (1989) *Carbohydr Res* 160: 303.
- Tako M, Kiriaki M (1990) *Agric Biol Chem* 54: 3079.

25. Smidsrod O, Haug A (1971) *Biopolymers* 10: 1213.
26. Lopes L, Milas M, Rinaudo M (1994) *Int J Biol Macromol* 16(5): 253.
27. Rinaudo M, Milas M (1978) *Biopolymers* 17: 2663.
28. Frollini E, Reed WF, Milas M, Rianudo M (1995) *Carbohydr.Polym.* 27: 129.
29. Urbani R, Brandt DA (1989) *Carbohydr.Polym.* 11: 169.
30. Yamakawa H, Fujii M (1974) *Macromolecules* 7: 128.
31. Campana S.(1990) Thesis. Universidade de São Paulo-São Paulo/Brasil.
32. Goring DAI, Rezanowich A (1960) *J.Coll.Sci.* 15: 472.
33. Hodgson DF, Amis EJ (1993) *Polyelectrolytes: Science and Technology.* Marcel Dekker Inc. New York.
34. Davis RM, Russel WB (1987) *Macromolecules* 20: 518.