

Influence of aggregates on the sedimentation properties of welan gum

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Summary

The water-soluble bacterial polysaccharides are used by industry as emulsifying, stabilizing, thickening and suspending agents, and eventually for their gelling properties. It has been observed that several of these properties may be influenced by the presence of disordered intermolecular associations (aggregates or microgels). The influence of aggregates on the sedimentation properties of welan gum were investigated by means of the falling sphere method. The velocity of the particle sedimentation is affected by the type and size of the aggregates.

Introduction

The great interest in the industrial production of microbial polysaccharides is due to their unique functionality, which has important applications mainly in the food, pharmaceutical, agricultural and oil recovery areas.

The search for useful microbial gums has yielded, in the last decade, a new group of bacterial polysaccharides developed by Kelco, Division of Merck Co.Inc., USA. The gellan family consists of gellan, welan, rhamsan, S-198 and S-657. All members of this polysaccharide family have the same acidic tetrasaccharide backbone sequence and, in the native state, O-acetyl groups as substituents (1-6).

Gellan, welan and rhamsan are produced commercially. Welan gum is synthesized by *Alcaligenes* ATCC31555 and is sold under the tradename BIOZAN (formerly known as S-130). Figure 1 shows the chemical structure of welan. Approximately two-thirds of the repeating units contain terminal α -L-rhamnopyranosyl groups, while the remainder has α -L-mannopyranosyl groups (3,7). It has been observed that these monosaccharide residues are randomly distributed on the main-chain (8). X-ray studies demonstrate that welan may adopt the same 3-fold double helical structure (9,10), as it was characterized for gellan (11). The ordered conformation of welan gum is much more stable than that of gellan (12,13), it has been recently reported that using high concentrations of DMSO as solvent, the polymer undergoes a rapid conformational transition to a disordered coil (14).

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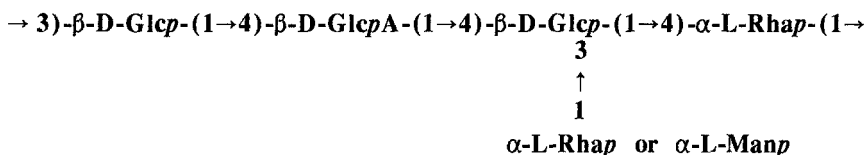


Figure 1. Molecular structure of welan gum.

Despite the structural similarities between gellan, welan and rhamosan (1-4), the aqueous solution properties of these polysaccharides are quite different (15-17). Welan solutions can suspend particles and stabilize emulsions like xanthan gum (15).

Potentiometric and rheological investigations suggested a very weak polyelectrolyte character for welan, in spite of its D-glucuronic acid content (12,16,18). Nevertheless, new rheological studies showed a typical polyelectrolyte behaviour for welan molecules (13). The divergences about solution behaviour of welan and other bacterial glycopolymers have been ascribed to the initial source of the polysaccharide (commercial product or un-pasteurized fermentation broth) and the method of purification employed. Aggregates of different sizes have been reported on gellan gum solutions (19).

In this work, welan commercial products (industrial powder) and samples of this polymer recovered from un-pasteurized culture-broth are analyzed in terms of the presence of aggregates and their influence on the sedimentation properties of welan. Sedimentation properties in distilled water were evaluated by falling sphere experiments. NaOH-Purified products were characterized and the results were compared to those obtained from commercial welan samples.

Experimental

The samples of welan were supplied by Kelco. The purification of welan from the un-pasteurized broth (WM) were accomplished by two different methods. In the first, approximately 30g of the broth (with $\approx 3\%$ polymer) were dissolved in 1000ml of distilled water, filtered through 8 and $3\mu\text{m}$ membranes and precipitated in isopropyl alcohol as Na salt form. The precipitate was then washed in water-isopropyl alcohol mixtures and dried under reduced pressure at 20°C . In the second method, an aqueous NaOH solution was added to 60g of the broth diluted in 1000ml of distilled water, to give a final concentration in NaOH of 0.1M and about 2g/l in welan. After stirring from 3 to 40 minutes at 80°C , the dispersions were neutralised with 1M HCl and centrifuged for 2 hours at 14000rpm. The supernatant was filtered through $0.2\mu\text{m}$ membrane and precipitated, washed and dried as previously described. Different commercial welan samples (A, B, C, D) were submitted to

the same methods of purification. The water content of purified and non-purified polymers were evaluated by thermogravimetry (Setaram G-70 termobalance) and taken into account in all solutions preparations. The water percent varied in the range 14-20%. The complete water elimination should be avoided in order to reduce the risk of irreversible aggregation.

Molecular weight determinations of NaOH-purified welan from the broth were achieved by using a Chromatix KMX-6 LALLS photometer. The value of $\bar{M}_w \approx 1 \times 10^6$ was determined for the samples submitted to the alkaline treatment for 20 and 40 minutes (WM-20 and WM-40). The presence of O-acetyl groups in welan was detected by infrared absorption with a FT-IR 1720X Perkin-Elmer spectrometer. All samples, purified and commercial, show an IR absorption at 1730cm^{-1} . The strong resistance to hydrolysis of the acetyl substituents in welan was verified by the presence of these groups in the NaOH-purified samples.

Viscosity measurements were carried out with a Contraves Low Shear-30 coaxial cylinders rheometer. All measurements were performed at 25°C . Sedimentation properties in distilled water were evaluated through the falling sphere method. The study was done with glass spheres of 4.1mm diameter and mass density $\rho = 2.5 \times 10^3 \text{Kg/m}^3$ suspended in glass graduated tubes of 12mm diameter. The tubes were filled with the welan solutions and then after 1 hour in rest at 25°C , the spheres were placed to fall. After 24 hours, the experiment was repeated in the same tube. The experiments were also performed at 25°C .

Results and Discussion

The filtration of welan water solutions through membranes of controlled pore dimensions was used to verify the presence of aggregates. Table 1 shows the viscosity values of the gum solutions before and after filtration through 8 and $3\mu\text{m}$ membranes. All samples have aggregate forms (20). The polymer reprecipitated from the broth without the NaOH-treatment (WM) and the D sample have aggregates of different sizes, since the viscosity continues decreasing after filtration through $3\mu\text{m}$. It can be observed that aggregates of large volumes may be present in unpasteurized welan fermentation broths. The A and B samples show the same strong reduction of viscosity after filtration through 8 and $3\mu\text{m}$ (>85%), which can be attributed to the presence of great volume aggregates. This kind of microgel seems to be present in C in lower quantity.

In Table 2 the results of the alkaline purification are listed. The viscosity of the solutions, of approximately 2g/l at 1.75s^{-1} and 25°C , was measured before and after filtration through $0.2\mu\text{m}$. The NaOH-treatment showed to be effective in the dissociation of aggregates of welan from the culture-broth. In all cases, the loss of viscosity after filtration through $0.2\mu\text{m}$ is not superior to

≈ 10%. The aggregates of the commercial product seem to be much more stable. The alkaline purification method produced only an improvement in the filtration through 3 μ m membrane (20). Concerning the viscosity of the purified samples from the broth, the values showed in Table 2 should not be compared among themselves, because the concentration of the initial welan solutions is not well determined.

TABLE 1. Viscosity values of 1 g/l welan water solutions at 1.75 s⁻¹ and 25°C after.

WELAN SAMPLE	Viscosity (cP) after filtration through membranes				
	Initial η (cP)	8 μ m pore	Viscosity loss (%) *	3 μ m pore	Viscosity loss (%) *
WM	344.43	213.49	38.0	142.69	58.5
A	107.01	14.88	86.0	13.18	87.6
B	111.52	8.29	92.5	5.09	95.4
C	83.17	66.84	19.6	65.68	21.0
D	78.84	48.33	38.6	41.17	47.7

*referred to the viscosity of the initial solution.

TABLE 2. Viscosity values of ≈ 2g/l alkaline purified welan water solutions, before and after filtration through 0.2 μ m.

WELAN SAMPLE	0.1M NaOH/80°C		η (cP) before filtration	η (cP) after filtration
	Time (min)			
WM-3	3		180.14	169.30
WM-10	10		321.92	287.73
WM-40	40		216.00	187.65
B-40	40		533.76	276.05*

* filtration through 3 μ m membrane.

The sedimentation properties were formerly evaluated using 5g/l welan water solutions. The glass sphere was placed on the surface of the solution and its displacement inside the graduated tube was measured at regular time intervals. Figure 2 presents the sedimentation velocity (v) of B, D and WM-10 samples. For the B and D commercial products, it can be observed that the movement of the sphere is not uniform; this irregular behaviour may be due to the presence of aggregates. After 24 hours, the same experiment shows a decrease in v that could be attributed to a better dissolution of the gum or to the swelling of aggregates, with consequent increase in viscosity. It can be seen that the difference after 24 hours is greater in B which has more stable aggregates (20,21). The NaOH-purified sample from the broth gives a constant rate of sedimentation and no difference was noticed after 24 hours (Figure 2-III).

Figure 3 shows the sedimentation rate for the B/3 sample (commercial product filtered through $3\mu\text{m}$) in water at 25°C . The removal of great volume aggregates by filtration through $3\mu\text{m}$ membrane eliminated the difference in v after 24 hours. In Figures 2 and 3, v initially decreases during the fall of the sphere inside the tube.

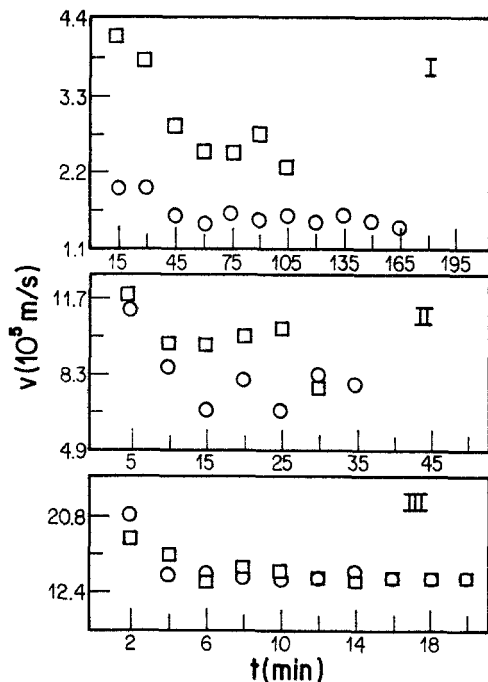


Figure 2.
Sedimentation velocity of a glass sphere in 5g/l welan in water at 25°C .
I-B, II-D, III-WM-10
□ - first day
○ - after 24 hours

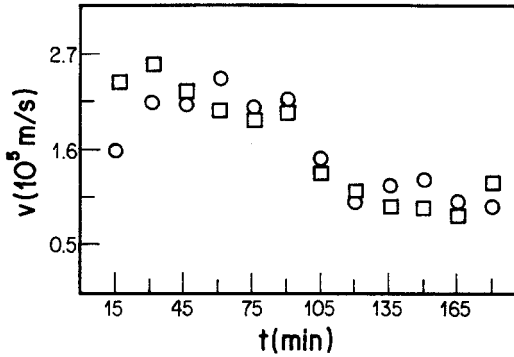


Figure 3. Sedimentation velocity of a glass sphere in 5g/l of B/3 solution in water at 25°C
 □ - first day
 ○ - after 24 hours

This behaviour could be explained by the decantation of aggregates in the tubes or by some wetting-surface problem at the glass spheres. To control these two factors, in the following experiments (described below), the viscosity of the solutions at the top and at the end portion of the tubes was measured after 24 hours and the glass sphere was immersed in each welan solution for approximately 15 hours, till the beginning of the sedimentation experiment.

In Figure 4, the sedimentation rates for B at 3.5g/l, D at 5g/l and WM-3 at 7g/l in water at 25°C are shown. These solutions have the same viscosity at low shear rates ($<0.1s^{-1}$) which correspond roughly to the shear rate caused by the glass sphere velocity. It is observed in Figure 4 that the decrease in v during the experiment was eliminated. The viscosity of the solutions from the top and the bottom of the tubes shows no evidence of decantation of aggregates. The decrease of v was certainly caused by the wetting-surface problem.

In conclusion, the velocity of the particle sedimentation is affected by the presence of aggregates; it is not regular and its average value depends on the solution viscosity at the shear rate imposed by the sedimentation stress. It was reported (22) that inhomogeneities in the polymeric solution can promote a great discrepancy between the measured and the Stoke's sedimentation viscosities. In a future communication, results of the sedimentation be-

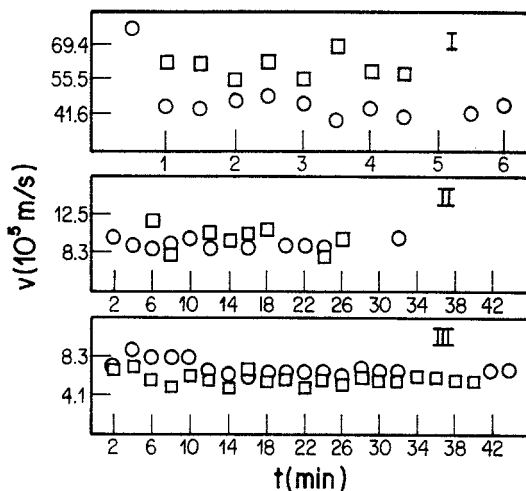


Figure 4. Sedimentation velocity of a glass sphere in welan water solutions at 25°C.

I-B (3.5g/l), II-D (5g/l), III-WM-3 (7g/l)

□ - first day

○ - after 24 hours

haviour of welan solutions will be investigated in relation to the Stoke's law.

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