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Extraction and solution properties of the galactomannan from the seeds of *Cassia javanica* L.

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Summary

The galactomannan from the seed of *Cassia javanica* L. was extracted in 26% yield and submitted to viscosity measurements. The intrinsic viscosity $[\eta]$ = 11.3 dl/g was determined at 25°C and used to calculate the viscosity average molecular weight, $\overline{M}_v = 1.5 \times 10^6$. Variation of the specific viscosity at zero shear rate as a function of the coil overlap parameter revealed two critical concentrations, C* = 0.54 and C** = 2.6 g/l, more frequently observed for rigid polysaccharides.

Introduction

Galactomannans are neutral polysaccharides, found as storage material in the seed endosperm of certain *Leguminosae* plants. Their chemical structure (Figure 1) consists of β-(1→4)-D-mannopyranosyl linear backbone, to which α-(1→6)-galactopyranosyl simple units are linked. They may be distinguished by the mannose/galactose ratio, galactose distribution and molecular mass.

Among several species whose seed endosperm is mainly constituted of galactomannan, *Ceratonia siliqua* and *Cyamopsis tetragonolobus* have been cultivated with the purpose of commercialization of their gums, locust bean gum and guar gum, respectively. These galactomannans are widely used as thickening, stabilising and coating agents in cosmetic, pharmaceutical, paper and food products.

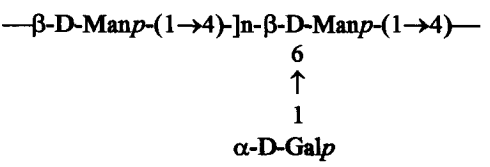

Figure 1: Molecular structure of galactomannas.

Due to their importance as thickening agents, rheological properties of galactomannans have been reported (1-5).

The aim of this work was to investigate the viscosity properties of the galactomannan extracted from the seeds of *Cassia javanica* L. This tree is a leguminous species cultivated in tropical regions worldwide, generally appreciated for the ornamental qualities of its pink flowers.

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2. Experimental

Green pods of *Cassia javanica* L. were collected; their seeds were separated manually, sun dried and milled. Soluble lipids and proteins were extracted with 2:1 v/v toluene-ethyl alcohol in a Soxhlet apparatus for 16h. Extraction of the polysaccharide was carried out in water at room temperature overnight. Hull and germ were removed by filtration. The filtrate was centrifuged at 7000 rpm for 2h. The gum, CJG, was recovered by precipitation in ethyl alcohol, successively washed with ethyl alcohol-water solutions of increasing ethyl alcohol concentrations and with pure ethyl alcohol, and dried under vacuum.

The inner part of the seed was examined with a Stereo Olympus Optical Microscope, model SZM10, equipped with a Olympus camera.

Stock solutions of different concentrations were prepared by stirring the dispersed polymer in distilled and deionized water at room temperature, and diluted as required. The actual concentration of each solution was determined after solvent evaporation in an oven at 100°C. Dilute solutions used for the determination of the intrinsic viscosity were filtered through 0.45μm membranes.

Viscosity measurements were carried out at 25°C, on a Contraves Low Shear 40 rheometer, equipped with coaxial cylinders of a MS-DIN 412 geometry, in the range of 10^{-4} to 10^{2} s^{-1} shear rate, and a Haake RS100 instrument with a cone-and-plate geometry (4° cone angle), in the 10-720s^{-1} range.

3. Results and discussion

In the average, the endosperm of the dry seed of *Cassia javanica* represents 52-54 % of the total weight of the seed is high when compared to 42-46% found for locust bean gum (6) and 35-42 % found for guar gum (7). After purification, the galactomannan yield reached 26 %. Figure 2 shows a photograph of the seed, cut transversely.

Soluble lipids, proteins and pigments represent 12.4 % of the seed. The purified dry CJG gum had a moisture content of 8.8 % and an ash content of 6.5 %.

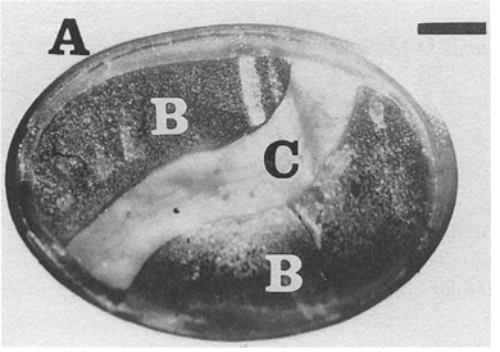

Figure 2: Transversal view of the seed of *Cassia javanica* L.; A, hull; B, endosperm; C, germ (scale bar = 1 mm).

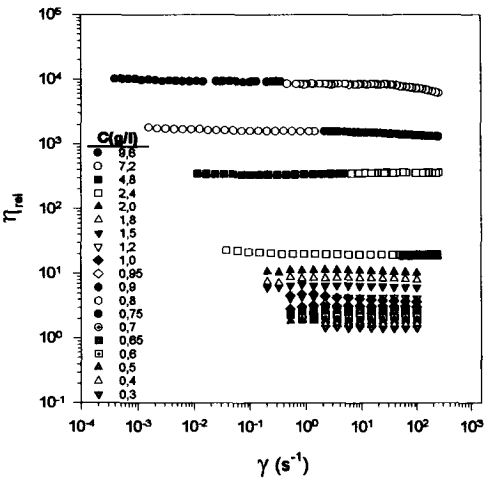

Figure 3: Experimental flow curves for CJG gum at concentrations in the range 0.3 to 9.6 g/L, in water at 25°C.

Flow curves (log η_{rel} plotted against log γ) obtained for CJG solutions over a wide range of concentrations (0.3 - 9.6 g/l) are shown in Figure 3. For the more concentrated solutions (C > 2.4 g/l), these curves result from a combination of data given by the Contraves Low Shear 40 and the Haake RS100 rheometers. More dilute solutions were investigated with the Contraves equipment.

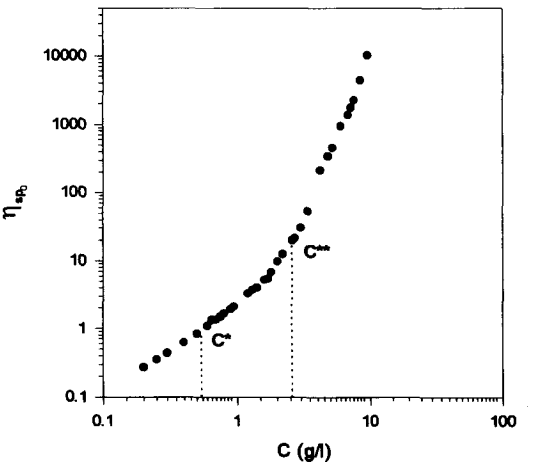

Figure 4: Dependence of the specific viscosity at zero shear rate on concentration for CJG gum in water, at 25°C.

In the range of shear stress used, a Newtonian behavior is observed for concentrations lower than 7 g/L. A smooth shear-thinning behavior is observed for the higher concentrations.

Figure 4 shows the dependence of η_o on concentration for CJG gum, with η_o expressed as the specific viscosity. In general, the intrisic viscosity is determined by extrapolation made for relative viscosity values between 1.2 and 2.0. The Huggins equation, $\eta_{sp}/C = [\eta] + k'[\eta]^2C$, was employed in the present case for concentrations with specific viscosities in the corresponding range, 0.2 and 1.0, and resulted in a high value for the Huggins constant, k' = 1.07. For good solvents, lower values of the Huggins constant have been reported, and values higher than 1.0 are attributed to macromolecular association. The measured viscosities for the lowest concentrations (lower than 0.5 g/l) were then used to determine the intrinsic viscosity of CJG gum by the Huggins equation, $[\eta] = 11.3$ dl/g, and the Huggins constant, $k' = 0.84$. The viscosity average molecular mass, $\overline{M}_v = 1.5 \times 10^6$ was calculated by the Mark-Houwink relation, with $K = 3.8 \times 10^{-4}$ and $a = 0.723$, taken from the literature (8).

More detailed observation of Figure 4 revealed two linear regions, defined by two critical concentrations, C* = 0.54 g/l and C** = 2.60 g/l, that limit the so-called dilute, semi-dilute and concentrated regimes. The existence of two critical concentrations is consistent with the concept of progressive compression of the coils with increasing concentration (9), and has been observed for a few other rigid polysaccharides (9,10), and for locust bean gum (11).

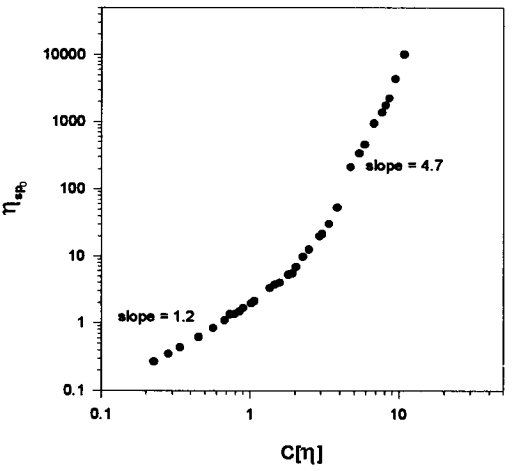

Figure 5: Dependence of the specific viscosity at zero shear rate on the coil overlap parameter for CJG gum in water, at 25°C.

The double-logarithmic plot of η_{spo} versus $C[\eta]$ is shown in Figure 5. The coil overlap parameter, $C[\eta]$, which represents the volume occupied by the macromolecule in solution has been extensively used to describe the flow properties of polymers in solution. For several randomly coiled polysaccharides (3,9), a unique curve is usually obtained that can be divided into two linear regions, with reduced critical concentrations

$C^*[\eta] \approx 4$. The dilute regime is characterized by the viscosity dependence on concetration with slopes of 1.2 - 1.4. For concentrated solutions, the slope increases to ≈ 3.4.

Galactomannans, however, were found to depart from this generalised behavior, with lower values of $C^*[\eta]$ (usually ≈ 2.5) (3), and slopes for the concentrated regime in the range 3.9 - 6.6 (3-5,8,11). These deviations have been attributed to specific intermolecular associations (hyperentanglements) between unsubstituted mannan regions of the galactomannan chains, in addition to physical entanglement of overlapping coils (3).

For CJG gum, the reduced critical concentration was found to be $C^*[\eta] = 0.60$. This result is in accord with the one reported for locust bean gum (11), $C^*[\eta] = 0.69$, a galactomannan with a low galactose content, but is much lower than the values usually reported for disordered polysaccharides.

In the dilute regime, $\eta_{spo} \approx C[\eta]^{1.2}$, with the exponent within the interval usually found for polysaccharides in general. According to Figure 5, the semi-dilute regime extends to $C^{**} = 2.93 / [\eta]$ and for higher concentrations, the logarithmic dependence of η_{spo} with $C[\eta]$ is linear with a slope of 4.7.

The low value of C^* obtained for CJG gum, the high values of η_{spo}, and their dependence on $C[\eta]$ are probably due to interactions, and indicate a galactomannan with a low galactose content and good thickenning properties.

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