

AN7003: Conformational properties of hyperbranched hetero-polysaccharides by SEC-MALS-IV

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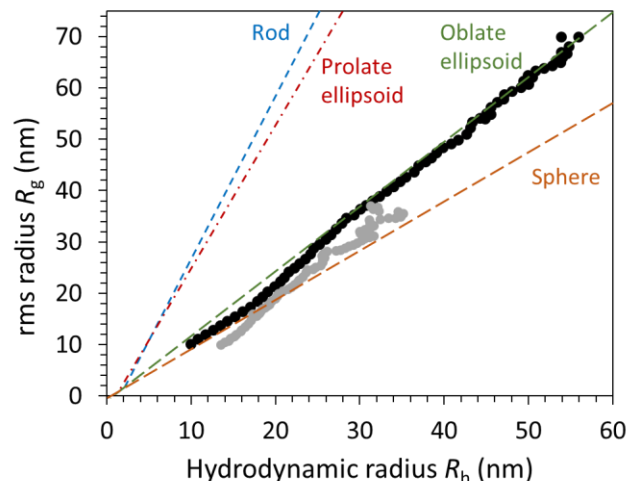
Summary

This note discusses the characterization and comparison of conformational properties of hyperbranched macromolecules from two Acacia gum varieties, *A. senegal* and *A. seyal*, using SEC-MALS-IV combining MALS with online viscometer and refractive index detector.

A. seyal macromolecules appeared more structured and compact than *A. senegal* ones. For both Acacia gums, the anisotropy of macromolecules increased with molecular weight. *A. senegal* macromolecules, however, were the most anisotropic. SEC-MALS-IV showed that the conformation of *A. seyal* macromolecules varied from spheres to oblate ellipsoids while *A. senegal* macromolecules varied from oblate ellipsoids to more anisotropic conformations, such as oblate and prolate ellipsoids.

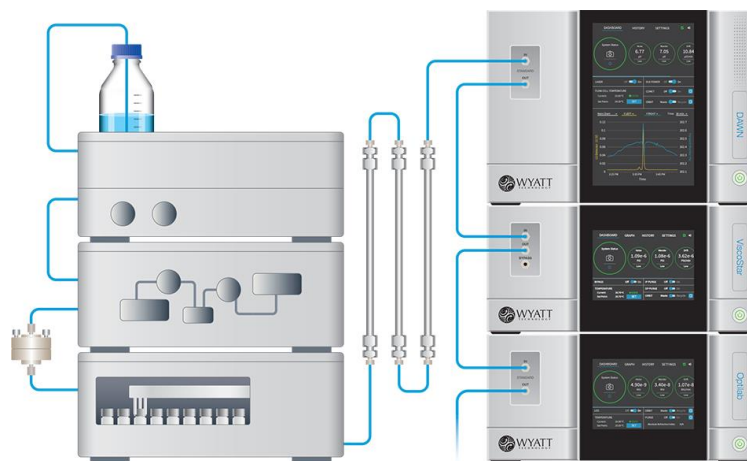
Introduction

Acacia gum is an edible dried gummy exudate obtained from the trunk and branches of *Acacia senegal* and *Acacia seyal* trees. Acacia gum macromolecules are complex and highly branched hetero-polysaccharides belonging to the arabinogalactan-protein (AGP) family, ubiquitous biopolymers found in plant kingdom. Acacia gum can be defined as a continuum of molecular species differing by their protein to sugar ratio, molar mass, charges, and hydrophobicity index. *A. senegal* and *A. seyal* gums differ by their chemical composition (sugar composition and protein content) as well as their degree of polysaccharide branching. They are composed of galactose, arabinose, rhamnose, glucuronic acid and a small amount of protein (0.5-3%). The sugar backbone is composed of 1,3-linked β -D-galactopyranosyl units.



Radius of gyration (R_g) as a function of hydrodynamic radius (R_h) for *A. senegal* (black) and *A. seyal* (gray) gums.

This application note focuses on the characterization of the conformational properties of both Acacia gums using size-exclusion chromatography (SEC) coupled inline to multi-angle light scattering (MALS), viscometric and a refractive index detection, i.e. SEC-MALS-IV.



Materials and Methods

SEC-MALS-IV experiments were performed using a Shimadzu HPLC system coupled online to a **DAWN[®] MALS instrument**, a **ViscoStar[®] differential viscometer** and an **Optilab[®] differential refractive index detector**. Acacia gum macromolecules were separated on a set of columns composed of one Shodex OHPAK SB-G pre-column followed by four columns in series (Shodex OHPAK SB 803 HQ, OHPAK SB 804 HQ, OHPAK SB 805 HQ and OHPAK SB 806 HQ).

Aqueous Acacia gum solutions at 1 mg/mL were injected and eluted through the system with 0.1 M LiNO₃ mobile phase containing 0.02% NaN₃ at a constant flow rate of 1 mL/min. Temperature was maintained at 30 °C in the column and instruments. Data were analyzed using **ASTRA[®]** software. Molecular weight (M), radius of gyration (R_g), intrinsic viscosity ($[\eta]$), and polydispersity index (M_w/M_n) were calculated using a refractive index increment (dn/dc) of 0.145 mL/g.

Results and Discussion

The M_w distributions (MWD) of *A. senegal* and *A. seyal* gums as a function of elution volume are shown in Figure 1. *A. senegal* and *A. seyal* gums consisted of macromolecules with M ranging from $1 \cdot 10^7$ g/mol to $1.2 \cdot 10^5$ g/mol and $4 \cdot 10^7$ g/mol to $1 \cdot 10^5$ g/mol, respectively. Along most of the elution volume, the MW of *A. seyal* was higher than that of *A. senegal*. Hence, it appears that *A. seyal* displayed higher M than *A. senegal* for the same hydrodynamic volume, suggesting a more compact structure of *A. seyal* macromolecules.

The structural and conformational features of the gums were further analyzed by the conformation plots of R_g vs M and $[\eta]$ vs M . In general, R_g and $[\eta]$ are related to the molecular weight as a simple power law relationship according to $R_g = K_g M^{\nu_g}$ and $[\eta] = K_\alpha M^\alpha$, where the parameters K_g and K_α are the corresponding constants.¹ ν_g and α depend on the overall shape of polymers, especially anisotropy, temperature, and polymer-solvent interactions that determine the chain density of polymers.

The log-log plot of R_g vs M for both gums is shown in Figure 2. This analysis considers only 50% and 30% of *A. senegal* and *seyal* macromolecules, respectively, due to the limitations of MALS measurements to R_g values above 10 nm. The plots cannot be described by a single slope,

which demonstrates the presence of biopolymers with different conformations and/or density. These deviations from a simple power law behavior are indicative of hyperbranched macromolecules with different branching density and/or length of branches,^{2,3} but also with different degrees of anisotropy.

The plot for *A. senegal* displayed two slopes with a value of 0.63 in the lower MW range and 0.51 in the higher MW range. Based on theoretical ν_g values for spheres (0.33), linear random coils (between 0.5 and 0.6 depending on the solvent quality) and rods (1)¹, it was tempting to ascribe the resulting exponent values to the presence of more or less extended linear random coils. However, such a conclusion would be misleading, as it was demonstrated that Acacia gum consists of hyperbranched polymers with a degree of branching of 78.2% and 59.2% for *A. senegal* and *seyal*, respectively.

Polymers with different shapes can display similar ν_g exponent values: a value of 0.5 can be found for linear random coils in Θ -conditions, thin discs, non-swollen randomly branched polymers, and star molecules in Θ -conditions.⁴ It was previously shown that all macromolecules from *A. senegal* gum exhibit oblate or triaxial ellipsoid shapes.⁵⁻⁷ Therefore, the ν_g exponents for *A. senegal* gum were likely due to the ellipsoidal shapes of macromolecules, which were indeed quite close to the thin disc theoretical ν_g .

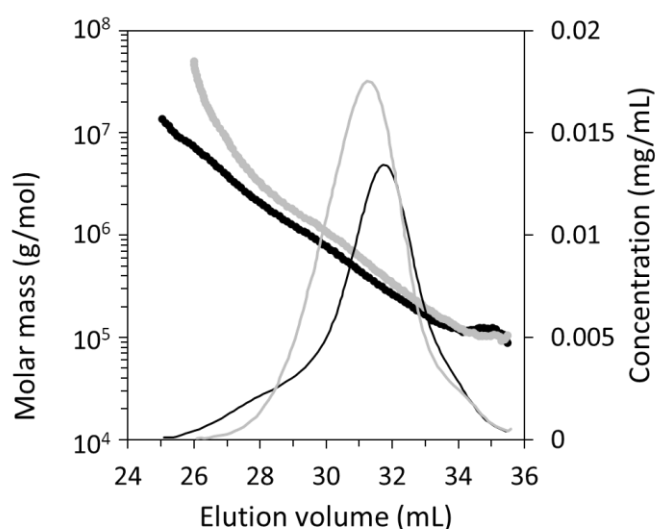


Figure 1: Molecular weight distribution (thick line) and concentration profile (thin line) of *A. senegal* (black) and *A. seyal* (grey) gums.

The R_g vs M conformation plot for *A. seyal* gum (Figure 2) also displayed two slopes, with ν_g values of 0.71 and 0.39, not so different from those previously obtained for *A. senegal* gum in quite similar M ranges. It can be noted that ν_g values decreased when increasing the M range. Similar trends can be observed for *A. senegal* gum, suggesting that larger macromolecules were more compact and/or less anisotropic than smaller ones. Alternatively, very small exponent values around or below 0.3 can also indicate the presence of aggregates. From our experience, above a MW of about $2 - 3 \cdot 10^6$ g/mol, aggregates of Acacia gum polymers are always present in solution. Other hyperbranched polysaccharides such as glycogen and dextran displayed lower ν_g value at higher M .^{2,3,8}

The analysis of Acacia gums using the Mark-Houwink-Sakurada (MHS) relationship, $[\eta]$ vs M , gave further information on the conformation and structure of macromolecules since more than 95% of macromolecules were covered by the analysis. The α power law exponent values theoretically range from 0 (sphere) to 1.8 (rod) with 0.5-0.8 intermediate values for flexible polymers depending on the solvent quality.⁹

MHS plots of both Acacia gums are given in Figure 3. The *A. seyal* MHS plot displayed one slope with an α exponent value of 0.28, suggesting a homogeneous distribution of macromolecule hydrodynamic volumes. On the other hand, the *A. senegal* MHS plot clearly displayed three slopes with α exponent values of 0.44, 0.78, and 0.44 for lower, intermediate and high molar molecular weight ranges, respectively. This indicates that macromolecules with M in the intermediate range displayed larger anisotropy or smaller chain density than those in the low and high M range. Furthermore, the α exponents indicated that *A. seyal* macromolecules adopted more compact conformation in water than *A. senegal* macromolecules. Similar α exponent values (around 0.3-0.5) were obtained for hyperbranched polysaccharides such as amylopectin, dextran, or glycogen and synthetic hyperbranched polymers with finely tuned structural characteristics.^{8,10-12} The theory on branched polymers and the recent numeric simulation on flexible polymers demonstrated that hyperbranching decreases the α exponent below 0.5, and a branching degree higher than 0.5 gave lower α values in the high M range than in the low range.^{13,14} Based on the effect of branching density on α , and assuming all other

structural parameters are identical, smaller α values for the most branched *A. senegal* gums were expected. However, exactly the opposite was found.

The differences in composition between Acacia gums might influence the conformation of gum macromolecules. The specific sugar composition of *A. seyal* gum, especially the lowest concentration of charged sugars and the highest content of long arabinose side chains distributed among sugar blocks, and polypeptide architectures with plasticizing properties, can promote a more compact conformation and result in a smaller α exponent for *A. seyal* gums than for *A. Senegal* gums.

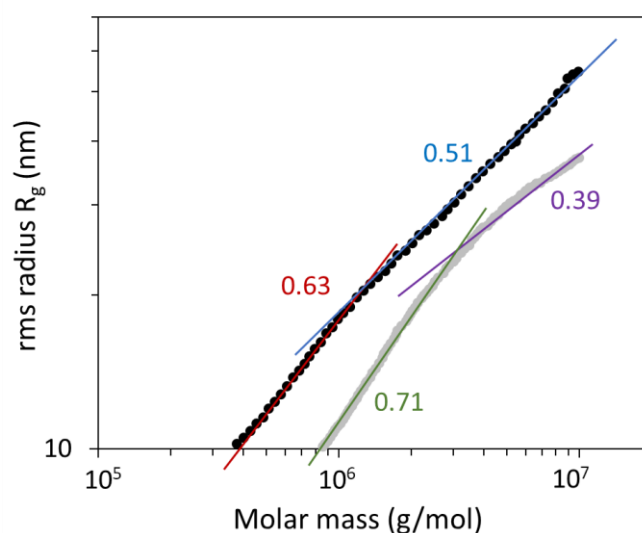


Figure 2: R_g conformation plot for *A. senegal* (black) and *A. seyal* (gray) gums.

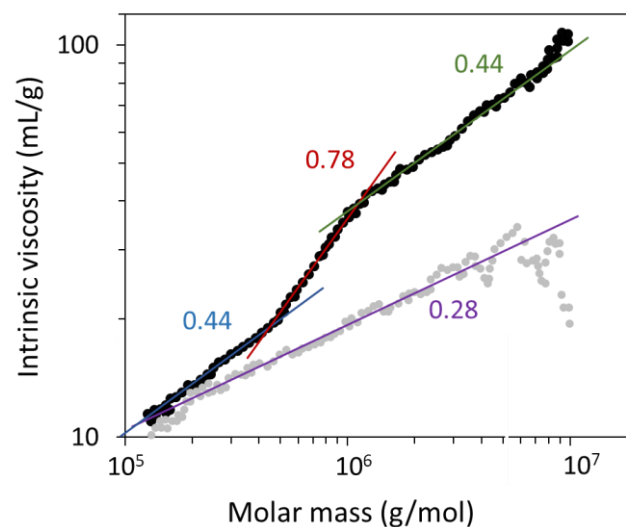


Figure 3: Mark-Houwink-Sakurada plot for *A. senegal* (black) and *A. seyal* (gray) gums.

Another way to consider the static (R_g) and hydrodynamic ($[\eta]$) parameters is to combine them in the ρ structural parameter, R_g/R_h .¹ R_h (the equivalent-sphere intrinsic viscosity-based hydrodynamic radius) was calculated according to the equation $R_h = 3[\eta]M/10\pi N_A$, where N_A is Avogadro's number. ρ , also named the asymmetry or anisotropy parameter, depends on the chain architecture and conformation, and is known to be affected by macromolecular flexibility and polydispersity.^{15,16} Theoretical ρ values are 0.778 for hard homogeneous spheres, 0.977 for dendrimers equivalent to Gaussian soft spheres, and between 1.08 and 1.33 for regular stars with many uniform-length chains. Hyperbranched polymers or regular stars with numerous polydisperse arms have ρ values of 1.22 and from 1.5 to 1.73 for random coil and linear chains, the latter ones increasing their value nearly 2-fold in good solvent conditions.¹ It was clearly observed from these theoretical values that ρ depends on polymer anisotropy, and values between 1 and 1.3 are usually found for branched and hyperbranched polymers.

The plot of ρ vs M for *A. senegal* and *A. seyal* gums is shown in Figure 4. Curves encompass 50% of macromolecules for *A. senegal* gum and 30% for *A. seyal*. For *A. senegal* gum, ρ values were first constant at 1.02, followed by an increase from 1.02 to 1.2, and then they remained constant. For *A. seyal*, all ρ values were lower than those for *A. senegal* gum. They increased from 0.75 to 1.02, and then did not further evolve. Depending on the MW, ρ values of both Acacia gums are compatible with the presence of more- or less-dense hyperbranched architectures with spheroidal, oblate ellipsoidal or triaxial ellipsoidal conformations.^{1,2,5-8,10} For both Acacia gums, the increase of ρ with M could be promoted by increasing anisotropy and/or by decreasing density. The increase of ρ with M is mainly due to larger macromolecules (or aggregates) being more anisotropic. A way to check this assumption is to plot R_g vs R_h and to compare experimental data with theoretical calculations of objects with increasing anisotropy, usually sphere, oblate and prolate ellipsoids, and rods.^{15,17} Figure 5 clearly shows that macromolecules from *A. seyal* displayed spheroidal conformations at low MW and more anisotropic conformations, close to oblate ellipsoids, at larger MW. In contrast, *A. senegal* macromolecules adopted oblate ellipsoid-like conformations at low MW, which was confirmed elsewhere,⁵ and more anisotropic conformations at larger

MW, varying between oblate and prolate ellipsoids, as demonstrated recently.^{6,7}

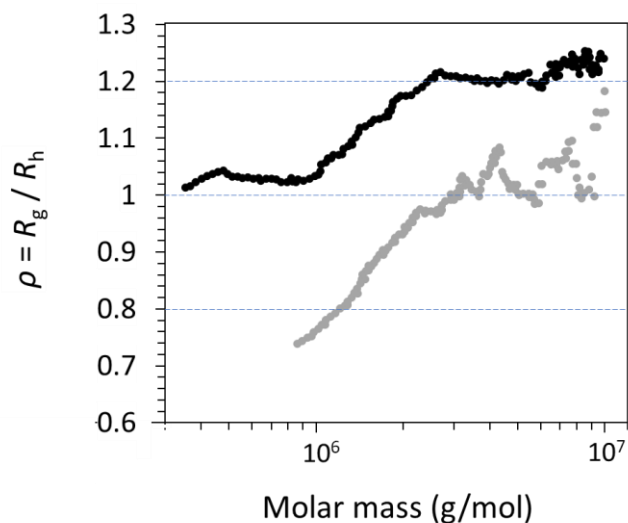


Figure 4: The ρ structural parameter (R_g/R_h) as a function of molecular weight for *A. senegal* (black) and *A. seyal* (gray) gums.

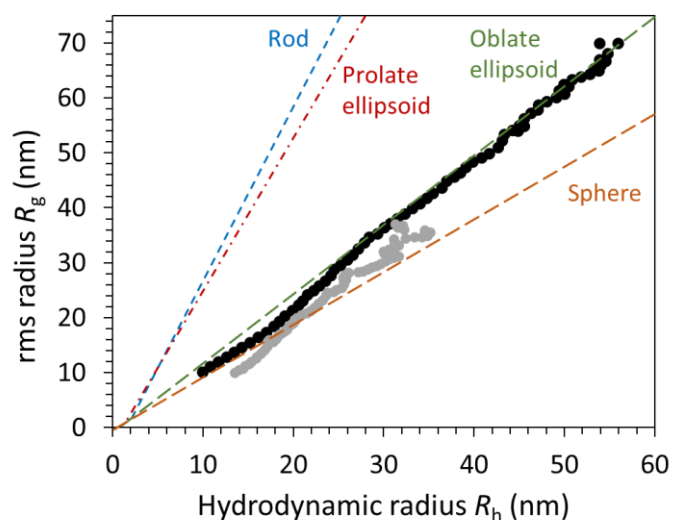


Figure 5: Radius of gyration (R_g) as a function of hydrodynamic radius (R_h) for *A. senegal* (black) and *A. seyal* (gray) gums.

Conclusions

SEC-MALS-IV using Wyatt instruments enables characterization of the conformational properties of hyperbranched hetero-polysaccharides, with structure and higher compactness represented by lower intrinsic viscosity. The measurements indicate anisotropy and its dependence on molecular weight; in this case, macromolecular conformation varied from spheres to oblate and prolate ellip-

soids. These results demonstrate the comprehensive information that may be obtained for such polymers by SEC-MALS-IV using Wyatt's DAWN, ViscoStar and Optilab.

For a detailed introduction to SEC-MALS-IV, visit www.wyatt.com/SEC-MALS. To learn about the products used in this application note, please visit www.wyatt.com/DAWN, www.wyatt.com/ViscoStar and www.wyatt.com/ASTRA.

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