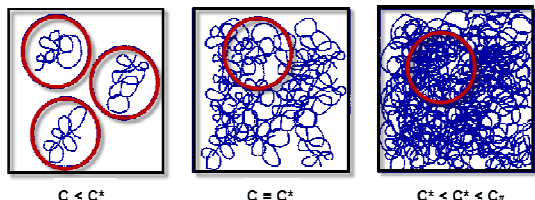# Combined Use of Rheometry and GPC/SEC for Characterising Ionic Polysaccharides

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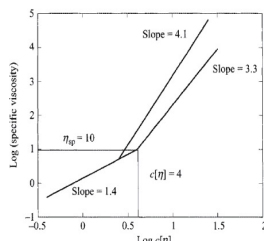*Malvern Instruments Ltd, Malvern, Worcestershire, UK*

Background

- Rheological properties of polysaccharides are very much dependent on their molecular weight and concentration in solution which are related to their hydrodynamic volume and hence intrinsic viscosity.

C < C*   C = C*   C* < C* < Cπ

- At low concentrations, the polymer chains are not in contact while at high concentrations, the polymer chains become entangled, contributing to a large increase in viscosity and onset of elasticity.

- The various regimes are dilute (c < c*), semi-dilute (c* < c < c#), and concentrated (c > c#).

Viscosity of Polysaccharide Solutions

- Solution viscosity is controlled primarily by the extent to which the polymer chains interpenetrate each other. This can be characterized by the coil overlap parameter c[η] which is the product of concentration and intrinsic viscosity.

- This is a dimensionless number that indicates the volume occupied by the polymer molecule in the solution.

- The illustration in Figure 1 shows dilute and concentrated regimes in terms of Log c[η] (coil overlap parameter) against Log η_{sp} = (η_0 - η_s)/ η_s (η_{sp} = specific viscosity).

- Previous work has shown a slope of 3.3 for entangled polysaccharide chains dissolved in good solvents and 4.1 for polymers with specific intermolecular associations. Below the critical overlap concentration the slope is 1.4 .

Morris et al. Carbohydrate Polymers 1 (1981) 5-21   **Figure 1 - Log η_{sp} vs. Log c[η]**

Experimental

- When using SEC (Size Exclusion Chromatography) and Rheology it is possible to characterise molecular properties and relate these to physical properties of the polymer in solution such as elasticity, viscosity and relaxation behaviour.

- In this work solutions of Sodium Hyaluronate were made up in Phosphate Buffer Solution (pH 7.4) at various concentrations and hydrated for 24 Hours prior to measurement.

- Intrinsic viscosity was determined using a Viscotek 4-capillary differential viscometer and absolute Molecular weight using a Viscotek LALS (Low Angle Light Scattering) detector following SEC analysis.

- Rheological measurements were made using a Kinexus Pro rheometer with cone and plate configuration in both oscillatory (viscoelasticity) and steady shear (flow behaviour) modes.

- All measurements were performed at 25℃.

Viscosity Dependence on Concentration

- SEC analysis determined the molecular weight of the polymer to be 2.8 MDa and the intrinsic viscosity [η] to be approx. 30 dL/g.

- The coil overlap parameter c[η] was plotted against the specific viscosity η_{sp} for a range of concentrations as shown in Fig 2.

- The behaviour is similar to that reported in the literature (Fig 1) with the transition from a dilute to semi-dilute regime occurring at c[η] ≈ 4.

- The slope in the dilute regime is higher than that reported for many polysaccharides with a slope of 1.67 compared with a value of 1.4 in Fig 1.
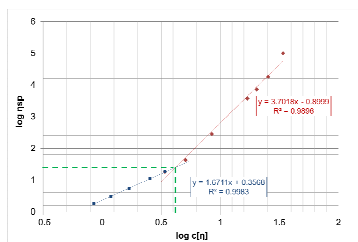
- In the semi-dilute regime we report a value of 3.7 .

Fig 2 – Variation of η_{sp} with c[η] which is representative of polymer volume in solution

Viscoelastic Properties

- Above the overlap concentration, polymer chains can entangle which causes significant onset of elasticity as shown in Figure 3.
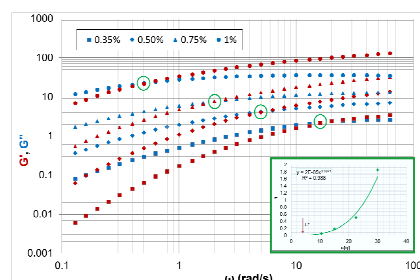

Fig 3 – G' and G" plotted as a function of angular frequency at four concentrations with G'/G" cross-over's ringed. Inset is relaxation time (1/crossover frequency) plotted against log [η].

- The cross-over in G' (elastic modulus) and G" (viscous modulus) signifies the start of the terminal region, where polymer chains have had sufficient time (1/ω) to free themselves from entanglements, allowing individual polymer chains to slip past one another.

- The inverse of the cross-over frequency is termed the relaxation time and is a measure of how long this disentanglement process takes.

- At times shorter than τ, G' exceeds G" indicating an elastically entangled network.

- At times greater than τ, G" exceeds G' indicating loss of elasticity consistent with disentanglement and translation of polymer chains.

Flow Properties

- When a uni-directional steady shear is applied to the polymer solution, these entanglements are disrupted.

- At low rates of shear, this disruption is slow enough to allow re-entanglement to occur with no net change in the extent of entanglement, giving a constant low shear viscosity.

- At higher shear rates the rate of disruption exceeds the rate of re-entanglement giving shear thinning behaviour.

- Plots of viscosity against shear rate are shown in Figure 4 for the same concentrations as in Figure 3 with all showing shear thinning behaviour.
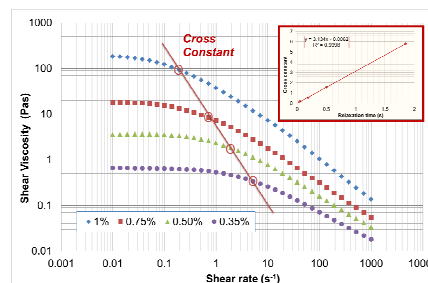

Fig 4 – Viscosity vs. shear rate curve for four polymer concentrations and inset the cross constant (k) plotted against relaxation time.

- This shear thinning behaviour can be described using the Cross model below with the Cross constant *K* equal to the inverse of the shear rate when η = η_0/2

$$\eta(\dot{\gamma}) = \frac{\eta_0}{1 + (K \cdot \gamma)^m}$$

- Since both relaxation time (Fig 3) and Cross constant (Fig 4) are related to the degree of molecular entanglements a strong correlation can be found between the two parameters, as shown in Fig 4 (inset).
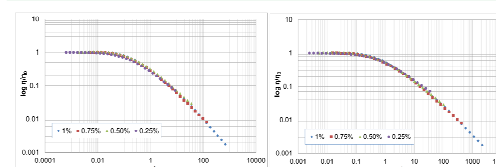
Generalised Flow Curve

Fig 5 – Generalised viscosity vs. shear rate curve for four concentrations using reduced variables based on relaxation time and cross constant.

- Since differences in flow behaviour are due to extent of entanglement, then plotting η/η_0 against reduced shear rate (using cross constant or relaxation time) allows all curves to be superimposed onto a single curve so long as entanglements are the primary mechanism of interaction.

Conclusions

Rheology and Size Exclusion Chromatography (SEC/GPC) are both well established methods for the characterization of polysaccharide solutions. SEC targets characterization on a molecular scale whereas rheology offers insights into properties on a more macroscopic scale. Linking the results of independent characterization technologies results in a much more complete picture of the samples under investigation.