

Branching in Polysaccharides by SEC³

Introduction:

Triple detection GPC (SEC³) analysis is generally used to determine molecular weight distributions. It can also be used to determine the degree of branching present in the sample if a linear reference sample is provided. In the following report two samples identified as branched polysaccharides and one sample identified as a corresponding linear polysaccharide were submitted for MWD and branching analysis by SEC³.

Experimental:

The samples were dissolved in 0.1M NaNO₃ heated to 65^oC for 4 hours then filtered with 0.2 μm Nylon filters. The two branched samples and the linear reference were then analysed via triple detection under the conditions and with the equipment stated. Low angle light scattering (Lals) was used to calculate the molecular weights due to the high R_h value for the linear reference. Right angle light scattering (Rals) would have required a correction to obtain a good value due to the anisotropic light scattering for molecules with R_h about 10nm. The UV signal was available but not used for this analysis.

GPC Run Conditions

Detector: Viscotek Model 302-050 TDA (Lals-Rals-UV-RI-Viscometer)

Autosampler: Viscotek GPCMax (integrated and software controlled degasser, pump and autosampler)

Solvent: Aqueous 0.1M NaNO₃ with 200PPM NaN₃

Column: ViscoGel GMPWXL

Temperature: 30^oC

Concentration: ≈ 1mg/mL

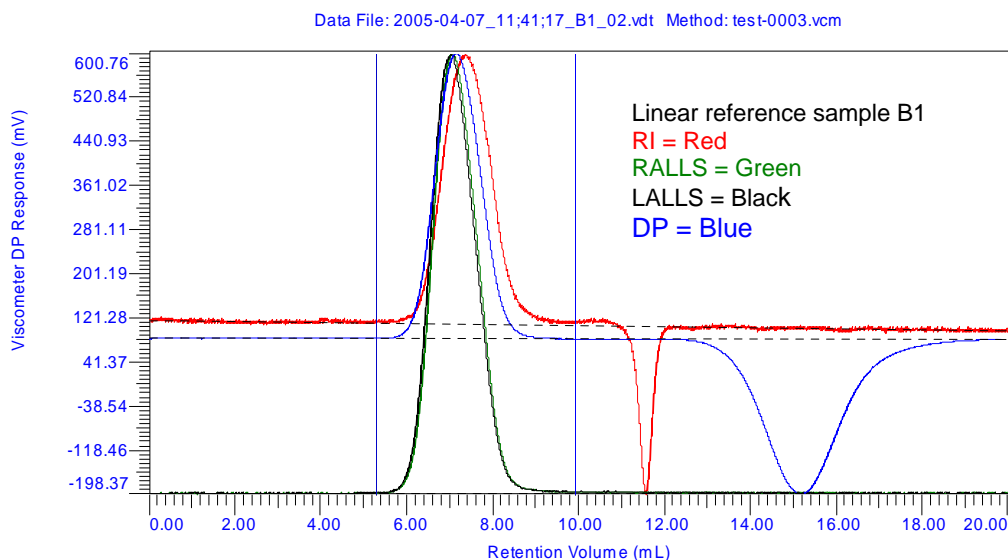
Injection Volume: 100 μL

Flow Rate: 1.0 mL/min

Software: OmniSEC 4.0

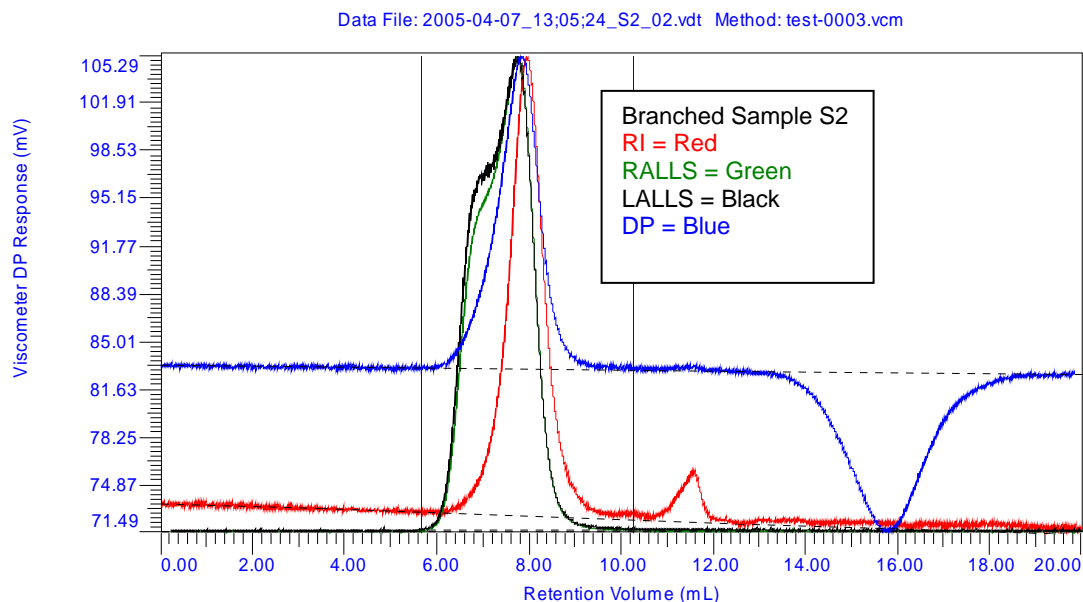
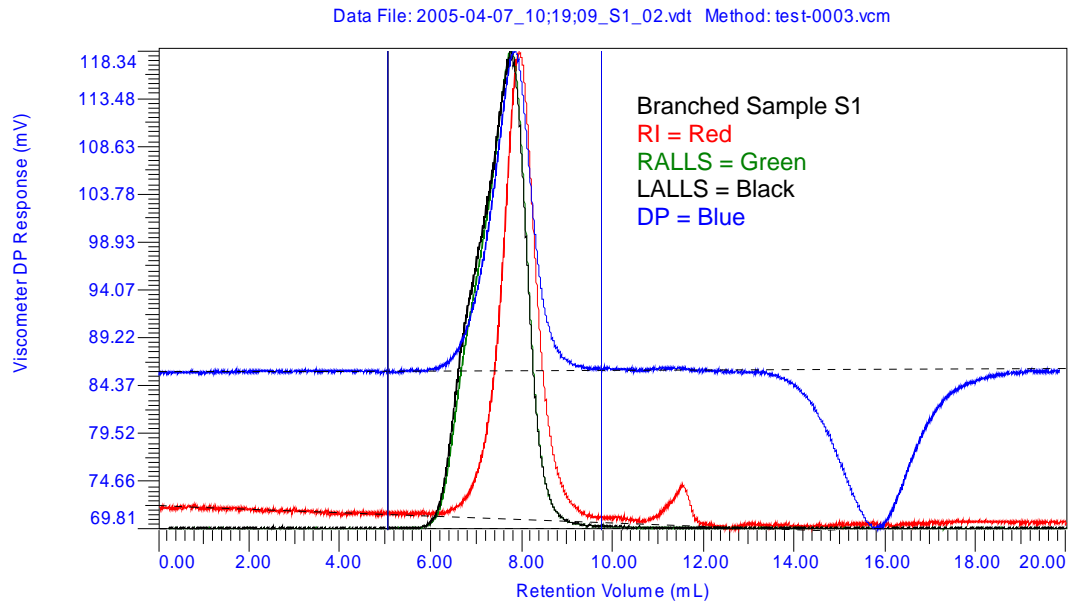
Results:

Figure 1 below is the triple chromatogram for the linear reference sample.



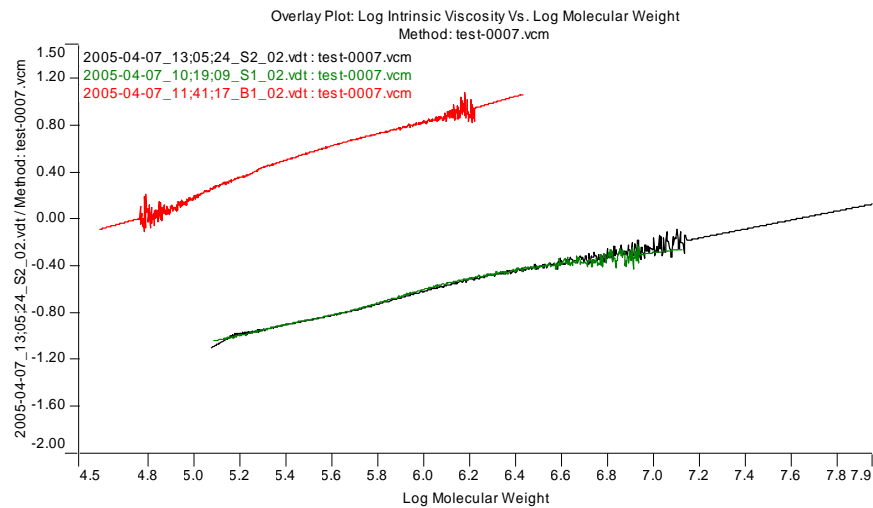
Note the shape of the two light scattering signals and the relative shapes of the viscometer DP and RI signals. They are relatively featureless gaussian type distributions that would normally be expected for linear polymers.

Figures 2 and 3 below are the triple detection chromatograms for the branched samples.



Compare the shapes of the light scattering DP and RI signals to those of the linear reference. Note the “pre-peak” on the light scattering signals and a slight shoulder on the DP channel. This looks like aggregation but is really branching. The highly branched (and thus higher molecular weight) species respond more per unit concentration to the light scattering detectors than to the concentration detector (RI).

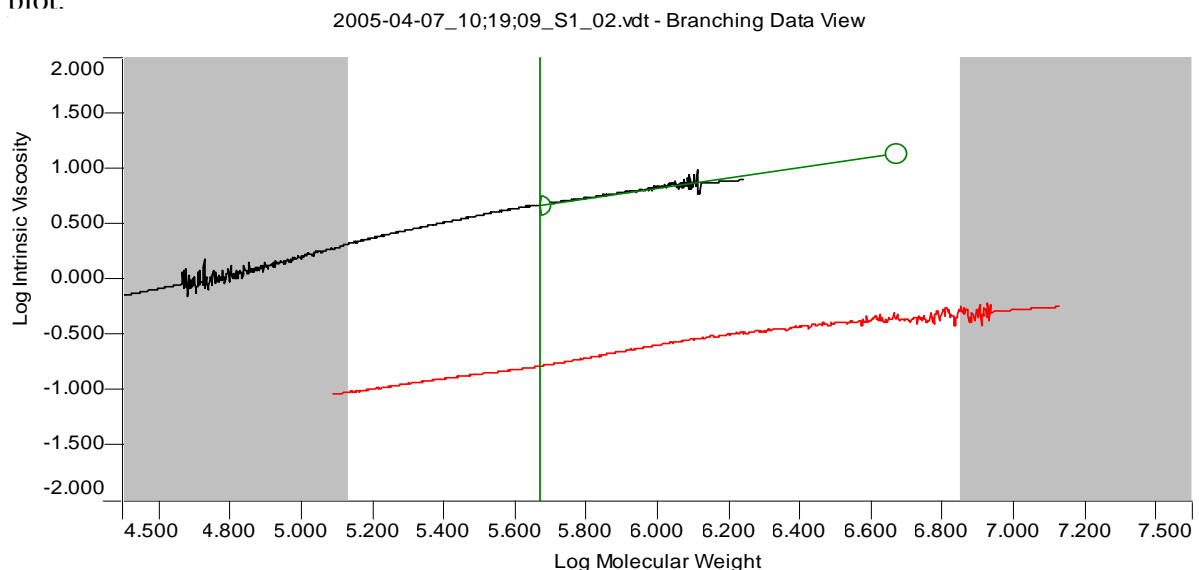
Figure 4 below is an overlay of the Mark-Houwink plots for these three chromatograms.



In order to determine branching for a sample two values must be known: the Mark-Houwink a and $\log [k]$ intrinsic viscosity vs. MW values for both the linear analogue of the sample and the same values for the sample itself.

It is evident in the Mark-Houwink plot that the distribution of linear reference sample does not extend to the high molecular weights that the samples do. However, OmniSEC allows an extrapolation of the data from the linear reference both up and down to cover the range of a branched sample. This allows a direct comparison of the sample with the linear reference.

Figure 5 below shows the overlay of Mark-Houwink plots for branched sample S1 and linear sample B1 in the Branching View in OmniSEC. The extrapolation tool (green line with handle) and also the calculation limits (between the grey areas) are overlaid on the B1 plot. The extrapolation tool allows the user to extend the slope of the linear sample Mark-Houwink plot either up or down in order that the linear Mark-Houwink plot will cover the entire range of the branched Mark-Houwink plot. The calculation limits define the range of data over which the calculations will be performed. In this case the extreme high portion of the distribution was excluded from the calculations because of the noise in the plot.



The branching number and branching frequency can be calculated according to the Zimm-Stockmayer equation for a polydisperse, randomly branched polymer. The high degree of branching present in these samples means that the sample at each elution volume is polydisperse.

$$[1] \quad g = \frac{6}{B_n} \left[\frac{1}{2} \left(\frac{2 + B_n}{B_n} \right)^{\frac{1}{2}} \ln \left(\frac{(2 + B_n)^{\frac{1}{2}} + B_n^{\frac{1}{2}}}{(2 + B_n)^{\frac{1}{2}} - B_n^{\frac{1}{2}}} \right) - 1 \right]$$

Where:

B_n is the number of branches

$$[2] \quad g = \left(\frac{IV_b}{IV_l} \right)^{\frac{1}{\varepsilon}}$$

ε is the shape factor (typically around 0.75)

Branching frequency (λ) normalizes B_n to molecular weight and the repeating units of the polymer.

$$[3] \quad \lambda(M) = \frac{RB_n}{M_w}$$

Where R is the repeat unit and M_w is molecular weight.

A repeat unit of 18200 Daltons was used for these calculations. This value is the equivalent of 100 glucose units and is a common value for repeat unit in polysaccharides.

Table I below gives the calculated values for these three samples.

Sample Id	M_w	M_n	M_z	IV	R_h	R_g	M-H a	M-H Log K	Branches/Molecule	Branching Frequency
B1	385,813	265,800	527,215	3.7923	27.682	39.991	0.602	-2.76	Linear reference	
S1	618,234	348,122	2,123,000	0.1642	10.899	Not Calc.	0.485	-3.541	1483	64.03
S2	837,299	378,052	7,819,000	0.1775	11.848	Not Calc.	0.461	-3.404	1467	56.24

Conclusions:

These samples are very highly branched; this is evident from the branching frequency, which is based on 100 glucose units. The branching frequencies of 64 and 56 may therefore be interpreted as equivalent to 64% and 56% of the glucose units having branches. This type of calculation is only possible with the utilisation of both light scattering and viscosity data. Mals cannot be used because molecules with R_h values below about 12nm do not exhibit enough anisotropic light scattering to reliably determine R_g values.

Key Words: GPC, Polysaccharides, Branching, Branching View, OmniSEC, Triple Detection, M_w , IV, Mark-Houwink, Zimm-Stockmayer