

GPC/SEC-Viscometry for Molar Mass and Structural Analysis of Carrageenan

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Abstract

GPC/SEC-viscometry enables measurement of true molar masses for carrageenan samples and helps to detect subtle differences even between samples with very similar molar mass distributions. Structural information is easily accessible with the Mark-Houwink plot.

Introduction

Carrageenans (also known as carrageenins) are used as food additives (e.g., as thickening and stabilizing agents). They are highly flexible molecules that form helical structures, giving them the ability to form a variety of different gels at room temperature. Since carrageenans are extracted from seaweeds, they are a vegetarian and vegan alternative to gelatin.¹

All carrageenans are high molecular weight polysaccharides consisting of galactose units and 3,6 anhydrogalactose, both sulfated and non-sulfated. Repetition units are joined by alternating alpha 1-3 and beta 1-4 glycosidic linkages.

There are three main commercial classes of carrageenan:

- **Kappa:** Forms strong, rigid gels
- **Iota:** Forms soft gels
- **Lambda:** Does not gel, mainly used to thicken dairy products

GPC/SEC-viscometry is the perfect method to detect even small differences between samples. The use of universal calibration enables determination of true molar masses. The additional results from the viscometer (e.g., Mark-Houwink coefficients K and α) provide structural information. A combination with an online multi-angle light scattering detector (MALLS) is also possible. This makes it possible to measure absolute molar masses if the refractive index increment, dn/dc , is precisely known.

Experimental

Table 1. Instrument and sample conditions.

	Conditions
Pump	Isocratic pump Flow rate: 1 mL/min Mobile phase: H ₂ O, 0.1 M LiNO ₃
Injection System	Autosampler Injection volume: 20 μ L
Columns	Agilent SUPREMA 10 μ m precolumn, 8 \times 50 mm (p/n SUA080510) Agilent SUPREMA 10 μ m linear XL, 8 \times 300 mm (p/n SUA083010LXL)
Temperature	23 °C
Sample Concentration	1.0 to 1.5 mg/mL
Calibration	Agilent ReadyCal-Kit Pullulan high (p/n PSS-PULKITR1H)
Detectors	Refractive index (RI) detector Differential viscometer Optional: Multi-angle light scattering detector (MALLS)
Software	Agilent WinGPC

Results and discussion

Sample preparation is extremely important for carrageenan characterization. Applying the correct temperature for a defined time interval using a strict SOP is required. In addition, the sample must be added slowly to the solvent while stirring. The samples must be injected immediately after the solution process is completed.

In this application note, two different carrageenans, Iota and Kappa, were investigated. A set of SUPREMA columns was used as stationary phase and aqueous 0.1 M lithium nitrate solution as the mobile phase. The molar masses were determined using two different methods:

- Conventional calibration with narrow pullulan molar mass standards yields apparent molar masses relative to pullulan.
- Universal calibration with an online differential viscometer based on pullulan molar mass standards yields true molar masses, the intrinsic viscosity, and the Mark-Houwink coefficient α .

The following figures show the results for both methods for Iota (blue) and Kappa (green) carrageenan.

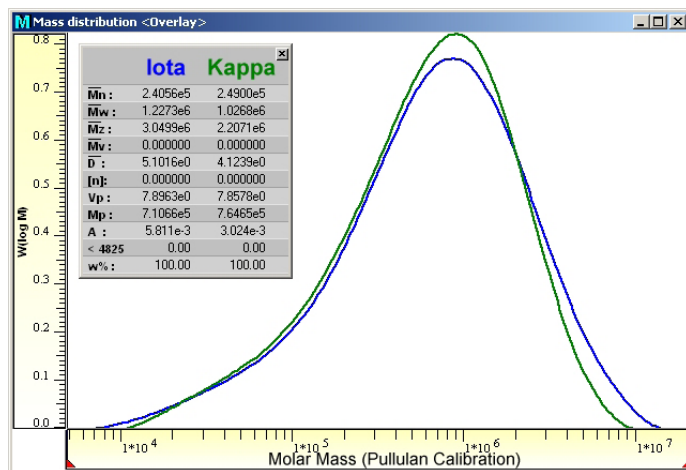


Figure 1. Molar mass distribution and averages for Iota and Kappa carrageenan from pullulan calibration.

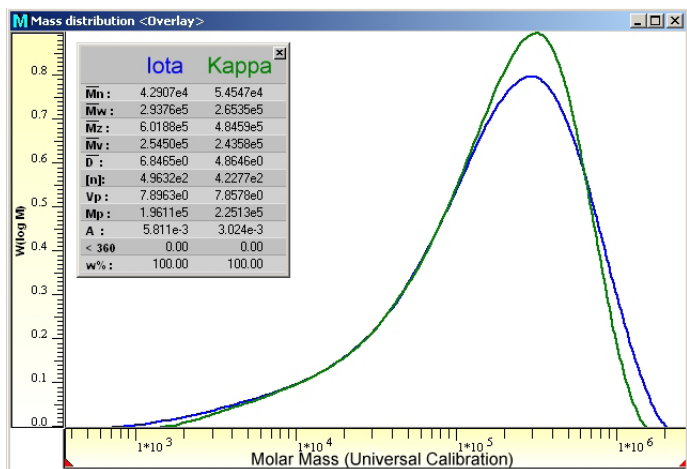


Figure 2. Molar mass distribution and averages for iota and kappa carrageenan from pullulan calibration.

Figure 1 shows the molar mass distribution and the molar mass averages obtained with conventional calibration with narrow pullulan molar mass standards.

Figure 2 shows the molar mass distribution and the molar mass averages obtained with universal calibration. The difference in the results is due to the fact that the molar masses obtained with conventional calibration are not the true results. Since carrageenan with a defined molar mass has a larger hydrodynamic volume than pullulan with the same molar mass, conventional calibration yields artificially high molar masses. It can be shown by GPC/SEC-viscometry that the true molar masses for both carrageenans are comparable and in the range of $M_w = 250,000$ to $300,000$ g/mol.

The Mark-Houwink plot comparison (Figure 3) shows subtle differences between the two samples other than just the molar mass distribution. Here, the logarithm of the molar masses from universal calibration (or from light scattering using the MALLS detector) is plotted versus the logarithm of the intrinsic viscosity measured online with a differential viscometer.

While the intrinsic viscosity increases linearly with the molar mass for the Iota carrageenan, a change of slope is measured for the Kappa carrageenan, reflecting a density and structure change.

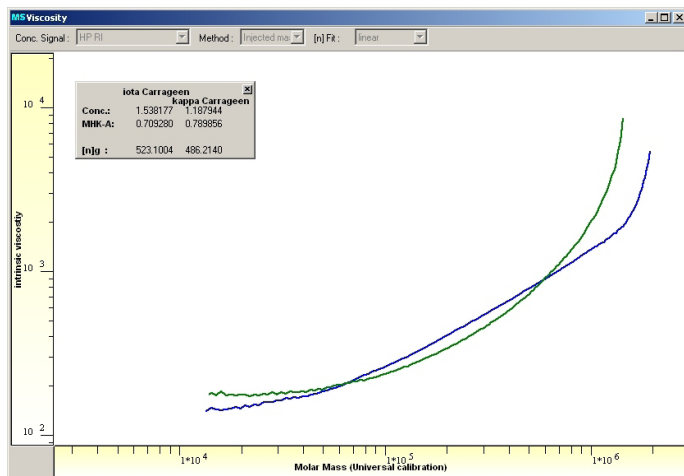


Figure 3. Mark-Houwink plot with intrinsic viscosity and Mark-Houwink coefficients for Iota (blue) and Kappa (green) carrageenan.

Further, the Iota carrageenan has an unexpectedly low Mark-Houwink coefficient α , showing that the molecules are less rigid and explaining the application difficulties this sample creates. The Iota carrageenan coefficient is in the range of the values for a random coil, while the Kappa coefficient tends towards more rigid structures like rods.

Conclusion

Reliable and robust GPC/SEC analysis of carrageenan is feasible using Agilent SUPREMA columns as stationary phase. Calibration with pullulan standards allows the determination of relative molar masses. The use of an online MALLS detector or a differential viscometer enables determination of true molar masses and carrageenan structural information.

Reference

- Haaris, P. Food Gels, **1990**, Springer Dordrecht.