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SEC-MALS analysis of hyaluronic acid and chondroitin sulphate

Your Challenge

- You deal with the separation and analysis of both high and low molecular weight polymer mixtures
- Current tools not delivering the sensitivity and accuracy your research demands?

Our Solution

EcoSEC Elite™ GPC system and LenS™3 MALS detector

- An optimized SEC-MALS solution for MW & R_g determination.

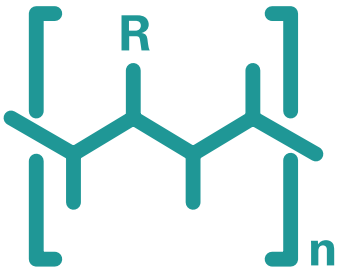
What was done?

- We analyzed Hyaluronic acid and chondroitin sulphate samples by SEC-MALS.

What was the result?

- SEC-MALS enabled accurate molecular weight, size, and impurity profiling of HA and CS.

SEC-MALS delivers precise MW and size for HA, CS, and blends—enabling reliable characterization and revealing impurities for advanced drug delivery and tissue engineering applications.



Analysis of hyaluronic acid

Your Benefit

Profiling of polymer mixtures and impurities with MALS detection

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Application Note

SEC-MALS characterization of hyaluronic acid and chondroitin sulphate

Hyaluronic acid or hyaluronan (HA) is a naturally occurring linear polysaccharide composed of alternating repeating D-glucuronic acid and D-N-acetylglucosamine units. HA contains between 500 to 50,000 monosaccharide units per molecule, thus has a molecular weight (MW) that can range from 10^4 to 10^7 Da with a dispersity (PDI) >1.3. This polysaccharide is widely distributed in mammalian cells and tissue but is primarily found in synovial fluid and loose connective tissues such as the umbilical cord, dermis, and arterial walls. HA plays a crucial role in the transport of macromolecules between cells, also known as tissue hydration due to its ability to swell, more specifically, its ability to absorb a large amount of water molecules. Moreover, the anti-bacterial properties of HA make it a potential candidate in the development of nanocomposites with chitosan for the application of wound and chronic ulcer dressing.

The physicochemical and biological properties give HA valuable features such as biocompatibility, viscoelasticity, lubricity, and immunostimulation. Hence, there is a high interest in using HA for drug delivery applications, as a surgical aid for ophthalmology, and for the treatment of arthritis in combination with chondroitin sulphate (CS). Moreover, HA, as a disaccharide formed from N-acetyl-b-galactosamine and D-glucuronic acid, provides elasticity to cartilage, cell development regulation, cell adhesion, etc. In combination, HA and CS are presently under investigation for tissue engineering applications.[1–3]

The unique physicochemical properties of HA are governed greatly by MW and distribution, requiring a reliable method for their analysis. Traditionally, the relative MW of HA has been determined using peak-position calibration (or conventional calibration) size exclusion chromatography (SEC) with a refractive index detector (RI) based on standards such as pullulan or dextran. However, to determine the true MW, we need to implement advanced detection — multi-angle light scattering (MALS). In this study, we utilized SEC-MALS for the analysis of HA (HA 1), two different grades of CS (CS 1 and CS 2), and HA samples mixed with the two grades of CS (HA 2 and HA 3).

Experimental Conditions:

System:	EcoSEC Elite™ (HLC-8420) GPC system
Columns:	2 × TSKgel® GMPW$_{XL}$
Mobile phase:	PBS buffer at pH 7.2 + 0.15 M NaCl
Flow rate:	0.5 mL/min
Injection vol.:	50 µL
Detectors:	RI and LenS™ 3 MALS detector
Software:	SECView™

The MALS detector was calibrated using a low polydispersity polyethylene oxide (PEO) with a MW of 23 kDa. A PEO solution with a concentration of 4 mg/mL in the mobile phase was injected at a volume of 50 µL. The specific refractive index increment (*dn/dc*) of the PEO standard is 0.132 mL/g. To determine the MW, signals from the low-angle detector at 10° (LALS) were used. The size, as the radius of gyration (R_g), was determined using signals from all three angles of the LenS3 MALS detector: LALS, right-angle light scattering (RALS) at 90°, and high-angle light scattering (HALS) at 170°. The *dn/dc* of the HA and CS samples used in this study were 0.155 mL/g and 0.137 mL/g, respectively.

Results and Discussion

Figure 1 shows the chromatograms obtained for the HA 1 samples from the RI detector and the three angles from the LenS3 MALS detector. To be able to use the LenS3 detector for the calculation of R_g above 50 nm, we have applied a new patented calculation method which allows the determination of larger Rg values with three angles by comparing the observed angular dissymmetry to that of various known conformations.[4] We obtained the weight average molecular weight M_w of HA in the range of 829 kDa to 8.4 MDa, while the R_g was in the range of 69 to 302 nm *(Figure 2)*. These results confirm that there are no structural changes within the sample, which would otherwise be reflected by a change in the slope of the distribution curves.

Figure 1. SEC-RI-MALS chromatogram of HA 1 sample

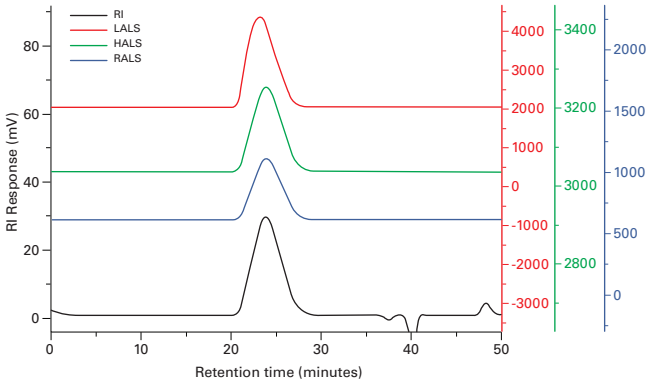
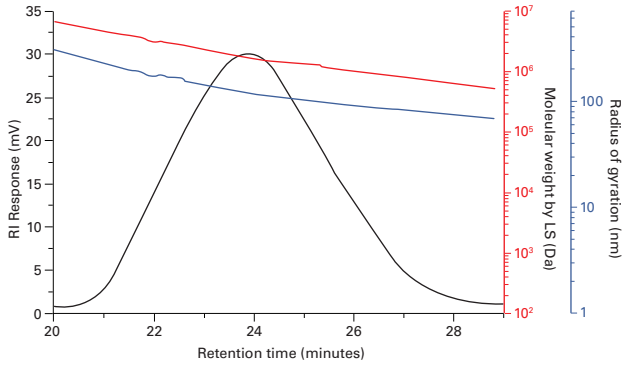
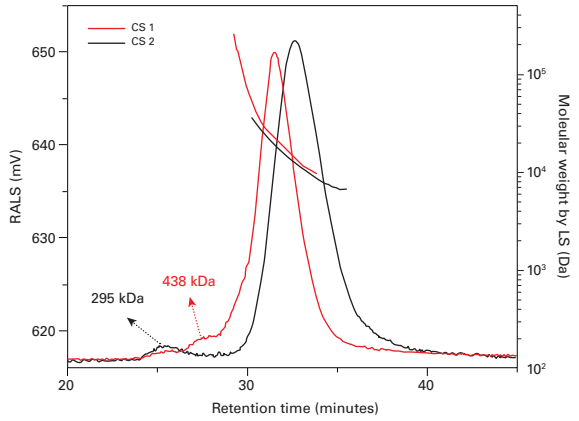


Figure 2. SEC-RI chromatogram, MW (red), and R_g (blue) distribution of HA 1 sample



From the elution profiles of CS samples, we can observe that CS1 elutes earlier than CS 2, indicating a higher M_w of CS 1 than CS 2, with 18 kDa and 11 kDa, respectively *(Figure 3 and Table 1)*. In the RALS chromatograms, due to the high sensitivity of the detector, we can also observe high M_w peaks considered impurities. In the case of CS 1, the impurity peak has a M_w of 438 kDa that slightly overlaps with the main peak and therefore provides a very high M_w for the CS 1 macromolecules eluted at the beginning of the distribution. In contrast to CS 1, in the CS 2 analysis, we determined the M_w of the impurity peak to be 295 kDa.

Figure 3. SEC-RALS chromatogram and MW distribution of CS1 (red) and CS 2 (black)



As described in the introduction, HA in combination with CS is incorporated in hydrogels, a polymeric network that absorbs large amounts of water and serves as tissue engineering constituents. We analyzed two HA samples (HA 2 and HA 3) containing different CS by SEC-RI-MALS. Well-separated HA and CS peaks can be seen in both HA 2 and HA 3 samples *(Figure 4)*. In the case of HA 2, both HA and CS peaks elute earlier compared to the corresponding peaks in HA 3, which indicates the presence of high M_w HA and CS. The HA segment in HA 2 sample shows a distribution from 6.1 MDa down to 169 kDa. The CS segment in HA 2 has a M_w of 17.6 kDa that corresponds to CS 1, whereas the CS segment in HA 3 showed a M_w of 10.4 kDa which corresponds to CS 2.

Figure 4. SEC-RI chromatogram and MW distribution of HA 2 (red) and HA 3 (black)

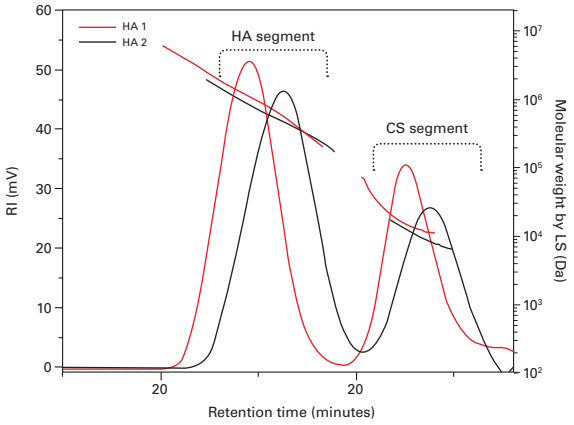


Table 1. Weight average MW (M_w), coefficient of variation (CV), polydispersity (Đ), and R_g of the HA, CS, and their combination samples

Sample		M_w (kDa)	CV (%)	Đ	R_g (nm)
CS 1		11,364	0.6	1.13	
CS 2		18,203	1.8	1.20	
HA 1		1,919,209	0.6	1.19	126
HA 2	HA segment	1,269,806	0.3	1.30	104
	CS segment	17,615	1.9	1.20	
HA 3	HA segment	563,869	0.1	1.34	69
	CS segment	10,464	3.3	1.70	

Conclusions

We successfully analyzed pure HA samples, two grades of CS samples, and HA samples mixed with different CS grades by SEC-RI-MALS using an EcoSEC GPC system and a LenS$_3$ MALS detector in combination with TSKgel GMPW$_{XL}$ columns. The pure HA samples were separated well, and accurate MW and R_g distribution analyses were performed. We observed high MW impurities in native CS, which we were able to identify thanks to the extreme sensitivity and minimal detector noise. In addition, we were able to separate CS and HA polymers in the mixture that allowed MW calculations of the two components individually. In conclusion, the use of the EcoSEC GPC system and LenS$_3$ MALS detector enables the determination of molecular weight averages using a method shown to be independent of polymer chemistry and architecture.

References:

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