APPLICATION NOTE

AN1303: Chondroitin sulfate analyzed by SEC-MALS-QELS

Summary

Chondroitin sulfate, a natural component of cartilage and connective tissue, is considered a helpful supplement for people suffering from arthritis. Different preparations of the product have different molecular properties, which are related in turn to therapeutic effectiveness. Such properties can be studied—and quantified—by light scattering analysis

Introduction

Two chondroitin sulfate samples were compared by means of a DAWN® multi-angle light scattering (MALS) instrument equipped with a WyattQELS™ quasi-elastic light scattering module (QELS, a.k.a. dynamic light scattering or DLS) and coupled to size- exclusion chromatography (SEC) for size-based separation. A downstream 3rd-party differential refractive index (dRI) detector served to measure concentration. MALS and dRI measurements are combined to calculate molar mass, absolutely, without reference to column calibration or elution properties. MALS can also determine molecular size (rms radius, R_g) from 10 – 500 nm for analysis of molecular conformation, while QELS is added to determine size (hydrodynamic radius, R_h) over a complementary range of 0.5- 240 nm. Data were collected and analyzed by ASTRA® software, including calculation of moments of the distribution such as the weight-average molar mass M_w and the polydispersity index M_w/M_n.

The addition of MALS and QELS to SEC overcomes the uncertainties associated with column calibration due to differences in elution properties between the sample and reference molecules: any discrepancies in terms of conformation, density and column packing interactions will provide erroneous results. Absolute analysis by MALS, based on first principles, also eliminates the need to perform frequent recalibration.

Results

Figure 1 shows chromatograms and molar masses obtained from MALS measurements for two samples, CS and CSLM. The DRI trace alone is shown for CSLM in red, while both DRI and MALS signals are shown for CS, in blue. The two samples exhibited differences in their molar mass distributions. Not all of these differences appear in standard SEC analysis, but are readily revealed in the SEC-MALS-QELS measurements.

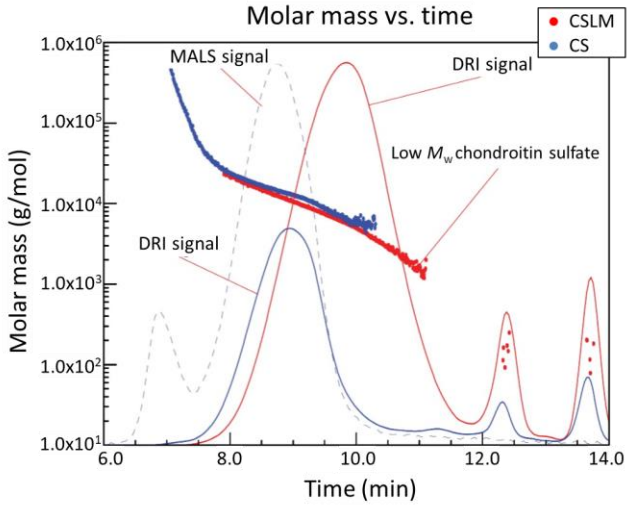

Figure 1. Molar mass versus time for two chondroitin sulfate samples measured by SEC with MALS detection. The dRI signals for both samples and the LS signal for one sample are superimposed.

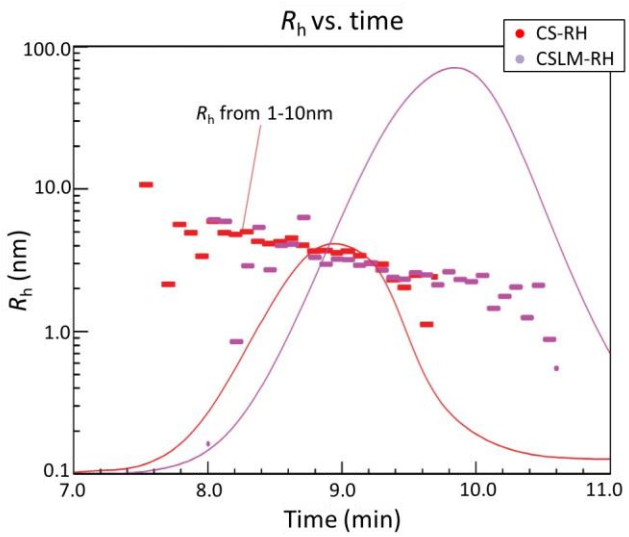

Figure 2. Hydrodynamic radius versus time superimposed with dRI signals for two samples measured by SEC with QELS.

MALS indicates that CS has a M_w of 14.8 ± 0.2 kDa and the polydispersity index (M_w /M_n) is 1.21 ± 0.03. The low molar mass chondroitin sulfate LMCS sample has a M_w of 6.9 ± 0.2 kDa and a polydispersity of 1.4 ± 0.1. MALS analysis in fact determines a full set of moments and polydispersities including M_n, M_w, M_z, M_w/M_n and M_z/M_w, as well as cumulative and differential molar mass distributions (not shown).

- Two additional peaks were observed in both samples at late elution times, though at different ratios to the main peak. The molar masses of these species are calculated by MALS as just a few hundred g/mol.
- In the CS sample, an early peak at 7 minutes is observed in the MALS trace but is barely present in the dRI trace. This high ratio of MALS to dRI is typical of high-molar-mass species, and in fact further analysis shows that this peak contains a high molar mass fraction, ranging from 40-500 kDa. The relatively low sensitivity of the 3rd-party dRI detector precluded analysis of the entire peak (the Wyatt Optilab® dRI detector, not used in this analysis, offers much higher sensitivity and would have enabled analysis of the entire high-M_w-fraction peak).

The size range for these chondroitin sulfate samples is less than the lower threshold for measurement of the rms radius R_g by MALS, 10 nm. Therefore, size measurements of CS and CSLM were accomplished using the WyattQELS module, embedded in the DAWN, which measures hydrodynamic radii down to 0.5 nm. QELS measurements are simultaneous with MALS measurements, and take place in the same flow cell with the same laser to illuminate the sample. Figure 2 shows the results for the size measurements of the two chondroitin sulfate samples. Hydrodynamic radii ranged from 1-10 nm.

Conclusions

The SEC-MALS-QELS method provides complete information about molar mass and radius of chondroitin sulfate, determining distributions and a full set of moments. The analysis indicated similarities and discrepancies between the two samples. The presence of a high-molecular-weight peak in CS was detected unequivocally by MALS, thanks to its high sensitivity to aggregates and high-molecular-weight species, but not by the concentration detector.

The fact that similar molar masses and sizes of each sample eluted at the same time is a good indication of similar conformation and chemical properties of CS and CSLM. Absolute macromolecular characterization by MALS and QELS provides rich, detailed information without the biases inherent in standard analytical SEC, for confidence in essential physiochemical properties.