Characterization of Hyaluronic Acid by AF4-MALS-RI equipped with Smart Stream Splitting

General Information ID0024

Application	Biopolymers, Cosmetics, Biologics
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Introduction

Hyaluronic acid (HA) is a ubiquitous, ultra-high molar mass polysaccharide found throughout the body and is a main component of the extracellular matrix. The pharmaceutical use of HA covers many areas including opthalmic surgery, osteoarthritis, wound care, dermal filling and other disciplines of medicine as well as finding many uses in high-value cosmetics. HA acts as a molecular "shock-absorber" and stabilizer for cells, and its visco-elastic properties are valuable for separating tissues and maintaining shape [1, 2, 3]. HA´s therapeutic effectiveness depends critically on the full range of molar masses present. Because of that, it is of paramount importance to characterize the complete molar mass distribution accurately. In many cases, a single intrinsic viscosity measurement is made and translated via a conversion factor into an average molar mass value [4]. This is prone to many potential errors and offers zero information on the range of molar mass values present.

The use of Gel Permeation Chromatography (GPC or SEC) to obtain a full molar mass distribution is possible, but for only a limited molar mass range due to the restricted upper size separation range of GPC columns. The sample must also be filtered prior to GPC analysis with the risk of loss of some larger species. In addition, the high sample viscosity requires very low concentrations to obtain accurate, repeatable measurements. This low concentration results in low detector signals, which in turn can make molar mass calculations by GPC difficult or unreliable.

An Asymmetrical Flow Field-Flow Fractionation (AF4) system coupled with Multi-Angle Light Scattering (MALS) and Refractive Index (RI) detection can be used to overcome all three of these issues and in this note we demonstrate the accurate and full measurement of the molar mass distribution for a complex HA sample.
The AF4 channel, unlike GPC columns, has no packing material limitations for the very largest molecules and does not require sample filtration prior to analysis. The low concentration and resulting low detector signals are mitigated in AF4 by using Smart Stream Splitting (S3) technology to enhance detector signals and achieve more accurate molar mass measurements [5]. During the separation the sample is usually located in the lower 5-10 % of the fractionation channel. Using Smart Stream Splitting by removing the sample free upper part of the channel flow, the sample concentration towards the detectors can be easily increased and thus the sensitivity of the fractionation (Figure 1).

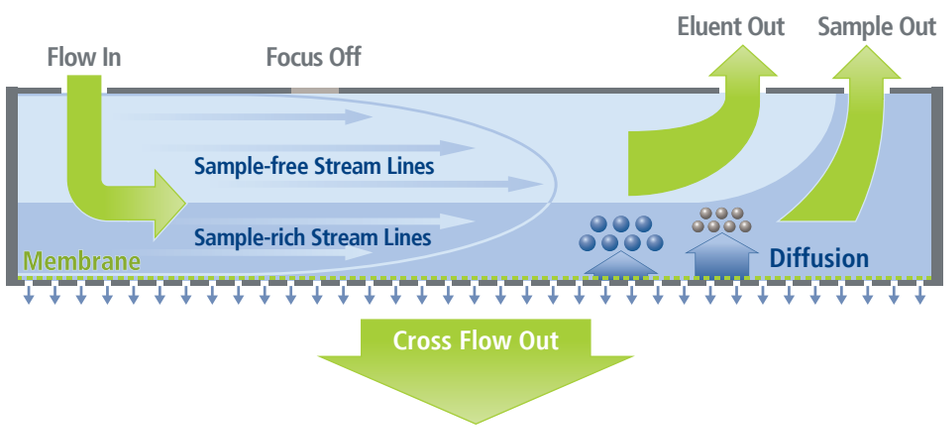

Figure 1: Schematic illustration of Smart Stream Splitting in AF4.

POSTNOVA

info@postnova.com
www.postnova.com

Postnova Analytics GmbH
86899 Landsberg, GERMANY
T: +49 8191 985 688 0

Postnova Analytics UK Ltd.
Worcestershire, WR14 3SZ, UK
T: +44 1684 585167

Postnova Analytics Inc.
Salt Lake City, UT 84102, USA
T: +1 801 521 2004

Postnova Northern Europe
00380 Helsinki, FINLAND
T: +358 9 8545 510

Experimental Details And Results

The HA sample was dissolved in 0.1 M $NaNO_3$, the same solution used as the AF4 eluent. The sample vial was placed on a shaker for 24 hours of gentle shaking. In order to avoid removing ultra-high molar mass polymers or aggregates from the sample, it was not filtered prior to injection into the AF4 system. An injection volume of 200 µL was used, at a sample concentration of 0.1 mg/mL. A 10 kDa RC membrane was used.

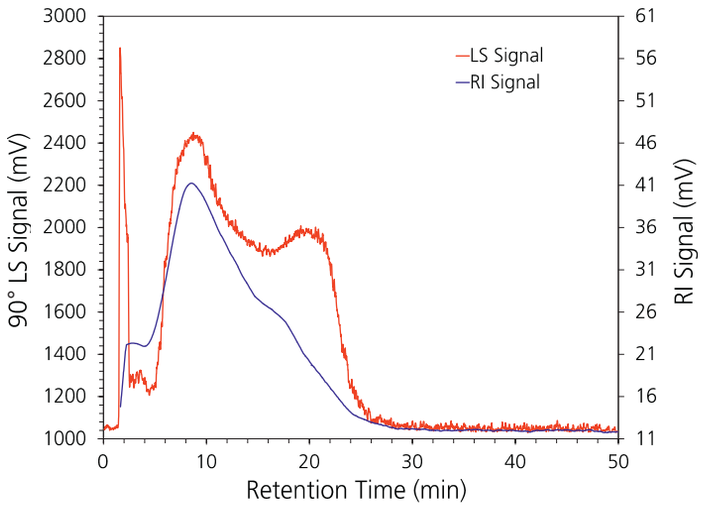

Figure 2: Overlay of 90° MALS (red trace) and RI (blue trace) signals of hyaluronic acid separated by AF4.

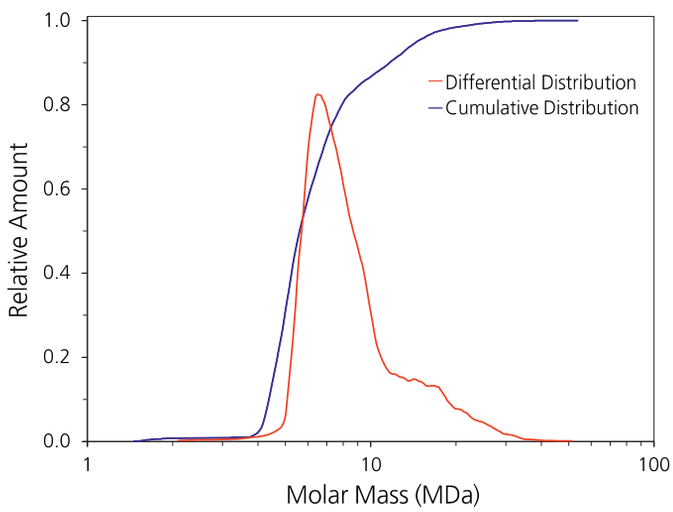

Figure 3: Molar Mass differential (red trace) and cumulative (blue trace) distributions for hyaluronic acid characterized by AF4-MALS.

Seen in Figure 2, the results of the measurement show a bimodal fractogram in both detectors. After the void peak (system peak @2 min) the main peak elutes, with a maximum at a retention time of about 10 minutes. This peak is followed by a smaller second peak with a maximum at a retention time about 19-20 minutes. The MALS signal for the second peak is larger relative to the RI signal than the MALS and RI signals for the first peak. This indicates that the second peak is made up of a higher molar mass fraction, generating much more light scattering than the smaller species in the first peak. The lower RI signal for the second peak indicates that the concentration of this larger molar mass fraction is lower than the concentration of molar mass fraction of the first peak, where the RI shows a higher signal. Using the MALS and RI data, molar mass distributions can be generated, as shown in Figure 3.

As seen in Figure 3, hyaluronic acid macromolecules were detected in the molar mass range of 4-13 MDa. The differential distribution in red has a distinct shoulder corresponding to the later-eluting, high molar mass fraction seen around 19-20 minutes in Figure 2. This peak is centered at about 11 MDa, much larger than the first peak which is centered at about 7 MDa.

Conclusion

AF4-MALS-RI-S3 is ideally suited for the accurate characterization of high molar mass hyaluronic acid, clearly overcoming the limitations of GPC analysis. AF4 provides a fast, gentle and nearly interaction-free separation without the interference of column stationary phase and without sample filtering prior to injection. AF4 can be easily coupled with many powerful detectors such as MALS, RI, UV, and more. With AF4 there is no size exclusion limit as in GPC, thus yielding more complete characterization of the sample. Lastly, the increase in detection sensitivity using Smart Stream Splitting enables more accurate characterization of the ultra-high molar mass polymers chains.

References

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POSTNOVA

info@postnova.com
www.postnova.com

Postnova Analytics GmbH
86899 Landsberg, GERMANY
T: +49 8191 985 688 0

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Salt Lake City, UT 84102, USA
T: +1 801 521 2004

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T: +358 9 8545 510