NovaSheet

Technology: AF4 - SLS/RI

Application: Hyaluronic Acid

Analysis Conditions / System Set-up

Sample:	Characterization of Hyaluronic Acid
Solvent/Eluent:	0.1 M NaNO3
Separation System:	Postnova AF1000 Series Assymmetric Flow FFF
Detector 1:	Postnova PN3000 Light Scattering Detector (SLS)
Detector 2:	Postnova PN3140 RI Detector

Hyaluronic Acid (HA) is a ubiquitous, ultra-high molecular weight polysaccharide, of particular importance in opthalmic surgery and other disciplines of medicine and therapy. HA acts as a molecular „shock-absorber" and stabilizer for cells and its visco-elastic properties are valuable for separating tissues and maintaining shape.

HA´s therapeutic effectiveness depends critically on the molecular weight: the higher the molecular weight, the longer its benefit. Because of that, it is of increasing importance to completely characterize the whole polymer and to know its complete range of molar mass.

Use of conventional analytical separation methods as GPC etc., is possible but for only a limited MW range. Especially when using GPC, the high and ultra-high molar masses are not accessible because of the limited separation range of the columns.

The difficult detection properties of hyaluronic acid required the use of our special detection enhancement capabilities, "Smart Stream Splitting" (patent pending). Only with this S3 technology were detection and Molar Mass calculations possible.

Sample Preparation

The sample was dissolved in 0.1M NaNO3, the same solution used as the eluent. The sample vial was placed on a shaker and measured after 24 hours of gently shaking. To get a "complete look" at the samples including aggregates etc., they were not filtered prior to injection into the AF4 system.

The results of the measurement show a bi-modal fractogram in both detectors. After the void peak (system peak @2 min) the main peak elutes, with a maximum at a retention time of ca. 10 min. This peak is followed by a smaller second peak whith a maximum at a retention time of 19 min (RI) and 20 min (SLS).

The SLS signal for the second peak is higher compared with the RI signal than the SLS signal for the first peak. That indicates, that the second peak is made up of a very high molecular weight fraction, with much more light scattering than the „smaller“ species in the first peak. The lower RI signals shows that the amount of this size fraction is smaller than the amount of size fraction of the first peak, where the RI shows a higher signal. Using the SLS and the RI data, molar mass calculations can be made as shown in Figure 2.

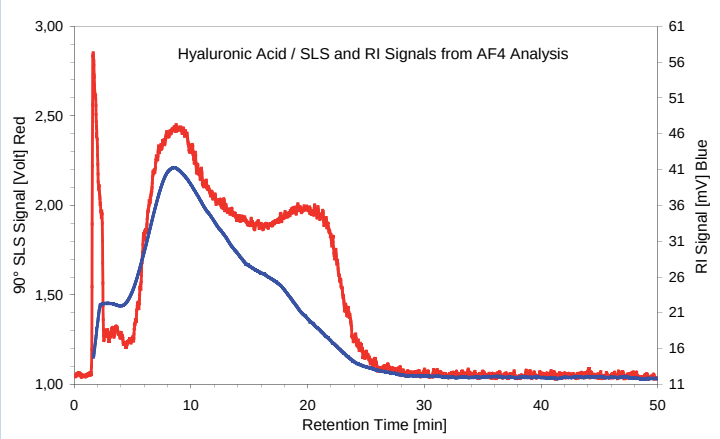

Fig 1: Overlay of 90° SLS (red) and RI (blue) signals

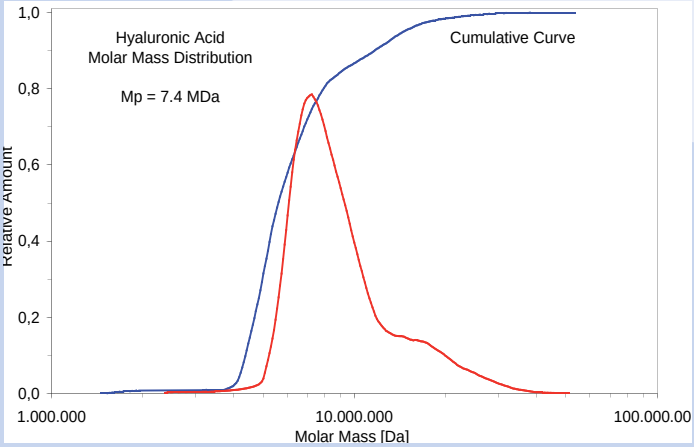

Fig 2: MW Distribution and cumulative mass plot

Ultra-high MW species were thus detected in the range of 4M – 13M Daltons using AF4-RI-SLS with Smart Stream Splitting (S3) technology.

In order to get a complete overview over the sample, Field-Flow Fractionation (FFF) is the method of choice. FFF uses an open flow channel for the separation without any stationary phase inside. Because of that FFF is able to separate all MW fractions - especially the high molecular weight fractions - and provide a complete MW distribution including the high molar mass fractions. This is important, as especially the high molar mass polymers are influential in the physical properties of the polymer. They may be present only in smaller amounts, but they effect the physical behavior of the polymer strongly.

Furthermore FFF separates with low or even no shear forces under very gentle conditions using solvents which are perfectly compatible with the sample. The solvents can be selected and adjusted due to the requirements of the sample and need not to be selected because of a special stationary phase or column requirements. Thus the most inert conditions can be applied to polymers when being characterized with FFF. In addition to that a variety of different detection system can be coupled on- and off-line to FFF. This enables to select the best suited detector for each application, to give the results needed.

Why use AF4 for Hyaluronic Acid Characterization?

- Very fast, gentle and interaction free separation without the effect of stationary phase.
- Separation of complex matrices without sample preparation or filtering prior to injection.
- Easy direct coupling of AF4 with many detection systems as on-line SLS, MS etc.
- No size exclusion limit as in GPC, thus yielding a complete overview of the sample.
- Increase of detection sensitivity by postnova´s patented S3 Technology enables characterization of ultra-high MW polymers which could not be analyzed with other systems before!