SEC Analysis of Hyaluronic Acid

Application Note

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Introduction

Hyaluronic acid is a high molecular weight linear polymer with repeating disaccharide units, each containing one residue of D-glucuronic acid and N-acetyl-D-glucosamine linked by glycosidic bonds. The unbranched chain forms a random coil with some stiffness. Hyaluronic acid has unique rheological properties because of its high molecular weight polyanionic character and a slight stiffness in its unbranched chain. Commercial preparations of hyaluronic acid are often isolated from the intercellular matrix of animal connective tissues, such as rooster combs, or fermented from bacteria. Rooster comb is the most common source. Hyaluronic acid is used in exclusive pharmaceutical products such as viscoelastic fluids in ophthalmological surgery and in viscosupplementary products for orthopaedic disorders. The application and effectiveness of hyaluronic acid is significantly affected by its molecular weight and molecular weight distribution, which can be readily determined by aqueous SEC. Four grades of hyaluronic acid received as ready-made solutions in saline were analyzed using Agilent PL aquagel-OH 40 and 60 15 µm columns. These columns were employed in order to avoid on-column shear degradation of these high molecular weight samples, and cover a molecular weight range from 10^4 to 10^7. The system was calibrated with narrow pullulan polysaccharide standards (Figure 1).

Verified for Agilent
1260 Infinity
GPC/SEC System

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Conditions

Samples: Four samples of hyaluronic acid
Columns: 1 x PL aquagel-OH 60 15 µm, 300 x 7.5 mm (p/n PL1149-6260) + 1 x PL aquagel-OH 40 15 µm, 300 x 7.5 mm (p/n PL1149-6240)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

Overlaid raw data chromatograms for the four hyaluronic acid samples are illustrated in Figure 2. Strong imbalance peaks were observed between the shipping solvent (saline) and the chromatographic eluent (nitrate buffer), but they were well resolved from the polymer peak.

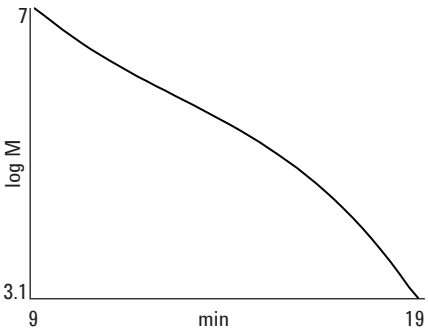

Figure 1. SEC calibration using pullulan standards

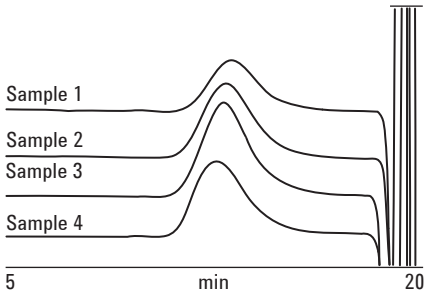

Figure 2. Raw data chromatograms of four hyaluronic acid samples

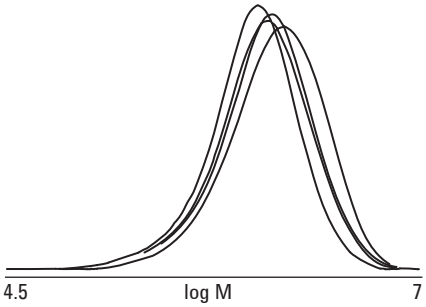

Figure 3. Overlay of the molecular weight distributions of four hyaluronic acid samples

Table 1. Mp, Mw, Mn and polydispersity values for the four hyaluronic samples

Sample	Mp	Mw	Mn	Polydispersity
1	1,677,000	1,680,050	1,084,400	1.5
2	1,444,400	1,465,700	932,200	1.6
3	1,351,400	1,398,500	882,500	1.6
4	1,201,100	1,210,800	826,800	1.5

Conclusion

SEC using PL aquagel-OH 40 and 60 15 µm columns accurately identified molecular weight distributions of four different samples of hyaluronic acid. Aqueous SEC with PL aquagel-OH columns provides information not only on the molecular weight of the polymer but also on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

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