Detailed Characterization of Sodium Hyaluronate using Aqueous Size Exclusion Chromatography with Advanced Detection

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Statement of Purpose: Sodium hyaluronate (HA) is a naturally occurring, non-branched polysaccharide that consists of alternately repeating disachharide units of D-glucuronic acid and N-acetylglucosamine linked by β 1-3 and β 1-4 glycosidic bonds. HA has attracted great interest and attention from various healthcare industries due to its unique properties.[1-2] For example, the recently launched multipurpose lens care solution Biotrue™ is inspired by the biology of your eyes™, and contains hyaluronic acid as a lubricant found naturally in your eyes. A more complete understanding of HA biopolymers has become increasingly critical in the healthcare industry as thorough characterization of raw materials, as well as finished products, helps promote product quality and process control.

Methods: On-line size exclusion chromatography with multi-angle light scattering (SEC-MALS)[3], SEC with triple detection (SEC-TD)[4], and single batch mode with MALS-dRI[5] were used to characterize HA raw materials. SEC-TD was also used to determine the molecular weight and concentration of HA in finished products, including marketed contact lens multipurpose solutions and contact lens packaging solutions. SEC-MALS work was performed using an Agilent 1200 series pump and autosampler, a Wyatt Optilab rEX as a concentration detector, and a HELEOS II MALS detector. SEC-TD analysis was done using a Polymer Laboratories Integrated PL-GPC50 Plus. Wyatt's Optilab and HELEOS detectors were utilized for the single batch mode analyses.

Results: The molecular weight and molecular weight distributions of HA raw materials determined by multiple techniques compared well with each other (within 15%). Solution properties, (RMS radius and the value of a) were also determined for HA raw materials. The values determined by MALS were significantly larger than those determined by triple detection. A wide range in molecular weight and concentration of HA used in currently marketed ophthalmic products was observed.

HA Lot	Mw (Da)	Mn (Da)	PD	Note
#1	1,346,000	818,000	1.65	SEC-TD
	1,550,000	900,000	1.71	SEC-MALS
	1,366,000	N/A	N/A	MALS
#2	1,675,000	1,105,000	1.52	SEC-TD
	1,915,000	1,210,000	1.60	SEC-MALS
	1,807,000	N/A	N/A	MALS

Table 1. Summary of molecular weights and molecular weight distributions of two lots of HA raw material using different analytical techniques

HA Lot	RMS radius (nm)	a (slope)	Note
#1	104.3	0.27	SEC-TD
	172.5	0.53	SEC-MALS
	168.5	N/A	MALS
#2	117.0	0.22	SEC-TD
	184.0	0.51	SEC-MALS
	195.0	N/A	MALS

Table 2. Summary of solution properties of two lots of HA raw material using different analytical techniques

Conclusions: We have shown that SEC with various advanced detection methods proves to be an effective tool to evaluate the solution properties of HA to ensure the quality of the products for specific claims.

References:

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