3rd International Conference and Exhibition on Advances in Chromatography & HPLC Techniques July 13-14, 2017 Berlin, Germany

Preparation and extensive characterization of hyaluronan with narrow molecular weight distribution

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The physicochemical properties and biological functions of hyaluronan (HA) are closely related to its molecular weight (MW) and molecular weight distribution (MWD). Therefore, it is crucially important to provide a reliable characterization of these parameters for proper use of HA and its degradation products in both chemical and clinical fields. In this study, we present a novel method for the preparation of HA fragments of defined size with narrow molecular weight distribution. The HA fractionation was performed using an anion-exchange chromatography and is applicable either after enzymatic or chemical hydrolysis of polymeric HA. Isolated fractions with a molecular weight ranging from 3000–420,000 g mol-1 were analyzed by size exclusion chromatography with multi-angle laser light scattering (SEC-MALLS). Hundred-milligram scale HA fragments were obtained from 5 g hyaluronan starting material. Independently on weight-average molecular weight (Mw), the polydispersity index (PDI) of the HA fractions was less than 1.23. The fractionation methodology can be easily up-scaled and is applicable on any negatively charged polymers. We have also found that PDI is insufficient to characterize almost monodisperse fractions and for proper material characterization we proposed a new characteristic termed "distribution angle Θ D", calculated from the slope of the cumulative molecular weight distribution curve. Compared to PDI, the distribution angle reflects more efficiently changes in size distribution and thus is highly recommended to be used along with Mw determination of any polymer. Apart from that, SEC separation conditions were exhaustively optimized with the great emphasis laid on the separation efficiency.



with narrow distribution. Illustration of novel parameter named "Distribution angle 66" is superimposed.

Biography

Martina Hermannova is currently working at Contipro (pharmaceutical company in the Czech Republic), where she is the Leader of 2 research groups focusing on Analytical Science, Department of Pharmacokinetics and Hyaluronan Fragments. She is also a member of Contipro Scientific Board. She has almost 15-years of experience in polymer separation and characterization by SEC-MALLS. In recent years, her research work focuses on elucidation of structural changes *in vitro* and *in vivo*.

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