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Letter to the Editor

Hyaluronan molecular weight and polydispersity in some commercial intra-articular injectable preparations and in synovial fluid

R. Mendichi[1] and L. Šoltés[2]

[1] Institute of Macromolecular Chemistry, National Research Council, IT-20133 Milano, Italy, e-mail: carmol@icm.mi.cnr.it

[2] Institute of Experimental Pharmacology, Slovak Academy of Sciences, SK-84216 Bratislava, Slovak Republic, e-mail: exfasolt@savba.sk

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Recently, Adam and Ghosh published an article in *Inflammation Research*, (2001) vol 50, pp 294–299 entitled "Hyaluronan molecular weight and polydispersity in some commercial intra-articular injectable preparations and in synovial fluid" [1]. In the introduction, the authors stated that "well-defined hyaluronan molecular weight standards are not commercially available". In Table 2 the molecular parameters for seven commercially available hyaluronan (HA) samples, determined by the authors on using a multi-angle laser-light scattering (MALLS) detector coupled to a gel-permeation chromatographic (GPC) system, are presented. By inspecting, for example, the HA sample polydispersities, the reader might assume that minimally four of the samples characterized are with regard to their M_w/M_n polydispersity values, 1.04, 1.05, 1.06, 1.08, classifiable as really very narrow – GPC standards/calibrants. Moreover, also further two HA samples would fully meet the criteria for GPC calibrants as to their M_w/M_n values, 1.15 and 1.29. Regarding their M_w parameters, these six HA samples – "GPC calibrants" – would cover the range of $2.34 \times 10^5 - 3.49 \times 10^6$ Da. However, on assuming that the M_w and M_n values established by GPC-MALLS are correct, it might have been desirable to (re)process the chromatographic records of the HA samples detected by using a UV photometer (441 UV absorbance detector), which they used for detecting on-line the sample concentration. (Since the UV light absorbance maximum of the HA samples is at ≈ 206 nm, the setting of 214 nm though not optimal may be satisfactory.) On processing the UV photometric records with calibration dependence of the GPC apparatus [logarithm of M_{peak} vs. elution volume; where the M_{peak} could be approximated by $(M_w \times M_n)^{1/2}$], another set of the output data could have been obtained. By comparing the molecular parameters for the seven commercially available HA samples established by the two qualitatively different approaches, the authors could either accept or refuse the validity of the molecular parameters of the seven HA samples. The finding of marked differences, or on the contrary, of a too close correspondence of the values M_{wi} and M_{ni} (GPC-MALLS) with those of M_{wj} and M_{nj} (GPC-UV), would have indicated whether the Superose 6 column used does or does not provide proper separation performance for the high molecular weight HA samples investigated.

We would like to comment on the above mentioned problem concerning the GPC analysis validity. The biological source of all seven HA samples investigated was obtained from rooster comb or by bacterial fermentation. It can be practically excluded that the polysaccharide, produced in vivo by any of the above biological systems, might be molecularly homogenous, monodisperse. At present, on producing the (commercially available) HA samples, a whole range of physico-chemical procedures has to be applied, such as protein enzymatic digestion, HA ion-pair precipitation, membrane/molecular ultrafiltration, HA non-solvent precipitation and/or lyophilization. Of these, at least the last mentioned procedure results in degradation of high molecular weight HA samples [2], and in the case of monodisperse polymers, this degradation is in turn invariably associated with an increase in their M_w/M_n parameters. Thus, we suppose that the true M_w/M_n values of the HA samples must be (markedly) higher than the reported ones, i.e. 1.04, 1.05, 1.06, 1.08 [1]. Along with a potentially improper separation performance of the Superose 6 column, another possible source of discrepancy might be the exploitation of the MALLS detector for the (on-line) estimation of the HA biopolymer M_n molecular weight values. There are namely several indications that the values of the M_w/M_n polydispersity parameters, determined by the GPC-MALLS system are frequently underestimated [3, 4]. The M_w/M_n values generated by exploiting the GPC-MALLS arrangement would thus require a more critical evaluation.

Correspondence to: R. Mendichi

References

[1] Adam N, Ghosh P. Hyaluronan molecular weight and polydispersity in some commercial intra-articular injectable preparations and in synovial fluid. Inflamm Res 2001; 50: 294–9.
[2] Tokita Y, Ohshima K, Okamoto A. Degradation of hyaluronic acid during freeze drying. Polym Degrad Stab 1997; 55: 159–64.
[3] Mendichi R, Giacometti Schieroni A, Grassi C, Re A. Characterization of ultra-high molar mass hyaluronan: 1. Off-line static methods. Polymer 1998; 39: 6611–20.
[4] Mendichi R, Giacometti Schieroni A. Fractionation and characterization of ultra-high molar mass hyaluronan: 2. On-line size exclusion chromatography methods. Polymer, paper submitted.