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Short communication

Long-term degradation study of hyaluronic acid in aqueous solutions without protection against microorganisms

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ABSTRACT

The degradation of hyaluronan (HA) of different molecular weights (M_w 14.3, 267.2 and 1160.6 kDa, measured for fresh solutions, before degradation) was studied in aqueous solutions by SEC-MALLS determination of molecular mass, polydispersity and conformation parameters. The solutions were stored either at laboratory or refrigerator temperatures for two months. After this period the weight average molecular weight decreased by 90% for 14.3 kDa, 95% for 267.2 kDa and 71% for 1160.6 kDa hyaluronan (room temperature) or 5.6% for 14.3 kDa, 6.2% for 267.2 kDa and 7.7% for 1160.6 kDa hyaluronan (refrigerator temperature).

The hyaluronan aqueous solutions studied did not contain sodium azide or other protectants against microorganisms, because the aim of our study was to assess the degradation in solutions to be used in medicine or cosmetics (without any compounds that are poisonous or toxic for the human body). The solvent used to prepare the samples was pure water.

The polydispersity of all the samples remained unaltered during the entire degradation at both temperatures. This indicates a non-random mechanism of degradation.

Hyaluronan (HA) is a linear natural polysaccharide of the glycosaminoglycans family. Its chemical structure comprises disaccharide units composed of β-D-glucuronic acid and *N*-acetyl β-D-glucosamine, which are alternately linked through (1 → 3) and (1 → 4) glycosidic bonds [→4)-β-D-GlcA-(1 → 3)-β-D-GlcNAc-(1→] (Lapcík, De Smedt, Demeester, & Chabrecek, 1998). Hyaluronan occurs naturally in the synovial fluid that surrounds the joints. It is one of the most important polysaccharides originated in mammalian bodies. Hyaluronic acid has many applications in medicine and cosmetics. The consistency of hyaluronan becomes thinner in individuals with osteoarthritis. Hyaluronic acid has been used for osteoarthritis treatment, by direct injection into the knee joint.

It is important to know details of hyaluronic acid degradation in aqueous solutions to be used in medicine and cosmetics, without any protection against microorganisms. In other words, it is important to know how long such solutions can be stored without significant degradation of the polymer.

Hyaluronic acid of different molecular weight plays different roles in the human body. High molecular weight hyaluronan organizes the extracellular matrix, while low molecular weight hyaluronan can be found in injured tissues or in tumours (Qhattal & Liu, 2011; Noble, 2002; Stern, Kogan, Jedrzejas, & Šoltés, 2007; Kogan, Šoltés, Stern, & Gemeiner, 2007). For this reason it is important to study the degradation of both high and low molecular weight hyaluronan in aqueous solutions without the addition of other compounds which prevent the growth of microorganisms (such as sodium azide).

In our study we used three hyaluronan samples of different molecular weight:

- HA 90–130 kDa (Producer determined weight average molecular weight M_w = 117 kDa)
- HA 300–500 kDa (Producer determined weight average molecular weight M_w = 458 kDa)
- HA 1750 kDa (Producer determined weight average molecular weight M_w = 1669 kDa)

All samples studied were produced by Contipro, Czech Republic. Many research teams have reported on studies of hyaluronic acid degradation (Lapcík et al., 1998; Tokita & Okamoto, 1995; Reed & Reed, 1989; Bothner, Waaler, & Wik, 1988; Reháková, Bakoš,

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Table 1
Number and weight average molecular weight and polydispersity (M_w/M_n) determined for fresh hyaluronan solutions.

Sample name	M_n kDa	M_w kDa	M_w/M_n
HA 90–130 kDa	10.6	14.3	1.35
HA 300–500 kDa	250.8	267.2	1.07
HA 1750 kDa	1132.3	1160.6	1.03

Soldán, & Vizárová, 1994; Vercruysse, Lauwers, & Demeester, 1995; Dřímalová, Velebný, Sasinková, Hromádková, & Ebringerová, 2005). However, there is little information on the stability of hyaluronan degradation in aqueous solutions stored in laboratories for various purposes.

Hyaluronan degrades in a non-random way when exposed to ultrasound (Vercruysse et al., 1995; Dřímalová et al., 2005). We observed the same for the long-term degradation of hyaluronan in aqueous solutions, in our previous studies (Mondek, Kalina, Simulescu, & Pekař, 2015; Simulescu, Mondek, Kalina, & Pekař, 2015; Mondek, Simulescu, & Pekař, 2014) and also in the present study; the polydispersity of all samples remained unaltered throughout the entire degradation, at both temperatures (room temperature and refrigerator temperature). This excludes the random scission mechanism of degradation.

We studied the degradation of hyaluronic acid by molecular weight determination, using the SEC-MALLS method (Zimm, 1948; Wyatt, 1993). All SEC-MALLS measurements were performed at 25 °C. The solutions of hyaluronic acid were prepared with pure water. The mobile phase used was 0.1 M $NaNO_3$ aqueous solution. All the measurements were repeated at least four times, for each period of degradation, as well as for the fresh solutions.

The SEC-MALLS method allows the determination of molecular weight, polydispersity and polymer conformation. The shape of the polymer can be obtained from a Mark–Houwink–Sakurada plot (Harding, 1992; Wagner & Verdier, 1978; Han, 1979; Hiemenz & Timothy, 2007; Harding, 1997). For molecular weight calculation the Zimm model was used (Zimm, 1948; Wyatt, 1993). The refractive index increment (dn/dc) value used to calculate the molecular weight was 0.165 mL/g (Huglin, 1989; www.wyatt.de, 2015).

When the fresh solutions of the samples were measured, lower molecular weight values were obtained for all samples studied (Table 1).

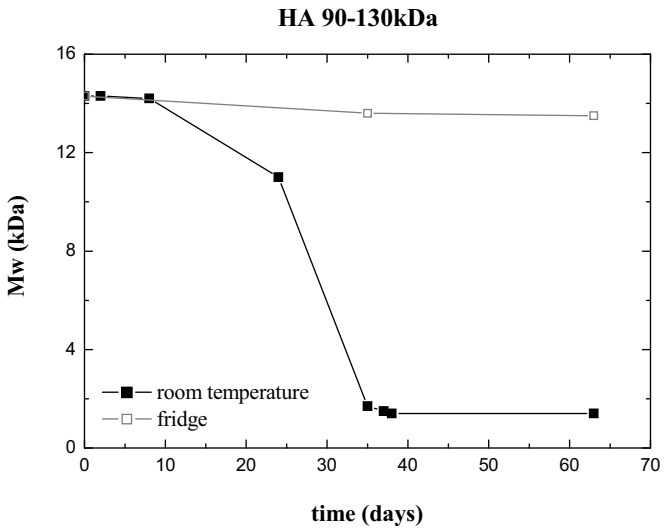

Fig. 2. The weight average molecular weight decrease of HA 90–130 kDa at room temperature and refrigerated.

The degradation of hyaluronan samples first occurred in the solid state during the storage. A significant difference was observed when the values obtained for the fresh measured solutions (Table 1) were compared with the weight average molecular weight values given by the producer. For example, for the highest molecular weight hyaluronan studied here (HA 1750 kDa), the mass loss in powder form during the storage was 34% of the initial value. It should be mentioned that all solid materials were kept at refrigerator temperature prior to analysis (4 °C). After measuring the fresh hyaluronic acid solutions, a quantity of the prepared solutions was kept at room temperature and another quantity was kept in the refrigerator. Several chromatograms obtained by using the SEC-MALLS method for the analyzed samples are shown in Fig. 1.

The difference in the intensity of the peaks shown in Fig. 1 was caused by M_w and concentration. We used a concentration of 5 mg/ml for HA 90–130 kDa and for HA 300–500 kDa samples, and a concentration of 1 mg/ml for HA 1750 kDa sample.

At room temperature the degradation showed three phases (Figs. 2–4):

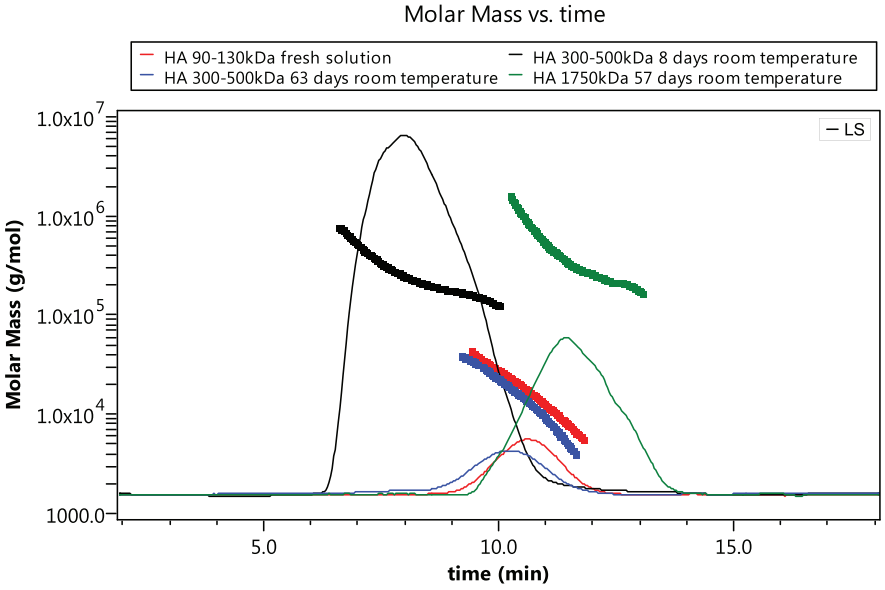

Fig. 1. Examples of chromatograms obtained with SEC-MALLS for some of the analyzed samples.