Polymer Testing 32 (2013) 1322–1325

Contents lists available at ScienceDirect

Polymer Testing

journal homepage: www.elsevier.com/locate/polytest

Material characterisation

Structural elucidation of hyaluronic acid gels after heat sterilisation

Andrea Szabó [a], Barnabás Szabó [b,c], Emese Balogh [d], Romána Zelkó [c,*], István Antal [d]

[a] Neustadt Apotheke, Kreyssigstr. 19, D-55118 Mainz, Germany
[b] Gedeon Richter Plc., Formulation R&D, Gyömrői Str. 19–21, H-1103 Budapest, Hungary
[c] University Pharmacy Department of Pharmacy Administration, Semmelweis University, Hőgyes Endre Str. 7–9, H-1092 Budapest, Hungary
[d] Department of Pharmaceutics, Semmelweis University, Hőgyes E. Str. 7–9, H-1092 Budapest, Hungary

ARTICLE INFO

Article history:
Received 9 July 2013
Accepted 17 August 2013

Keywords:
Hyaluronic acid
Hydrogel
Heat sterilisation
Positron annihilation lifetime spectroscopy

ABSTRACT

The influence of heat sterilisation and hyaluronic acid (HA) concentration on the micro- and macrostructure of HA hydrogels was investigated. HA hydrogels of different concentrations were prepared and heat sterilised. The microstructures of the polymer gels were characterised by positron annihilation lifetime spectroscopy (PALS) based on their ortho-positronium lifetime values and distributions, while their macrostructures were characterised by rheological measurements. As expected, the heat sterilisation modified both the micro- and macrostructures of the gels. The HA concentration was also observed to influence the hydrogel structure. At a concentration of 7.5 mg/ml HA, the thermal treatment did not cause significant microstructural changes, and the viscoelastic properties of the treated gels were similar to those of the untreated samples.

1. Introduction

Hyaluronic acid (HA), hyaluronan or sodium hyaluronate are of importance in epidermal pathology, tissue engineering, ophthalmic surgery, drug delivery systems, pulmonary and joint pathologies and aesthetic surgery [1]. HA is the most important substance in the synovial fluid of articular joints, acting as a lubricant for the cartilage and regulating the viscosity of the synovial fluid. Healthy joints contain approximately 2.26 g/l HA [2]. If the joints are in motion, HA has a low viscosity in the synovial fluid; at rest, it is very viscous.

HA macromolecular chains are built from D-glucuronic acid and *N*-acetyl-D-glucosamine disaccharides. A molecule of 10 million Da contains 25,000 disaccharide units in the chains, which are held together by hydrophobic bonds (Romagnoli et al., 2008). The polysaccharide chains are linear and unbranched and roll up into a coil conformation. These coils can straighten, and this behaviour is the mechanism of action behind viscosupplementation. The length of the polysaccharide chains and the M_w of the HA are very different in various tissues. In normal tissues, a molecule of HA (10 million Da) has a thickness of 1 nm and a length of 25 μm [3]. In the biomatrix, HA has an M_w in the range of 6 to 12 million Da [4]. The molecular weight of HA is approximately 7 million Da in healthy joints and 4.8 million Da in unhealthy joints [5]. The viscoelastic properties of HA under 1 million Da are negligible because of the M_w in inflamed joints (4.8 million Da). For that purpose, cross-linked HA is in demand (such as Hylan G-F 20, with an M_w of 6-7 million Da) for intra-articular injections [6].

Due to the chemical structure of the polymer, HA shows interesting rheological properties. The viscoelastic

* Corresponding author. Tel./fax: +36 1 2170927.
E-mail address: zelko.romana@pharma.semmelweis-univ.hu (R. Zelkó).

0142-9418/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymertesting.2013.08.006

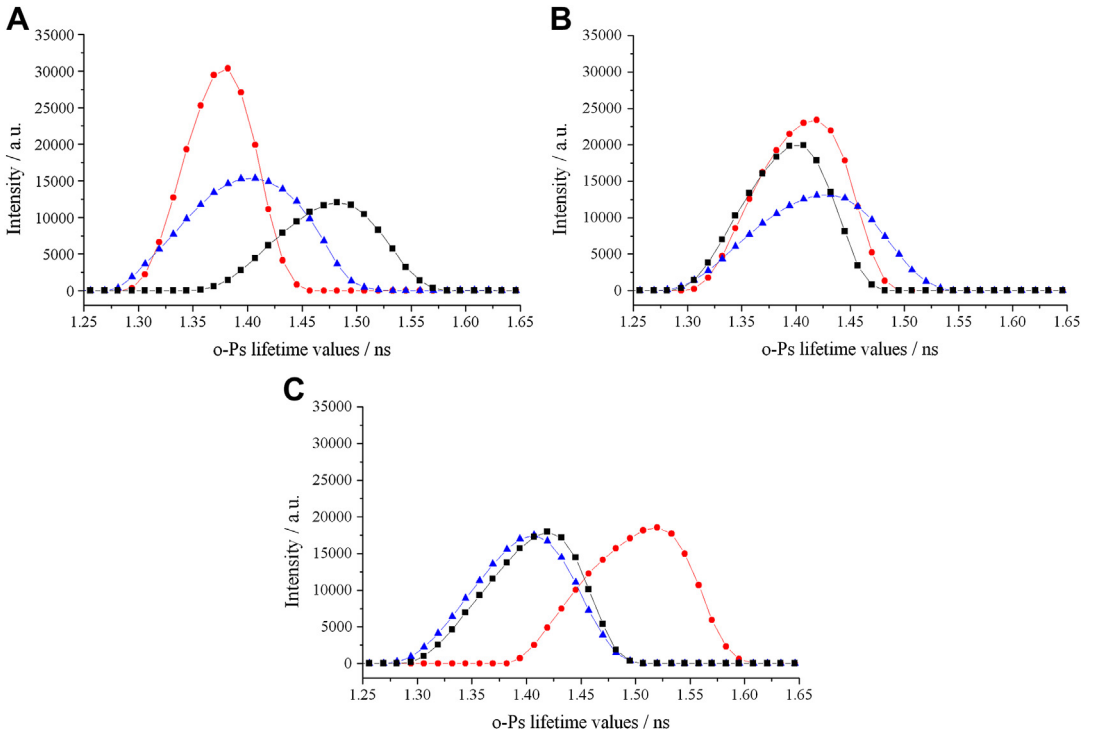

Fig. 1. Distribution curves of the HA gels of different concentrations (black: untreated sample, red: treated sample, blue: one week after the treatment) A – 5 mg/ml HA B – 7.5 mg/ml HA C – 10 mg/ml HA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

behaviour of HA in solution changes with exposure to different shear rates. The shear rate is dependent on the dynamic elastic and viscous moduli. The crossover point of these two moduli can be used for the rheological characterisation of HA. Above this frequency, the solution has elastic properties, and below this frequency it shows viscous behaviour [4]. HA is a very unstable molecule. If HA in solution is warmed to 100 °C, the bonds between the chains are damaged, and the M_w and viscosity decrease. Experiments with "ready-to-use pre-filled" HA syringes showed that, by autoclaving for 20 min, the M_w decreased from 1.4 million Da to 0.8 million Da. This is the reason that sterilisation of HA in solution is performed by sterile filtration, and the solid HA by gamma radiation (5–10 kGy) [5]. However, data from simulated sterilisation conditions suggest that higher concentrations of HA may have a protective effect on the stability of the long-chain molecule, such that heat treatment would not denature the hydrogel [7].

Variation of the free volume with temperature in the sol-gel transition of 2% (w/w) κ-carrageenan showed the sol-gel transition to be an onset of the decreasing percentage of the free volume [8]. Positron annihilation lifetime spectroscopy (PALS) is able to give important information about the free volume properties of polymers related to their molecular dynamics, volume relaxation and physical ageing.

Despite the experiences concerning the thermal instability of HA under heat sterilisation, the primary aim of the present study was to track the changes in the macro and supramolecular structure of HA gels caused by the heat effect of autoclaving. In addition, the correlation between the free volume changes of HA macromolecules measured by PALS and the rheological properties of HA gels of various concentrations was determined.

2. Materials and methods

2.1. Preparation of HA gels

HA sodium salt of low molecular weight (M_w = 1.500 kDa) and of pharmaceutical grade was obtained from Gedeon Richter Ltd., Hungary. It was dissolved at different concentrations using high intensity

Table 1
Summarised results from RESOLUTION computer code (o-Ps lifetimes in ns).

Concentrations	Untreated	Treated	After 1week	Dried	Treated, dried
5 mg/ml	1430 ± 15	1380 ± 11	1389 ± 11	1102 ± 37	1093 ± 40
7.5 mg/ml	1393 ± 11	1396 ± 11	1405 ± 13	1095 ± 21	1094 ± 25
10 mg/ml	1400 ± 12	1465 ± 12	1390 ± 12	1118 ± 23	1131 ± 16