Journal of Pharmaceutical and Biomedical Analysis 104 (2015) 12–16

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Microstructural analysis of the fast gelling freeze-dried sodium hyaluronate

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ARTICLE INFO

Article history:
Received 21 August 2014
Received in revised form 27 October 2014
Accepted 3 November 2014
Available online 11 November 2014

Keywords:
Sodium hyaluronate (NaHA)
Freeze-drying
Fast gelling
Viscoelasticity
Positron annihilation lifetime spectroscopy

ABSTRACT

Although sodium hyaluronate is a very unstable and heat sensitive molecule, it can remain relatively stable during the freeze-drying process. Aqueous sodium hyaluronate (NaHA) gels were prepared and the obtained samples were freeze-dried. The freeze-dried NaHA samples showed fast gelling ability meanwhile preserved their initial viscoelasticity even after reconstitution. The microstructure of gels obtained from raw substance and freeze-dried NaHA samples was characterized with positron annihilation lifetime spectroscopy and X-ray diffraction patterns while their functionality-related macrostructural properties were tested based on their rheological behavior. The presence of phosphate salts improved the formation of ordered supramolecular structure retaining water in the free volume holes of the polymer chains characterized with decreased ortho-positronium lifetime values. This property may be advantageous in the development of a freeze-dried NaHA injection dosage form.

1. Introduction

Hyaluronic acid (HA) is essential in the synovial fluid of articular joints. It is the lubricant for the cartilage and responsible for the viscosity of the synovium. In physiological conditions, HA appears in the form of sodium salt, negatively charged and highly hydrophilic [1]. HA is built from polysaccharide chains that are linear and unbranched and roll up to coils. These coils can straighten out. This behavior of the macromolecule serves as the basis of the viscosupplementation [2,3]. The viscous and elastic behavior of HA solution are changed by different shear rates. This network, when sheared by flow or oscillation movements, can dissipate the energy in viscous flow or store it as elastic deformation [4]. This shear rate (frequency in Hz) is depending on dynamic elastic and viscous moduli. The crossover point of these two moduli can use for the rheological characterization of HA. Above this frequency the solution has elastic properties and below this frequency it has viscous behavior [3]. The more suitable viscosupplementation product is the one with an elastic component similar to or greater than that of healthy young synovial fluid [5].

HA is a very unstable and heat sensitive molecule, the molecular mass and the viscosity may be decreased due to the damage of bonds in the polymeric chains [6]. It is well known that hyaluronates are susceptible to degradation under a variety of conditions such as acid hydrolysis [7,8], oxidative depolymerization reactions [9,10], and sonication [11]. Sterility for the injection dosage forms of the hyaluronic acid derivatives can be achieved only by sterile filtration of the solution or with the application of sterile solid HA treated by gamma irradiation (5–10 kGy) [12]. The investigations of Szabó et al. [13] showed that the heat sterilization modified both of the micro- and macrostructures of the NaHA gels depending on the concentration and therefore it is not a suitable method to achieve the sterility.

Freeze-drying process, also lyophilization is a treatment to get chemically stable and sensitive substances more sustainable and it should be taken into consideration that it is also able to reduce the number of the bacteria in the formulations because of the very low temperature during the treatment. However the freeze-drying and subsequent rehydration of thermosensitive polymer gels, like hydroxypropyl cellulose (HPC), could alter the microstructural properties of the gels in a way that leads to rapid shrinking rates [14]. Earlier data indicated that lyophilization of hyaluronates as the free acid, in contrast to the sodium salt form (NaHA), can have a detrimental effect on their physical characteristics [15].

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http://dx.doi.org/10.1016/j.jpba.2014.11.005
0731-7085/

The data of Doherty et al. [15] indicate that the free acid form of medium molecular mass hyaluronate exhibited marked changes in molecular mass during lyophilization whereas the NaHA appeared unchanged. Measurements showed that the evolution of carbohydrate radicals on freeze-drying of HA is more than three times that of NaHA, which explained that HA is structurally less stable than NaHA [16]. Further, the calculations of Tokita et al. suggest that HA is much more labile against hydrogen abstraction as compared to NaHA. The results of Wedlock et al. suggest that the structural stability of HA against a distortion induced by freeze-drying is lower than that of NaHA [17]. Concerning the stability of hyaluronate during freeze-drying, significant concentration effects would occur with respect to the hyaluronate, and it is possible that a combination of concentration effects and shifts in the effective pH of the amorphous reaction matrix could contribute to the apparent changes in molecular mass. Furthermore, this hypothesis concerning concentration and pH effects is consistent with the observed stability of NaHA to lyophilization as the pH of those solutions was near neutral [15]. The changes of the NaHA microstructure meanwhile of the lyophilization were studied by positron annihilation lifetime spectroscopy (PALS) that gives information about the free volume of the polymer systems. This measurement can be used for the analysis of amorphous materials like NaHA [18].

The aim of the study was to evaluate the relationship between the functionality-related macrostructural characteristics of high molecular weight freeze-dried NaHA with their microstructural properties. The functionality of NaHA was tested with its viscoelastic behavior and gel forming ability, while microstructural analysis was performed through morphological study with scanning electron microscopy, evaluation of supramolecular ordering by positron annihilation lifetime spectroscopy and X-ray powder diffraction.

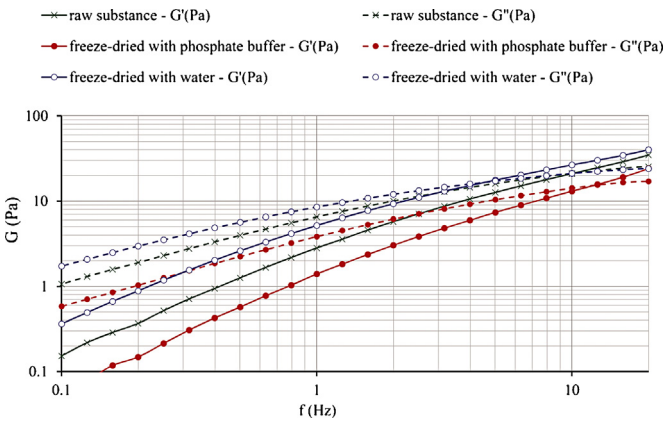

Fig. 1. Effect of freeze-drying on the viscoelastic behavior of NaHA gels.

2. Materials and methods

2.1. Preparation of gel samples

Pharmaceutical grade NaHA (M_w = 1.500 kDa) was obtained from Gedeon Richter Ltd., Hungary. NaHA gel samples of 10 mg/ml concentration were prepared allowing 24 h for swelling in sodium phosphate buffer adjusted to the physiological range (218–286 mOsm/l, pH 7.44–7.45).

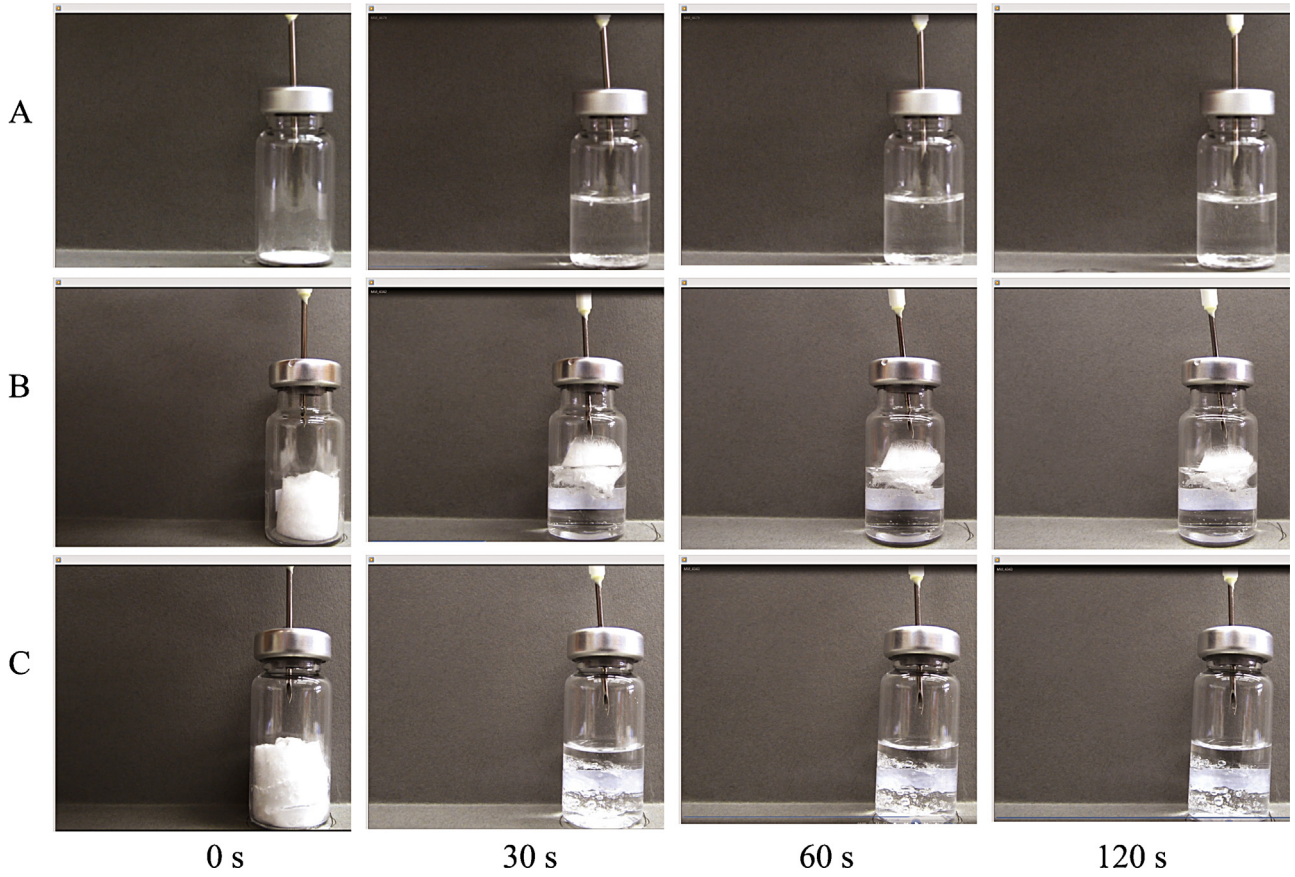

Fig. 2. Tracking of the dissolution and gelling of freeze-dried NaHA during reconstitution with water (A, raw substance in powder form; B, freeze dried gel prepared with water; C, freeze dried gel prepared with aqueous phosphate buffer).