Carbohydrate Polymers 153 (2016) 275–283

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

# Optimization of hyaluronan-based eye drop formulations

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ARTICLE INFO

*Article history:*
Received 26 April 2016
Received in revised form 22 July 2016
Accepted 25 July 2016
Available online 29 July 2016

*Keywords:*
Hyaluronan
Eye drops
Viscosity
Mucoadhesiveness
Corneal epithelial cells

ABSTRACT

Hyaluronan (HA) is frequently incorporated in eye drops to extend the pre-corneal residence time, due to its viscosifying and mucoadhesive properties. Hydrodynamic and rheological evaluations of commercial products are first accomplished revealing molecular weights varying from about 360 to about 1200 kDa and viscosity values in the range 3.7–24.2 mPa s. The latter suggest that most products could be optimized towards resistance to drainage from the ocular surface. Then, a study aiming to maximize the viscosity and mucoadhesiveness of HA-based preparations is performed. The effect of polymer chain length and concentration is investigated. For the whole range of molecular weights encountered in commercial products, the concentration maximizing performance is identified. Such concentration varies from 0.3 (wt%) for a 1100 kDa HA up to 1.0 (wt%) for a 250 kDa HA, which is 3-fold higher than the highest concentration on the market. The viscosity and mucoadhesion profiles of optimized formulations are superior than commercial products, especially under conditions simulating *in vivo* blinking. Thus longer retention on the corneal epithelium can be predicted. An enhanced capacity to protect corneal porcine epithelial cells from dehydration is also demonstrated *in vitro*. Overall, the results predict formulations with improved efficacy.

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## 1. Introduction

Topical applications currently represent the main route of administration of drugs used to treat many eye disorders, including dry eye, conjunctivitis, post-operative inflammation, etc. and eye drops are the formulation of choice for the delivery (Almeida, Amaral, Lobão, & Lobo, 2013; Davies, 2000; Di Colo, Zambito, Zaino, & Sansò, 2009; Van Santvliet & Ludwig, 2004). One of the main challenges associated with the use of conventional topical ophthalmic formulations is the short retention time of the components on the ocular surface. After instillation, there is drainage of the exogenous substances, mainly due to blinking and lachrymation that lowers the efficacy. Frequent instillations would be necessary to maintain the drug concentration in the tear film at a pharmacological level; although, this would worsen patient compliance and lead to ocular and systemic side effects (Almeida et al., 2013; Davies, 2000 Davies, Farr, Hadgraft, & Kellaway, 1991; Di Colo et al., 2009; Ludwig, 2005; McKenzie & Kay, 2015; Séchoy et al., 2000; Snibson et al., 1990; Van Santvliet & Ludwig, 2004). Introduction of mucoadhesive polymers is one of the most used strategies to prolong the contact time of the preparation with the corneal/conjunctival epithelium (Davies et al., 1991; Davies, 2000; Di Colo et al., 2009; Ludwig, 2005; Séchoy et al., 2000; Snibson et al., 1990). The incorporation of macromolecules increases the formulation viscosity; therefore, the drainage rate from the pre-corneal area is reduced. Moreover, mucoadhesive macromolecules are able to intimately interact with the mucin layer, covering the corneal and conjunctival surfaces of the eye. This adhesive capacity further prolongs precorneal retention, improving the ocular bioavailability of the active agent (Davies et al., 1991; Davies, 2000; Di Colo et al., 2009; Ludwig, 2005; Séchoy et al., 2000; Snibson et al., 1990).

Both the mucoadhesiveness and viscosity of the preparations are mainly dependent on polymer molecular weight and concentration; therefore, these parameters have to be adjusted for optimal performance (Di Colo et al., 2009). When tuning the formulation, limits concerning viscosity must be considered. It has been reported that final viscosity should not exceed 30 mPas; otherwise, discomfort due to blurred vision and foreign body sensation occurs, resulting in a faster elimination due to reflex tears and blinks (Oechsner & Keipert, 1999; Pires et al., 2013). Thus, an ideal

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http://dx.doi.org/10.1016/j.carbpol.2016.07.106
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preparation should have the maximal contrast to drainage without excessively increasing viscosity.

Hyaluronic acid sodium salt (hyaluronan, HA) is commonly used as a bioavailability-enhancer in eye drops (Ludwig, 2005; Liao, Jones, Forbes, Martin, & Brown, 2005; Tong, Petznik, Yee, & Tan, 2012; Zambito & Di Colo, 2011). In the presence of HA, the precorneal residence times of pilocarpine, timolol, aceclidine, tropicamide, arecoline, gentamicin, and tobramycin were prolonged (Bernatchez, Tabatabay, & Gurny, 1993; Liao et al., 2005). In addition to its viscosifying and mucoadhesive properties, HA has other beneficial effects on the corneal epithelium, including: 1) protection against dehydration, 2) reduction of healing time, 3) reduction of the inflammatory response caused by dehydration, and 4) lubrication of the ocular surface (Aragona, Di Stefano, Ferreri, Spinella, & Stilo, 2002; Di Colo et al., 2009; Guillaumie et al., 2010; Ludwig, 2005; Tong et al., 2012; Zambito & Di Colo, 2011; Zheng, Goto, Shiraishi, & Ohashi, 2013;). Due to this clinical efficacy, HA is largely used in ophthalmology not only as an excipient but also as the main component of the artificial tear substitutes commonly prescribed for the treatment of dry eye disease (Aragona et al., 2002; Johnson, Murphy, & Boulton, 2006; Ludwig, 2005; McDonald, Kaye, Figueiredo, Macintosh, & Lockett, 2002; Snibson et al., 1990; Zheng et al., 2013). High-performing HA-based eye drop formulations are of great clinical interest.

There are limited scientific data on HA-containing products for topical ophthalmic use. These include the HA concentration range generally used (0.1–0.4 wt%; the HA concentration is also indicated in the package inserts of the commercialized products) and the biopolymer weight average molecular weight ($M_w$), which varied from 155 to 1400 kDa in 11 commercial products (Guillaumie et al., 2010; Johnson et al., 2006; Liu, Harmon, Maziarz, Rah, & Merchea, 2014; McDonald et al., 2002). No viscosity or mucoadhesiveness data are reported. To address this, we performed hydrodynamic and rheological characterizations on six additional products in this study and found most available formulations do not exhibit optimal viscosity. Therefore, we aimed to determine novel, optimized formulations by varying the HA $M_w$ and concentrations (considering the range of molecular weights commercially used) to maximize the mucoadhesiveness and viscosity while maintaining the latter within suitable limits. Such formulations are expected to exhibit the maximum practical retention on the corneal epithelium *in vivo*. We determined the viscosity and mucoadhesion profiles of selected preparations and their capacity to protect the corneal epithelium against dehydration *in vitro* using porcine corneal epithelial cells. The preparations were also compared with commercial products.

## 2. Materials and methods

### 2.1. Materials

Hyaluronic acid sodium salt, lot. N. 02622 (HA1100) and hyaluronic acid sodium salt, lot. N. 11004 (HA250) were kindly provided by Altergon srl (Italy). Hyaluronic acid sodium salt (HA800 and HA500) were produced as described below. Six commercial HA-based formulations indicated for the treatment of dry eye syndrome were evaluated in this work: *Bluyal* (SOOFT italia S.p.A., Fermo, Italy, multi-dose bottle, 8 mL, HA 0.15%), *Blugel* (SOOFT italia S.p.A. Fermo, Italy, multi-dose bottle, 8 mL, HA 0.30%), *Hyabak* (Laboratorios Thea, Barcelona, Spain, multi-dose bottle, 10 mL; HA 0.15%,) *Artelac Splash* MDSC (Fabrik GmbH, Berlin, Germany multi-dose bottle, 10 mL HA 0,24%,) *Hyalistil Bio* (S.I.F.I S.p.A., Catania, Italy, multi-dose bottle, 10 mL, 0,2%), and *Octilia Natural* (C.O.C. Farmaceutici S.r.l., Bologna, Italy, 10 single-dose vials x 0.5 mL). Mucin (from porcine stomach type II, cat. N. M2378), $Na_3PO_4$ (cat. N. 342483), $NaH_2PO_4{\cdot}2H_2O$, cat. N. 71505, $Na_2HPO_4{\cdot}2H_2O$ (cat. N. 71643), EDTA (ethylenediaminetetraacetic acid disodium salt dihydrate, cat. N. E5134), and sodium hydroxymethylglycinate (Cat. N. CDS003712) were all purchased from Sigma-Aldrich (Milan, Italy). Dulbecco's Phosphate Buffered Saline (PBS) without calcium and magnesium was purchased from Lonza Sales Ltd, (Switzerland, cat. N. BE17-516F).

### 2.2. HA800 and HA500 preparation

HA800 and HA500 samples were prepared by hydrolysing a HA powder (lot. N. 08748 $M_w = 1584 \pm 100$ kDa; $M_w/M_n = 1.70$) under heterogeneous acid conditions, as reported elsewhere with slight modifications (D'Agostino et al., unpublished). In brief, a certain amount of the HA powder was dispersed in ethanol (93% v/v) (ethanol/HA 10 mL/g). The dispersion was pre-warmed at 65 °C and HCl (37 wt%) was added under vigorous stirring, resulting in a 0.2 M HCl final concentration. The hydrolysis was carried out for 50 min to obtain HA800 and for 110 min to obtain HA500. Reactions were stopped by adding $Na_3PO_4$ (0.35 M) until neutralized, while cooling in an ice/water bath. Products were purified by washing in ethanol/water (8/2 v/v) to remove phosphate salts. Purification was monitored using conductivity measurements: a conductivity in the range of 30–40 μS/cm was the target value. Samples were then treated with pure ethanol and dried under vacuum at 40 °C. The resulting sodium hyaluronate powders will be referred to as HA800 and HA500.

### 2.3. Hydrodynamic characterization of HA using a SEC-TDA system (Viscotek)

The HA samples and commercial products were characterized using SEC-TDA (Size Exclusion Chromatography-Triple Detector Array) equipment by Viscotek (Lab Service Analytica, Italy). A detailed description of the system and its analytical conditions were reported elsewhere (La Gatta, Schiraldi, Papa, & De Rosa, 2011; La Gatta, De Rosa, Marzaioli, Busico, & Schiraldi, 2010). The molecular weight ($M_w$, $M_n$, $M_w/M_n$), molecular size (hydrodynamic radius-$R_h$), and intrinsic viscosity ([η]) distributions of samples were derived. Each sample was analyzed in triplicate; results were reported as means ± SD. The Mark-Houwink-Sakurada (MHS) curves (log [η] vs log $M_w$) were also directly obtained (La Gatta et al., 2010, 2011).

### 2.4. Rheological evaluation

Rheological measurements were carried out using a Physica MCR301 oscillatory rheometer (Anton Paar, Germany) equipped with a coaxial cylinders geometry (CC27-SN7969; measuring cup diameter/measuring bob diameter: 1.0847 according to ISO 3219; gap length 39.984 mm; sample volume 19.00 mL) and a Peltier temperature control.

#### 2.4.1. Viscosity measurements

The HA1100, HA800, HA500, and HA250 powders were dissolved at different concentrations (in the range 0.15–1.5 wt%) in $NaH_2PO_4{\cdot}2H_2O$ (2.2 g/L), $Na_2HPO_4{\cdot}2H_2O$ (9.5 g/L), sodium hydroxymethylglycinate (0.04 g/L), EDTA (1.1055 g/L), and NaCl (4.3 g/L) in $H_2O$. This was the composition of the buffer (pH 7.4) in commercial products. The dynamic viscosity of the samples was registered as a function of shear rate (1–1000 $s^{-1}$) at 35 °C, using 50 measuring points and no time setting. From each flow curve, the value of zero-shear viscosity ($\eta_0$, viscosity in the range of Newtonian plateau) was obtained. Each solution was prepared in triplicate and each resulting sample was analyzed once, therefore, three flow curves were registered for each HA solution. The $\eta_0$ values reported were the mean values of the three measurements. For all solutions tested,

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