European Journal of Pharmaceutics and Biopharmaceutics 94 (2015) 342–351

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research Paper

In vitro and in vivo ocular safety and eye surface permanence determination by direct and Magnetic Resonance Imaging of ion-sensitive hydrogels based on gellan gum and kappa-carrageenan

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ARTICLE INFO

Article history:
Received 1 April 2015
Revised 20 May 2015
Accepted in revised form 8 June 2015
Available online 14 June 2015

Chemical compounds studied in the article:
Alginate (CID 6850754)
Kappa-carrageenan (CID 11966249)
Gellan-gum (CAS Number 71010-52-1)

Keywords:
In situ gelling system
Ion-activated ophthalmic hydrogel
Polysaccharides
Gellan gum
Kappa-carrageenan
Ocular toxicity
In vivo ocular surface permanence
Magnetic Resonance Imaging
Real-Time Cell Analyzer System
Het-Cam

ABSTRACT

Gellan gum, kappa-carrageenan and alginates are natural polysaccharides able to interact with different cations that can be used to elaborate ion-activated in situ gelling systems for different uses. The interaction between fluid solutions of these polysaccharides and cations presents into the tear made these biopolymers very interesting to elaborate ophthalmic drug delivery systems. The main purpose of this study is to evaluate the ability of mixtures of these polymers to obtain ion-activated ophthalmic in situ gelling systems with optimal properties for ocular use. To achieve this purpose different proportion of the biopolymers were analyzed using a mixture experimental design evaluating their transparency, mechanical properties and bioadhesion in the absence and presence of simulated tear fluid. Tear induces a rapid sol-to-gel phase transition in the mixtures forming a consistent hydrogel. The solution composed by 80% of gellan gum and 20% kappa-carrageenan showed the best mechanical and mucoadhesive properties. This mixture was evaluated for rheological behavior, microstructure, cytotoxicity, acute corneal irritancy, ex-vivo and in vivo ocular toxicity and in vivo corneal contact time using Magnetic Resonance Images (MRI) techniques. Result indicates that the system is safe at ophthalmic level and produces an extensive ocular permanence higher than 6 h.

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1. Introduction

Due to the particular anatomy and physiology of the eye it is a challenge to achieve a high pharmacological concentration on ocular surface and anterior chamber since the high corneal clearance rate after drop instillation produces a rapid clearance of the drugs. For this reason, the main goal on the ocular treatment is to try to achieve high concentrations without ocular surface damage. With this aim, different strategies have been developed since the last

Abbreviations: GG, deacylated gellan gum; KC, kappa-carrageenan; AL, alginate; RTCA, xCELLigence Real-Time Cell Analyzer System; CI, cell index; NCI, normalized cell index; HET-CAM, Hen's Egg Test Chorion allantoic Membrane; CAM, chorioallantoic membranes; MRI, Magnetic Resonance Imaging; H, hardness; A, adhesiveness; B, bioadhesión work; G″, loss moduli; G′, storage moduli; SLF, simulated eye tears; SEM, scanning electron microscopy.

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http://dx.doi.org/10.1016/j.ejpb.2015.06.003
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decades, the main purpose was to increase the cornea permeability and contact time with the ocular surface. Several drug delivery systems have been studied to satisfy both conditions [1]. Some polymeric ocular formulations have been studied to improve the viscosity and/or mucoadhesivity, being hydrogels the most remarkable. Hydrogels are aqueous three-dimensional hydrophilic polymeric networks cross-linked by physical or chemical bonds, with the ability to swell in aqueous solutions inducing a liquid–gel transition. After their administration, ocular hydrogels enhances the viscosity resulting in a decrease of lacrimal drainage. Also, if mucoadhesive polymers are used, interaction between polymers and ophthalmic mucosa takes place increasing hydrogel permanence on the ocular surface. Normally, ocular hydrogels behaves as viscous solutions that do not undergo modifications after administration. This property can difficult their administration and produces discomfort to the patients (more often lachrymation and blurred vision). To overcome these drawbacks the use of in situ gelling systems has been proposed, based on the use of smart polymers which are able to recognize changes in the environmental conditions modifying their conformation as results of the stimulation [2]. As a consequence of the external stimuli, in situ gelling polymers respond undergoing a sol–gel transition that produces the hydrogel formation.

In the case of ocular in situ-forming gels sol–gel transition must be induced by ocular surface conditions such as surface eye temperature [3], tear pH [4] or ionic environment changes [5]. Ion-activated in situ gelling systems have great potential as ocular drug delivery systems due to the presence of mono and divalent cations such as Na^+, K^+, Mg^{2+} and Ca^{2+} in eye tear. Some of the polysaccharides have the ability to interact with ions being candidates for obtaining ion-sensitive systems. Gellan gum (GG) [6–8] has been proposed to increase ocular bioavailability in the ophthalmic formulation because of its reticular properties due to eye tears divalent cations and calcium. Gellan gum is a high molecular weight and linear anionic heteropolysaccharide produced by aerobically bacteria *Aeromonas* (*Pseudomonas*) *elodea*, which is composed of a tetrasaccharide repeating unit of glucose, glucuronic acid and rhamnose [9,7]. Carrageenan's are another group of natural polymers which can be proposed for use in the ophthalmic formulation [10,11]. These biopolymers have attracted particular interest because they exhibit sulfonic acid groups in their structure. They are extracted from red algae, appearing as sulfated galactans soluble in water [12]. They are linear polysaccharides extracted from connected blocks of poly-β-D-mannuronic acid and α-L-guluronic poly in different proportions and with different sequences, which explains molecular weight differences between the currently marketed [5,12]. These carrageenan's are classified by the degree of sulphation. Kappa-carrageenan (KC) is suitable for hydrogels formation due to its rheological, texture and ionic gelation in situ properties, mainly triggered by monovalent ions. Despite they have good biocompatibility [13] and have been extensively used in food and cosmetics industries, this sulfated biopolymers has been poorly explored in the biomedicine field, and, only a small amount of topical drugs incorporate it in their formulation [14]. Several authors have studied the combination of gellan gum and alginate (AL) ophthalmic formulation [15,8,16], chitosan [4] or Pluronic® [17,18], using different proportions and concentrations. Furthermore, this kind of matrix has also been used to incorporate a variety of active ingredients such as indomethacin [7], perfloxacin [6], gatifloxacin [19] or timolol [20]. Due to the high sulfonic acid content, the incorporation of kappa-carrageenan in ocular gels may enhance their bioadhesiveness; the presence of sulfonic acid groups in the polymers can increase the bioadhesion allowing interactions with the mucosal tissues.

In this experimental study we present a novel matrix for ophthalmic topical drug delivery. We have used a mixture of three polymers (GC, CK and AL); a preselected optimal parameter for ophthalmic formulation and resulting in the selection of a gel containing the best possible characteristics is produced. Meanwhile, other parameters such as security and irritating cytotoxic ability of polymers are studied. Subsequently, the best hydrogel is characterized by studying its rheology, microstructure, and surface retention on ocular safety in vivo (bid administration for three months), ex vivo (immunohistochemistry of treated corneas) and in vitro (cell viability of stromal keratocytes in contact with the hydrogel).

2. Material and methods

Deacylated gellan gum (GG) (Kelcogel® CG-LA) and kappa-carrageenan (CK) (GENUGEL® carrageenan GC-130) were a generous gif of CPKelco®, and alginate (AL) (Keltone® LVRC) from FMC biopolymers®. Simulated eye tears (SLF) were prepared as previously used by Ceulemans and Ludwig [21] using the following electrolytes: calcium chloride, magnesium chloride, sodium chloride, sodium bicarbonate and potassium chloride, which are of analytical grade and were purchased from Scharlab SL (Barcelona, Spain). The water used for preparing the formulations was obtained by ion exchange, distillation and passage through a Milli-Q.

2.1. Selection composition of hydrogel formulations with experimental mixtures' design

Experimental design of mixtures using Statgraphics Centurion XVI Version 16.1.15 was used to select and optimize the in situ gel formulations using the proportions of solutions of 1% GG, 0.5% CK and 1% AL in the mixtures as input variables (Table 1). Initially 1% solutions of the polysaccharides were prepared but due to the extremely high viscosity of the 1% CK solution, we have decided to use a 0.5%. To prepare the mixtures, each polysaccharide was dispersed in hot water (50 °C), and the volume of each (Table 1) of those is taken and mixed together maintaining the system under stirring until full homogenization.

To evaluate the in situ gelification properties and bioadhesiveness, we have studied the mechanical properties (hardness, adhesiveness and cohesiveness), bioadhesivity (maximum detachment force and bioadhesion work) and transparence. These properties were also evaluated after dilution of the mixtures in artificial tear fluid in the proportions of 90:10 and 75:25 of polymer: tear (V/V). All measurements were made six-fold. Results were analyzed using Statgraphics Centurion XVI, selecting the input variables with significant effect (analysis of variance of the model) on the response variables. The optimization of composition was made taken into account the next objectives, minimization of the hardness and adhesiveness in the absence of tear in order to facilitate the hydrogel administration and maximization of the remaining parameters.

Table 1
Experimental mixture design used to optimize the ion-sensitive hydrogel composition.

	Volume GG 1%	Volume CK 0.5%	Volume AL 1%
1	0.33	0.33	0.33
2	1	0	0
3	0	1	0
4	0.33	0.67	0
5	0.67	0	0.33
6	0	0.33	0.67
7	0.67	0.33	0
8	0	0	1
9	0	0.67	0.33
10	0.33	0	0.67
11	1	0	0
12	0.67	0.33	0
13	0.67	0	0.33

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