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Formulation and evaluation of cefuroxim loaded submicron particles for ophthalmic delivery

Gabriela Andrei[a], Cătălina A. Peptu[a,*], Marcel Popa[a], Jacques Desbrieres[b], Cristian Peptu[c], Fotios Gardikiotis[d], Marcel Costuleanu[d], Dănut Costin[d], Jean Charles Dupin[b], Arnaud Uhart[b], Bogdan I. Tamba[d]

[a] Gheorghe Asachi Technical University of Iasi, Department of Natural and Synthetic Polymers, Prof. dr. docent Dimitrie Mangeron street, Iasi 73, Romania
[b] Université de Pau et des Pays de l'Adour, Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux, IPREM, Hélioparc Pau Pyrénées, 2 Avenue P. Angot, Pau Cedex 09,64053 France
[c] "Petru Poni" Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda 41A, Iasi 700487, Romania
[d] "Grigore T. Popa" University of Medicine and Pharmacy, University Str., Iaşi 16, Romania

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ABSTRACT

Chitosan gelatin particles could be the ideal candidate for intraocular drug delivery due to their desirable properties. Double crosslinking in double emulsion has been used as an original and reliable method for particles preparation and their morphology has been optimized considering the main synthesis parameters such as polymers ratio, crosslinker amount, stirring speed, tensioactive amount and ionic crosslinking time, respectively. The particles have been analyzed for their physical–chemical properties (swelling degree, drug loading and release capacity, surface characteristics, *etc.*), the enzymatic degradation properties along with *in vivo* ocular investigations (ocular biodistribution, *in vivo* drug release). In the present study cefuroxim was used as a model drug, which is generally used in the prophylaxis of postoperative endophthalmitis following cataract surgery after intraocular administration. The present study proved that the dimensions and the physical–chemical properties of the particles can be modulated (by varying the preparation parameters) to facilitate the administration, the biodistribution and the drug release in the specific segment of the eye. This experimental study demonstrated also the ability of fluorescent nanoparticles to penetrate ocular tissues close to the administration site (intravitreal injection) and especially their tendency to migrate deep in the retina at time intervals of 72 h.

1. Introduction

The diseases of the posterior segment of the eye (glaucoma, diabetic retinopathy and age-related macular degeneration, *etc.*) are responsible for compromising eyesight of a large number of subjects. The therapeutic efficiency of pharmacological treatments in this area is limited mainly due to the difficulty of the active substance to reach the target tissues. That is the reason the delivery of therapeutic doses of drugs to the tissues in the posterior segment of the eye, however, represents a significant challenge. When a topical path is chosen, there are a lot of barriers (cornea, lens, haematoaqueous and haematoretinal barriers) which stop the medication access to the vitreous, the retina and the choroid. On the other hand, if the oral pathway is selected, the amount of drug reaching the posterior segment of the eye coming from general blood flow is very low (Alarcón and Martínez, 2006). Also, therapeutic drug levels cannot be maintained for longer periods in target tissues.

In the last years, many pharmaceutical formulations have been used for the treatment of ocular diseases. Micro and nanotechnology ophthalmic formulations represent one of the approaches which are currently being designed for both anterior and posterior segment drug delivery. Several carriers, such as micro/nanoparticles, nanosuspensions, liposomes, nanomicelles and dendrimers have been developed for ocular drug delivery, some of them showing promising results for improving ocular bioavailability (Patel et al., 2013). Nanomicelles are carrier systems able to formulate therapeutic agents into clear aqueous solutions with high drug encapsulation efficiency. They are easy to prepare and present a small size and they have been proved to enhance the

* Corresponding author.
E-mail address: catipeptu@tuiasi.ro (C.A. Peptu).

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bioavailability of the therapeutic drugs in ocular tissues (Trivedi and Kompella 2010). Nanosuspensions, a promising strategy for delivery of hydrophobic drugs, are colloidal systems of submicron drug particles stabilized by polymer or surfactant and they present some important advantages for ocular delivery such as sterilization, ease of eye drop formulation, less irritation, increase precorneal residence time and enhancement in ocular bioavailability of drugs which are insoluble in tear fluid (Patravale and Kulkarni, 2004). Liposomes, vesicular systems composed of aqueous core enclosed by phospholipid bilayers of natural or synthetic origin, were intensively explored over the years for the ophthalmic drug delivery applications. Liposomes are biodegradable and biocompatible and they can enhance the permeation of poorly absorbed drug molecules by binding to the corneal surface and improving residence time. Liposomes are able to encapsulate both the hydrophilic and hydrophobic drug molecules. In addition, liposomes can improve pharmacokinetic profile, enhance therapeutic effect, and reduce toxicity associated with higher dose. Liposomes have been widely investigated for the treatment of both anterior and posterior segment eye disorders (Mishra et al., 2011). Micro/nanoparticles based systems with an appropriate morphology can be designed to ensure low irritation, adequate bioavailability, and ocular tissue compatibility. For ophthalmic delivery, particles are generally composed of lipids, proteins, natural or synthetic polymers such poly(alkyl cyanoacrylates)(PACA) (Kreuter, 1993), poly(caprolactone) (PCL) (Gagandeep et al., 2014), poly(lactic acid) (PLA) (Nagarwal et al., 2011), poly(lactic-*co*-glycolic acid) (PLGA) (Wagh and Apar, 2014), chitosan (CS) (Jayaraman et al., 2012), poly(acrylic acid) (PAA) (De et al., 2003; Hornof et al., 2003) and hyaluronic acid (Apaolaza et al., 2014). Among these polymers, chitosan is most widely studied due to its unique properties besides the well known biocompatibility and biodegradability—the chitosan is positively charged and hence it binds to negatively charged corneal surface and thereby improves precorneal residence and decreases clearance. These are the main reasons to explain why chitosan was chosen as the main polymer for our research. In order to better modulate the particles morphology we introduced a second polymer, gelatin, which presents free amine groups and gives similar chemical reactions as chitosan. Moreover, gelatin is well known for its properties being intensively used in advanced drug delivery field (Elzoghby, 2013). Previous studies on chitosan- gelatin hydrogel films and particles were already published by our research group (Cadinoiu et al., 2011; Peptu et al., 2010).

The purpose of this study was to develop and characterize a new ophthalmic formulation of cefuroxim, a drug generally used in the prophylaxis of postoperative endophthalmitis following cataract surgery after intraocular administration (Delyfer et al., 2011), using polymer based nanoparticles. Particles were synthesized using a double crosslinking in double emulsion technique and the effects of crosslinkers amount and concentration, ionic crosslinking time, surfactant amount, stirring speed, polymer ratio on their morphology and physical–chemical properties (swelling degree, drug loading and release capacity, surface characteristics, *etc.*). The optimum considered particles has been analysed from the point of view of the enzymatic degradation properties along with *in vivo* ocular investigations (ocular biodistribution, *in vivo* drug release).

We consider that our work bring original elements from many points of view: (i) it was developed an improved method comparing to the one previously reported (Peptu et al., 2010); (ii) the *in vivo* evaluation of cefuroxim release was performed by HPLC-MS, the method being developed and validated by the authors; iii) to our knowledge, this is one of the few papers which include preparation of the polymer particles, optimization of the preparation method, physical–chemical characterization of the particles and also from the point of view of biomaterial characteristics and *in vitro* and *in vivo* tests of the new polymer-drug nanosystems specially designed for targeting the posterior segment of the eye.

2. Materials and methods

2.1. Materials

Chitosan (CS)—low molecular weight (Sigma–Aldrich), molecular weight—70,000 (determined by viscosimetry), deacetylation degree 91%, Gelatin type A (Sigma–Aldrich), glutaraldehyde (25%—Sigma–Aldrich), sodium fluorescein (Sigma–Aldrich), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC) (Fluka), Sodium triphosphate pentabasic (TPP) (Sigma–Aldrich), sorbitan monooleate—Span 80 and Polyoxyethylenesorbitan

Table 1
Varying parameters of microparticles preparation.

Sample code	CS/G (w/w)	TPP concentration (%)	NH_3/TPP (mol/mol)	NH_3/GA (mol/mol)	TPP crosslinking time (h)	Ultraturrax speed, (rpm)	Surfactant, (% in respect with phase volume)	Mean diameter in acetone (microns)
C1	1/1	5	2/1	14/1	1	9000	2	0.967
C2		15						4.154
C3		1						5.116
C4		10						6.3
C5		5	4.7/1					2.547
C6			11.74/1					0.667
C7			1.175/1					1.466
C7.1					2			2.224
C7.2					3			0.648
C 7.3					4			0.755
C7.4				28/1				0.225
C 7.5				7/1				0.742
C7.6				14/1		15,000		0.889
C7.7						9000	3	0.12
C7.8							4	0.166
C7.9				7/1	4	15,000	3	0.225
C 7.9.1	1/0							0.345
C7.9.2	3/1							0.42
C7.9.3	1/3							0.637
C8	1/0		5/1	14/1	1	9000	2	0.82
C9	3/1							0.341
C10	1/1							0.637
C11	1/3							1.190