OpenNano 2 (2017) 28–36

Contents lists available at ScienceDirect

OpenNano

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

Cross-linked chitosan-dextran sulphate vehicle system for controlled release of ciprofloxaxin drug: An ophthalmic application

Chetan Chavan[a], Preetam Bala[a], Kavita Pal[b], S.N. Kale[a,*]

[a] Department of Biosciences and Technology, Defence Institute of Advanced Technology (DIAT), Girinagar, Pune 411025, India
[b] Translational Research Laboratory, Advance Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai 410210, India

ARTICLE INFO

Keywords:
Chitosan
Nanoparticles
Ophthalmic
Ciprofloxacin
Sustained release

ABSTRACT

The major challenge associated with conventional eye-drop is the rapid drug loss due to precorneal defence barrier. In this context, controlled-release system of ciprofloxacin-conjugated chitosan (CS)-Dextran sulphate (DS) nanoparticles (NPs) have been synthesized, to increase the bioavailability. The formulated drug delivery vehicle was evaluated for its therapeutic value in the simulated tear fluidat pH 7.4. Ophthalmic microbes were tested with this formulation, to confirm the drug efficacy; which showed conducive therapeutic values of both MIC and MBC. Ocular irritancy test was performed using HET-CAM test, which showed that the CS-DS system did not yield any vascular response, offering it to be a non-irritant to the ocular surface. The release studies showed monotonous controlled-release for duration of 21 h. A fine cross-linking between CS and DS has been demonstrated to form CS-DS NPs and their interaction with drug has been evaluated using conventional characterization tools.

1. Background

Human eye is constantly exposed to a variety of pathogens and irritants and the infections occur when the normal defence mechanisms of the eye gets compromised. There are various infections with number of causes such as bacterial, viral and so on [1], among which bacterial eye infections are most common and are known as microbial keratitis. In extreme cases, bacterial infection may also damage the cornea, eventually leading to vision loss and blindness [2]. The predominant ocular pathogens belong to *Streptococcus sp*., *Pseudomonas aeruginosa*, *Staphylococcus aureus*families, involved in microbial keratitis.

Conventional drugs for combating these infections are antibiotics; mostly in the form of eye drops, suspensions and ointments. However, more than 90% of the marketed ophthalmic formulations are in the form of eye drops [3]. The inherent problems associated with the topically applied drugs is this that they get easily washed off from the eye by various mechanisms, such as, lacrimation, tear dilution and tear turnover; resulting in low ocular availability of the drug. Moreover, human cornea, comprising of epithelium, substantia propria and endothelium are negative in charge due to sialic acid residues, also restricts the ocular entry of drug molecules [4]. As a result of these factors less than 5% of administered drug enters the eye. This result in more amount of drug administration and that too, more number of times. Another problem with these commercial antibiotics are burning or stinging of the eye, momentarily, when drops are administered and occasional allergic reactions that include swelling or redness [5]. Many efforts in ophthalmic drug delivery are being made, not only to prolong the contact time of the vehicle at ocular surface, but, to simultaneously slow down the elimination of the drug and increase its corneal penetration.

* Correspondence to: Department of Applied Physics, Defence Institute of Advanced Technology (DIAT), Pune 411025, India.
E-mail addresses: kavitapal80@gmail.com (K. Pal), sangeetakale2004@gmail.com (S.N. Kale).

http://dx.doi.org/10.1016/j.onano.2017.04.002
Received 27 January 2017; Received in revised form 27 April 2017; Accepted 27 April 2017
Available online 03 May 2017

Several new materials are being proposed as ophthalmic drug delivery vehicles for sustained release of drug molecule, such as: polymers [6]; microparticles [7]; nanoparticles [8]; liposomes [9]; collagen shields [10]; ocular inserts/discs [11]; dendrimers [12]; and transcorneal iontophoresis [13]. Among the different strategies explored so far, the use of colloidal polymer systems have shown an assured degree of success [14]. Another strategy aims to increase the residence time of drugs in the precorneal area, using mucoadhesive polymers [15,16]. Polymeric particles can also be used to target diseases in the posterior segment of the eye such as age-related macular degeneration, cytomegalovirus retinitis, diabetic retinopathy *etc* [17]. Within the polymeric particles, drug of interest can be dissolved/entrapped/adsorbed or covalently attached [18]. These colloidal particles can be applied in the liquid form just like eye drops and reduce discomfort caused by application of semisolid ointments. They are patient friendly due to low application rate, extended duration of retention in the extraocular portion without blurring vision. In summary, a mucoadhesive polymeric particle, loaded with the drug of interest can serve as a better alternative to commercially available eye drops.

Among various polymers, the cationic polysaccharide chitosan (CS) exhibits several favorable biological properties, such as biodegradability, non-toxicity, biocompatibility, and mucoadhesiveness [19–21]. Ionic interaction between the positively charged amino groups of chitosan and the negatively charged sialic acid residues in mucus helps in mucoadhesion [22]. These properties make it a novel versatile biopolymer, which fulfils the requirements for its application in the area of management of ophthalmic infections. There are few reports in the literature on the use of CS for ocular drug delivery. Motwani et al. [23] have reported chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery of drug gatifloxacin. In another study, they have described about PLGA - levofloxacin nanoparticles for sustained ocular drug delivery [24]. Campos and co-workers evidenced that the presence of the chitosan significantly prolonged the corneal contact time of tobramicin, following topical instillation to rabbits [25]. In the present study, mucoadhesive polymeric nanoparticles for sustained release of drug into the eye offering improved drug availability and prolonged residence time of drug, has been synthesized and characterized for topical insertion into the eye, which will take care of ocular infections.

A cross-linked composition of CS has been made using Dextran sulphate (DS), to incorporate drug Ciprofloxacin. Ciprofloxacin (Cipro) is a fluoroquinolone antibiotic that is very effective against wide range of microorganisms. The formed composition has been carefully characterized for their chemical structure, morphology, drug-loading and stability using conventional techniques such as Fourier Transform Infrared Spectroscopy (FTIR), thermogravimetric analysis (TGA), Scanning electron Microscopy (SEM) and particle size analyzer. The improved release time of drug into a tear solution (simulated tear fluid composition, with appropriate pH) has been studied using UV–visible Spectroscopy (UV–vis). Antimicrobial studies have been done on nine different organisms to ensure the efficacy of the drug, even after its encapsulation in the polymer matrix. The eye-irritancy test has been done to evaluate qualitative irritancy potential of test substance on ocular surface, using *Hen's Egg Test Chorioallantoic Membrane (*HET-CAM) assay. It is important to mention that hen's eggs is a borderline case between *in vivo* and *in vitro* system and does not conflict with ethical and legal aspects, especially the animal protection laws [26]. The details of these methods are mentioned below. The results showed that the formulated chemical system works as a good drug delivery vehicle, which helps the drug to be released in controlled fashion, without being detrimental to the original properties of the drug.

2. Method

2.1. Materials

Chitosan (85% deacetylated), dextran sulphate sodium salt (MW ca 40,000) and glacial acetic acid (99.7% MW 60.05) we repurchased from Alfa Aesar and ciprofloxacin were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and as purchased. STF (Simulated Tear Fluid) solution was prepared as per given in Ref. [27]. Standard ophthalmic microbial cultures used in study were*Acinetobacter calcoaceticus*(2886), *Bacteroides fragilis* (5273), *Corynebacterium glutamicum* (2168), *Escherichia coli* (5346), *Flavobacterium devorans* (2581), *Pseudomonas aeruginosa* (2200), *Staphylococcus aureus* (5345), *Nocardia calcarea* (2086) and *Bacillus subtilis*(2920)obtained from NCIM, Pune.

2.2. Preparation of bare nanoparticle (CS-DS NPs) and ciprofloxacin loaded chitosan nanoparticles (Cipro-NPs)

CS-DSNPs were fabricated by using inotropic gelation method. CS and DS stock solution was prepared by dissolving CS in 0.1% acetic acid to make final concentration of 1 mg/ml and by dissolving DS in distilled water to make final concentration of 0.3 mg/ml, respectively. DS solution was added drop-wise in 1 mg/ml CS solution in the ratio of 3:4 on sonicator for 30 min at room temperature. Similarly, for drug loaded nanoparticles ciprofloxacin was dissolved in CS solution to achieve final concentration 1 mg/ml and similar steps were followed as mentioned above. The resultant nanoparticles were centrifuged at 12,000 rpm for 10 min with a rotor (Kubota 7780-AG-506R, Japan) and subsequently washed.

2.3. Spectrophotometric analysis

2.3.1. Determination of drug entrapment efficiency

The percent drug entrapment efficiency quantified by UV–vis. Cipro-NPs were purified from the solution by centrifugation at 12,000 rpm, at room temperature. The concentration of free ciprofloxacin in the supernatant was measured by UV–vis (Specord Plus, Analytic Jena, Germany) signature at 281 nm with preconstructed calibration curve established by testing known serial concentrations of ciprofloxacin. The drug entrapment efficiency (% EE) was determined using the equation: