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# Development of chitosan nanoparticles coated with hyaluronic acid for topical ocular delivery of dexamethasone

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ABSTRACT

The present study involved design of dexamethasone-sodium phosphate (DEX) loaded mucoadhesive chitosan nanoparticles for topical ocular delivery to improve its precorneal retention and corneal permeability. The chitosan-sodium tripolyphosphate nanoparticle (CS-NPs) was developed through ionotropic-gelation technique. The developed CS-NPs were coated with hyaluronic-acid (HA) to make discrete, free-flowing NPs and to improve their mucoadhesive characteristics. The particle-size, zeta-potential and polydispersity-index were determined by Malvern-Zetasizer. The average size of the CS-NPs ranged from 305.25 ± 14.29 nm (without HA-coating and before freeze-drying) to 400.57 ± 15.23 nm (HA-coated and after freeze-drying). Due to the polyanionic nature of HA, reversing of zeta-potentials from +32.55 ± 4.15 to −33.74 ± 3.45 was observed. Polydispersity-indices varied from 0.178 ± 0.067 (before freeze-drying of HA-coated F2) to 0.427 ± 0.028 (after freeze-drying of HA-coated F2). The encapsulation and loading capacity of around 72.95% and 14.51% respectively were found in optimized CS-NPs. In simulated tear fluid 75.84% cumulative amount of released drug was detected and the *in-vitro* release results suggested the mechanism of drug release was Fickian-diffusion type. The clarity, pH, refractive index, surface tension and viscosity of the suspensions of DEX-CS-NPs were found promising for ocular use. Stability study on nanoparticles revealed no significant changes were observed in particle-size, encapsulation, drug release and physicochemical characteristics at 25 °C for 3-months storage.



## 1. Introduction

Dexamethasone, 9α-fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1, 4-pregnadiene-3, 20-dione, a synthetic derivative of the glucocorticoid, is a highly potent and long-acting glucocorticoid. Among the corticosteroids used in ocular therapy, dexamethasone sodium phosphate (DEX) was found to have the highest potency and effectiveness [1]. The actions of dexamethasone are mediated by binding of the drug molecules to corticosteroid receptors present in the sensitive cells, and such receptors are present in human trabecular meshwork cells and in rabbit iris ciliary body tissues. DEX inhibits phospholipase-A2, hence inhibits the prostaglandins synthesis and other agents which mediate inflammation. Therefore, the swelling and pain of inflammatory conditions is decreased. DEX ophthalmics are used to treat inflammations caused by infections, injury, surgery, or other conditions in the eyes. Though, DEX can have serious systemic side effects, so the controlled, continuous local delivery of DEX at the targeted site via nanocarriers could be a mean to circumvent the side effects and accomplish the goal of suppressing the ocular inflammatory conditions [2]. In rabbit and human studies, DEX has shown high concentrations on the surface of the eye [3] and resulted an effective penetration and delivery into the anterior and posterior chambers [4] of the eye when applied topically using nanoparticles as carrier system [5,6].

Because of the strong defensive barriers like corneal epithelial layer, aqueous-vitreous humors, blood-aqueous humor barrier and blood-retinal barrier limit the entry of hydrophilic or hydrophobic moieties through various routes of administration in to the eyes, which in turn causes very low bioavailability of drugs [7]. Only 2–7% of drugs are bioavailable if administered topically in to human eyes due to the natural protective mechanism of eyes like, (i) the naso-lacrimal drainage and absorption in to the systemic circulation (ii) spillage of administered dose due to very low capacity of human cul-de-sac and (iii) blinking, basal and reflex tearing, tear dilution and metabolism of drugs by tear enzymes [8,9]. Due to these protective mechanism of eye and low penetration of the carriers through tight junctions of corneal epithelium, there is a challenge of effective transport of topically applied drugs to the anterior and posterior chambers and corneal stroma of eyes [10,11]. Hence, for the administration of drugs on ocular surface, the development of

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a suitable delivery system becomes obligatory to attain effective drug concentrations in to the eyes for a prolonged time after the instillation of dose.

The drainage rate can be reduced by increasing the viscosity [12,13], by using the mucoadhesive polymer based nanoformulations the ocular bioavailability of drugs can be improved [14]. Mucoadhesive polymers are supposed to decrease the drug drainage rate when adhere to the ocular surface [15]. Chitosan is a hydrophilic mucoadhesive biodegradable polymer and are anticipated to retain and stabilize tear fluids on the surface of eye, hence reducing the drainage and prolonging the precorneal retention and increasing the contact time on ocular surface of drug loaded nanoparticulate formulations [16], while the unbound or free drug molecules can diffuse in the tear film and can be removed from the precorneal area [8].

With this idea, we developed chitosan nanoparticles coated with hyaluronic acid for the delivery of dexamethasone in to the eyes. Chitosan is a natural polysaccharide and has been proved a non-toxic, biodegradable and biocompatible [17], and approved Generally Recognized as Safe (GRAS), also it was found to have broad-spectrum antimicrobial activity [18] against gram-positive, gram-negative bacteria as well as antifungal activity [19–21]. Therapeutic potency of chitosan have been reported also in terms of inhibition of microorganisms' growth and relieving pain [22], also promotes hemostasis and epidermal cell growth [23]. The use of chitosan in pharmaceutical area, is increased due to its biocompatibility, susceptibility to enzymatic hydrolysis, and intrinsic physiological activity and nontoxicity [24]. Due to its biological and physicochemical properties chitosan is involved in a wide variety of biomedical applications in drug delivery, drug targeting, wound healing, tissue engineering, and in other area of nanobiotechnology [25]. Hence, chitosan was chosen as nano-carrier for the delivery of dexamethasone in to eyes. Chitosan was reported to boost the intraocular drug penetration by binding with corneal epithelial surface and causes reversible loosening of the tight junctions of corneal epithelium. It has ability to sustain the delivery of drugs and has very low ocular irritation potential, so considered as one of the ideal polymer of biological origin for ophthalmics [26], and has been exploited in development of ocular products like nanoemulsions [27] and nanocapsules of indomethacin [28]; nanoparticles of cyclosporine-A [29], microspheres of ofloxacin [14] and acyclovir [30]. Previously many researchers reported the CS-NPs and liposome-CS-NP complexes [31] interaction and penetration to the corneal and conjunctival epithelial layers [29,32].

Hyaluronic acid (HA) is one of the material of choice in terms of biodegradability and biocompatibility, hence was used to coat the CS-NPs. HA has been reported as protein repellant to coat the surfaces of polymeric nanoparticles. The formation of interfacial HA-CS complexes exploited by Richert, 2004 [33], was truly the technique that we have explored in the present study. The use of HA to coat the CS-NPs was based on its capacity to improve cellular targeting.

Besides, biodegradability, biocompatibility and mucoadhesive property, HA is associated in many other processes, e.g. HA improves the corneal and conjunctival epithelial cells regeneration by interacting with the CD44 receptors which are expressed in the human corneal and conjunctival epithelia [34,35] and helps in receptor-mediated internalization and biodegradation of hyaluronan [36–38]. The coating of HA on CS-NPs expedite the cellular uptake of NPs by receptor-mediated endocytosis. Moreover, HA encourage wound healing on corneal surfaces by regulating the regeneration and migration of epithelial cells, this regulation is attained by binding of HA to CD44 and hyaluronan-mediated motility receptors present on the corneal and conjunctival epithelial surfaces, and such receptor-mediated processes might accompanying the cellular uptake of HA-coated-CS-NPs.

Hence, the goal of the present study was to develop and evaluate the potential of a nanocarrier, consisting of CS and TPP, coated with HA, designed for the targeted and intracellular delivery of dexamethasone sodium phosphate (DEX) into the eyes, because the HA coated CS-NPs has a strong ability to interact with the CD44 and hyaluronan receptors present on the corneal and conjunctival epithelial surfaces. Apart from this characteristic, the strong mucoadhesive characteristics of HA-coated CS-NPs loaded with DEX, would encourage ocular bioadhesion and hence prolonged drug retention in the eyes to suppress the ocular inflammatory conditions.

## 2. Materials and methods

### 2.1. Materials

Dexamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$; MW 516.40), high purity, molecular weight ($M_v$) 140K–220 K, deacetylated chitin (Degree of acetylation: ≤40 mol%) and the cross linker sodium tripolyphosphate (TPP) and sodium dihydrogen phosphate were purchased from Sigma Aldrich (St. Louis, MO, USA). Acetic acid glacial was purchased from BDH Limited (Poole, England), hyaluronic acid (HA) 200 kDa was obtained from Medipol SA (Lausanne, Switzerland). The RC-dialysis membrane of MWCO 12–14 kDa obtained from (Spectra Por, Spectrum Laboratories Inc., Rancho Dominguez CA, USA). Mannitol was purchased from Qualikems Fine Chem Pvt. Ltd, Vadodara, India. Methanol and acetonitrile (HiPerSolv CHROMANORM for HPLC grade) were purchased from BDH, PROLABO®, LEUVEN, EC. Purified water was obtained by Milli-Q® water purifier (Millipore, France). All other chemicals used were of analytical grade and the solvents used were of HPLC grade.

### 2.2. Methods

#### 2.2.1. Formulation of chitosan nanoparticles (CS-NPs)

The CS-NPs was prepared by ionotropic gelation of chitosan (CS) with sodium tripolyphosphate (TPP) as crosslinking agent, which is a small ion with a triple negative charge at the physiologically acceptable pH range by adopting a reported method [39]. Chitosan (Degree of acetylation: ≤40 mol%) was dissolved in aqueous solution of acetic acid (1%, v/v) to get the concentrations in the range of 0.2, 0.4, 0.6 and 1.0 mgmL$^{-1}$. Finally, 10 mg of dexamethasone sodium phosphate (DEX) was added in to the CS solution. TPP was dissolved in deionized water and the pH of the solution was maintained to 7.2 with 0.1 M sodium dihydrogen phosphate buffer. Thereafter, 6 mL of TPP solution at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mgmL$^{-1}$) were added to 12 mL of CS solution at the addition rate of 1.5 mL min$^{-1}$, under magnetic stirring at 500 rpm for 120 min at 25 °C temperature [40].

#### 2.2.2. Surface coating of CS-DEX nanoparticles with hyaluronic acid (HA)

The surface coating of prepared CS-DEX nanoparticles was done by using HA. About 25 mg of CS-DEX nanoparticles was dispersed in 2.5 mL of 0.1 M acetic acid-acetate buffer (pH 5). The obtained nano-dispersions was added drop by drop to 2.5 mL of 0.1 M acetic acid-acetate buffer (pH 5) containing HA (200 KDa) at 0.5, 1, 2, 5, 10 and 20 mg mL$^{-1}$ concentrations under the continuous magnetic stirring at 1000 rpm for 45 min. The obtained nano-dispersions were subjected to ultrafiltration against deionized water using pre-activated and pretreated 12–14 KDa, MWCO dialysis membrane [41,42].