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Sorbitan ester nanoparticles (SENS) as a novel topical ocular drug delivery system: Design, optimization, and *in vitro/ex vivo* evaluation

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ABSTRACT

We explored the potential of two types of sorbitan ester nanoparticles (SENS) as novel tools for topical ocular drug delivery. The optimized SENS formulation (SENS-OPT) consisted of nanoparticles (NPs) of 170.5 nm, zeta potential +33.9 mV, and cyclosporine loading of 19.66%. After hyaluronic acid (HA) coating, the resulting SENS-OPT-HA NPs had a particle size of 177.6 nm and zeta potential of −20.6 mV. The NPs were stable during 3 months of storage at different temperatures and did not aggregate in the presence of protein-enriched simulated lacrimal fluid. There was no toxicity to cultured human corneal epithelial (HCE) cells when exposed to NPs up to 0.4% (w/v). Both NPs were effectively internalized by HCE cells through active mechanisms. Endocytosis of SENS-OPT NPs was caveolin-dependent whereas SENS-OPT-HA NP endocytosis was mediated by HA receptors. HA-receptor–mediated endocytosis may be responsible for the higher cellular uptake of SENS-OPT-HA NPs. After cyclosporine incorporation into the NPs, corneal penetration of this immunosuppressive drug by loaded SENS-OPT NPs was 1.3-fold higher than the commercial reference formulation Sandimmun®. For cyclosporine-loaded SENS-OPT-HA NPs, the penetration was 2.1-fold higher than for Sandimmun®. In *ex vivo* stimulated lymphocytes, both formulations demonstrated the same reduction in IL-2 levels as Sandimmun®.

1. Introduction

Most drugs used in ocular therapies are formulated as topically-applied dosage forms (i.e., eyedrops) because topical administration is a simple and non-invasive route that achieves high levels of patient compliance. However, conventional dosage forms present several pharmaceutical issues that limit therapeutic efficacy due to low drug bioavailability, the most evident drawback. The underlying reason is that the eye is a highly protected organ composed of numerous nearly impermeable anatomical and physiological barriers that prevent access of foreign substances such as drugs (Patel et al., 2013). In fact, intraocular drug bioavailability achieved with conventional topical dosage forms is generally less than 5% (Reimondez-Troitino et al., 2015).

One of the most studied drugs in ocular drug delivery (ODD) is the immunosuppressive agent cyclosporine A (CsA). This cyclic undecapeptide is effective in the treatment of various ocular surface disorders in which the immune system is activated as occurs in dry eye disease (Rhee and Mah, 2017) or after corneal graft transplantation (Ziaei and Manzouri, 2015). However, the choice of an appropriate vehicle for topical administration of CsA represents a big challenge for scientists due the unfavorable physicochemical (poor water solubility) and biopharmaceutical properties (low permeability) (Lallemand et al., 2017).

In the recent decades, multiple CsA formulations have been developed, including emulsions, microparticles, *in situ* gelling systems, drug-loaded contact lenses, and nanotechnology-based systems (Lallemand et al., 2017). The colloidal nature of nanocarriers not only improves the solubility of CsA, it also improves key biopharmaceutical properties such as formulation residence time and drug penetration through cornea. Li et al. reported that in rabbit corneas cationic chitosan-coated

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liposomes had prolonged drug retention and higher levels of CsA compared to uncoated liposomes (Li et al., 2012). Polymeric chitosan-coating of CsA-loaded lipid and polymeric nanoparticles (NPs) also showed promising results by improving the intraocular availability of this drug (Battaglia et al., 2012; Sandri et al., 2010; Hermans et al., 2012; De Campos et al., 2001). Other cationic nanosystems that have been evaluated for ODD include cationic Eudragit® polymers, sterylamine, didecyldimethylammonium bromide, and cetyltrimethylammonium bromide (CTAB) (Aksungur et al., 2011; Li et al., 2008; Leonardi et al., 2015; Fangueiro et al., 2016). As alternatives to cationic nanosystems, other mucoadhesive formulations have been proposed. Hyaluronic acid (HA)-coated colloidal systems have gained attention lately for topical administration because of their technological features. In addition to its mucoadhesiveness, HA coating enhances nanoparticle uptake by corneal cells due to specific targeting of HA-receptors (Contreras-Ruiz et al., 2011). In a recent study, Kalam observed that dexamethasone ocular bioavailability obtained with chitosan NPs and HA-coated chitosan NPs was 1.83- and 2.14-fold higher respectively than with the drug solution alone (Kalam, 2016). This author attributed this difference to the presence of the HA coating that may allow a targeted receptor-mediated endocytosis by corneal cells.

Recently, our group patented novel types of NPs based on the cheapest available materials in the pharmaceutical market, sorbitan esters (Sanchez et al., 2013). Based on our patented NPs, the objective of the present work was to optimize and evaluate two novel NP formulations specifically designed for topical ODD. As a drug model, we selected CsA because of its proven anti-inflammatory efficacy in the treatment of common eye diseases. We employed a Box-Behnken experimental design to optimize the physicochemical properties of the initial prototype formulations. According to the design outcome, we selected a cationic optimized formulation (SENS-OPT) for further study. Additionally, an anionic optimized formulation was prepared based upon the SENS-OPT by coating it with HA (SENS-OPT-HA). The developed NPs were characterized physicochemically and evaluated in terms of biocompatibility, cellular uptake, corneal penetration, and immunosuppressant activity.

2. Materials and methods

2.1. Materials

Sorbitan monooleate (Span® 80), d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), CTAB, HA, Nile red, filipin, chlorpromazine, concanavalin A (Con A), NaOH, benzalkonium chloride (BZK), 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt (XTT), and simulated lacrimal fluid (SLF) components were purchased from Sigma-Aldrich (Madrid, Spain). High performance liquid chromatography (HPLC)-grade acetonitrile (ACN) was from VWR (Barcelona, Spain). Cyclosporine A (CsA) was obtained from LC Laboratories (Woburn, MA, USA). Sandimmun® was obtained from the local hospital pharmacy. Alamar Blue® reagent was from Bio-Rad (Madrid, Spain). Dulbecco's Phosphate Buffered Saline (DPBS), DMEM/F12 culture medium and other cell culture reagents were purchased from Invitrogen-Gibco (Inchinnan, UK). Pierce® BCA Protein Assay Kit was from Thermo Fisher Scientific (Waltham, MA, USA), and IL-2 enzyme-linked immunosorbent assay (ELISA) kit was purchased from Diaclone (Besançon, France).

2.2. Preparation of SENS

NPs were prepared according to our previously patented method (Sanchez et al., 2013). Briefly, all NP components, including Span® 80, TPGS, and CTAB, were dissolved in 30 mL of absolute ethanol. For drug-loaded preparations, CsA was added to this mixture. This organic phase was then poured steadily over an aqueous phase (ultrapure water, 60 mL) under continuous magnetic stirring (500 rpm, 15 min), leading to the spontaneous formation of SENS. Immediately, the ethanol was removed by a rotating evaporator, and the formulation was subsequently concentrated to a final volume of 10 mL.

Table 1
Variables of Box-Behnken experimental design.

Independent variables	Levels under study		
	Low	Medium	High
Span® 80 amount (% w/v)	2.00	2.50	3.00
TPGS amount (% w/v)	0.10	0.20	0.30
CsA amount (% w/v)	0.30	0.48	0.65
Dependent variables	**Established constraints**		
Particle size (nm)	Minimize		
Zeta potential (mV)	Maximize		
Entrapment efficiency (%)	Maximize		
Drug loading (%)	Maximize		

Span® 80, sorbitan monooleate; TPGS, d-α-tocopheryl polyethylene glycol 1000 succinate; CsA, cyclosporine A.

2.3. SENS optimization by Box-Behnken experimental design

A three-factor, three-level Box-Behnken experimental design was performed to develop an optimized formulation for topical ODD. A design matrix was built with combinations of three factors (independent variables, Table 1) consisting of low, medium, and high concentrations of Span® 80, TPGS, and CsA. These concentration ranges were selected by evaluating the results of preliminary experiments. In those experiments, critical formulation parameters were considered, such as a preferred particle size under 200 nm, zeta potential as far as possible from electroneutrality, and the highest drug payloads that did not compromise nanosystem stability. The cationic agent CTAB was constantly kept at the lowest possible level in all formulations to avoid potential biocompatibility issues. Fixed objectives (dependent variables, Table 1) were selected to minimize particle size at the same time as maximizing zeta potential, entrapment efficiency, and final drug loading.

The design matrix was constructed using Statgraphics® Centurion XVI (Statpoint Technologies, Inc., Warrenton, VA, USA) software, according to which a total of 15 experimental runs, including 12 factorial points and 3 center points, were required. The composition of these formulations and the corresponding experimental responses observed for each variable were recorded (Table 2). Statgraphics® software statistically fitted each response to the most appropriate polynomial model and provided response surface plots where the relationships between factors and responses were graphically represented. A one-way analysis of variance (ANOVA) determined if each observed response could be attributed to significant ($p \leq 0.05$) individual effects or interaction effects of the three factors.

As a final step in experimental design, a multiple variable optimization process was implemented. The resulting optimized formulation was identified as SENS-OPT. To demonstrate design validity, a SENS-OPT formulation and two checkpoint formulations (SENS-HIGH and SENS-LOW) were prepared in triplicates. The predicted and experimental values of responses were compared, and the percentage of prediction error was determined as follows:

$$\%\text{Bias} = (\text{Experimental value} - \text{Predicted Value})/\text{Predicted Value} \times 100.$$

2.4. Preparation of SENS-OPT-HA

An anionic optimized formulation was prepared by the surface coating of SENS-OPT with HA. The positive surface charge provided by CTAB allowed polymer adsorption. SENS-OPT-HA was prepared by