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Novel liposome-based and *in situ* gelling artificial tear formulation for dry eye disease treatment

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ABSTRACT

Purpose: Artificial tears are widely used in the treatment of dry eye disease, although current formulations do not closely resemble natural tears. The purpose of this study was the design and characterization of a novel *in situ* gelling artificial tear formulation, containing both lipid and aqueous components, in order to resemble natural tears and replenish the tear film.

Methods: Liposomes, containing phosphatidylcholine, cholesterol, vitamins A and E, were prepared by the thin-film hydration method. The aqueous phase of the formulation was comprised of gellan gum, hydroxypropyl methylcellulose, levocarnitine, electrolytes (sodium chloride and potassium chloride), trehalose, and borates. The artificial tear was characterized in terms of liposome size, pH, surface tension, and viscosity. *In vitro* tolerance studies were performed in a human epithelial carcinoma cell line (HeLa) and a murine macrophage cell line (J774). *In vivo* tolerance was assessed in rabbits.

Results: Liposomes presented a unimodal distribution with a mean size of 200.1 ± 4.4 nm. The resulting surface tension was 53.4 ± 1.1 mN/m (at 33 °C) and the pH was 7.6 ± 0.1. The viscosity of the formulation presented a mean value of 4.0 ± 0.1 mPa s within the shear rate interval of 200–1000 s^{-1} at 33 °C. Cell viability remained higher than 90% in both cell lines. No discomfort or clinical signs were observed in rabbits.

Conclusions: The liposome-based and *in situ* gelling artificial tear formulation presented good tolerance and suitable properties for topical ophthalmic administration. It may be beneficial in the treatment of dry eye disease.

1. Introduction

Dry eye disease (DED) is a highly prevalent health problem affecting approximately 5–35% of the world's population [1]. It is defined as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability [2]. DED is associated with a significant adverse impact on vision-related quality of life, causing difficulties in daily activities [3,4]. It can be caused by various factors such as meibomian gland dysfunction, low blink rate, ocular surface disorders and contact lens wear, among others, resulting in the alteration of the tear film [2].

Artificial tears are widely used for mild to moderate dry eye, and can be prescribed in combination with other therapies for moderate to severe dry eye. Numerous artificial tear formulations with a diverse range of components are currently available in the market; however, most of these formulations do not quite resemble the composition of natural tears [5]. Furthermore, they have a short ocular residence time and require repeated daily administration.

An ideal artificial tear formulation should contain both aqueous and lipid components to mimic natural tears, and have certain physicochemical properties such as hypotonicity or isotonicity, neutral to slightly alkaline pH, suitable surface tension and viscosity [6]. A surface tension near the physiological range of natural tears, that is reported to be 40–46 mN/m [7,8], provides a good spreading of the formulation

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over the ocular surface. For artificial tears that follow Newtonian behavior, the desirable viscosity would be in the physiological range of 1.0–8.3 mPa s [9,10]; whereas for those that follow non-Newtonian behavior, a dynamic viscosity in the same range would be suitable. Additionally, an ideal artificial tear formulation should be well tolerated on the ocular surface, provide extended ocular residence time, and be preservative-free. This study aimed at designing a novel liposome-based and *in situ* gelling artificial tear formulation having such properties to replenish the tear film, with potential beneficial effects for the treatment of DED.

2. Materials and methods

2.1. Materials

Phosphatidylcholine obtained from soy lecithin (Phospholipon® 90 G) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol, retinyl acetate (vitamin A), DL-α-tocopherol acetate (vitamin E), gellan gum (Gelzan™ CM, Gelrite®), boric acid, sodium tetraborate anhydrous, and benzalkonium chloride were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Hydroxypropyl methylcellulose was purchased from Abarán Materias Primas S.L. (Madrid, Spain), levocarnitine from Fagron Ibérica SAU (Barcelona, Spain), and D-(+)-trehalose dihydrate from ThermoFisher (Kandel) GmbH (Karlsruhe, Germany). Sodium chloride and potassium chloride were supplied by Merck KGaA (Darmstadt, Germany), and chloroform (stabilized with ethanol) by Panreac Química SLU (Barcelona, Spain).

2.2. Animals

Six male New Zealand white rabbits (purchased from San Bernardo Farm, Navarra, Spain), weighing between 3.0 to 3.5 kg, were used for this study. The animals were housed individually with free access to food and drinking water, in a room with an alternating 12-h light/dark cycle. The room was maintained at 22 °C with 50% relative humidity. The animal experiments were performed in accordance with the EU Directive 2010/63/EU for animal experiments, U.K. Animals (Scientific Procedures) Act 1986, and ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. The study protocol was approved by the Animal Experimentation Ethics Committee of the Complutense University of Madrid and by the Community of Madrid (approval reference: PROEX 011/16).

2.3. Preparation of the artificial tear formulation

Liposomes were prepared by the thin-film hydration method described by Bangham et al. [11] and modified by Vicario-de-la-Torre et al. [12]. 400 mg of phosphatidylcholine (PC), 50 mg of cholesterol (Chol), 4 mg of vitamin E (vit E), and 400 ng of vitamin A (vit A) were dissolved in 25 mL of chloroform. The solvent was evaporated in a rotary evaporator (Büchi Rotavapor R-205, Massó Analítica S.A., Spain). The resulting dry lipid film was hydrated with 10 mL of an aqueous medium composed of 1.68% trehalose, 0.84% boric acid (H_3BO_3), and 0.08% sodium tetraborate anhydrous ($Na_2B_4O_7$). To obtain a homogenous population of lipid vesicles, the liposomal suspension was sonicated (Ultrasons-H, J.P. Selecta S.A., Barcelona, Spain), and extruded (Lipex® Extruder, Transferra Nanosciences Inc., Burnaby, BC, Canada), being forced through polycarbonate filters (Nuclepore™, Whatman International Ltd., Kent, UK) with a pore size of 0.2 μm.

The liposomal suspension was diluted with solutions of gellan gum, hydroxypropyl methylcellulose (HPMC), levocarnitine (L-carnitine), sodium chloride (NaCl) and potassium chloride (KCl) to obtain the final artificial tear formulation (Table 1). The preparation was performed under aseptic conditions. The aqueous medium, polymer and electrolyte solutions underwent steam sterilization, whereas the L-carnitine solution was sterilized by filtration.

Table 1
Composition of the artificial tear formulation.

Formulation component	Concentration (% weight/volume)
PC (liposomes)	0.5
Gellan gum	0.25
HPMC	0.12
L-carnitine	0.1
Vit E	0.005
Vit A	0.0005
NaCl	0.05
KCl	0.05
Trehalose	1.68
H_3BO_3	0.84
$Na_2B_4O_7$	0.08

2.4. Characterization of the artificial tear formulation

The particle size and the size distribution profile of liposomal nanoparticles were determined at room temperature by dynamic light scattering (Microtrac® Zetatrac™ Particle Size & Zeta Potential Analyzer, Microtrac Europe GmbH, Meerbusch, Germany).

The pH of the formulation was determined at room temperature with a calibrated pH meter (GLP 22, Crison Instruments S.A., Barcelona, Spain). The surface tension was measured with a tensiometer (Krüss K-11 Tensiometer, Krüss GmbH, Hamburg, Germany) according to the Wilhelmy plate method. Measurements were performed at 33 °C, which corresponds to the temperature of the ocular surface. The viscosity of the artificial tear was determined with a rheometer (HAAKE RheoStress 1, ThermoFisher Scientific), using the parallel plate system (plate diameter 60 mm, separation 0.5 mm) at 33 °C. The shear rate was increased in 20 steps from 0 to 1000 s^{-1}. All measurements were performed in triplicate.

2.5. In vitro tolerance studies

The *in vitro* tolerance of the formulation was assessed by means of cell viability measurements in a human epithelial carcinoma cell line (HeLa) and a murine macrophage cell line (J774). Cellular reduction of the tetrazolium salt MTT to formazan [13] was evaluated after cells were exposed to the formulation for 2 h.

2.6. In vivo tolerance studies

The *in vivo* tolerance studies were performed in six male New Zealand white rabbits; 30 μL of the artificial tear was administered in the right eye of each animal every 30 min for 6 h, whereas the contralateral eye received the same volume of isotonic saline solution and served as control. Macroscopic evaluation of the ocular surface and the clinical signs was performed before the first instillation, and at 3, 6, and 24 h after the first instillation, in compliance with the protocol described by Enríquez de Salamanca et al. [14]. Corneal opacity, conjunctival alterations, discharge, eyelid swelling, and animal discomfort as evident by intense blinking were examined and graded from 0 to 2.

3. Results

3.1. Characterization of the artificial tear formulation

The results obtained in the characterization of the physicochemical properties of the artificial tear are reported in Table 2. Data were expressed as mean ± standard deviation (SD).

The liposomal nanoparticles of the formulation showed a unimodal size distribution (Fig. 1). The rheological properties of the artificial tear followed Newtonian behavior (Fig. 2).