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Research Paper

# Biopharmaceutical profile of pranoprofen-loaded PLGA nanoparticles containing hydrogels for ocular administration

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ABSTRACT

Two optimized pranoprofen-loaded poly-L-lactic-co glycolic acid (PLGA) nanoparticles (PF-F1NPs; PF-F2NPs) have been developed and further dispersed into hydrogels for the production of semi-solid formulations intended for ocular administration. The optimized PF-NP suspensions were dispersed in freshly prepared carbomer hydrogels (HG_PF-F1NPs and HG_PF-F2NPs) or in hydrogels containing 1% azone (HG_PF-F1NPs-Azone and HG_PF-F2NPs-Azone) in order to improve the ocular biopharmaceutical profile of the selected non-steroidal anti-inflammatory drug (NSAID), by prolonging the contact of the pranoprofen with the eye, increasing the drug retention in the organ and enhancing its anti-inflammatory and analgesic efficiency. Carbomer 934 has been selected as gel-forming polymer. The hydrogel formulations with or without azone showed a non-Newtonian behavior and adequate physicochemical properties for ocular instillation. The release study of pranoprofen from the semi-solid formulations exhibited a sustained release behavior. The results obtained from *ex vivo* corneal permeation and *in vivo* anti-inflammatory efficacy studies suggest that the ocular application of the hydrogels containing azone was more effective over the azone-free formulations in the treatment of edema on the ocular surface. No signs of ocular irritancy have been detected for the produced hydrogels.



## 1. Introduction

Pranoprofen is a non-steroidal anti-inflammatory drug (NSAID) which can be used as a safe and effective alternative anti-inflammatory treatment following strabismus and cataract surgery [1–3]. This drug has the beneficial effect of reducing the ocular signs and symptoms of dry eye and decreasing the inflammatory markers of conjunctival epithelial cells [4]. Its efficacy is equivalent to moderate-potency corticosteroids, but it has improved safety profile. It should be considered for the treatment of chronic conjunctivitis of presumed nonbacterial origin [5]. Although this drug has shown high anti-inflammatory and analgesic efficiency, the pharmaceutical use of pranoprofen is limited due to its inadequate biopharmaceutical profile. Pranoprofen has a short plasmatic half-life, low water solubility and is unstable in aqueous solution, particularly when exposed to light [6,7]. Pranoprofen is commercially available as eye-drops (0.1% m/V). However, this conventional

*Abbreviations:* PF, pranoprofen; NPs, nanoparticles; HG, hydrogel; PF-F1NPs and PF-F2NPs, optimize pranoprofen nanoparticles; HG_PF-NPs-Azone and HG_PF-NPs, nanoparticles incorporated into hydrogel with and without azone, respectively; Z-Ave, average particle size; PI, polydispersity index; ZP, zeta potential; EE, entrapment efficiency; PVA, polyvinyl alcohol; cPF, PF concentration; cPVA, PVA concentration; PLGA, poly-L-lactic-co glycolic acid; cPLGA, PLGA concentration; SA, arachidonic acid sodium; PBS, phosphate buffer solution; BR, Bicarbonate Ringer; $Q_P$, amounts of drug permeated across cornea; $Q_R$, amounts of drug retained in the cornea.

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dosage form cannot be considered optimal in the treatment of ocular diseases due to the fact that upon instillation most of the drugs are removed from the surface of the eye, by various mechanisms (tear dilution and tear turn over). Moreover, the relatively impermeable corneal barrier restricts the entry of foreign substances. As a result, less than 5% of the administered drug penetrates the cornea and reaches intraocular tissue [8]. Polymeric NPs are one of the colloidal systems that have been most widely studied over the past few decades with the objective of improving drug targeting of tissues and organs and increase drug bioavailability across biological membranes. Biodegradable polymers, such as poly (lactic-co-glycolic) acid (PLGA), have been widely used in drug delivery research, in part due to their approval by the FDA for use in humans and they can effectively deliver the drug to a target site with a controllable degradation [9]. PLGA can be used such as matrix to load different drugs for topical administration [10–12].

Different drug delivery systems have been studied in order to improve drug targeting of tissues, increase drug bioavailability across biological membranes or reducing its toxicity. For topical application of nanoparticle suspensions, several of these systems have been dispersed in semi-solid vehicles such as hydrogels or cream [13,14]. Among the gelling agents, carbomer has been extensively used for design topical formulations [15–17]. In addition, to improve the permeability of drugs through the ocular barriers, different enhancers have also been tested. Azone is one of the most widely studied penetration enhancers which can be used as a safe and effective penetration enhancer for human use in the range of 1–10% [18]. In previous studies, we have formulated pranoprofen in PLGA nanoparticles (PF-NPs) using the solvent displacement technique [19]. A $2^4$ central composite factorial design has been applied to study the main effects and interactions of four factors on average particle size (Z-Ave), polydispersity index (PI), zeta potential (ZP) and entrapment efficiency (EE). The factors studied were PF concentration (cPF), PVA concentration (cPVA), PLGA concentration (cPLGA) and aqueous phase pH. From a total of 26 formulations obtained by factorial design, two optimum formulations (PF-F1NPs and PF-F2NPs) were selected for further investigation here [20]. The aim of this study was designed semi-solid formulations containing pranoprofen loaded-PLGA nanoparticles for ocular administration. Carbomer 934 was selected to disperse the optimized PF-NP suspension because of the bioadhesive properties, low or no toxicity, rheological characteristics and biocompatibility of the hydrophilic polymer. Polyacrylic acid hydrogels such as Carbomer 934, polycarbophil and carboxymethylcellulose have been reported as the most appropriate bioadhesive polymers for ocular drug delivery [21]. Additionality, the high viscosity of the carbomer hydrogels ensures the prolonged retention improving the ocular bioavailability of some drugs [22]. The optimized PF-F1NP and PF-F2NP suspensions were dispersed into blank hydrogels (HG_PF-F1NPs and HG_PF-F2NPs) or in hydrogels containing 1% azone (HG_PF-F1NPs-Azone and HG_PF-F2NPs-Azone) in order to improve the biopharmaceutical profile of pranoprofen in the eye, by increasing is ocular retention and improving the anti-inflammatory and analgesic efficiency. The ultimate aim of the developed formulations is to improving the patient's compliance to the pharmacological treatment by reducing the application frequency. In this study, azone was selected as permeation enhancer with the purpose to improve the permeability of pranoprofen from PF-NPs based HG through the ocular barriers. Azone is one of the most widely studied penetration enhancers for hydrophilic and lipophilic drugs. As a penetration enhancer, azone is more effective at low percentages (1–3%), and it has also been reported to be of low irritancy and very low toxicity [23]. The mechanism of azone may be related to some changes in the epithelial cell junctions of the cornea, which are nevertheless reversible in cornea structure [24,25].

The physicochemical properties and the rheological behavior of HG_PF-NP formulations have been characterized. The physical stability of the nanoparticles incorporated into hydrogels has also been evaluated. *In vitro* release profile and *ex vivo* corneal permeation of pranoprofen from the semi-solid formulations, as well as their *in vitro e in vivo* ocular tolerance and the anti-inflammatory efficacy have also been assayed.

## 2. Materials and methods

### 2.1. Materials

Pranoprofen and Oftalar® were kindly supplied by Alcon Cusi (Barcelona, Spain); PLGA Resomer® 753S was obtained from Boehringer Ingelheim (Ingelheim, Germany). Polyvinyl alcohol (PVA) with 90% hydrolyzation and Arachidonic acid sodium (SA) were obtained from Sigma Aldrich (St. Louis, USA). Gel-forming polymer (Carbomer 934) was obtained from Fagron Ibérica. The purified water used in all the experiments was obtained from a MilliQ System. All the other chemicals and reagents used in the study were of analytical grade.

### 2.2. Methods

#### 2.2.1. Preparation of pranoprofen-loaded nanoparticles

The nanoparticles have been produced by the solvent displacement technique, described by Fessi et al. [19]. PLGA (90 mg or 95 mg) and pranoprofen (10 mg or 15 mg) were dissolved in 5 mL of acetone. This organic phase was poured, under moderate stirring into 10 mL of an aqueous solution of PVA (5 mg/mL or 10 mg/mL) adjusted to the desired pH value (4.5 or 5.5). The acetone was then evaporated and the dispersed nanoparticles were concentrated to 10 mL under reduced pressure (Büchi B-480 Flawil, Switzerland). Table 1 shows the composition of the optimized pranoprofen-loaded nanoparticles.

#### 2.2.2. Mean particle size and zeta potential

The mean particle size (Z-Ave) and the zeta potential (ZP) of the nanoparticles were determined by photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25 °C using disposable quartz cells and disposable folded capillary zeta cells (Malvern Instruments, Malvern, UK), respectively. For all measurements, the samples were diluted with MilliQ water (1:20). The reported values are the mean ± SD of at least three different batches of each formulation.

#### 2.2.3. Encapsulation efficiency

The encapsulation efficiency (EE) of pranoprofen in the nanoparticles was determined indirectly by measuring the concentration of the free drug in the dispersion medium. The non-encapsulated pranoprofen was separated using a filtration/ centrifugation technique with Ultracel-100K (Amicon® Ultra, Millipore Corporation, Billerica, MA) centrifugal filter devices at 3000 rpm for 30 min at 4 °C (Heraeus, Multifuge 3 L-R, centrifuge. Osterode, Germany). Each sample was diluted with MilliQ water (1:20) prior to filtration/centrifugation. The EE was calculated using the following equation:

**Table 1**
Composition of the optimized pranoprofen-loaded nanoparticles.

| PF-NPs | cPF (mg/mL) | cPVA (mg/mL) | cPLGA (mg/mL) | pH |
|---|---|---|---|---|
| PF-F1NPs | 1.5 | 10.0 | 9.5 | 5.5 |
| PF-F2NPs | 1.0 | 5.0 | 9.0 | 4.5 |