Design of Nanosuspensions and Freeze-Dried PLGA Nanoparticles as a Novel Approach for Ophthalmic Delivery of Pranoprofen

GUADALUPE ABREGO,[1,2] HELEN L. ALVARADO,[1,2] MARIA A. EGEA,[1] ELIZABETH GONZALEZ-MIRA,[1] ANA C. CALPENA,[2] MARIA L. GARCIA[1]

[1]Department of Physical Chemistry, Faculty of Pharmacy, University of Barcelona, Barcelona 08028, Spain
[2]Department of Biopharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Barcelona 08028, Spain

Received 12 February 2014; revised 23 June 2014; accepted 3 July 2014

Published online 4 August 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24101

ABSTRACT: Pranoprofen (PF)-loaded poly (lactic-*co*-glycolic) acid (PLGA) nanoparticles (NPs) were optimized and characterized as a means of exploring novel formulations to improve the biopharmaceutical profile of this drug. These systems were prepared using the solvent displacement technique, with polyvinyl alcohol (PVA) as a stabilizer. A factorial design was applied to study the influence of several factors (the pH of the aqueous phase and the stabilizer, polymer and drug concentrations) on the physicochemical properties of the NPs. After optimization, the study was performed at two different aqueous phase pH values (4.50 and 5.50), two concentrations of PF (1.00 and 1.50 mg/mL), three of PVA (5, 10, and 25 mg/mL), and two of PLGA (9.00 and 9.50 mg/mL). These conditions produced NPs of a size appropriate particle size for ocular administration (around 350 nm) and high entrapment efficiency (80%). To improve their stability, the optimized NPs were lyophilized. X-ray, FTIR, and differential scanning calorimetry analysis confirmed the drug was dispersed inside the particles. The release profiles of PF from the primary nanosuspensions and rehydrated freeze-dried NPs were similar and exhibited a sustained drug delivery pattern. The ocular tolerance was assessed by an HET-CAM test. No signs of ocular irritancy were detected (score 0). © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3153–3164, 2014

Keywords: pranoprofen; polymeric drug delivery system; nanoparticles; factorial design; poly (lactic/glycolic) acid (PLGA); freeze-drying/lyophilization; stability; biodegradable polymers

INTRODUCTION

Pranoprofen (PF) is a nonsteroidal anti-inflammatory drug that can be used as a safe and effective alternative anti-inflammatory treatment following strabismus and cataract surgery.[1–3] PF 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 a better 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 PF is limited because of its inadequate biopharmaceutical profile. PF has a short plasmatic half-life, low water solubility, and is unstable in aqueous solution, particularly when exposed to light.[6,7] PF is commercially available such as eye drops (PF 0.1%). However, this conventional dosage form cannot be considered optimal in the treatment of ocular diseases because of the fact that most of the drug is removed from the surface of the eye, following the instillation, 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 tissues.[8] Polymeric nanoparticles (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 United States Food and Drug Administration for use in humans and they can effectively deliver the drug to a target site with a controllable degradation.[9]

The instability of the NPs after a long storage period is one of the main problems that limit the use of these systems. To ensure the long-term preservation of the physicochemical integrity of these colloidal systems, the water contained in the formulations must be removed. Lyophilization or freeze-drying is one of the procedures commonly employed to this end. Freezing is the first step of the lyophilization process. The liquid suspension is frozen and then the water is removed by sublimation and desorption under vacuum. Lyophilization could generate considerable stresses that could destabilize NPs, and modify their physicochemical characteristics. Thus, to avoid such possible modifications, cryoprotectants or lyoprotectants are usually added to the formulation.

Polyvinyl alcohol (PVA) is a hydrophilic, biocompatible polymer used as surfactant to produce stable NPs.[10] PVA forms a hydrophilic layer at the NP surface, which provides stability to the colloidal system and improves their resistance to freezing as well as their redispersion after freeze-drying.[11–13]

The objective of this study was to develop and optimize a new delivery system for PF-loaded PLGA NPs, suitable for the ocular route, prepared by the solvent displacement technique.[14] After selecting the critical formulation variables that affect mean particle size, zeta potential (ZP), and drug loading

Correspondence to: Ana C. Calpena (Telephone: +34-934024560; Fax: +34-934024563; E-mail: anacalpena@ub.edu)

Journal of Pharmaceutical Sciences, Vol. 103, 3153–3164 (2014)

efficiency, a four-factor five-level central rotatable composite design was employed to plan and perform the experiments. The physical stability of the NPs was also evaluated. As an attempt to improve the stability of the systems, lyophilization studies were carried out. Furthermore, the physical state of the PF and possible interactions between the drug and the polymer were studied, as well as *in vitro* release and ocular tolerance of the optimized vehicle.

MATERIALS AND METHODS

Materials

Pranoprofen and Oftalar® were kindly supplied by Alcon Cusi (Barcelona, Spain); PLGA Resomer® 753S was obtained from Boehringer Ingelheim (Ingelheim, Germany); PVA with 90% hydrolization was obtained from Sigma–Aldrich (St. Louis, Missouri). 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.

Methods

Preparation and Optimization of PLGA NPs

The NPs were obtained by the solvent displacement technique described by Fessi et al.[14] PLGA (80–100 mg) and PF (0–20 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 (5–25 mg/mL) adjusted to the desired pH value (2.5–6.5). The acetone was then evaporated, and the NPs dispersed were concentrated to 10 mL under reduced pressure (Büchi B-480 Flawil, Switzerland).

A factorial design is frequently used to plan research because it provides maximum information, requiring the minimum number of experiments.[15] A four factor, five-level central composite rotatable design 2^4 + principal was used to study the main effects and interactions of four factors on average particle size (*Z* Ave), polydispersity index (PI), ZP, and entrapment efficiency (EE). This central composite design consisted of three groups of design points, including two-level factorial axial and center design points. The factors or independent variables studied were PF concentration (cPF), PVA concentration (cPVA), PLGA concentration (cPLGA), and aqueous phase pH. They were studied at five different levels coded as $-\alpha$, -1, 0, 1, and $+\alpha$. The value of alpha (2) was calculated to meet the design rotatability (Table 1).

Effects and interactions between factors were calculated. To determine the effect of a factor, x, (Ex) the following expression was used:

$$\mathrm{E}x = \frac{\sum x(+) - \sum x(-)}{n/2} \quad (1)$$

Table 1. Factors and their Corresponding Levels in Experimental Design

Factor	−2	−1	0	+1	+2
cPF (mg/mL)	0.00	0.50	1.00	1.50	2.00
cPVA (mg/mL)	5.00	10.00	15.00	20.00	25.00
cPLGA (mg/mL)	8.00	8.50	9.00	9.50	10.00
Aqueous phase pH	2.50	3.50	4.50	5.50	6.50

where Σx (+) is the sum of the factors at their highest level (+2), Σx (−) is the sum of the factors at their lowest level (−2), and $n/2$ is half of the number of measurements used in the calculation.

Interactions between factors were also calculated. To estimate an interaction between two factors, one has to calculate the effect of the first factor at the lowest level of the second factor and subtract this from the effect of the first factor at the highest level of the second factor. An interaction between two factors is symbolized as factor1 × factor2.

The experimental responses studied were the result of the individual influences and interactions of the four factors. The responses were modeled by the following polynomial equation:

$$\begin{aligned} Y = {} & \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \\ & + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 \\ & + \beta_{12} X_1 X_2 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 \\ & + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_2 \end{aligned} \quad (2)$$

where Y is the measured response, β_0 is the intercept term, β_is (for i = 1–4) are the linear effects, β_{ij}s the quadratic effects, β_{ij}s (for ij = 1–4, $i < j$) the interaction between the ith and jth variables.

ANOVA identified the significance of the effects and the interactions between them. p Values of less than 0.05 were considered to be statistically significant.

According to the composite central design matrix, generated by Statgraphics Plus 5.1 (Sigma Plus) Software, a total of 26 experiments, including 16 factorial points, eight axial points, and two replicated center points, are summarized in Table 2.

Physicochemical Characterization of the NPs

Particle Size and ZP. Z Ave and ZP of the NPs 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), respectively. In all the determinations, 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. PCS is referred to as dynamic light scattering and quasi-elastic light scattering when it is used to determine rapidly the particle diameter and size distribution expressed as the PI. The movement of particles in water is inversely proportional to their size, which can therefore be identified by analyzing the time dependency of the light intensity fluctuations caused by scattering off the particles when they are illuminated with a laser beam.[16] The ZP of NPs is a measure of the electrical charge at the surface of nanospheres, and is an indirect measure of their physical stability. ZP was calculated using the Helmholtz–Smoluchowski equation.[17]

Entrapment Efficiency. The EE of PF in the NPs was determined indirectly by measuring the concentration of the free drug in the dispersion medium. The nonentrapped PF was separated using a filtration/centrifugation technique with Ultracel–100K (Amicon® Ultra; Milipore Corporation, Billerica, Massachusetts) centrifugal filter devices at 1814 g. 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