Ocular Delivery of Flurbiprofen Based on Eudragit® E-Flurbiprofen Complex Dispersed in Aqueous Solution: Preparation, Characterization, *In Vitro* Corneal Penetration, and Ocular Irritation

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ABSTRACT: A novel ophthalmic formulation based on the ionic complexation between Eudragit E 100 (EU) and flurbiprofen (**FB**) is proposed. The selected complex composition, named EU–$FBH_{50}Cl_{50}$, had the basic groups of EU completely neutralized with equal molar amounts of **FB** and HCl. This complex, obtained in the solid state, exhibited a high aqueous compatibility producing a colloidal dispersion with a high positive electrokinetic potential, in which more than 99% of FB was ionically condensed with EU. In bicompartimental Franz cells, FB diffusion from the complex was very slow. However, dispersion in 0.9% NaCl increased the FB release through an ionic exchange, providing an optimal constant rate of delivery. Corneal FB permeation from 0.1% EU–FBH_{50}–Cl_{50} dispersed in 0.9% NaCl solution was substantially more effective compared with 0.1% **FB** solution, EU–FBH_{50}–Cl_{50}(Dex), or Tolerane® (a marketed formulation). This complex formulation was shown to be innocuous for rabbit ocular tissues because no irritant effects were evidenced. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3859–3868, 2014

Keywords: flurbiprofen; Eudragit; complex; ophthalmic solution; Permeability; Polymeric drug delivery systems; Controlled release

INTRODUCTION

The effective incorporation of drugs into the inner structures of the eye needs to overcome its efficient mechanism of precorneal drug elimination and achieve adequate drug permeability through the corneal epithelium.

Novel strategies that have been applied to the design of optimized ophthalmic formulations include retention (mucoadhesion) on the eye surfaces, sustained release, and permeation enhancement. On the basis of these techniques, different ocular drug delivery systems such as liposomes, microemulsions, and nanoparticles, among others, have been developed.[1,2] However, although all these formulations have some important advantages, they are quite complicated to manufacture, with the aqueous solutions still being the most convenient system for ophthalmic formulations.

The topical use of NSAIDs(Non-steroidal anti-inflamatory drugs) in ophthalmology is limited, as most of the NSAIDs are weakly acidic drugs that ionize at the pH of the lachrymal fluid, and therefore have limited permeability through the anionic cornea, which has an isoelectric point (p*I*) of 3.2.[3] Although reducing the pH of the formulation increases the unionized fraction of the drug which in turn enhances permeation, NSAIDs are acidic and consequently inherently irritant, with a further reduction in the pH of the formulation intensifying their irritation potential as well as decreasing their aqueous solubility. As a result, it is therefore difficult to formulate topical NSAID formulations that are comfortable when applied to the eye. Nevertheless, NSAIDs seem to be a safe and effective alternative to corticosteroids in the topical management of ocular inflammations and these drugs are currently used topically in the inhibition of intraoperative miosis, management of postoperative inflammation, treatment of seasonal allergic conjunctivitis, prevention and treatment of cystoid macular edema, and in the control of pain after photorefractive keratectomy. NSAIDs have also been found to be useful in decreasing bacterial colonization of contact lenses and prevent bacterial adhesion to human corneal epithelial cells.

The NSAID, flurbiprofen (FB), is a nonselective inhibitor of prostaglandin biosynthesis in humans and is indicated for the acute or long-term treatment of the signs and symptoms of gout, osteoarthritis, rheumatoid arthritis, and sunburn.[4,5] Furthermore, it is currently used as a first-line ophthalmic medication for the inhibition of miosis induced during the course of cataract surgery, as it inhibits cyclooxygenase.[6] The prevention of inflammation mediator release in the anterior eye segment can also decrease the postoperatory time after intraocular surgery.[7–9]

Flurbiprofen, 2-(2-fluorobiphenyl-4-yl) propionic acid, is a weak acid (p*K*a = 4.2),[10] practically insoluble in water (2.70×10^{-2} mg/mL at 25°C), with high lipophilicity (log PC = 4.24)[11] and low molecular weight (244.26). It is usually formulated as aqueous solutions of sodium flurbiprofen (FBNa), but **FB** solutions of concentrations greater than 0.2% (w/v) are irritating.[12] ***FBNa*** is more soluble but much less permeable than the acid species ***FBH***. Therefore, an increase in the apparent

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solubility of neutral species ***FBH*** may render the formulation more efficient regarding the amount of **FB** available for absorption.

In previous works, we have reported some results related to increasing the apparent solubility of anionic drugs by means of their complexation with the cationic acrylate polymer Eudragit E® (EU).[13] EU[14] is a cationic polymer based on dimethylaminoethyl methacrylate and other neutral methacrylic acid esters. This polymer may be considered an electrolyte of high molecular weight containing numerous basic groups. Complexation of EU with acidic drugs (AH) results in a noticeable increase in the apparent aqueous solubility of these types of drugs at pH (~5) in which the nonionized species exert solubility control. These acid base interactions are schematized as follows:

$$\mathrm{EU} + AH \rightleftarrows EUH^{+} + A^{-} \rightleftarrows [\mathrm{EUH}^{+}A^{-}] \qquad (1)$$

The determination of the degree of counter ionic condensation revealed a remarkable affinity between *AH* and EU. In particular, for NSAIDs, this affinity was higher for compounds without an alpha methyl group.[15]

The concentration of the species involved in the equilibrium depends on the characteristics of the medium (pH, ions, and solvent). In addition, the basic groups of EU can be neutralized not only by the drug but also with a second anionic species (i.e., ***Cl***$^-$), which may improve the aqueous compatibility of the complex. In fact, the introduction of ***Cl***$^-$ as a second counter ion yields highly aqueous compatible systems that exhibit properties, which may be useful to improve the biopharmaceutical performance of these types of drugs.

In this article, we report the results obtained from studies addressing the physical and chemical properties of the EU–FBH_{50}–Cl_{50} complex in aqueous dispersion, the effect of formulation variables on drug release, and the potential of complexation to improve FB corneal permeability. The irritation and safety of the EU–FBH_{50}–Cl_{50} complex using a slightly modified version of the Draize test[16] were also evaluated, and a histological examination was performed.

Different species such as ***FBH***, ***FB***$^-$, and ***Cl***$^-$ are highlighted by means of bold italic letters to differentiate these from generic compounds such as **FB**.

Table 1. Composition, pH, and Osmolality of Test Formulations (mean ± SD, $n = 3$)

Formulations	Vehicle	pH	Osmolarity (Osmol/kg)
Flurbiprofen	PBS	6.82 ± 0.02	0.302 ± 0.024
Tolerane®	Sodium tetraborate tetrahydrate, EDTA disodium salt, boric acid, ß-cyclodextrin, purified water	7.66 ± 0.01	0.292 ± 0.034
EU–FBH_{50}–Cl_{50}	NaCl (0.9%)	4.87 ± 0.03	0.289 ± 0.045
EU–FBH_{50}–Cl_{50}	Dextrose (5%)	5.53 ± 0.03	0.287 ± 0.055

MATERIALS AND METHODS

Materials

Poly(butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate) 1:2:1 (Eudragit® E100; Pharmaceutical Grade, Rohm, Germany) was a gift from Etilfarma S.A. (Buenos Aires, Argentina). Acetone (PA grade; Cicarelli, Santa Fe, Argentina), sodium lauryl sulfate, sodium chloride (NaCl), dextrose (PA grade; Cicarelli), cyclohexane (CH) (Sintorgan, Buenos Aires, Argentina), 1 N hydrochloric acid (Anedra, San Fernando, Argentina), and **FB** were donated by Quimica Luar S.A. (Córdoba, Argentina). FB (0.1% w/v) Tolerane; (Alcon®, Buenos Aires, Argentina), phenobarbital 100 mg/mL;(Fada Pharma®, Buenos Aires, Argentina), and 0.25% fluorescein sodium salt ,Solution of Grant,(Alcon®, Buenos Aires, Argentina) were used for assays. Phosphate buffer saline PBS solution at pH 6.85 was prepared according to the method of Zimmer et al.[17] Simulated tear fluid (based on the electrolyte composition of tear fluid[18] was prepared as follows: $NaHCO_3$ 0.218 g, NaCl 0.678 g, $CaCl_2{\cdot}2H_2O$ 0.0084 g, KCl 0.138 g, and ultrapure water 100 g. The osmolality of the simulated tear fluid was 287.5 ± 5.4 mmol/kg, and the pH of the fluid was around 8.

Methods

Preparation of EU–FBH_{50}–Cl_{50} Complex and Formulations

Before complexation, EU was milled and sieved through 40 and 70 mesh sieves, and the equivalents of amino groups per gram of EU (3.10×10^{-3}) were assayed by acid base titration. The complexes were prepared by dispersing 1 g of EU and the appropriate amount of FB necessary to neutralize 50% of the amino groups of EU in 15 mL of acetone. After the remaining basic groups of the polymer were neutralized with 1.0 N HCl, the solvent was evaporated under vacuum at room temperature. In the notation EU–$FBH_{50}Cl_{50}$, "50" indicates the percentage of basic groups of EU neutralized by ***FBH*** and ***Cl***.[13,19] Two EU–FBH_{50}–Cl_{50} aqueous dispersions having 0.1% **FB** were prepared by adding either dextrose [EU–FBH_{50}–Cl_{50}(Dex)] or 0.9% NaCl solution [EU–FBH_{50}–HCl_{50}(NaCl)], respectively. A marketed formulation (Tolerane®, 0.1% **FB**), whose design was based on complexation of **FB** with β-cyclodextrin (β-CD),[20–22] was comparatively assayed. In addition, a solution of 0.1% **FB** in an isotonic phosphate buffer (PBS) at pH 6.85 was also assayed. The composition of the formulations is shown in Table 1.

Partition Equilibrium with CH

Aqueous dispersions of EU–FBH_{50}–Cl_{50} at 0.1% FB were shake flask partitioned with CH at a CH/aqueous dispersion ratio of 2. The concentration of **FB** in CH was spectrophotometrically assayed at 276 nm (molar absorptive 2.14×10^4), and the pH was recorded before extraction and at equilibrium. In addition, an experiment with 0.9% NaCl incorporated into the aqueous phase was also performed. In the same way, the partition equilibrium CH/water of **FB** was measured in order to obtain the true partition coefficient (PCt), with 1.998×10^{-5} M of **FB** solution in CH being partitioned with water or NaCl at a ratio of 2 and the pH at equilibrium being measured. Each sample was assayed in triplicate ($n = 3$).

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Osmolarity and pH

An Osmomat 030-D Cryoscopic Osmometer Printer Ganatec apparatus, using 0.9% NaCl solution as the reference (0.303 Osmol/kg) and a Hanna HI 112 instrument, was used to determine osmolarity and pH, respectively. Each sample was assayed in triplicate ($n = 3$).

Surface Tension

The surface tension (γ, dina cm^{-1}) of the solvents of the formulations (dextrose and NaCl) and the EU–FBH_{50}–Cl_{50} aqueous dispersions were measured using the DuNoüy ring method at 25°C with a thermostated TS Surface Tensiomat 21heta (Cole Parmer). Each sample was assayed in triplicate ($n = 3$).

Electrokinetic Potential (ξ)

A particle microelectrophoresis apparatus (Marck II, Rank Brothers Ltd.,Cambridge, UK), with a 10 cm length between the electrodes and 50.0 ± 0.5 V of potential at a controlled temperature of 25°C, was used to determine the electrokinetic potential. Each sample was assayed in triplicate ($n = 3$).

Particle Size

The aqueous dispersions were measured by photon correlation spectroscopy (PCS) using a DelsaNano-C instrument (Beckman Coulter, Osaka, Japan). Hydrodynamic diameters (d_H) were calculated from diffusion coefficients values, using the cumulants method (DelsaNano 2.20 software, Beckman Coulter, Osaka, Japan). The PCS measurements were carried out at a 165° scattering angle and a laser diode of 658 nm. All measurements were performed in triplicate at 25°C, which allowed the instrument to automatically optimize signal intensity of the sample.

Ex Vivo Transcorneal Permeation

Transcorneal permeation experiments were performed in a modified diffusion chamber of acrylic plastic, consisting of donor and receptor compartments of 1.0 and 4.0 mL volumes, respectively.[23] No significant adsorption of the tested formulations into the diffusion chamber surface was observed over the 2 h period. Before use, the PBS receptor solution was aerated with a mixture of 95% O_2 and 5% CO_2.

Albino rabbits were sacrificed according to the protocols. First, they were anesthetized with phenobarbital and euthanized with a mixture of 10% O_2 and 90% CO_2 in a hermetic chamber. Then, the corneas, with a 2 mm ring of sclera, were immediately excised and mounted in the diffusion chamber. A 4 mL aliquot of the receptor solution was added to the endothelial side, and 1.0 mL of the test solution was added to the epithelial side.

The formulations were freshly prepared, with the temperature of the chamber being maintained at 35.0 ± 0.5°C, by means of a thermostatic water bath. Sample aliquots from the receptor chamber were withdrawn at 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min and immediately replaced by previously aerated fresh receptor medium. Samples were filtered through a 0.45-μm membrane, and the filtrate was kept at 4°C until being analyzed by UV. The area available for permeation in the cell was 0.785 cm^2.

The permeation parameter of **FB** was calculated by plotting the amounts of drug permeated through the excised cornea (mg/cm^2) versus time (seconds).[24,25] The steady-state flux (J) values across excised cornea were evaluated from the linear ascents of the permeation graphs by means of the following relationship:

$$J = dQ/dtA \ [\mu g/(cm^2 s)]$$

where Q indicates the quantity of substance crossing the cornea, A is the corneal area exposed, and t is the time of exposure. The permeation coefficient P was calculated using the following equation:

$$P = J/C_0(cm/s)$$

The apparent permeability coefficient was calculated using the following equation:

$$P_{app} = dQ/dt \times 1/(A \times C_0)$$

where C_0 represents the initial drug concentration in the donor compartment.

In previous studies, we were able to corroborate that at the end of the study the final concentration of **FB** was at least 20-fold below the maximal solubility in the acceptor medium. Each sample was assayed in quadruplicate ($n = 4$).

In Vitro Drug Release Studies

These assays were performed with the aim of obtaining information about the FB release kinetic from the formulations, using the previously described diffusion cells. In this case, a semipermeable membrane compounded by cellulose acetate (Sigma®-12000) was placed between the donor and receptor compartments. Different mathematical models may be used for inferring release patterns. In this case, we observed that the experimental data fitted very well with the Korsmeyer equation, which represented the best model for describing the dissolution of the drug. So, the following equation was used:

$$\text{ft} = \frac{M_t}{M_\infty} = kt^n \qquad (2)$$

where ft is the ratio of absolute cumulative amount of the drug released at time t and at infinite time, α is a constant incorporating structural and geometric characteristics of the carrier, and n is the release exponent, indicative of the drug release mechanism. If $n = 0.5$, the release is governed by Fickian diffusion. For $n = 1$, the molecules are released by surface erosion, both mechanisms play a role in the release if n has a value between 0.5 and 1. In all cases, the fit was carried out using the Kaleida Graph v4 software.

Ocular Irritation Test

Evaluation with the Draize Method. The potential ocular irritancy and/or damaging effects of the developed formulation in comparison with Tolerane® (commercial formulation) and SDS solution in 2% (w/w) PBS (positive control) were evaluated using a slightly modified version of the Draize test.[16] The assay was carried out in 12 eyes of six male albino white rabbits weighing 2–2.5 kg. A volume of 50 μL of the test formulations was instilled into the conjunctiva sac of each eye (the rabbit's conjuctival sac capacity is ~30 μL). A separate control group of six rabbits received normal saline solution (NaCl 0.9% w/v) in

Table 2. Score for Potential Corneal Injury

Score Value	Formulation Effects
0–8	No irritation
9–20	Mild irritation
21–40	Mild-to-moderate irritation
41–60	Moderate irritation
61–80	Severe injury
81–110	Very severe injury

each eye. Pre- and postexposure evaluations of the eyelids, conjunctiva, cornea, and iris were performed by external observations under adequate illumination, with additional information being provided by examination using slit lamp biomicroscopy (Kowa SL-14). For each observation, one drop of fluorescein salt (0.25%) was instilled to reveal the potential corneal injury. The rating of ocular irritation or damage was scored (Table 2) for each observation at 30, 60, 120, and 180 min.

Histological Examination

According to the results described above and aiming to examine the effects on corneal structure and integrity, 30 min after the instillation, where the maximum irritation occurred, the animal was sacrificed, the corneas were removed, and histological examination was performed. For comparison, the effect of 0.9% NaCl and SDS solutions on 2% (w/w) PBS was also evaluated in the same experimental conditions.

After incubation, the corneas were washed with PBS and immediately fixed with 8% (w/w) formalin solution. The material was dehydrated with an alcohol gradient, put into melted paraffin, and solidified into a block form. Cross sections (<1 μm) were cut, stained with hematoxylin and eosin, and microscopically observed for any pathological modifications.[26]

Animals

White New Zealand normotensive rabbits (IOP average = 11.39–0.92 mmHg) weighing 2–2.5 kg were used. The rabbits were provided with food and water ad libitum in a temperature-controlled room (21°C–5°C) and exposed to 12-h light–12-h dark cycles.

All experimental procedures conformed to the Association for Research in Vision and Ophthalmology resolution about the use of animals in research and the European Communities Council Directive (86/609/EEC). The Institutional Care and Use Committee of the School of Chemistry of Córdoba University, Córdoba, Argentina, reviewed and approved the protocols (HCD 342/09). After a week of adaptation in the facilities, the animals were admitted to the experimental session.

RESULTS AND DISCUSSION

Ionization Equilibrium

As deduced from Eq. (1), in aqueous EU–FBH_{50}–Cl_{50} dispersions, **FB** is distributed as free species $\boldsymbol{FB^-}$, $\boldsymbol{FBH}$, and condensed with EU as $EUH^+\boldsymbol{FB^-}$. Then, the total drug molar concentration $[\mathbf{FB}]_{w/NaCl}$ is distributed as:

$$[\mathbf{FB}]_w = [FB] + [FBH] + [EUH^+FB] \text{ or } [\mathbf{FB}]_{NaCl} = [FB^-Na^+] + [FBH] + [EUH^+FB]$$

The proportions in which such species are distributed in EU–FBH_{50}–Cl_{50} dispersions were determined according to previously described methods by Jimenez-Kairuz et al.[27] through the selective extraction of **FB** by an appropriate organic solvent.

According to the possible ionization of amino groups [R–N–$(CH_3)_2$] of the EU and its interaction with **FB**, the following speciation equilibriums could be expected:

$$[EU]_{total} = [EUH^+] + [EU] + [EUH^+FB^-] \text{ or } [EU]_{total} = [EUH^+Cl^-] + [EU] + [EUH^+FB^-]$$

Then, according to the equilibrium derived from Eq. (1), the affinity constant of ion-pair formation (K_{ip}) is given by

$$K_{ip} = [EUH^+FB^-]/[EU][FBH] \text{ or } K_{ip} = [EUH^+FB^-].Ka/[EU][H^+][FB^-]$$

The measured values of $\boldsymbol{K_{ip}}$ (Table 3) are indicative of the high affinity of EU–FB complexes. In dextrose solution, we were able to determine a log $\boldsymbol{K_{ip}}$ value of 14.09 for this complex.

The determination of the species distribution of an aqueous dispersion of EU–FBH_{50}–Cl_{50} revealed that the predominant species corresponding to equilibrium (1) was the counter-ionic condensed complex $[EUH^+\boldsymbol{FB^-}]$ (see Table 3).

On the other side, in the case of the complexes dispersed in NaCl medium, the affinity remains very high although the relative concentration of $[\boldsymbol{EUH^+FB^-}]$ diminished. It may be also noted that the concentration of $\boldsymbol{FBH}$ species is noticeably higher in NaCl solution than in dextrose solution, which could has implication in the observed high permeation in the in vivo assay [see the section *Ex Vivo FB Permeation (Franz Cells, Rabbit Cornea)*]. Consequently, the macromolecular complex was considered to be a reversible drug reservoir. In fact, as also reported in Table 3, equilibrium (2) was partially shifted to the right by the addition of NaCl as follows:

$$[EUH^+FB^-] + NaCl \rightleftarrows EUH^+ + Cl^- + FB^- + Na^+$$

Table 3. Species Distribution and log K_{ip} of Eu–FBH_{50}–Cl_{50} Complexes of EU–FBH_{50}–Cl_{50} in Aqueous Media and 0.9% NaCl Solution (mean ± SD, $n = 3$)

	Stoichiometric Composition		Species Distribution (%)			
Formulations	[EU] (eq/L)	$[FB]_T$ (M)	[*FBH*]	$[FB^-]$	$[EUH^+FB^-]$	*log* K_{ip}
EU–FBH_{50}–Cl_{50}(Dex)	7.74×10^{-3}	3.86×10^{-3}	0.12 ± 0.01	0.55 ± 0.03	99.33 ± 4.32	14.09
EU–FBH_{50}–Cl_{50}(NaCl 0.9%)			0.49 ± 0.07	19.55 ± 5.36	78.95 ± 5.36	13.42

With regard to the formulation selected for comparison (Tolerane®), FBH is solubilized by mean of the complexation with CD. Under normal conditions, the large and very hydrophilic CD molecule is no able to penetrate biological membranes although may acts as penetration enhancer.[28] CD may improve ocular bioavailability of drugs by keeping the water-insoluble drug molecules in solution and deliver them to the surface of the corneal barrier where they are able to partition into the eye.

The ionization equilibrium of CD–FB complex is slightly affected by pH changes, where lower pHs favored complex formation as a consequence of the higher concentration of nonionized FB.[29] On the other hand, as expected, this equilibrium is not influenced by the presence of electrolytes in the media. In this way, from this point of view, this kind of complexes is not comparable with the EU–FB complexes. So, a study focused on the equilibrium behavior of CD–FB complex exceeds the aim of this study. In this case, we were only interested in the comparison of the *in vivo* effectiveness of EU–FB complexes compared to a commercial formulation.

Osmolarity

The osmolarity of lachrymal fluid varies between 280 and 293 mOsm/kg. Solutions with an osmolarity lower than 100 mOsm/kg or higher than 640 mOsm/kg have the tendency to cause irritation, with the original osmolarity of lachrymal fluid being restored within 1–2 min.[30] The aqueous dispersions of EU–FBH_{50}–Cl_{50} were isosmotic (see Table 1).

pH

The EU–FBH_{50}–Cl_{50} complexes, dispersed in both 0.9% NaCl solution as well as in 5% dextrose solution, showed slightly acidic pHs (4.87 ± 0.03 and 5.53 ± 0.03, respectively) (Table 1). Taking into account these pH values, a potential irritating effect over the conjunctival mucosa would be expected.[3] However, the negligible irritant effect observed for the formulations [see the section *Ex Vivo FB Permeation (Franz Cells, Rabbit Cornea)*] might have been a consequence of the high complexation ratio of FB with EU, which ranged from 78% to 99.5%, depending on the dispersion media.

Surface Tension

The modification of surface tension of lachrymal fluid may be one of the main reasons for tear film destabilization. Therefore, the surface tension was measured for 5% dextrose (65 ± 0.5 mN/m) and 0.9% NaCl (56.933 ± 0.002 mN/m) solutions, both with and without FB, and the results are shown in Table 4. Both aqueous media revealed relatively high surface tensions, which decreased when the complexes were dispersed. This effect may have been a consequence of the slight surfactant effect of the polymer. Thus, EU–FBH_{50}–Cl_{50}(Dext) (49.17 ± 0.02 mN/m) and EU–FBH_{50}–Cl_{50}(NaCl) (43.70 ± 0.30 mN/m) had similar values of surface tension to that of the lachrymal fluid, which was previously reported to be about 40 and 50 mN/m in human.[31]

Particle Size and Electrokinetic Potential

The particle size of each formulation ranged from 470 to 550 nm, all measurements showed a polydispersity index between 0.1 and 0.3. Such high indexes could be attributed to nonspherical colloidal structures produced by random ionic interaction. Similar results were reported by Dillen et al. and Palena et al.[32,33]

The electrokinetic potential of the aqueous dispersions of EU–FBH_{50}–Cl_{50} was high and positive (see Table 4). As it is well known, high electrokinetic potential values are indicative of significant electrostatic repulsions between the colloidal particles, leading to higher stability conditions.[34]

In Vitro FB Release (Franz Cells, Semipermeable Membrane)

In order to obtain information about the release kinetic of FB from the formulations, we evaluated the drug diffusion in a Franz cell model through a synthetic semipermeable membrane. A commercial formulation and control solution were also included in the study for comparison (Fig. 1).

Free **FB**, which was prepared at 0.1%, exhibited a faster diffusion rate than EU–FBH_{50}–Cl_{50} (NaCl), EU–FBH_{50}–Cl_{50}(Dex), and Tolerane®. As expected, EU–FBH_{50}–Cl_{50}(NaCl) produced a higher **FB** release in comparison with EU–FBH_{50}–Cl_{50}(Dex), whose release was almost negligible. Taking into account that in the complex EU–FBH_{50}–Cl_{50}, the drug (FB) showed a high affinity for the polymer (EU), where the predominant species (>99%) was [EUH^+***FB***$^-$]; this behavior may be explained by this complex being able to modulate FB release in aqueous media interchange mechanism with the electrolytes (NaCl) present in the medium. In this way, the presence of ***Cl***$^-$ ions accelerated FB delivery. Actually, FB release is substantially influenced by Cl^- ions according to the results showed in Figure 2. The table given in the figure shows the values of ionic strength (***I***, mol·L^{-1}), pH, and Cl^- concentration (M) at which the assays were performed. Rate release was proportional to the amount of Cl^- present in the medium, whereas apparently the ionic strength had no influence.

In contrast, when the complex was dispersed in a nonionic media such as dextrose solution, the release ratio was very low.

With regard to Tolerane®, the inclusion of **FB** into β-CD seemed to be efficient for raising its solubility and exhibited a release pattern similar to EU–FBH_{50}–Cl_{50}(NaCl).

Kinetics of Drug Release

For a drug delivery system, an n value of 0.43 corresponds to Fickian diffusion of the drug, whereas n values equal to or higher than 0.85 correspond to a case II transport (relaxation controlled delivery). Intermediate values ranging from 0.43 to 0.85 indicate anomalous transport.

Table 4. Surface Tension (γ), Particle Size (d_H), Polydispersity Index (PI) and Zeta Potential (ξ) of Formulations (mean ± SD, $n = 3$)

Formulations	γ (dina cm^{-1})	d_H (nm)	PI	ξ (mV)
EU–FBH_{50}–Cl_{50}(Dex)	49.17 ± 0.02	475.90 ± 40.89	0.227 ± 0.023	44.14 ± 0.15[a]
EU–FBH_{50}–Cl_{50}(NaCl)	43.70 ± 0.30	549.70 ± 89.97	0.253 ± 0.024	

[a]Measurements were carried out in an aqueous medium.

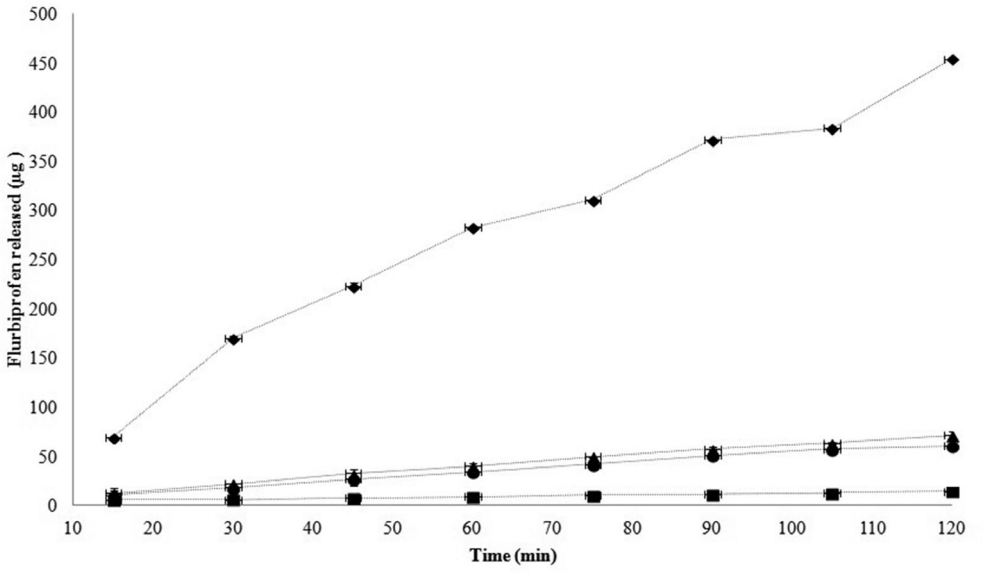

Figure 1. Release profiles of (♦) 0.1% flurbiprofen, (●) Tolerane®, (■) EU–FBH_{50}–Cl_{50} in 5% dextrose solution, and (▲) EU–FBH_{50}–Cl_{50} in 0.9% NaCl solution (mean ± SD, $n = 3$).

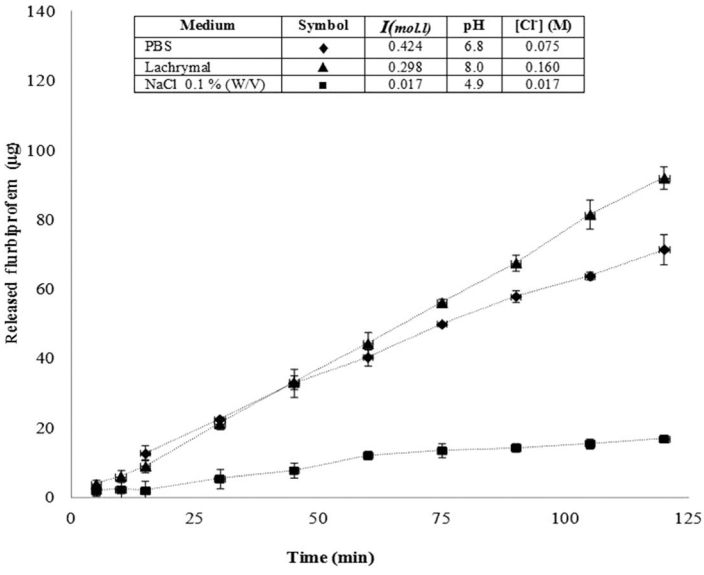

Figure 2. Release profiles of EU–FBH_{50}–Cl_{50} in (♦) PBS buffer, (■) 0.1% (w/v) NaCl solution, and (▲) simulated lachrymal fluid (mean ± SD, $n = 3$). The inserted table informs the values of ionic strength (***I***, mol. L^{-1}), pH, and Cl^- concentration (M) at which the assays were performed.

The FB release from complexes showed a good fit with the Korsmeyer–Peppas model, presenting correlation coefficients higher than 0.99 (Table 5). The n values ranged from 0.43 to 0.85 indicating that the release of the drug was explained by the anomalous transport of FB from the complexes.

Ex Vivo FB Permeation (Franz Cells, Rabbit Cornea)

In this study, excised corneas were incorporated into the Franz cells and placed between the donor and the acceptor compartments. Then, the permeation of the four formulations was evaluated under the same experimental conditions described above [see the section, *In Vitro FB Release (Franz Cells, Semipermeable Membrane)*]. These results are shown in Figure 3 and summarized in Table 5, where it can be observed that EU–FBH_{50}–Cl_{50}(NaCl) exhibited the fastest permeation rate, which remained practically constant over time and with the apparent permeability being even higher than that for the 0.1% **FB** solution. This reveals that EU–FBH_{50}–Cl_{50}(NaCl) provided higher concentrations of ***FBH*** species in the immediate vicinity of the cornea surface. As presented in Table 3, although ***FBH*** concentration is still low compared with the condensed complex [$\boldsymbol{EUH^+FB^-}$]; in the case of the complex dispersed in 0.9% NaCl solution, this concentration is four time higher than in the case of glucose solution. In addition to this, the pH of the medium (see Table 1) in which the complexes are dispersed are rather acid, leading to the prevalence of nondissociated species [***FBH***].

Moreover, it appears that the ionic equilibrium was responsible for the sustained provision of the drug onto the cornea

Table 5. Transcorneal Permeability of Flurbiprofen from Various Formulations (mean ± SD, $n = 4$)

Formulations	(µg) Permeation[a] (120 min)	Flux (µg)/(cm^2s)10^2	Apparent permeability P_{app}, (cm/s)10^5
Flurbiprofen (0.1%)	92.76 ± 3.08	1.79 ± 0.02	1.79 ± 0.10
Tolerane®	31.55 ± 1.61	0.50 ± 0.35	0.50 ± 0.35
EU–FBH_{50}–Cl_{50} (Dex)	28.02 ± 2.39	0.36 ± 0.04	0.36 ± 0.35
EU–FBH_{50}–Cl_{50}(NaCl)	166.53 ± 4.33	3.27 ± 0.03	3.27 ± 0.31

[a] Permeation *in vitro* using rabbit cornea.

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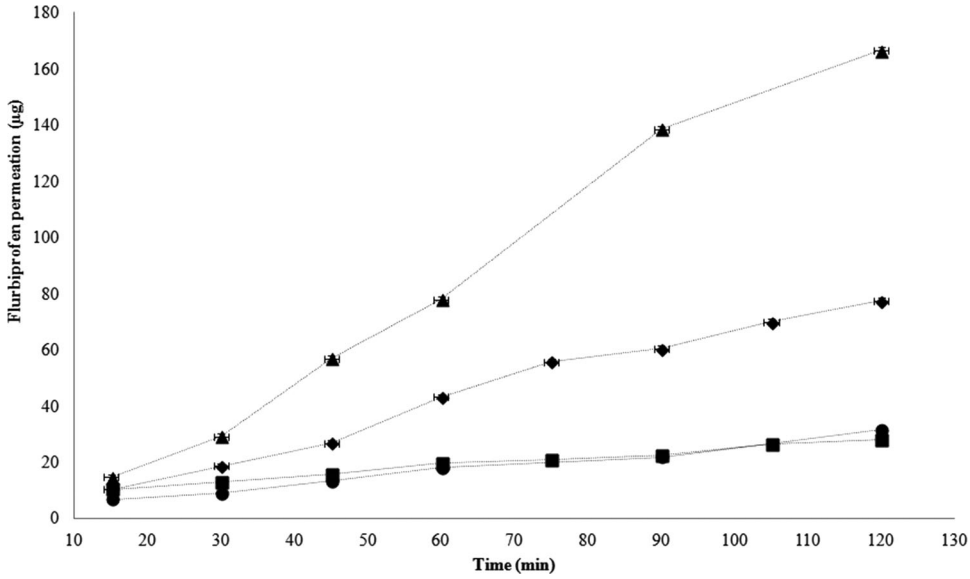

Figure 3. Permeation profiles of (♦) 0.1%flurbiprofen; (●) Tolerane®, (■) EU–$FBH_{50}Cl_{50}$ in 5% dextrose solution, and (▲) EU–$FBH_{50}Cl_{50}$ in 0.9% NaCl solution (mean ± SD, $n = 4$).

surface. In contrast, the FB permeation from Tolerane® was lower than in the case of EU–FBH_{50}–Cl_{50}(NaCl). Probably, this fact can be attributed to the high affinity of the FB–CD complex, which may hinder the absorption of free FBH. In addition, this formulation possessed a basic pH (7.66, Table 1). Therefore, as a consequence of the ionic equilibrium, FB was rapidly ionized to the ionic species (FB^-) after its release from the complex.

Table 6 shows a comparison of the diffusion and permeation rates of FB solution, Tolerane®, EU–FBH_{50}–Cl_{50}(NaCl), and EU–FBH_{50}–Cl_{50}(Dex). Conversely, the PE complexes exhibited faster permeation than diffusion release. This behavior may be ascribed to the interaction of the positively charged PE carrier system with negative mucoproteins, thus leading to a higher concentration of the complex at the mucosa boundary and also at the epithelium surface of the cornea. This type of interaction has already been reported in the literature,[35] which should provide a higher concentration of the carrier near the absorption target than in the bulk.

It was also reported that the interaction of EU with membranes was mediated by electrostatic attractions between the cationic charges of the polymer and the electronegative groups of cell surfaces. Moreover, hydrophobic association of the polymer with membrane structures may also be involved.[36,37] According to observations from recent investigations in our laboratory, the complex $(Eudragit–Enalapril\ Maleate)_{30}$ exhibits a higher permeability compared to an equivalent solution of enalapril maleate in everted rat intestine.

On the other hand, it is well known that the positively charged amino groups of EU are able to interact with negatively charged mucus gel layer,[38] thus increasing intimate contact with the membrane. In addition, it was reported that the cationic polymer may affect the electronegativity of the cell

Table 6. Kinetic Values Obtained from *In Vitro* and *Ex Vivo* Plots of Flurbiprofen Formulations

Test	Formulations	Tolerane®	Eu–FBH_{50}–Cl_{50}(NaCl)	Eu–FBH_{50}–Cl_{50}(Dex)	Flurbiprofen (0.1%)
			Korsmeyer–Peppas model		
In vitro	k (min^{-n})	0.105 ± 0.016	**0.053 ± 0.010**	**0.102 ± 0.036**	0.403 ± 0.016
	N	0.854 ± 0.033	0.815 ± 0.016	0.548 ± 0.080	0.740 ± 0.050
	R^2	0.9947	0.9987	0.9959	0.9843
Ex vivo	k (min^{-n})	0.056 ± 0.018	**0.145 ± 0.018**	**0.220 ± 0.032**	0.035 ± 0.007
	R^2	0.9781	0.99232	0.9832	0.9963
			Higuchi's plot		
In vitro	k ($min^{-1/2}$)	0.498 ± 0.028	**0.57841 ± 0.029**	**0.125 ± 0.005**	3.710 ± 0,163
	R^2	0.8687	0.8891	0.9120	0.9087
Ex vivo	k ($min^{-1/2}$)	0.237 ± 0.0186	**1.227 ± 0.184**	**0.247 ± 0.004**	0.6749 ± 0,067
	R^2	0.8516	0.70541	0.9814	0.7489
			Zero-order plot		
In vitro	k (min)	0.054 ± 0.001	**0.063 ± 0.001**	**0.013 ± 0.001**	0.403 ± 0,016
	R^2	0.9795	0.9720	0.7058	0.9217
Ex vivo	k (min)	0.026 ± 0.001	**0.143 ± 0.006**	**0.916 ± 0.002**	0.076 ± 0,075
	R^2	0.9560	0.9725	0.5903	0.9844

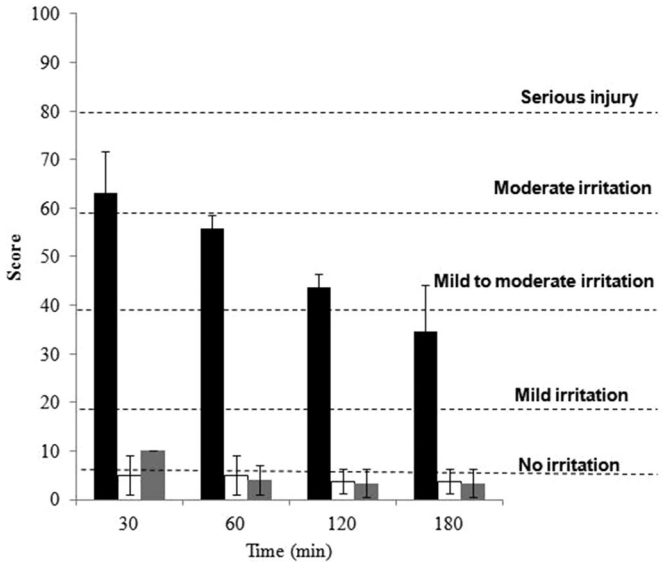

Figure 4. *In vivo* irritation study in albino rabbits of (■) 2% sodium dodecylsulfate, (▨) EU–FB_{50}–Cl_{50}, and (□) Tolerane® using a modified Drize test (mean ± SD, $n = 12$).

surface, leading to the disorganization of the membrane and thus rendering it permeable to antibiotics.[39] On the basis of these antecedents, it would be expected that this kind of interaction may affect the permeation properties of the membrane interacting with EU, although more studies are necessary to clarify this issue.

Ocular Irritation Tests

Evaluation with the Draize Method

This test was based on an objective medical evaluation of lesions caused by formulations in different tissues of the eye, using a procedure designed to assign scores to potential ocular irritancy and/or damaging effects. EU–FBH_{50}–Cl_{50} dispersed in NaCl solution, and Tolerane® and 2% SDS solution (SDS, positive control) were assayed. Also, the possible influence of complex concentration on toxicity was evaluated. These results are shown in Figure 4.

Both complex formulations were revealed to be nonirritants because they presented a score lower than 10. As expected, the SDS solution produced a noticeable irritation with a score higher than 60. In all cases, the irritation was highest at 30 min after instillation, and then the irritant effect decreased with time. Consequently, the concentration of complexes used in this study was high enough to interact reversibly with the eye tissues, thus affecting drug permeation without pernicious effects.

Histological Examination

The cross sections of corneas after the administration of different formulations are shown in Figure 5. The epithelium and stroma structures were apparently unchanged when NaCl solution was administered (Fig. 5a). A typical stratified epithelial layer can be recognized by the appearance of a bulge at the nuclei of the basal columnar cells and by the squamous surface cells. When the corneal epithelium was exposed to SDS solution (Fig. 5b), the structure of epithelium was destroyed as superficial epithelial cells detached from the tissue assembly of corneas treated with EU–FBH_{50}–Cl_{50}(NaCl). As shown in Figure 5c, there were no morphological or structural changes, with neither the structure nor the integrity of the corneas being visibly affected. The above results reveal that the EU–$FBH_{50}Cl_{50}$(NaCl) formulation had a good biocompatibility.

CONCLUSIONS

By complexation of FB with EU, it was possible to improve the dispersion and increase in the apparent solubility of the drug. The studies revealed that the predominant species at equilibrium was the ionic pair [EUH^+FB^-], which in the presence of NaCl generated an ionic interchange. This behavior ruled the release rate of FB from the complex dispersed in NaCl solution, which was clearly evidenced in the *in vitro* release experiments. This dispersion revealed the slowest drug release in comparison with the other formulations evaluated. However, when drug permeation was evaluated *in vivo*, the complex dispersed in saline solution was able to penetrate the cornea faster than comparative solutions. As explained above, this could be attributed to polymer–mucose interactions.

Finally, the complex EU–FBH_{50}–Cl_{50}(NaCl) exhibited low ocular irritation when evaluated by the Draize test and histological examination. The results reported in this article suggest that ophthalmic formulations designed through this

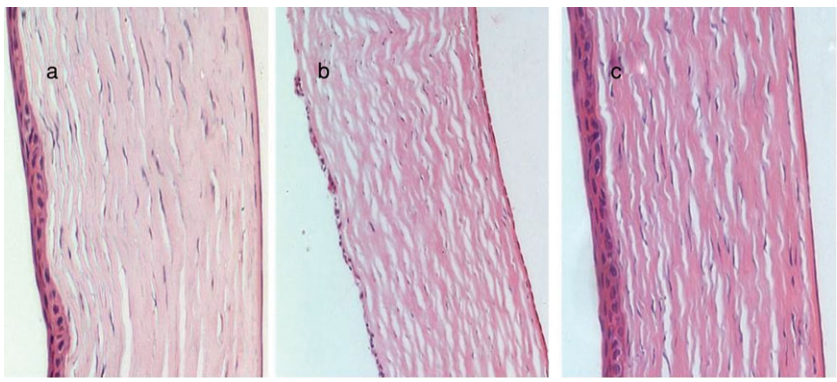

Figure 5. Histological cross sections of excised rabbit cornea showing epithelium and stroma stained with hematoxylin & eosin: (a) 0.9% NaCl solution, (b) 2% (w/w) SDS solution, and (c) EU–$FBH_{50}Cl_{50}$ in 0.9% NaCl solution.

strategy may be very advantageous for the ocular administration of NSAIDs.

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