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Gel-forming erodible inserts for ocular controlled delivery of ofloxacin

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Abstract

A new application of high molecular weight (400 kDa) linear poly(ethylene oxide) (PEO) in gel-forming erodible inserts for ocular controlled delivery of ofloxacin (OFX) has been tested in vitro and in vivo. Inserts of 6 mm diameter, 20 mg weight, medicated with 0.3 mg OFX, were prepared by powder compression. The in vitro drug release from inserts was mainly controlled by insert erosion. The erosion time scale was varied by compounding PEO with Eudragit L100 (EUD) 17% neutralized (EUDNa17) or 71% neutralized (EUDNa71). The insert erosion rate depended on the strength of interpolymer interactions in the compounds, and on the hydrophilic-hydrophobic balance of compounds. Immediately after application in the lower conjunctival sac of the rabbit eyes, the inserts based on plain PEO, PEO–EUDNa17 or PEO–EUDNa71 formed mucoadhesive gels, well tolerated by the animals; then the gels spread over the corneal surface and eroded. The gel residence time in the precorneal area was in the order PEO–EUDNa71 < PEO < PEO–EUDNa17. Compared to commercial OFX eyedrops, drug absorption into the aqueous humor was retarded by the PEO–EUDNa71 inserts, and both retarded and prolonged by the PEO–EUDNa17 inserts, while C_{max} (maximal concentration in the aqueous) and AUC_{eff} (AUC in the aqueous for concentrations > MIC) were barely altered by either insert type. On the other hand, C_{max}, AUC_{eff} and t_{eff} (permanence time in the aqueous at concentrations > MIC) were strikingly increased by plain PEO inserts with respect to commercial eyedrops (5.25 ± 0.56 vs. 1.39 ± 0.05 μg ml^{-1}; 693.6 vs. 62.7 μg ml^{-1} min; and 290 vs. 148 min, respectively). Bioavailability increase has been ascribed to PEO mucoadhesion and/or increased tear fluid viscosity.

Keywords: Ocular insert; Ocular drug delivery; Ocular bioavailability; Ofloxacin; Controlled drug delivery; Poly(ethylene oxide); Mucoadhesive polymer

1. Introduction

High molecular weight (10^2–8×10^3 kDa) linear poly(ethylene oxide)(PEO) has shown a great potential as a material for controlled drug delivery systems. Matrix tablets based on PEO can be

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manufactured readily, thanks to the good compressibility of this polymer (Yang et al., 1996). The polyether chains of PEO can form strong hydrogen bonds with water; therefore, when solid matrices are brought into contact with an aqueous medium, the polymer tends to hydrate, forming a superficial gel which eventually erodes as the polymer dissolves. Drug release from such matrices may be controlled by polymer swelling or erosion, or drug diffusion in the hydrated gel, or by these processes altogether. Hence, a variety of release patterns can be obtained, depending on the PEO molecular mass and the drug physicochemical properties. Several studies on PEO-based controlled-release matrices for oral application have been reported (Apicella et al., 1993; Cappello et al., 1994; Kim, 1995; Moroni and Ghebre-Selassie, 1995; Yang et al., 1996; Kim, 1998). Also, applications of PEO as a carrier or component of oral, mucosal and transdermal drug delivery systems are documented by numerous patents (Union Carbide Corporation, 1997). On the other hand, to the authors' knowledge no ophthalmic applications of PEO have yet been reported, even if good mucoadhesive properties (Bottenberg et al., 1991) and lack of irritancy to the rabbit eye (Union Carbide Corporation, 1996) point to this polymer as an interesting candidate material for controlled-release erodible ocular inserts. Indeed, the research and development of new ocular polymeric ophthalmic drug-delivery systems is desirable, as they show promise of improving drug bioavailability and decreasing side effects, with respect to conventional eyedrops.

The foregoing considerations have prompted the present study, aimed at evaluating the biocompatibility of PEO-based solid inserts in the rabbit eye, their ability to release the potent broad-spectrum fluoroquinolone antibiotic ofloxacin (OFX) at controlled rates, and their potential to increase the bioavailability of this drug in the aqueous humor with respect to the commercial OFX eyedrops. The latter dosage form is commonly used to treat external ocular bacterial infections, such as conjunctivitis and keratitis. Nevertheless, advantage could be taken of OFX transcorneal penetration into the aqueous for antibiotic prophylaxis in cataract surgery or treatment of endophthalmitis. A low, if not null, swelling degree of the insert, and drug release kinetics controlled by insert erosion in the conjunctival cul-de-sac are main prerequisites of the inserts under study. In order to modulate insert swelling and erosion rate, PEO was blended with Eudragit L100 (EUD), 17% neutralized (EUDNa17) or 71% neutralized (EUDNa71) with sodium hydroxide. EUD, which is a copolymer of methacrylic acid and methyl methacrylate, is expected to interact with PEO essentially via hydrogen bonding between the unionized carboxyls of the former and the ether oxygens of the latter (Bekturov and Bimendina, 1981; Kharenko and Kemenova, 1995; Haglund, et al., 1996; Ozeki et al., 1998). Hence, it was thought that the interpolymer interactions, and thereby, the swelling degree and erosion rate of the PEO–EUD compound could be modulated via the EUD neutralization degree. Plain PEO, and the polymer compounds PEO–EUDNa17 (1:1 w/w) and PEO–EUDNa71 (1:1 w/w), each containing 1.5% w/w OFX, were compressed into matrix tablets, the characteristics of which, relevant to controlled release, were studied in vitro. The matrices were then evaluated for their biocompatibility in the rabbit eye and for OFX bioavailability in the aqueous, using the commercial collyrium Exocin® as a reference.

2. Materials and methods

2.1. Materials

The following commercially available materials were used as received. Ofloxacin (OFX)(Sigma, St. Louis, MO), Exocin® eyedrops (Allergan), poly(ethylene oxide), M_w 400 kDa (PEO) (Polyox® WSR N–3000, gift from Union Carbide Italia S.r.l., Milan, Italy), Eudragit L100 (EUD) (gift from Rofarma Italia S.r.l., Milan, Italy), Tegiloxan 300000T (TEG) (gift from Goldschmidt Italia S.r.l., Cremona, Italy). TEG is a liquid silicone having a kinematic viscosity of 3×10^5 cStokes.

Buffer substances and all other chemicals or solvents were of reagent grade.

2.2. Partial neutralization of EUD: preparation of EUDNa17 and EUDNa71

EUD was treated with NaOH to an around 17% (EUDNa17) or 71% (EUDNa71) neutralization degree, by adding, dropwise, NaOH 0.25 N to a stirred suspension of 1 g EUD in 100 ml water, under pH control, with care never to exceed pH 7 during the addition. In the case of EUDNa17, the addition was terminated as soon as the suspension turned into a stable milky dispersion, of pH 6.6 and neutralization degree 16.46%. In the case of EUDNa71, the addition was terminated as soon as the suspension turned into a clear solution, of pH 6.8 and neutralization degree 70.94%. The neutralization degree was calculated from the NaOH milliequivalents added, and the EUD acidity index of 315 mg KOH/g and mean molecular mass of 135 kDa (Eudragit Handbook, Röm Pharma GmbH, Weiterstadt, Germany). EUDNa17 and EUDNa71 were obtained as powders by evaporating the respective aqueous dispersion and solution to dryness under reduced pressure, vacuum drying the residue at 60°C to a constant weight, and finally grinding the solid in a mortar.

2.3. Preparation of PEO–EUDNa compounds

PEO–EUDNa17 and PEO–EUDNa71 compounds, each in the 1:1 wt ratio, were obtained in the form of fine powders by the following coevaporation procedure. A 18 ml volume of a solution containing 0.25 g PEO and 0.25 g EUDNa, prepared as will be specified below, was dispersed portionwise, by levigation, into 7.5 g TEG, under an air stream to favor solvent evaporation. After dispersion of the last portion, the levigation-evaporation process was continued up to a constant weight of the resulting suspension. The coevaporate powder was collected by dissolving the TEG with excess petroleum ether, decanting the liquid, repeatedly washing the powder with petroleum ether to complete TEG removal, vacuum drying the powder, and finally, passing the powder through a 106 μm sieve.

The preparation procedure of the polymer solution to be dispersed into TEG depended on the solubility properties of polymers. To prepare the solution of PEO and EUDNa17, PEO was first suspended in 4.5 ml methanol and next dissolved by adding 4.5 ml chloroform to the suspension. A solution of EUDNa17 in 9 ml methanol was added to the PEO solution, and the mixture was stirred until an apparently homogeneous solution was obtained. To prepare the solution of PEO and EUDNa71, the two polymers were added to 9 ml methanol under stirring, next, 3.5 ml water were added to completely dissolve the solids, then, 5.5 ml methanol were added to reduce the viscosity of the solution.

2.4. Loading of PEO and PEO–EUDNa compounds with OFX

OFX was adsorbed onto the surface of PEO powder, sieve-sized to the 180–250 μm range, by wetting the powder, portionwise, with a 0.8 mg/ml OFX solution in absolute ethanol–methanol 2:1 v/v, while mixing with a spatula and letting the solvent evaporate. Following the addition of a solution volume corresponding to 1.5% w/w OFX on a PEO basis, the powder was vacuum dried to a constant weight.

Medicated PEO–EUDNa coevaporates were prepared by dissolving the appropriate drug amount in the methanol used to prepare the polymer solution to be dispersed into TEG (see section 2.3). The nominal OFX load in the coevaporate powder was 1.5% w/w.

The actual drug load in PEO and PEO–EUDNa compounds was determined spectrophotometrically at 286 nm, following dissolution of powders in pH 7.4 phosphate buffer and filtration through a 0.45 μm pore size membrane. The load values, as determined for three batches of each compound, were $1.56 \pm 0.15\%$ w/w, for PEO; $1.55 \pm 0.09\%$ w/w, for PEO–EUDNa17; and $1.47 \pm 0.13\%$ w/w, for PEO–EUDNa71. None of the determined values was significantly different from the nominal load.

2.5. Preparation of inserts

Powders were compressed by a hydraulic press into flat faced tablets of 6 mm diameter, 0.8–0.9

mm thickness and 20 mg weight, by applying a force of 1000 kg. The nominal drug dose in the medicated inserts was 0.3 mg, a dose corresponding to 2x50 μl Exocin® eyedrops. Non-medicated inserts containing 0.5% w/w sodium fluorescein, as a tracer for the in vivo tests to be described in Section 2.10.1, were prepared from PEO, PEO–EUDNa17 and PEO–EUDNa71 powders. The tracer was dispersed into the powders by wetting, portionwise, with an appropriate volume of a 0.05% w/v sodium fluorescein solution in absolute ethanol, while mixing and letting the solvent evaporate, then vacuum drying to a constant weight.

2.6. Kinetic measurements in vitro

The kinetics of OFX release from inserts and insert swelling and erosion were measured in vitro. All inserts formed a soft, tacky gel on hydration, with shape and volume changes. In order to control at best the insert surface in contact with the dissolution medium, each insert was tightly inserted into a 3 mm deep cylindrical cavity, of exactly the same diameter as the insert, bored at the centre of a 4 mm thick Teflon disk of 11 mm diameter. At time $t = 0$, two disks, each containing an insert, were immersed, with the exposed insert surface in upward position, into 50 ml of pH 7.4, 0.0026 M phosphate buffer, made isotonic with sodium chloride, contained in a jacketed beaker (internal diameter, 6.5 cm; internal height, 9 cm) thermostated at 37°C, stirred by a paddle stirrer (diameter, 4.9 cm; paddle width, 0.7 cm) operated by a synchronous motor at 60 rpm. The paddles were at a 3 mm distance over the disks.

To obtain the percentage dose released versus time data, at appropriate intervals, samples of dissolution medium were spectrophotometrically analysed for the drug at 286 nm after filtering, as described in Section 2.4. Sink conditions in the receiving phase were always maintained.

To obtain the percentage insert eroded versus time data, after a pre-established elution time each disk was withdrawn, vacuum dried and weighed (sensitivity, 10^{-5} g), to determine the undissolved insert weight. This procedure was repeated for different elution times.

2.7. Differential scanning calorimetry (DSC) measurements

A Pyris DSC6 differential scanning calorimeter (Perkin–Elmer), connected to an MC480 cooler circulator (FTS, Stone Ridge, NY), was used. Samples of 8–10 mg were scanned in sealed aluminum pans in the 0–100°C temperature interval, at a heating rate of 10 K/min, with nitrogen purge.

2.8. Solubility measurements

An excess OFX was equilibrated with the solvent at 37°C, then the suspension was filtered with a syringe equipped with a 0.45 μm pore size membrane filter, and the filtrate, after appropriate dilution with pH 7.4, 0.0026 M phosphate buffer, was analyzed spectrophotometrically for the drug, as described in Section 2.4. By this procedure, the drug solubility values in the buffer (solubility, 2.57 mg/ml), in a 2.5% w/v PEO solution in the buffer (solubility, 2.86 mg/ml), or in a 2.5% w/v PEO–EUDNa17 solution in the buffer (solubility, 2.79 mg/ml) were compared, in order to evidence and compare OFX-polymer interactions. The above concentration of the polymer solutions was the highest allowing filtration with the above described apparatus.

2.9. Mucoadhesion tests

The mucoadhesive potential of PEO and that of the PEO–EUDNa17 compound were compared by measuring the work required to detach the unit surface of sample from a mucous substrate, consisting of a 25% w/w aqueous dispersion of hog gastric mucin, spread uniformly on wet filter paper. The measurements were performed as described by Saettone et al., 1989. The samples were disks of 0.68 mm thickness, 13 mm diameter and 100 mg weight, obtained by compression of the polymer powder with a force of 4500 kg. For testing, each disk was hydrated for a pre-established time, by immersion in artificial tear fluid, containing Mg^{++} 0.50 mM ($MgCl_2$), Ca^{++} 0.72 mM ($CaCl_2$), K^+ 26.00 mM ($KHCO_3$), HCO_3^- 26.00 mM, Na^+ 132.28 mM (Na_3PO_4, NaCl) and

Cl^- 132.44 mM. The diameter of the hydrated disk was measured with calipers, in order to calculate the hydrated surface area, then the disk was placed between the upper and lower mucous surfaces in the testing cell. All detachment tests were carried out after 1 min of contact, at 30°C. The force versus elongation curves were analysed with KaleidaGraph® software (Synergy Software, Reading, PA).

2.10. Animal tests

Male, New Zealand albino rabbits, 2.5–3.0 kg (Pampaloni rabbitry, Fauglia, Italy) were used. They were treated as prescribed in the publication 'Guide for the care and use of laboratory animals' (NIH Publication No 92–93, revised 1985). The animals were housed in standard cages, in a light-controlled room at 19 ± 1°C and 50 ± 5% relative humidity, with no restriction of food or water. During the experiments, the rabbits were placed in restraining boxes, where they could move their eyes and heads freely. All experiments were carried out under veterinary supervision, and the protocols were approved by the ethical-scientific committee of the University.

2.10.1. Evaluation of biocompatibility and residence time of inserts in the precorneal area

Non-medicated inserts, based on PEO, PEO–EUDNa17 or PEO–EUDNa71, containing 0.5% w/w sodium fluorescein as a tracer, were used for these tests. One insert of each type was applied in the lower conjunctival sac of each eye of at least two rabbits. Following insertion, all devices formed a superficial gel and adhered to the application site within 5 min. The behavior of inserts after 10, 60 and 180 min from insertion was evaluated on the basis of direct visual observation using a slit lamp. The lamp was also used to detect irritation signs, such as conjunctival/corneal edema and/or hyperhemia. Fluorescence at the rabbit nose, due to lacrimation, was checked under illumination with a long wave (366 nm) lamp. Each remark, reported in Table 1, refers to all of the inserts of each type. None of them caused important irritation signs. All measurements were made by the same operator

2.10.2. Measurement of OFX transcorneal penetration

One PEO, PEO–EUDNa17 or PEO–EUDNa71 insert, containing a nominal dose of 0.3 mg OFX, or a 100 μl volume (two 50 μl drops, corresponding to 0.3 mg OFX) of the reference Exocin® eyedrops, was applied in the lower conjunctival sac of one eye of each rabbit. The reference drops were instilled at a 5 min interval, with care to avoid any spillage from the eye. After a pre-established time from administration, the rabbits were anaesthetized by i.m. administration of 30 mg/kg ketamine and 5 mg/kg xylazine, then 50–80 μl of aqueous humor were aspirated from the anterior chamber, using a 1.0 ml insulin syringe fitted with a 29 gauge needle (B–D, Micro-Fine U-40 insulin, Beckton Dickinson, Dublin, Ireland). At least six animals were used for each time point. The aqueous humor samples were immediately frozen and stored at -18°C.

Table 1
In vivo behavior of non-medicated inserts, based on PEO, PEO–EUDNa17 or PEO–EUDNa71.

Insert material	Time, min	Insert in conjunctival sac	Gel on corneal surface
PEO	10	Partially gelled	Absent
	60	Completely gelled	Thin film
	180	Completely eroded	Absent
PEO–EUDNa17	10	Partially gelled	Absent
	60	Completely gelled	Thick film
	180	Completely eroded	Thin film
PEO–EUDNa71	10	Completely gelled	Thick film
	60	Completely eroded	Thin film
	180		Absent

For analysis, each sample was mixed with an equal volume of acetonitrile, then it was centrifuged (13000 rpm, 15 min) and 20 μl of the supernatant were analyzed by HPLC. The HPLC apparatus (Perkin–Elmer) consisted of Series 4 pump, 20 μl Rheodyne injector, LC 290 UV detector and 1020 LC Plus integrating system. The column (Macherey–Nagel 250 × 4 mm, Düren, Germany) was packed with Nucleosil® 100 – 5 C_{18} (5 μm). The mobile phase (flow rate 1.0 ml/min) was methanol–acetonitrile–citric acid 0.4 M (3:1:10). The UV detection was set at 294 nm. The OFX retention time was 6.8 min. The limit of quantitation was 0.12 μg/ml.

2.10.3. Pharmacokinetic analysis

The area under the concentration in the aqueous humor vs. time curve and over the level of 0.5 μg/ml ($MIC_{90\%}$ for the less resistant ocular pathogens; Taravella, et al., 1999)(see Fig. 4), coded AUC_{eff}, was calculated by means of the linear trapezoidal rule (Kaleidagraph, Synergy Software).

3. Results and discussion

3.1. Interpolymer interaction in the PEO–EUDNa compounds

In a previous paper, the lowering of the crystallinity degree of PEO in PEO–EUD compounds, compared to plain PEO, was taken as a proof, as well as a measure, of the PEO interaction with EUD (Carelli, et al., 2000). The relative crystallinity degree, RCD, of PEO in the PEO–EUD compounds was reported as the ratio of the enthalpy of fusion per unit PEO mass, for the PEO–EUD compounds to that for plain PEO, as determined by DSC. Following the same line, in the present study the RCD and the lowering of the peak temperature with respect to plain PEO, ΔT_p, were determined for each of the PEO–EUDNa17 and PEO–EUDNa71 compounds. The measurements of the enthalpy of fusion of the PEO crystallites in the coevaporate compounds were enabled by the absence of EUDNa signals in the temperature range scanned. Some time after complete removal of solvent was required by POE to attain the crystallinity degree corresponding to thermodynamic equilibrium. In fact, constant fusion enthalpy values could not be obtained before 10–15 days from coevaporate drying. RCD and ΔT_p values were determined as the means of at least three samples taken from different batches of each coevaporate. For both compounds analyzed, RCD values significantly less than one and negative ΔT_p values were found, indicating some interpolymer interactions in both cases. Such interactions are stronger in the PEO–EUDNa17 compound ($RCD = 0.20 \pm 0.05$; $\Delta T_p = -9.1 \pm 0.8$°C) than in the PEO–EUDNa71 compound ($RCD = 0.72 \pm 0.07$; $\Delta T_p = -6.5 \pm 1.2$°C). DSC measurements, carried out with medicated polymer compounds, showed that a 1.5% OFX load in the inserts did not significantly alter the interpolymer interactions. The non-neutralized PEO–EUD complex was not used as an insert material, since it would not erode in lacrimal fluid, due to an insufficient buffering capacity.

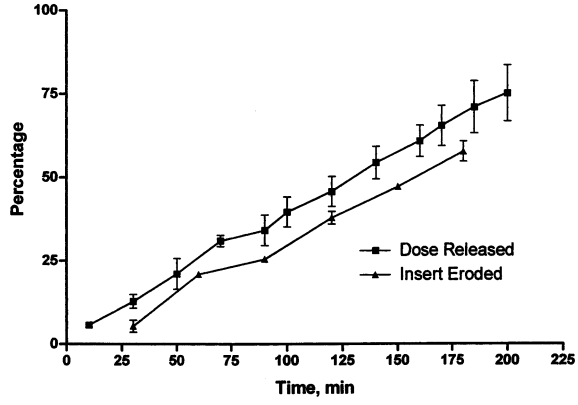

Fig. 1. In vitro drug release and insert erosion kinetics for inserts based on PEO, medicated with 1.5% (0.3 mg) OFX. Each data point is the mean ± S.D. of at least three values.

3.2. Kinetic measurements in vitro

To gain information on the drug release mechanism from inserts, the kinetics of drug release and insert erosion were measured in vitro for inserts based on PEO, PEO–EUDNa17 or PEO–EUDNa71. As shown in Figs. 1–3 in no case

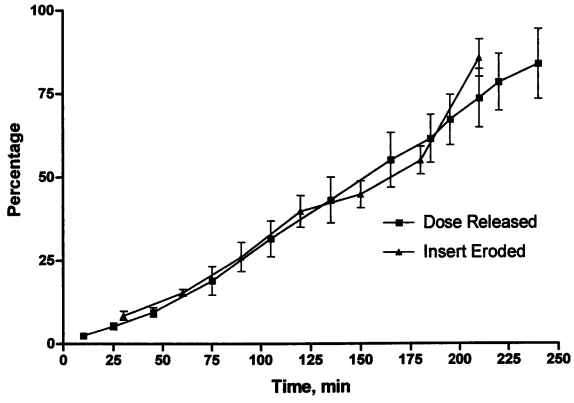

Fig. 2. In vitro drug release and insert erosion kinetics for inserts based on the PEO–EUDNa17 compound, medicated with 1.5% (0.3 mg) OFX. Each data point is the mean ± S.D. of at least three values.

important differences between the drug release and the insert erosion pattern for the respective insert types were observed. This suggests that, with the present systems, the release time scale can be controlled by controlling the insert erosion rate. This could actually be done by modulating the PEO–EUDNa interpolymer interactions and hydrophilic-lipophilic balance via the EUD neutralization degree, as clearly shown by a comparison between the kinetic data for the PEO–EUDNa17 insert (Fig. 2) and those for the PEO–EUDNa71 insert (Fig. 3). However, PEO

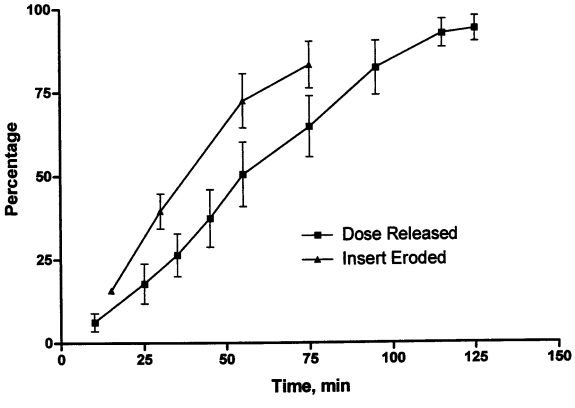

Fig. 3. In vitro drug release and insert erosion kinetics for inserts based on the PEO–EUDNa71 compound, medicated with 1.5% (0.3 mg) OFX. Each data point is the mean ± S.D. of at least three values.

admixture with partially neutralized EUD could speed up, but not significantly retard insert erosion and drug release with respect to plain PEO, even using the more interactive and less hydrophilic EUDNa17. The release and erosion kinetics were of apparent pseudo-zero order in all cases illustrated in Figs. 1–3. As can be seen in Fig. 1, with the PEO insert the fraction released is always higher than the corresponding fraction eroded, and the excess of the former over the latter is virtually constant in time. This is in accord with the hypothesis that, in this system, the release mechanism consists of an erosion-controlled phase, preceded by an initial, short phase, where the release is controlled by drug diffusion in the swollen matrix. This hypothesis is in agreement with literature information about the mechanism of drug release from poly(ethylene oxides) (Apicella et al., 1993). The data for the PEO–EUDNa71 insert, shown in Fig. 3, indicate an insert erosion faster than drug release. This apparently odd finding can be explained by an inhomogeneous dissolution of this polymer compound, one component of which would dissolve faster than the other, while the dissolution of the more slowly dissolving component would be controlling drug release. In any case, the release patterns in Figs. 1–3 indicate that OFX release from all the insert types under study is essentially controlled by superficial erosion, with a minor role played by drug diffusion in the swollen matrix.

3.3. Animal tests

The behavior of non-medicated inserts based on PEO, PEO–EUDNa17 and PEO–EUDNa71 in rabbit eyes is described in Table 1. In all cases, the inserts adhered almost instantly to the application site, then they were gradually transformed into gels, which spread over the corneal surface and finally eroded. From the remarks reported in Table 1 it appears that these phenomena were faster with the PEO–EUDNa71 insert than with the PEO or PEO–EUDNa17 insert, in agreement with the faster erosion of the former observed in vitro. On the other hand, the observations in vivo concerning PEO and PEO–EUDNa17 indicate a longer permanence of the latter on the corneal

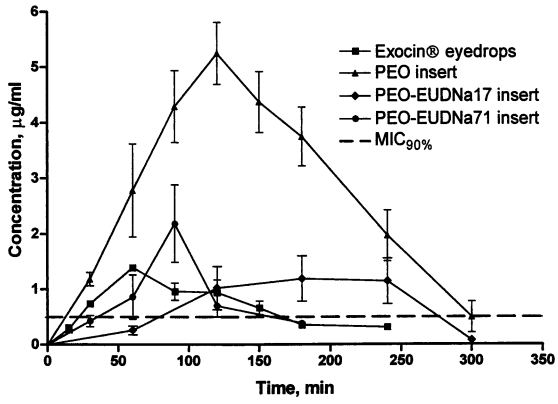

Fig. 4. Profiles of OFX concentration in the aqueous humor of rabbits, following topical administration of 0.3 mg OFX by different vehicles. Each data point is the mean ± S.E. of at least six values obtained with different animals.

surface, even though these two systems showed no significantly different erosion rates in vitro.

The OFX concentration profiles in the aqueous, following administration of a 0.3 mg dose by the systems under study are compared in Fig. 4, while the relevant pharmacokinetic data are listed in Table 2. The profile for the PEO–EUDNa17 insert, illustrated in Fig. 4, shows a plateau, typical of a controlled-release system producing zero-order transcorneal penetration kinetics. With such a system, a constant drug concentration in the aqueous is attained when the constant penetration rate equals the rate of elimination from the aqueous. In fact, this insert showed pseudo-zero order release kinetics in vitro, as observed in Table 2. The plateau extends up to about 4 h, in agreement with the observation, reported in Table 1, that the gelled polymer was still present on the corneal surface 3 h after application. All of the concentration values corresponding to the plateau are over the $MIC_{90\%}$ for the less resistant ocular pathogens; still higher plateau concentrations might presumably be obtained by increasing the drug load in the insert. With the PEO–EUDNa71 insert the concentration profile shows a sharp peak at a t_{max} shorter than that for the PEO–EUDNa17 insert (Fig. 4 and Table 2). This is in agreement with the faster erosion and release shown by the former both in vitro and in vivo, (cf. Figs. 2 and 3, and Table 1). The AUC_{eff} and AUC_{rel} values in Table 2 indicate, for the inserts based on the PEO–EUDNa compounds, effective bioavailabilities of about the same magnitude as the reference eyedrops. On the other hand, AUC_{eff} for the insert based on plain PEO is one order of magnitude greater than the reference, while C_{max} exceeds 4 μg/ml, ($MIC_{90\%}$ for the more resistant ocular pathogens; Taravella et al., 1999), and t_{eff} is about doubled with respect to the reference eyedrops. This striking difference in bioavailability between the PEO and PEO–EUDNa17 inserts cannot be explained on the basis of the release and erosion data in vitro, which are substantially similar. The much higher bioavailability of the PEO insert could result from a much higher rate of transcorneal drug penetration and/or a much lower rate of drug elimination from the precorneal area. PEO was actually seen,

Table 2
Pharmacokinetic parameters for transcorneal penetration into aqueous humor after ocular administration of 0.3 mg OFX via the commercial eyedrops Exocin®, or ocular inserts based on PEO–EUDNa71, PEO–EUDNa17, or plain PEO.

Vehicle	C_{max}[a] ± S.E. (μg ml^{-1})	t_{max}[b], min	AUC_{eff}[c] (μg ml^{-1} min)	t_{eff}[d], min	AUC_{rel}[e]
Exocin®	1.39 ± 0.05	60	62.75	148	1
PEO–EUDNa71	2.19 ± 0.70	90	66.82	132	1.06
PEO–EUDNa17	1.19 ± 0.41	180	98.39	194	1.57
PEO	5.25 ± 0.56	120	693.61	290	11.05

[a] Maximal OFX concentration in the aqueous humor.
[b] Time to reach C_{max}.
[c] Area under the concentration in the aqueous vs. time curve and over the $MIC_{90\%}$ level (cf. Fig. 4).
[d] Time of permanence of the concentration in the aqueous at values > $MIC_{90\%}$.
[e] Ratio of AUC_{eff} to the value for the reference Exocin®

as indicated in Table 1, to completely dissolve in the rabbit eye in a shorter time compared to PEO–EUDNa17, thus granting a higher release rate. This, however, is insufficient, per se, to justify the higher bioavailability of PEO, since the PEO–EUDNa71 compound was dissolved in still shorter a time than PEO, yet, as indicated in Table 2, its AUC_{eff} was of about the same magnitude as that of PEO–EUDNa17. As a further hypothesis, a strong binding of OFX by EUDNa, reducing the drug transcorneal penetration rate, and hence, its availability with respect to the PEO system, might be envisaged. This hypothesis was checked by comparing the OFX solubility values in phosphate buffer pH 7.4 (solubility, 2.57 mg/ml), or in this buffer containing the same 2.5% w/v concentration of PEO (solubility, 2.86 mg/ml), or PEO–EUDNa17 (solubility, 2.79 mg/ml). Such values show that the OFX interactions with the EUDNa17 dissolved in the tear fluid could be no stronger than the OFX–PEO interactions. The possibility of OFX forming an insoluble complex with EUDNa17 was excluded by the observation that the OFX contained in the PEO–EUDNa17 compound, after dissolution of the compound in pH 7.4 phosphate buffer and subsequent filtration through a 0.45 μm pore membrane, as described under 2.4, showed an UV spectrum superimposable to that of pure OFX dissolved in the same solvent at the same concentration (not reported). The above findings rule out the hypothesis of substantial EUDNa17–OFX interactions. The cause of the higher OFX bioavailability in the aqueous produced by PEO compared to the PEO–EUDNa17 compound might be an enhancement of the corneal permeability of the drug via some mechanism involving adhesion of the PEO gel to the mucin layer of the corneal epithelium. In fact, the mucoadhesive potential of PEO, assessed in vitro as described in Section 2.9, was stronger than that of the PEO–EUDNa17 compound, and the difference tended to increase with increasing polymer hydration, as shown in Fig. 5. Mucoadhesive polymers, such as, e.g. chitosan, hyaluronic acid, or poly(acrylic acid) have been reported to increase the permeability of epithelial barriers (Lehr et al., 1992, 1994; Lehr 1996; Artursson et al., 1994; Illum et al., 1994). In particular, the transcorneal penetration of gentamicin into the aqueous humor was increased by a mucoadhesive polycarbophil gel formulation (Lehr et al., 1994). The authors speculated that the prolonged and intensified contact of the mucoadhesive formulation would temporarily weaken the barrier properties of the corneal epithelium, thus facilitating drug penetration. A temporary opening of the tight junctions of the outermost cell layer of the corneal epithelium might be the mechanism. Another possible explanation of the higher bioavailability allowed by PEO compared to the PEO–EUDNa17 compound could be a higher viscosity of the tear fluid in the presence of the PEO compared to the PEO–EUDNa17 gel, which could limit the lacrimal drainage and slow down drug elimination from the precorneal area to a greater extent in the former case.

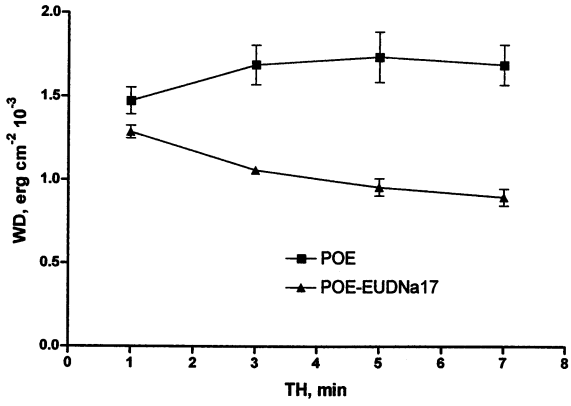

Fig. 5. Results of the mucoadhesion tests. Work of detachment (WD) vs. time of hydration (TH). Each data point is the mean ± S.E. of at least eight measurements.

4. Conclusions

The ocular inserts based on PEO or PEO–EUDNa compounds are able to form in situ mucoadhesive gels, well tolerated by the rabbit eye. OFX release from inserts is essentially controlled by the erosion of the resulting gels. This can be modulated by modulating the PEO–EUDNa interpolymer interactions and hydrophilic-lipophilic balance via the EUD

neutralization degree. By this means, different profiles of OFX concentration in the aqueous vs. time have been obtained. In particular, the insert based on the PEO–EUDNa17 compound yielded a profile typical of a zero-order controlled delivery system. This insert has the potential to provide an effective and time-constant drug concentration in the aqueous, with a reduced number of applications. A similar potential was also shown by the insert based on plain PEO, which showed the additional, remarkable advantages of increasing the OFX effective availability in the aqueous by one order of magnitude with respect to the commercial eyedrops, and of providing intraocular drug levels above the $MIC_{90\%}$ for the more resistant pathogens. PEO may produce such effects by enhancing the corneal permeability to the drug and/or by increasing the viscosity of the tear fluid in contact with the PEO gel, thus decreasing the rate of drug clearance from the precorneal area. The above findings open new prospects for ocular applications of poly(ethylene oxide)s, and warrant further work, presently underway, aimed at evaluating and comparing the properties of poly(ethylene oxide)s of different molecular sizes, relevant to ocular drug delivery.

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