Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 273 (2004) 37–44
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Effect of chitosan and of *N*-carboxymethylchitosan on intraocular penetration of topically applied ofloxacin

G. Di Colo*, Y. Zambito, S. Burgalassi, I. Nardini, M.F. Saettone

Department of Bioorganic Chemistry and Biopharmaceutics, University of Pisa, Via Bonanno 33, Pisa 56126, Italy

Received 25 June 2003; received in revised form 5 December 2003; accepted 12 December 2003

Abstract

The effects of chitosan hydrochloride (Ch-HCl) and of *N*-carboxymethylchitosan (CMCh), formulated in ophthalmic solutions, on the ocular pharmacokinetics of ofloxacin were studied in rabbits. The carboxymethylation of a chitosan of high molecular mass (1460 kDa) and deacetylation degree (89.9%) introduced 0.84 *N*-carboxymethyl groups per repeating unit. Aqueous solutions containing 1% (w/v) of either polymer showed a pseudoplastic rheologic behaviour, and, when instilled in rabbit eyes, produced no irritation. The kinetics of drug disappearance from tear fluid and the profiles of drug concentration in the aqueous humour versus time were determined and interpreted in the light of a pharmacokinetic model and of drug–polymer binding. Ch-HCl significantly enhanced intraocular drug penetration with respect to an isoviscous drug solution containing poly(vinyl alcohol) and to commercial ofloxacin eyedrops. This effect, which resulted in about 190% increase of the peak concentration in the aqueous, was ascribed to an increased corneal permeability. The polyanionic CMCh failed to enhance intraocular drug penetration. It nevertheless increased precorneal drug retention in virtue of its viscosity and of ofloxacin binding. Consequently, the residence time at concentrations higher than the MIC_{90} and the bioavailability of the antibiotic in the aqueous were increased by about 150 and 240%, respectively, with respect to the reference vehicle.

Keywords: Ofloxacin; Chitosan; *N*-carboxymethylchitosan; Ocular drug delivery; Pharmacokinetics; Absorption enhancer

1. Introduction

Biocompatible enhancers of intraocular drug penetration are of extreme interest, as they can enable topical, non-invasive treatment of endophthalmic affections. Chitosan, a well-known cationic polymer of natural origin, has shown an excellent ocular compatibility, and also the ability to interact with the negatively charged conjunctiva and cornea (Felt et al., 1999). Chitosan was found to enhance the permeability of intestinal and nasal epithelia by opening the tight junctions between cells, thereby favouring paracellular drug transport (Artursson et al., 1994; Illum et al., 1994). The above findings prompted us to verify whether chitosan can also enhance drug penetration through such a layered structure as the corneal epithelium. This hypothesis was put to test in a previous study, where the presence of chitosan hydrochloride (Ch-HCl) in erodible ocular inserts based on poly(ethylene oxide) (PEO) was actually found to increase the peak concentration of ofloxacin in the aqueous humour of rabbit eyes well over the peak produced by Ch-HCl-free inserts (Di Colo et al., 2002b). Since a synergistic activation of the Ch-HCl effect by the mucoadhesive PEO insert could not be excluded a priori, it appeared of interest to ascertain

* Corresponding author. Tel.: +39-050-24000; fax: +39-050-43321.
E-mail address: giadic@farm.unipi.it (G. Di Colo).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2003.12.018

whether and to what extent Ch-HCl alone would act as a transcorneal penetration enhancer for ofloxacin, when administered via eyedrops. It should be considered, indeed, that although ocular inserts offer unquestionable biopharmaceutical advantages over the traditional eyedrops, the latter are much better tolerated and more convenient for the patient. Felt et al. (2001) reported that co-administration of ofloxacin and chitosan in eyedrops resulted in increased antibiotic bioavailability and time of efficacy in tear fluid compared to commercial eyedrops and ascribed this effect to the high viscosity of the chitosan solution. These results are of relevance to the treatment of external ocular infections. The purpose of the present study, on the other hand, was to verify whether and to what extent chitosan, added to ofloxacin eyedrops, is able to improve transcorneal antibiotic transport, thereby improving topical treatment of endophthalmic infections. Indeed, although ofloxacin can penetrate through the corneal barrier into the aqueous humour (Beck et al., 1999; Cekic et al., 1999), an enhancement of the penetration rate could increase the antibiotic effectiveness in the topical treatment of endophthalmitis. In the present study, polymer effects on corneal permeability were deduced from the effects on the drug ocular pharmacokinetics, determined in rabbit eyes, with reference to an isoviscous drug solution containing poly(vinyl alcohol) (PVA) and to commercial ofloxacin eyedrops. In order to ascertain the relevance of the polycationic nature of Ch-HCl, *N*-carboxymethylchitosan (CMCh), which is a polyanion at the physiological pH of the tear fluid, was also tested. Assessment of the CMCh effects is of particular interest, since this polymer was claimed to behave as an intestinal absorption enhancer (Thanou et al., 2001).

2. Materials and methods

2.1. Materials

Ofloxacin, chitosan (minimum 85% deacetylated), glyoxylic acid, sodium borohydride were purchased from Sigma. Poly(vinyl alcohol) (Polyviol W 48/20, MW 90 kDa, 99% hydrolysed) was purchased from Wacker-Chemie GmbH. Buffer substances and all other chemicals or solvents were of reagent grade.

Commercial chitosan was characterised by determining its molecular weight by capillary viscometry (Ostwald capillary viscometer, Series 200) and its deacetylation degree by IR spectroscopy (Mattson 3000 FTIR spectrophotometer), following the procedures described by Khalid et al. (1999). An average viscometric molecular weight of 1460 kDa and a deacetylation degree of 89.9% resulted from the analysis. The water content of commercial chitosan, as determined by desiccation at 100 °C was 12.3% (w/w).

2.2. Synthesis and characterization of *N*-carboxymethylchitosan

The method of synthesis of CMCh was taken from the literature and modified (Muzzarelli et al., 1982; Thanou et al., 2001). Chitosan (1.5 g) was dissolved in an aqueous 0.7% (v/v) acetic acid solution (pH 3.7). Following filtration to remove traces of non-dissolved material, glyoxylic acid (2.067 g) was added and the mixture was stirred at room temperature for 90 min (pH 2.7). Then the pH was brought to 4.5 by addition of 1 M NaOH over 30 min, after which stirring was continued for further 45 min. According to Muzzarelli et al. (1982), pH 4.5 is favourable for formation of the imine at room temperature. Subsequently, the imine was reduced by adding dropwise a 5% (w/v) aqueous solution of sodium borohydride and leaving the mixture 1 h under stirring at room temperature, after which the polymer was precipitated by an excess of ethanol and collected by filtration under vacuum. To note that the glyoxylic acid and sodium borohydride equivalents used for the reaction were in a 3:1 excess with respect to the repeating unit equivalents calculated for the chitosan amount subjected to the reaction. After washing with ethanol/water and drying, the product was pulverised by a ball-mill, then subjected to a second carboxymethylation step using the procedure described above. CMCh was purified by dissolution in water (pH 9) and subsequent precipitation by acidification to pH 5. The polymer was collected from the milky suspension by centrifugation at 5000 rpm. However, the product insolubilized at pH 5 underwent an ageing phenomenon and could not be re-dissolved at alkaline pH after about 1 week of storage. Therefore, for storage freshly prepared CMCh was converted into its sodium salt and the solution (pH 10.5) was lyophilised to yield a product soluble

after at least 6 months from its preparation (code, CMCh/10.5).

CMCh was characterised by IR spectroscopy and alkalimetry. The polymer sample used for the IR spectrum was insolubilized by adding an excess of absolute ethanol to an aqueous polymer solution brought to pH 12 with 0.1 M NaOH. The resulting suspension was centrifuged at 5000 rpm, the sediment was let to dry in a stream of ambient air, then it was ball-milled and subsequently vacuum dried at 60 °C (code, CMCh/12). The IR spectrum of a disk, obtained by pressing a CMCh/12–KBr 1:9 w/w powder mixture by a Perkin-Elmer press, was recorded.

An alkalimetric curve was constructed by dissolving 0.8 g of a CMCh/10.5 sample, vacuum dried at 60 °C, in 100 ml of pre-boiled water, bringing the solution to pH ∼ 2 with 0.1 M HCl (76 ml), and titrating with 0.1 M NaOH under nitrogen.

2.3. Preparation of the solutions for the in vivo tests

The following isotonic solutions were used for the in vivo tests:

- Solution A: 0.2% (w/v) ofloxacin, 1% (w/v) Ch-HCl in saline, pH 4.7;
- Solution B: 0.2% (w/v) ofloxacin, 1% (w/v) CMCh/10.5 in 0.13 M phosphate buffer pH 7.4;
- Solution C: 0.2% ofloxacin, 5.5% (w/v) PVA in 0.13 M phosphate buffer pH 7.4.

The osmolality of solutions, as measured by a micro-osmometer (Hermann Roebling, Berlin), ranged between 305 and 315 mOsm/kg. The Ch-HCl used to prepare Solution A was a fine powder obtained by spray-drying a 0.5% Ch-HCl aqueous solution as described previously (Di Colo et al., 2002b).

2.4. Viscometry

Rheograms of Solutions A, B and C were recorded at 35 °C with a Haake RS1 rheometer, equipped with the co-axial cylinders Z40 (rotor) and Z41 (stator). Data were acquired and analyzed by the Rheo Win Pro software (Haake).

2.5. Determination of drug–polymer interactions

A previously described method, based on the dynamic dialysis technique (Di Colo and Zambito, 2002a), was used to determine the drug–polymer interactions in Solutions A, B and C at 35 °C. Drug flux through a porous cellulose membrane (Spectra/Por®, molecular weight cutoff, 3500 Da, Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) under quasi-steady state conditions was measured in the presence or absence of the polymer in the donor phase. For this purpose, dialysis cell, apparatus and procedure similar to those described by Bottari et al. (1975) were used. The initial ofloxacin concentration in the donor (0.2%) was lower than solubility. Sink conditions were ensured in the receptor medium, which contained the same salts at the same concentrations as the donor, in order to prevent volume variations due to osmosis. At intervals the receptor was spectrophotometrically analysed for ofloxacin at 286 nm. The regression for the fitting of dialysis data, expressed as drug concentration in the donor versus time, to first-order kinetics was always highly significant ($r^2 \geq 0.999$, $n \geq 13$). In all cases the dialysis data were independent of the donor phase stirring speed, which demonstrated that the membrane was the only effective diffusional barrier to drug transport from donor to receptor phase. Under the above experimental conditions, the ratio of the first-order rate constant obtained in the presence of polymer in the donor to that obtained in the absence of polymer was a measure of the drug–polymer interactions.

2.6. Animal tests

Male, New Zealand albino rabbits of 2.5–3.0 kg 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 heads and eyes freely. All experiments were carried out under veterinary supervision, and the protocols were approved by the ethical-scientific committee of the University. Solutions A, B and C were tested. Three drops (50 μl each), corresponding to 0.3 mg ofloxacin, which is the dose usually applied by the commercial eyedrops, were instilled at 1 min intervals into the lower conjunctival sac of both eyes, with care to avoid spillage.

For determination of the kinetics of drug disappearance from the precorneal area, at intervals tear fluid samples were collected from the lower marginal tear strip using 1.0 μl disposable glass capillaries (Drummond 'Microcaps', Fisher Scientific, St. Louis MO, USA). The samples were transferred into microtubes and the capillaries flushed with 1.0 μl water. After further dilution with 100 μl water the samples were stored at −18 °C before HPLC analysis. Kinetic data were obtained from six eyes.

For measurement of ofloxacin penetration into aqueous humour, after pre-established times from drug administration rabbits were anaesthetised, then 60–80 μl of aqueous humour were aspirated from the anterior chamber, using a 1.0 ml insulin syringe fitted with a 29 gauge needle (B-D, Micro-Fine U-100 insulin, Beckton Dickinson, Dublin, Ireland). At least six animals were used for each time point. The aqueous humour samples were stored at −18 °C. For analysis, each sample was mixed with an equal volume of acetonitrile, then it was centrifuged and 20 μl of the supernatant were analysed by an already described HPLC method (Di Colo et al., 2002b). The area under the concentration in aqueous humour versus time curve and over the level of 0.5 μg/ml (MIC_{90} for the less resistant ocular pathogens; Taravella et al., 1999), coded AUC_{eff}, was calculated by the linear trapezoidal rule (Kaleidagraph, Synergy Software). The pharmacokinetics determined for Solutions A, B and C were compared with those for the commercial eyedrops Exocin®, determined previously (Di Colo et al., 2001). Significance of differences was evaluated by the Student's *t*-test.

3. Results and discussion

3.1. Characterisation of CMCh

The IR spectrum of CMCh/12 (not reported) corresponded to that reported by Muzzarelli et al. (1982) for their carboxymethylated chitosan insolubilized at pH 12. In particular, the spectrum showed the bands at 1580 and 1400 cm^{-1}, attributed to the carboxylate ion. The alkalimetric curve for CMCh is shown in Fig. 1. The curve shows two inflections of which the first corresponds to the neutralisation of the free acidity of HCl and the attainment of the CMCh isoelectric

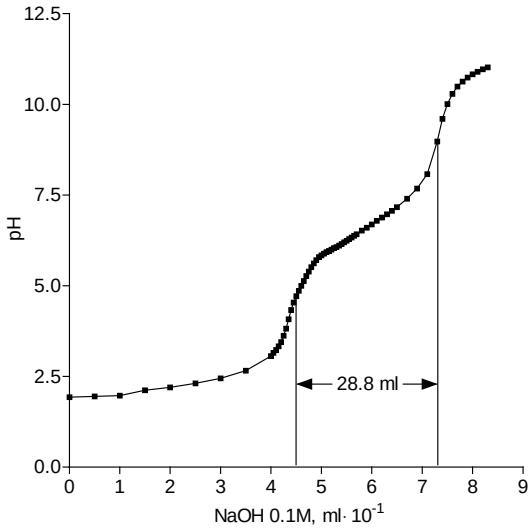

Fig. 1. Alkalimetric curve for CMCh/10.5 at 25 °C.

point (pH ∼ 4.6), while the second corresponds to the complete titration of the carboxyl group. The distance between the two inflections represents the NaOH volume needed for such a titration, which corresponds to 3.60 mmol/g. From here and from the theoretical molecular weight of the repeating unit of CMCh/10.5, calculated assuming a full *N*-carboxymethylation, considering that the carboxymethyl group is in the sodium salt form and that 10.1% of the amino group is acetylated, an approximate *N*-substitution degree was calculated. This allowed computation of a more closely approximated repeating unit molecular weight. Then the computation was repeated until the constant values of 232 and 0.84 were obtained for the repeating unit molecular weight and the number of *N*-carboxymethyl groups per repeating unit, respectively. The latter value is in substantial agreement with those determined by different methods for carboxymethyl derivatives of chitosans of different molecular weights and deacetylation degrees (Muzzarelli et al., 1982; Thanou et al., 2001).

3.2. Formulation of ophthalmic solutions

The ofloxacin concentration in commercial eyedrops is 0.3%, i.e., very close to the drug solubility (3.23 mg ml^{-1} at 25 °C and pH 7, according to Ross and Riley (1990)). In order to shorten the time for complete drug dissolution during the preparation

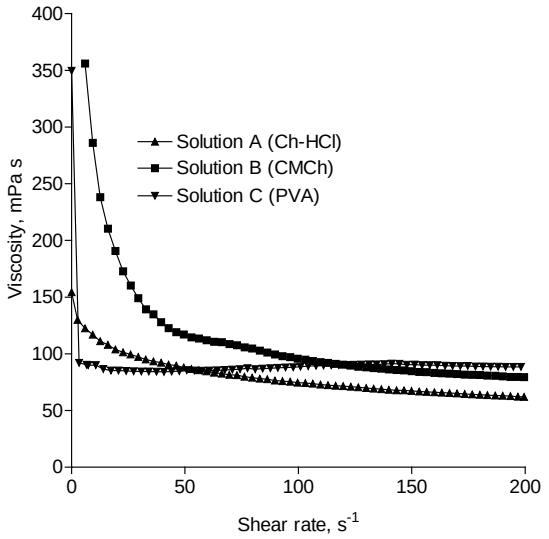

Fig. 2. Viscosity properties of Solutions A–C.

of Solutions A–C, a concentration of 0.2% (w/v) ofloxacin was used in the present study. Nevertheless, the dose instilled in the in vivo tests was the same for the test solutions and the reference commercial eyedrops. The solutions containing CMCh or PVA were buffered at the physiological pH 7.4 of the tear fluid. Since chitosan is insoluble at such a pH, Ch-HCl was dissolved in saline. The viscosity properties of Solutions A–C, shown in Fig. 2, were studied as they could be of relevance to the biopharmaceutic performances of solutions. It is known, indeed, that a higher viscosity of eyedrops increases the drug bioavailability by increasing the preocular residence time. Both chitosan derivatives showed a pseudoplastic behaviour, appropriate for ophthalmic solutions. The PVA concentration in Solution C was adjusted to obtain viscosity values similar to those for Solutions A and B. In fact, at shear rates >50 s^{-1} the viscosity values of the three solutions were similar, so differences in ocular pharmacokinetics could not be ascribed to differences in solution viscosity. Solutions A–C could easily be instilled as eyedrops despite their relatively high viscosity.

3.3. Drug–polymer interactions

The drug–polymer interactions could affect the biopharmaceutical behaviour of solutions. Such interactions were measured by the ratio of the dynamic dialysis rate constant determined in the presence of polymer in the donor to that determined in the absence of polymer. Such a ratio equals 1 in the absence of drug–polymer interactions, and decreases with increasing interaction strength (Di Colo and Zambito, 2002a). The mean values ± S.D. ($n = 3$) of the ratio for Solutions A–C were 0.989 ± 0.003, 0.748 ± 0.006 and 0.757 ± 0.004, respectively. The values indicate a virtual absence of drug interactions with Ch-HCl and significant interactions with CMCh and PVA. The interactions may consist in hydrogen bonding between the hydroxyl groups of CMCh or PVA and the unionised amino functions of ofloxacin, which could be favoured at pH 7.4. On the other hand, at the pH 4.7 of the Ch-HCl solution the amino functions of the drug should mostly be in the ionised form and hydrogen bonding should be substantially reduced.

3.4. Animal tests

Solutions A–C when instilled in rabbit eyes were biocompatible and caused no apparent irritation signs, such as conjunctival/corneal edema and/or hyperaemia. The decline of concentration in tear fluid over time following instillation of these solutions is shown in Fig. 3. The minimal detectable drug concentration in tear fluid was 3.5 μg/ml, due to the necessity to dilute the withdrawn samples at least 1:100 v/v. Small differences among the rates of drug disappearance from tear fluid appear from the figure for the three solutions, reflecting the small differences among the respective viscosity values, illustrated in Fig. 2. Such values were high enough to ensure the presence of drug in tear fluid at measurable concentrations (>3.5 μg/ml) after at least 1.5 h from instillation.

In order to understand the polymer effects on drug penetration into the aqueous humour, which essentially occurs via the cornea, reference should be made to the following expression of the time to attain peak concentration (t_{max}) in the cornea, following topical application of a drug solution (Makoid and Robinson, 1979):

$$t_{max} = \frac{\ln((K_{ca} + K_{pe})/K_{ah})}{K_{ca} + K_{pe} - K_{ah}} \quad (1)$$

where K_{pe}, K_{ca} and K_{ah} are the rate constants for precorneal drug elimination, corneal drug absorption and

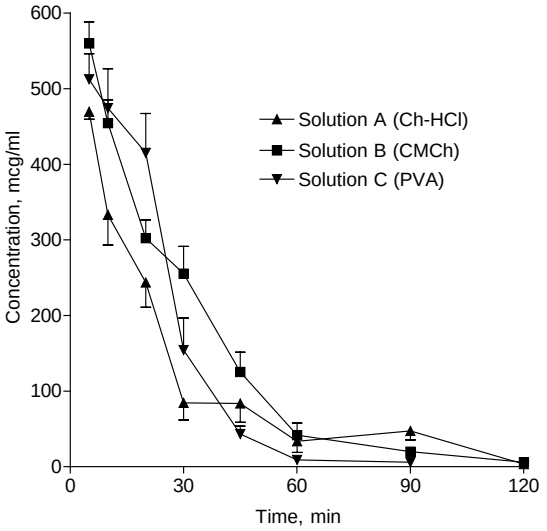

Fig. 3. Kinetics of ofloxacin disappearance from tear fluid following topical administration of 0.3 mg drug by Solutions A–C. Each data point is the mean ± S.E. of six eyes.

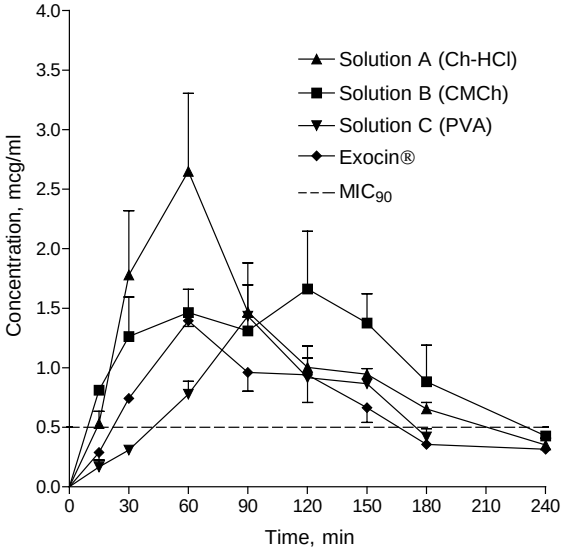

Fig. 4. Ofloxacin concentration in the aqueous humour vs. time, following topical administration of 0.3 mg drug by Solutions A, B or C. Each data point is the mean ± S.E. of at least six values obtained with different animals. The data for the reference Exocin® have been reproduced for comparison from Di Colo et al. (2001). The dotted line represents the MIC_{90} level.

drug transfer from cornea to aqueous humour, respectively.

K_{pe} is determined by tear fluid drainage and turnover, and non-productive absorption. Then, according to Eq. (1), a reduction of K_{pe} caused, e.g., by a reduced tear fluid drainage should prolong the time to peak in the cornea and, consequently, that in the aqueous, since the latter is an open compartment connected in series with the former. In agreement with the above theory, a prolongation of the time to attain pilocarpine concentration peak in the aqueous was actually observed when K_{pe} was reduced by plugging the drainage duct (Makoid et al., 1976).

Hence, Eq. (1) is a sound basis whereon to interpret the effects of the polymers under study on the pharmacokinetics of ofloxacin in the aqueous humour. The relevant concentration–time profiles are shown in Fig. 4, while the pharmacokinetic data are listed in Table 1. Data obtained previously following instillation of an equal drug dose by Exocin® eyedrops (Di Colo et al., 2001) are also reported for reference. As can be seen, PVA produced an increase of t_{max} with respect to the reference (from 60 to 90 min), which

Table 1
Pharmacokinetic data for transcorneal penetration into aqueous humour after administration of 0.3 mg ofloxacin by Exocin® or Solution A (containing Ch-HCl) or Solution B (containing CMCh) or Solution C (containing PVA)

Vehicle	C_{max} ± S.E. ($\mu g\,ml^{-1}$)	t_{max} (min)	AUC_{eff} [a] ($\mu g\,ml^{-1}$ min)	t_{eff} [b] (min)
Exocin®[c]	1.39 ± 0.05	60	62.3	148
Solution A	2.65 ± 0.66	60	160.8	200
Solution B	nd[d]	nd[d]	149.2	224
Solution C	1.43 ± 0.45	90	57.1	132

[a] Area under the experimental concentration in the aqueous humour vs. time curve and over the MIC_{90} level.
[b] Residence time in the aqueous at concentrations higher than the MIC_{90}.
[c] Data from Di Colo et al. (2001).
[d] Not detectable (plateau).

can be ascribed to the increased viscosity of Solution C, causing a reduction of tear fluid drainage, and hence, of K_{pe}. However, the prolonged precorneal drug residence did not result in any increase of concentration peak (C_{max}) nor of bioavailability in the aqueous (AUC_{eff}). This was probably due to the significant binding of ofloxacin by PVA, discussed in Section 2.5, which reduced K_{ca}, the corneal absorption rate constant. Ch-HCl in Solution A produced a C_{max} significantly higher than that relative to the reference, at the same t_{max} of 60 min. These findings, if interpreted in the light of Eq. (1), suggest that the reduction of K_{pe} was compensated for by a substantial increase of K_{ca}, so as to leave t_{max} substantially unvaried. Then, Ch-HCl in Solution A produced an increase of the corneal absorption rate constant, most probably by enhancing the corneal permeability. The polycationic nature of this polymer could play a fundamental role in opening the tight junctions between corneal epithelial cells, thereby favouring paracellular drug transport, as it was the case with intestinal and nasal epithelia (Artursson et al., 1994; Illum et al., 1994). The permeability of the conjunctiva could also be enhanced by Ch-HCl. However, the conjunctiva is highly vascularized, therefore most of the drug permeated through it was presumably removed by the blood circulation before it could enter the aqueous humour and the inner ocular tissues. On the other hand, CMCh, which is a polyanion at the pH 7.4 of Solution B, failed to significantly enhance the drug levels in the aqueous over the C_{max} for the reference, as can be seen in Fig. 4. Interestingly, however, the data points in the figure show a plateau of concentration values in the interval 30–150 min. This pattern points to zero-order transcorneal absorption kinetics which, although intriguing, is hardly explainable on the basis of the experimental evidence available at present. As a hypothesis, CMCh contained in Solution B might mediate a pseudo-steady-state transcorneal transport, possibly via the interactions with the drug discussed in Section 2.5 and the polymer adhesion to the corneal mucus. However, such a mucoadhesion, if any, did not significantly enhance the transcorneal penetration rate, although it could prolong drug residence at the absorption site. Despite the different effects of Ch-HCl and CMCh on ofloxacin ocular pharmacokinetics, these polymers increased the drug bioavailability with respect to the reference by about the same factor, as shown by the AUC_{eff} values in Table 1.

4. Conclusions

A pharmacokinetic model has been used to demonstrate that Ch-HCl is able to significantly increase ofloxacin transcorneal penetration rate, via an enhancement of corneal permeability. Most probably, this effect is linked to the polycationic nature of Ch-HCl, allowing it to strongly interact with the negatively charged corneal surface. Presumably, however, the permeability enhancing effect is temporary, since chitosan requires pH $\leq$ 5 for dissolution, hence it is supposed to precipitate some time after instillation, as the physiological pH of the tear fluid is gradually restored. Therefore, a more intense transcorneal penetration-enhancing effect is expected of polycationic chitosan derivatives soluble at the physiological pH of the tear fluid. This hypothesis was confirmed by preliminary results obtained with the quaternary ammonium derivative *N*-trimethylchitosan, in course of publication. On the other hand, CMCh, which is a polyanion at the physiological pH of the tear fluid, failed to significantly enhance intraocular drug penetration. This chitosan derivative, however, showed the ability to prolong the precorneal drug retention, by virtue of its viscosity-increasing effect, of its ability to bind ofloxacin and, probably, of its mucoadhesive properties. As a result, the residence time at concentrations higher than the MIC_{90} and the bioavailability of the antibiotic in the aqueous were increased with respect to the reference commercial eyedrops. Also, CMCh-mediated zero-order ofloxacin absorption, leading to a time-constant effective antibiotic level in the aqueous. Since this polymer, which is a polyanion in the tear fluid, is potentially able to bind cationic drugs, it may prolong the precorneal residence of, e.g., aminoglucoside antibiotics at effective antimicrobial concentrations, thus allowing reduction of the frequency of instillation.

Acknowledgements

The present investigation was supported by the Italian Ministry of University and Research (MIUR, COFIN 2002).

References

Artursson, P., Lindmark, T., Davis, S.S., Illum, L., 1994. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2). Pharm. Res. 11, 1358–1361.

Beck, R., van Keyserlingk, J., Fischer, U., Guthoff, R., Drewelow, B., 1999. Penetration of ciprofloxacin, norfloxacin and ofloxacin into the aqueous humour using different topical application modes. Graefes Arch. Clin. Exp. Ophthalmol. 237, 89–92.

Bottari, F., Di Colo, G., Nannipieri, E., Saettone, M.F., Serafini, M.F., 1975. Evaluation of a dynamic permeation technique for studying drug–macromolecule interactions. J. Pharm. Sci. 64, 946–949.

Cekic, O., Batman, C., Totan, Y., Yasar, U., Basci, N.E., Bozkurt, A., Kayaalp, S.O., 1999. Penetration of ofloxacin and ciprofloxacin in aqueous humour after topical administration. Ophth. Surg. Lasers 30, 465–468.

Di Colo, G., Burgalassi, S., Chetoni, P., Fiaschi, M.P., Zambito, Y., Saettone, M.F., 2001. Gel-forming erodible inserts for ocular controlled delivery of ofloxacin. Int. J. Pharm. 215, 101–111.

Di Colo, G., Zambito, Y., 2002a. A study of release mechanism of different ophthalmic drugs from erodible ocular inserts based on poly(ethylene oxide). Eur. J. Pharm. Biopharm. 54, 193–199.

Di Colo, G., Zambito, Y., Burgalassi, S., Serafini, A., Saettone, M.F., 2002b. Effect of chitosan on in vitro release and ocular delivery of ofloxacin from erodible inserts based on poly(ethylene oxide). Int. J. Pharm. 248, 115–122.

Felt, O., Furrer, P., Mayer, J.M., Plazonnet, B., Buri, P., Gurny, R., 1999. Topical use of chitosan in ophthalmology: tolerance assessment and evaluation of precorneal retention. Int. J. Pharm. 180, 185–193.

Felt, O., Gurny, R., Buri, P., Baeyens, V., 2001. Delivery of antibiotics to the eye using a positively charged polysaccharide as vehicle. AAPS PharmSci 3 (article 34). http://www.pharmsci.org.

Illum, L., Farraj, N.F., Davis, S.S., 1994. Chitosan as a novel nasal delivery system for peptide drugs. Pharm. Res. 11, 1186–1189.

Khalid, M.N., Ho, L., Agnely, F., Grossiord, J.L., Couarraze, G., 1999. Swelling properties and mechanical characterization of a semi-interpenetrating chitosan/polyethylene oxide network. Comparison with a chitosan reference gel. S.T.P. Pharma Sci. 9, 359–364.

Makoid, M.C., Robinson, J.R., 1979. Pharmacokinetics of topically applied pilocarpine in the albino rabbit eye. J. Pharm. Sci. 68, 435–443.

Makoid, M.C., Sieg, J.W., Robinson, J.R., 1976. Corneal drug absorption: an illustration of parallel first-order absorption and rapid loss of drug from absorption depot. J. Pharm. Sci. 65, 150–152.

Muzzarelli, R.A.A., Tanfani, F., Emmanueli, M., Mariotti, S., 1982. *N*-(carboxymethylidene)-chitosans and *N*-(carboxymethyl)-chitosans: novel chelating polyampholytes obtained from chitosan glyoxylate. Carbohydr. Res. 107, 199–214.

Ross, D.L., Riley, C.M., 1990. Aqueous solubilities of variously substituted quinolone antimicrobials. Int. J. Pharm. 63, 237–250.

Taravella, M.J., Balentine, J., Young, D.A., Stepp, P., 1999. Collagen shield delivery of ofloxacin to the human eye. Cataract. Refract. Surg. 25, 562–565.

Thanou, M., Nihot, M.T., Jansen, M., Verhoef, J.C., Junginger, H.E., 2001. Mono-*N*-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. J. Pharm. Sci. 90, 38–46.