ELSEVIER

International Journal of Pharmaceutics 246 (2002) 17–24

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Release kinetics and up-take studies of model fluoroquinolones from carbomer hydrogels

A.P. Vilches, A. Jimenez-Kairuz, F. Alovero, M.E. Olivera, D.A. Allemandi, R.H. Manzo *

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina

Received 8 November 2001; received in revised form 10 April 2002; accepted 20 June 2002

Abstract

Hydrogels of carbomer (C) loaded with model slightly soluble fluoroquinolone antimicrobials (AMFQ), norfloxacin (I) and ciprofloxacin (II) were prepared to evaluate their physical and delivery properties. Thus, dispersions of 0.25% of C loaded with 0.2–0.5 mol equivalents of AMFQ and 0.2–0.5 mol equivalents of NaOH yielded pseudoplastic hydrogels with a high negative electrokinetic potential and good physical stability. Concentration of AMFQ in the hydrogels was, respectively, 7.2 and 34 times higher than I and II aqueous solubility, indicating a high increase in aqueous compatibility. Release of AMFQ in bicompartimetal Franz type cell occurred by zero order kinetics. Delivery rate constant (k_0) was five to six times higher as water was replaced by NaCl solution as receptor medium. Release in agar dishes revealed that, even under high dilution, delivery remains modulated. Intestinal absorption flux coefficient in everted rat intestine (k_U) were measured with reference solutions (RS) of free AMFQ (k_U^{RS} II $> k_U^{RS}$ I) and with hydrogels (H), in which the pattern was reversed since k_U^H I $> k_U^H$ II. As expected k_U^H II was 0.55 times lower than k_U^{RS} II. However, k_U^H I was 1.37 times higher than its reference, which cannot be explained from the analysis of k_0 and k_U^{RS} alone. Hydrogels C–AMFQ behave as a reservoir of AMFQ able to deliver it at a constant rate and would be useful to design topical and or systemic dosage forms.

Keywords: Fluoroquinolones; Carbomer hydrogels; Compatibility; Ion pair interaction; Up-take; Drug delivery; Zero order release

1. Introduction

Fluoroquinolones (AMFQ) are a very useful group of antibiotics, which are widely used to treat numerous diseases (Hopper and Wolfson, 1993; Reynolds, 1999). Most of the AMFQ in current use have a piperazine ring attached in position 7. Norfloxacin (I) and ciprofloxacin (II) are currently regarded as model compounds of the series (Fig. 1).

In aqueous solution, such compounds exist mainly in their zwitterionic form owing to the acid/base interaction between the basic nitrogen of the piperazine and the carboxylic acid group (Fig. 1). Such interaction also determines the low

* Corresponding author. Tel.: +54-351-433-4163 Int.112; fax: +54-351-433-4127

E-mail address: rubmanzo@dqo.fcq.unc.edu.ar (R.H. Manzo).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00333-2

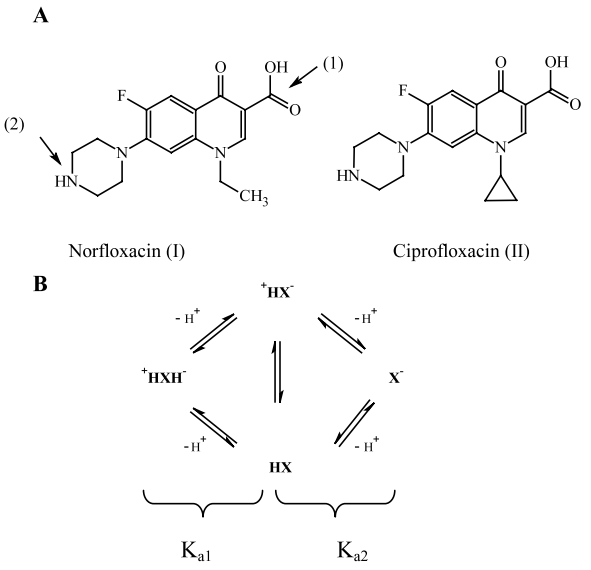

Fig. 1. (A) Structure of norfloxacin (I) and ciprofloxacin (II) with protonation sites (1) and (2). (B) Acid base interaction of I and II. K_{a1} and K_{a2} are the two macroscopic acid dissociation constants corresponding to deprotonation of carboxylic acid group and deprotonation of the nitrogen of piperazine ring, respectively. The four species are designated as follows: $^{+}$HXH, cation; X^{-}, anion; HX, neutral and $^{+}HX^{-}$ zwiterionic. I: $pK_{a1} = 6.22$ (Tackás-Novák et al., 1990), 6.30 (Ross and Riley, 1992) $pK_{a2} = 8.51$ (Tackás-Novák et al., 1990); 8.38 (Ross and Riley, 1992); II: $pK_{a1} = 6.00$ (Tackás-Novák et al., 1990), 6.15 (Yu et al., 1994), $pK_{a2} = 8.80$ (Ross and Riley, 1992); 8.66 (Yu et al., 1994).

aqueous solubility of these compounds at pH close to 7 (Tackás-Novák et al., 1990; Mazuel, 1991; Ahumada et al., 1993; Fallati et al., 1994). This is the main factor, which prevents the design of liquid dosage forms, such as parenteral and ophthalmic solutions, because the aqueous compatibility of these drugs occurs at rather basic or acid pH (Allemandi et al., 1999).

The purpose of this work was: (a) to use the acidic polyelectrolyte carbomer (C) as a carrier to be loaded with I or II in order to enhance their aqueous compatibilities and (b) to get stable colloidal dispersions able to deliver AMFQs at controlled rate. This hypothesis arose from previous results obtained with C loaded with lidocaine (L) (Jimenez-Kairuz et al., 2002) and metoclopramide (M) (Jimenez-Kairuz et al., 2001), in which it was shown that a high fraction of the loaded basic drug is under the form of ionic pairs with the carboxylic groups of C. Such interaction determines in great extension the equilibrium and delivery properties of these systems.

2. Materials

2.1. Chemicals

Carbomer (Carbopol® 934P NF) was kindly supplied by BF-Goodrich, USA. Norfloxacin USP XXII (Marshing and Co. Ltd., Denmark), ciprofloxacin USP XXII (Amifarma, Spain), trometamine p.a. (Tris buffer, Anedra, Argentine), NaCl p.a. (Anhedra, Argentine), anhydrous dextrose p.a. (Cicarelli, Argentine), HCl and NaOH soln. (Titrisol®, Merk, Germany) were used.

2.2. Preparation of hydrogels

(C–AMFQ)Na hydrogels were prepared by neutralizing a 0.25% dispersion of C with the appropriate amount of NaOH solution followed by addition of an aqueous dispersion of I or II under constant stirring.

3. Methods

Rheology measurements were made at 25 °C in a Haake viscometer VT 500 equipped with a MV1 sensor. Data reported correspond to 21 rpm.

Electrophoretic mobility (μ) and electrokinetic potential (δ) were assayed in a Rank Brothers Mark II electrophoresis apparatus, equipped with a cylindrical cell with 10 cm between electrodes at 25 °C and $\pm$50 V; δ was calculated as: $\delta = 128$ s/cm^2. μ (Martin, 1993). Samples were prepared by diluting the hydrogels 20 or 25 times with water.

Physical stability of hydrogels was assessed by placing them in stoppered glass columns, 30 cm high and 3 cm wide, provided with a tap at the lower extreme. The columns were kept in the dark at room temperature and, at appropriate time intervals samples were withdrawn from both ends of the column (upper and lower), conveniently

diluted and assayed at 272 (maximum absorbance of I and II) and 500 nm (transparency) in a spectrophotometer.

Kinetic release was measured in Franz cells, containing as sample compartment a cylindrical tube of glass (55 mm diameter), provided with a cellulose membrane (Sigma, 12000d) attached to the bottom. After loading the cylinder with 20 ml of hydrogel, the sample cell was vertically fitted into a longer diameter vas containing 500 ml of a receptor medium and a magnetic stirring bar at the bottom. The sample cylinder was immersed to a depth of 4 mm from the surface of the receptor medium. The device was immersed in a 37 °C constant temperature bath attached to a magnetic stirrer. Samples (3 ml) were withdrawn from the receptor medium at selected time intervals and spectrophotometrically assayed at 272 nm after addition of one drop of NaOH and proper dilution with water. The internal volume was kept constant upon addition of equivalent amounts of fresh medium.

In vitro antibiotic susceptibility test: two reference strains were used in this study; *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. Minimum inhibitory concentration (MIC) was determined by a standard macrodilution method with Müeller–Hinton Broth (Merck Química Argentina S.A.I.C., Argentina), according to guidelines of the National Committee for Clinical Laboratory Standards. The inoculum size was approximately 5×10^5 CFU/ml. The lowest concentration of antimicrobial agent that prevented visible growth of the organism after 18–20 h at 37 °C was determined to be the MIC.

Microbiological diffusion assay: the cylinder–plate assay described in USP XXIV (US Pharmacopeia XXIV, 2000) was modified and adapted to evaluate the potency of $(C–AMFQ)_{30}Na_{30}$ hydrogels in a comparative assay to free AMFQs. Müeller–Hinton agar plates were inoculated with *E. coli* suspension standardized to 0.5 McFarland turbidimetric patrons. Six standardized stainless steel cylinders were placed on the inoculated agar using a guide to ensure even spacing on a radius of 2.8 cm. The cylinders were filled alternatively with dilutions of free AMFQs solutions or $(C–AMFQ)_{30}Na_{30}$. The experiment was done in triplicate, filling at least nine cylinders with each antibiotic dilution. The plates were incubated at 35 °C for 16–18 h. The cylinders were removed and the diameter of each zone of growth inhibition was measured. The relative potency of $(C–AMFQ)_{30}Na_{30}$ to free AMFQ was calculated from a standard curve using a log transformation of data.

Everted rat intestine permeation: a buffer pH 7.40 ± 0.05 with osmotic pressure of 287 ± 1 mOsm/kg was prepared with: Tris buffer 4.8×10^{-2} M, HCl 4.2×10^{-2} M, dextrose 9×10^{-3} M and NaCl 9.6×10^{-2} M. Stock solutions of I, $(C–I)_{30}Na_{45}$, II, and $(C–II)_{30}Na_{45}$ were appropriately diluted with buffer to be used as mucosal solutions. Male CR Wistar albino rats weighing between 295 and 350 g were selected. Food was removed about 18 h prior to trial, but water was allowed ad libitum. The rats were sacrificed with ether and the entire small intestine was immediately removed via a midline incision of the abdomen and placed into the buffer solution. After discarding the first 15 cm of the proximal end, the intestine was rinsed with 20–30 ml of buffer, then sleeved onto a glass rod and everted, according to the method of Crane and Wilson (1958). Two 10 cm segments were measured after stretching the entire intestine with a 10 g weight. Each everted intestinal segment was ligated at the distal end, mounted onto a glass cannula and ligated at the proximal end so that 10 cm of the intestine were available for absorption. The entire device was placed at 37 °C into a flask containing 70 ml of mucosal solution. Air was constantly bubbled through the mucosal solution at a rate of 3 bubbles per s. One ml of buffer solution (drug free) was then placed into the serosal compartment. This compartment was sampled after 15 min once and then every 10 min for 85 min. The entire serosal volume was removed at the sampling time. Buffer solution (1 ml) was then introduced into the serosal compartment as a rinse, immediately removed and added to the sample, then diluted with buffer to a final volume of 5 ml. Next, another 1 ml of buffer was placed into the serosal compartment to be withdrawn at the next sampling interval. Serosal concentrations were spectrophotometrically analyzed at 272 nm and

cumulative amounts of drug transferred per unit concentration of drug in the mucosal solution were plotted for each intestinal segment, as a function of time. Permeability flux coefficient (k_U) were obtained from the slope of the straight line obtained by applying minimum square method to the experimental points and is informed as the average of each set of measurements.

4. Results and discussion

Mixing an aqueous dispersion of C with either I or II at different molar ratios yielded opaque dispersions (C–AMFQ) of low viscosity. However, as it is shown in Fig. 2, the addition of increasing amounts of NaOH to such dispersions produced a progressive increase in transparency and viscosity, yielding pseudoplastic hydrogels. It should be noted that, at the same time, the addition of NaOH produced only small increases of pH (Fig. 3). Such a relative high buffer capacity ($\beta = \mathrm{d(NaOH)/dpH} \cong 8 \times 10^{-3}$) is due to the overlapping of the acid base equilibrium depicted in Fig. 1B with the following ones:

$$\mathrm{R{-}COOH + HO^- Na^+ \rightleftarrows R{-}COO^- + H_2O + Na^+} \quad (1)$$

where R–COOH represents carboxylic groups of C and

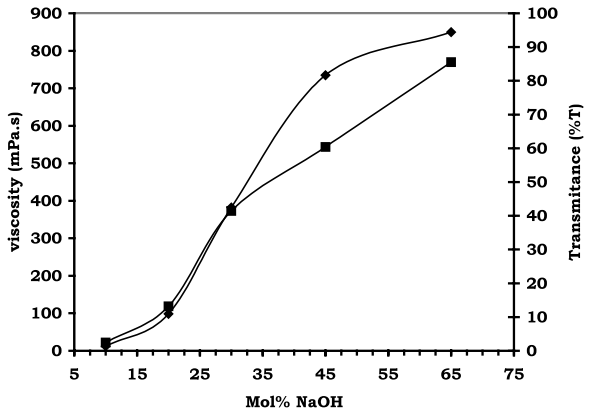

Fig. 2. Variation of viscosity (■) and transmittance (◆) of $(C{-}I)_{30}$ upon addition of NaOH.

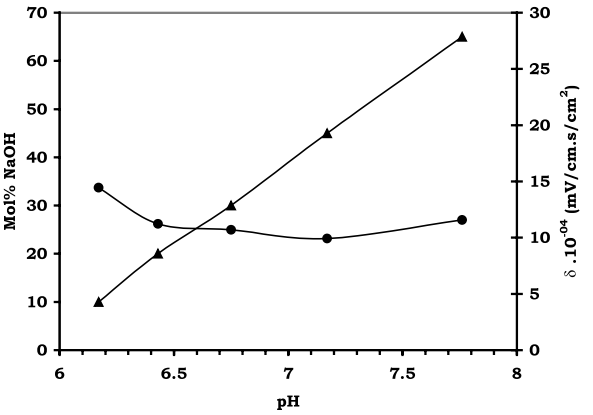

Fig. 3. Relationships between: NaOH added (▲) or electrokinetic potential δ (●) and pH for $(C{-}I)_{30}$.

$$\mathrm{R{-}COOH + XH \rightleftarrows R - COO^-} \; {}^{+}\mathrm{HXH \rightleftarrows R{-}COO^-} \; {}^{+}\mathrm{HX^- + H^+} \quad (2)$$

where XH = I, II.

The scattered colloidal particles of C–AMFQ exhibit a high negative δ that remains essentially unchanged upon addition of NaOH (Fig. 3). This result suggests that Na^+ counter ions remain close enough to the particles to migrate with them.

The analysis of the properties described above allowed to select for further studies the composition $(C{-}AMFQ)_{30}Na_{45}$, in which 30 and 45 mol% of AMFQ and NaOH, respectively, were used to neutralize the carboxylic groups of C. Such composition exhibited a pH of about 7.2 for both I and II hydrogels.

4.1. Physical stability and compatibility

As can be observed in Fig. 4, the hydrogel of I showed an increase in turbidity throughout the first 20 days; afterwards it remained stable. Additionally, non-significant differences in drug content were apparent between samples taken from the upper and lower extremes of the test glass columns containing the hydrogels of I and II. This result indicates that particles remained homogeneously dispersed along the column.

It should be remarked that the physical stability observed in the hydrogels indicates not only the stability of the colloidal dispersions, but also the absence of a precipitate of free AMFQ.

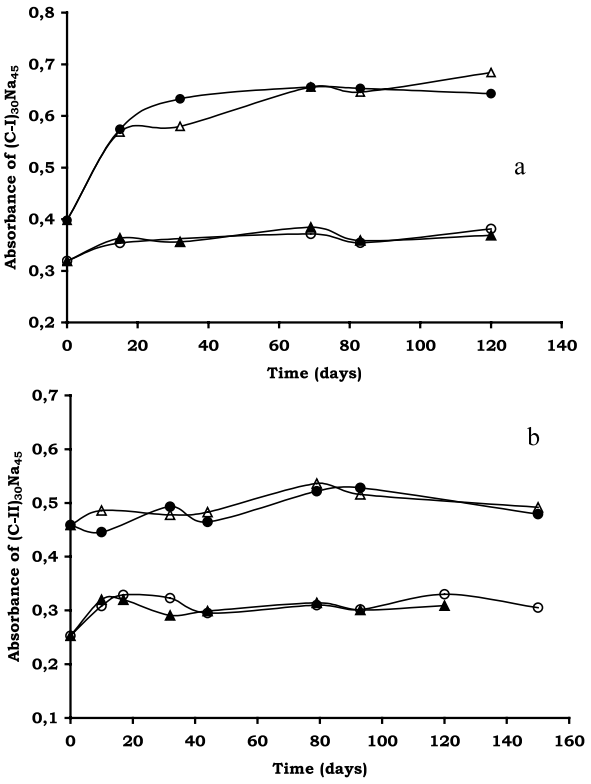

Fig. 4. Variation of absorbance of $(C–I)_{30}Na_{45}$ (a) and $(C–II)_{30}Na_{45}$ (b) against time. At 272 nm: upper samples (○); bottom samples (▲). At 500 nm: upper samples (△), bottom samples (●).

In both hydrogels the concentration of AMFQ was 6.2×10^{-3} M, which is higher than the respective aqueous solubility of I or II at the same pH (Ahumada et al., 1993; Fallati et al., 1994). In fact, as the ratios between concentration of AMFQ in the hydrogels and the aqueous solubility at the same pH of each AMFQ were calculated, the values of 7.2 and 34 for I and II were obtained. These results indicate an important increase in the compatibility of I and II as a consequence of the interaction with the polyelectrolite.

As previously mentioned, in systems (C–L) and (C–M), a high fraction of drug (higher than 83%) is under the form of ionic pairs with $R–COO^-$. If this would be the case in the system C–AMFQ, the concentrations of free AMFQ species in the surrounding aqueous medium would be reduced. Consequently, according to Eq. (3), the increase in the compatibility of I and II in the hydrogels, would arise from the fact that the concentration of the lowest soluble species (XH) remains below its intrinsic solubility (Ahumada et al., 1993; Fallati et al., 1994).

$$[AMFQ] = [XH] + [^+HX^-] + [X^-] + [HXH^+] + [(R–COO^- {}^+HXH)] + [(R–COO^- {}^+HX^-)] \quad (3)$$

where $(R–COO^- \cdot {}^+HXH)$ and $(R–COO^- \cdot {}^+HX^-)$ represent ionic pairs.

It should be noted that the pH on the surface of the colloidal particles of hydrogel is lower than the bulk pH, because the negative charges accumulated there attract hydrogen ions (and also HXH^+) from the surrounding medium and repel hydroxyl ions (Schott and Young, 1972), providing an extra contribution to enhance the aqueous compatibility of the AMFQ.

4.2. Kinetics of AMFQ release

4.2.1. Franz cell

Delivery properties of hydrogels were studied using water and 0.9% NaCl as receptor media. The solution of NaCl was selected to simulate saline properties of biological fluids. As water was the receptor medium, AMFQs were slowly delivered from their hydrogels by zero order kinetics. Delivery was only 7% after 6 h. However, as NaCl solution was used instead of water, zero order kinetics was also observed, but the rate constant (k_0) increased 6.09 and 5.22 times for hydrogels of I and II, respectively, and about 35% of AMFQ was delivered in 6 h (Table 1 and Fig. 5). Such an increase in rate delivery may be attributed mainly to the diffusion of Na^+ and Cl^- ions from the receptor to the hydrogel compartment promoting ionic exchange with AMFQ charged species attached to C. This result is consistent with the view that a high proportion of AMFQ is under the form of ionic pairs. Thus, if delivery occurs through the Fickian diffusion of free species, and their concentrations are low because of the high proportion of ionic pairs, then, rate delivery should remain slow as was

Table 1
Zero order delivery rates of AMFQ from their hydrogels in a Franz cell

Hydrogel	Receptor medium	No. of experiments	k_o[a] (mg/h)
$(C–I)_{30}Na_{45}$	Water	3	0.345±0.011
	Soln. NaCl	3	+2.10±0.05
$(C–II)_{30}Na_{45}$	Water	3	0.433±0.005
	Soln. NaCl	3	2.26±0.07

[a] Zero order release rate constant.

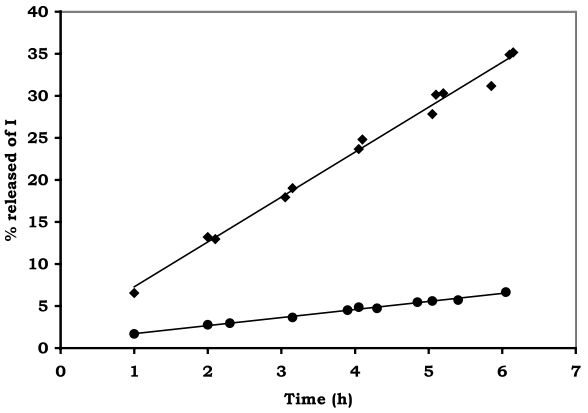

Fig. 5. Release rate of I from $(C–I)_{30}Na_{45}$ in a Franz cell against water (●) or NaCl solution (◆) as receptor media.

observed with water receptor medium. This result suggests that the dissociation of ion pairs is the slow step that controls the rate delivery. As Na^+ and Cl^- diffuse into the hydrogel, Na^+ promotes the exchange of cationic and zwiterionic species (HXH^+ and $^-XH^+$) attached to C, while Cl^- acts as a counter ion to diffuse with them. Both mechanisms contribute to raise the rate as was observed.

Unlike the zero order kinetics observed here, C loaded with basic drugs possessing only one ionizable group under the conditions assayed, such as procaine (Realdon et al., 1998), L (Jimenez-Kairuz et al., 2002), or M (Jimenez-Kairuz et al., 2001), exhibited delivery rates proportional to the square root of time ($t^{1/2}$) (Higuchi, 1961). The system $(C–AMFQ)_{30}Na_{45}$ exhibits higher β than the other ones. This factor contributes to keep more stable the pH and concomitant equilibria (Eqs. (1)–(3)) during the progress of delivery, which in turn would contribute to the zero order that is observed here but not in the other systems.

4.2.2. Microbiological assays

Cylinder–plate assay provides complementary information on delivery properties of C–AMFQ systems under high-diluted conditions. Inhibition zones produced by the diffusion of I or II from their diluted hydrogels were proportional to their concentrations (Fig. 6) although diameters were 0.8 times lower than those of the free AMFQ solutions. Therefore, even under high dilution, AMFQ release remains modulated by their interactions with the polyelectrolyte. On the other hand, MIC of $(C–AMFQ)_{30}Na_{45}$ of I and II against reference strains of *E. coli* and *S. aureus*, measured after 18 h of contact in the liquid medium, did not reveal significant differences from their respective solutions of free AMFQs (Table 2).

4.2.3. In vitro intestinal absorption

In order to determine how much the absorption characteristics of I and II are affected when they are in the colloidal system under diluted conditions, their permeability flux coefficients (k_U) in everted rat intestine were determined and are reported in Table 3.

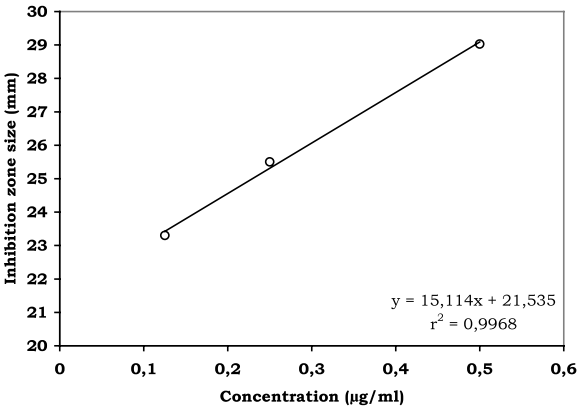

Fig. 6. Inhibition grown zones size against $(C–II)_{30}Na_{45}$ concentration.

Table 2
Minimum inhibitory concentrations (MIC) of AMFQ free and loaded in hydrogels

Microorganism	MIC (μg/ml)			
	I	$(C–I)_{30}Na_{45}$	II	$(C–II)_{30}Na_{45}$
S. aureus ATCC 29213	1	1–2	0.25–0.5	0.25–0.5
E. coli ATCC 25922	0.06–0.125	0.06–0.125	0.015	0.015–0.03

It is known that AMFQs are absorbed by passive diffusion through intestinal membrane (Level, 1988). Consequently, the uncharged species XH should have the greatest ability to pass across the membrane. It is also known that the lipophilicity of XH species of II is higher than that of I, as it is accounted for the partition coefficient (PC) methodology (Fallati et al., 1996).

As it is reported in Table 3 for reference solutions of free AMFQ, compound II, having the highest lipophilicity, also exhibited the highest permeability flux coefficient (k_U^{RS}) that was 1.64 times than that of I.

On the other hand, although delivery experiments measure the diffusion to the receptor compartment of all species depicted in Fig. 1, k_0 of the hydrogel of II was also higher than that of I in either cases water or NaCl solution as receptors.

Then, if both k_0 and k_U^{RS} are higher for II than for I, the same pattern would be expected for the intestinal absorption from their hydrogels (k_U^H). However, a reverse order was observed since k_U^H of II was only 0.65 times than that of I.

It should be noted that k_U^H of II was 0.55 times lower than its respective k_U^{RS}. This result is consistent with that observed in the cylinder–plate assay. However, in the case of I k_U^H was 1.37 times higher than its reference. The enhanced rate of absorption observed in this case may arise from the bioadhesive properties of the hydrogel. There are many reports on such effects with polyelectrolyte systems (Poelma and Tukker, 1987; Sanders et al., 2000). No additional efforts were made to explain the reasons for which that behavior occurs with the hydrogel of I but not with that of II since it was considered outside the primary goals of this work. Nevertheless, this point will be further addressed.

Finally, results analyzed support the conclusion that loading C with a favorable composition of a model AMFQ like I or II, together with an appropriate proportion of Na^+, yields physically stable dispersions in which the aqueous compatibility of the AMFQ is significantly enhanced. Such dispersions behave as a molecular matrix of AMFQ, able to deliver the drug at a constant rate (zero order kinetics) in contact with a biological fluid-like solution.

Ion pairing between AMFQ and C seems to be the main interaction that determines the enhanced aqueous compatibility and releasing properties.

Modulation of delivery rates also occurs with diluted dispersions, as it was apparent from microbiological cylinder–plate assay and absorption in everted rat intestine.

Table 3
In vitro permeability flux coefficients in everted rat intestine of AMFQ loaded in hydrogels or as free AMFQ solutions

Hydrogel or reference solution	Number of experiments[a]	Molar mucosal concentration $\times 10^4$	k_U^H or k_U^{RS} (g/min)[b]
$(C–I)_{30}Na_{45}$	3	5.80	3.53 ± 0.05
I	3	5.80	2.57 ± 0.14
$(C–II)_{30}Na_{45}$	3	5.58	2.31 ± 0.07
II	3	5.58	4.22 ± 0.55

[a] Eight time points each, $r^2 > 0.992$ in every case.
[b] k_U, permeability flux coefficient; H, hydrogel; RS, reference solution.

The analysis of the set of kinetic and lipophilic parameters k_0, k_U^{RS}, k_U^H and PC was consistent for II but not for I. With the last there was a failure in the prediction of, k_U^H from the analysis of such parameters alone and would be necessary further complementary efforts to account for the enhanced absorption measured.

In summary, the system (C–AMFQ)Na would be useful to design topical and/or systemic controlled release dosage forms.

Acknowledgements

Financial support from Grants of CONICET (Arg.), CONICOR (Arg.), and SECYT-UNC.

References

Ahumada, A.A., Seeck, J., Allemandi, D.A., Manzo, R.H., 1993. The pH-solubility profile of norfloxacin. STP Pharm. Sci. 3, 250–253.

Allemandi, D.A., Alovero, F., Manzo, R.H., 1999. Formulation of a neutral solution of ciprofloxacin upon complexation with aluminum. Il Farmaco 54, 758–760.

Ahumada, A.A., Seeck, J., Allemandi, D.A., Manzo, R.H., 1993. The pH-solubility profile of norfloxacin. STP Pharm. Sci. 3, 250–253.

Fallati, C.S., Ahumada, A.A., Manzo, R.H., 1994. El perfil de solubilidad de la Ciprofloxacina en función del pH. Acta Farm. Bonaer. 13, 73–77.

Fallati, C.S., Mazzieri, M.R., Manzo, R.H., 1996. Lipophilicity of zwitterionic and non-zwitterionic fluoroquinolone antibacterials. STP Pharm. Sci. 6, 162–165.

Higuchi, T., 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 50, 874–877.

Hopper, D.C., Wolfson, J.S., 1993. Quinolone Antimicrobial Agents, second ed.. American Society for Microbiology, Washington, DC.

Jimenez-Kairuz, A., Allemandi, D.A., Manzo, R.H., 2001. Equilibrios y cinética de liberación de metoclopramida (M) desde hidrogeles de carbomer (C), XIII Simposio Nacional de Química Orgánica, Córdoba, Argentina, 13 Nov.

Jimenez-Kairuz, A., Allemandi, D.A., Manzo, R.H., 2002. Mechanism of lidocaine release from carbomer- lidocaine hydrogels. J. Pharm. Sci. 91, 267–272.

Lebel, M., 1988. Pharmacokinetics of oral fluoroquinolones. ISI Atlas of Sciences: Pharmacology, 2, pp. 196–201.

Martin, A., 1993. Colloids. In: Physical Pharmacy, fourth ed.. Williams & Wilkins, Baltimore, pp. 393–422.

Mazuel, C., 1991. Norfloxacin. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 20. Academic Press, New York, pp. 557–600.

Poelma, F.G.J., Tukker, J.J., 1987. Evaluation of a chronically isolated internal loop in the rat for the study of drugs absortion kinetic. J. Pharm. Sci. 76, 433–436.

Realdon, N., Ragazzi, E., Dalzotto, M., Dallafini, G., 1998. Possibilities of conveying a cationic drug in carbomer hydrogels. Drug Dev. Ind. Pharm. 24, 337–343.

Reynolds, J., 1999. Actibacterials. In: Martindale, The Extra Pharmacopeia, 32nd ed., Pharmaceutical Press, London, pp. 185–189, 233.

Ross, D., Riley, C., 1992. Physicochemical properties of the fluoroquinolone antimicrobials. II. Acid ionization constants and their relationship to structure. Int. J. Pharm. 83, 267–272.

Sanders, N.N., De Smedt, S.C., Demeester, J., 2000. The physical properties of biogels and their permeability for macromolecular drugs and colloidal drug carriers. J. Pharm. Sci. 89, 835–849.

Schott, H., Young, C.Y., 1972. Electrokinetic studies of bacteria I: effect of nature, ionic strenght, and pH buffer solutions on electrophoretic mobility of *Streptococcus faecalis* and *Escherichia coli*. J. Pharm. Sci. 61, 182–187.

Tackás-Novák, K., Noszál, B., Hermecz, I., Kersztúri, G., Podanyi, B., Szasz, G., 1990. Protonation equilibria of quinolone antibacterials. J. Pharm. Sci. 79, 1023–1028.

US Pharmacopeia XXIV, 2000. US Pharmacopeial Convention, Rockville, MD, pp. 1823–1829 and 1837–1847.

Yu, X., Zipp, G., Ray Davidson, G.W., III, 1994. The effect of temperature and pH on the solubility of quinolone compounds: estimation of heat of fusion. Pharm. Res. 11, 522–527.