

Ocular Bioerodible Minitablets as Strategy for the Management of Microbial Keratitis

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PURPOSE. Evaluation in volunteers of ciprofloxacin-containing ocular gelling minitables with prolonged release properties.

METHODS. The irritation potential of ciprofloxacin-containing bioadhesive powder mixtures, used to prepare ocular bioerodible minitables, was evaluated with a slug mucosal-irritation test. The tear pharmacokinetic profiles of ciprofloxacin were determined in six healthy volunteers after topical administration of a minitab and a single eye drop in the lower fornix. The drug concentrations in the tear samples collected were measured by using a validated HPLC method. Each volunteer was asked to give an evaluation of the preparations applied by answering a standard questionnaire.

RESULTS. The results of the mucosal-irritation test demonstrated the nonirritating properties of the bioadhesive powder mixtures. The ocular minitab, applied in the fornix was in general well tolerated by the healthy volunteers. The mean tear concentration of ciprofloxacin was 33.0, 135.2, and 33.7 $\mu\text{g/g}$ at 30, 300, and 480 minutes after application of the minitab. Mean tear levels of 84.7, 45.6, and 8.4 $\mu\text{g/g}$ were obtained at 5, 30, and 60 minutes after application of an eye drop.

CONCLUSIONS. Due to their prolonged drug release properties, the ocular minitables containing ciprofloxacin can be considered as a promising drug delivery system to be used in the treatment of ulcerative bacterial keratitis. (*Invest Ophthalmol Vis Sci.* 2004;45:3229-3233) DOI:10.1167/iovs.04-0206

Bacterial keratitis is a potentially blinding condition. In more than 80% of cases, the infections are caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Pseudomonas aeruginosa*.^{1,2} Standard initial treatment consists of frequent instillation of eye drops with a broad-spectrum antibiotic. The drop application schedule requires strict discipline from the patient or care provider. At present, the two following regimens are commonly used: combination therapy with fortified antibiotics (e.g., an aminoglycoside with vancomycin hydrochloride) or monotherapy with a fluoroquinolone (e.g., ciprofloxacin).³

Ophthalmic solutions, however, have poor bioavailability because of rapid precorneal clearance, induced lacrimation, and normal tear turnover.⁴ Therefore, daily frequent instillation of the solution is necessary to achieve a therapeutic effect. The frequent use of concentrated solutions may damage the ocular surface. Systemic absorption of the drug drained through the nasolacrimal duct systems can cause side effects.^{5,6} Various methods were investigated to increase the drug bioavailability by prolonging the contact time between drug and corneal-conjunctival epithelium. The first strategy developed was the viscosity increase of the vehicle by addition of viscolyzers to the formulation. Only a small improvement of the retention time of the drug in the fornix was obtained.^{7,8} Highly viscous gels and ointments on the other hand provided a sustained contact with the eye surface, but caused a sticky sensation and blurring of vision and induced reflex blinking due to irritating properties.⁹ Another approach to optimizing bioavailability was the implementation of the mucoadhesive concept. In this method, suitable polymers interact with the mucus layer that coats the external surface of the eye.^{10,11} The use of films or inserts was proposed, to allow drug release over a long period.¹² Inserts and collagen shields were very effective because of less frequent administration and absence of additives, which often cause adverse effects on corneal epithelial wound healing, leading to toxic keratitis manifested as punctate lesions with a persistent epithelial defect. Insoluble inserts had the disadvantage that they had to be removed manually, as they are not eliminated naturally (e.g., Ocuser; Akorn, Buffalo Grove, IL). Soluble inserts that dissolve or erode gradually after administration offer several advantages (i.e., a simple design), employing ingredients that are well adapted for ophthalmic use and an easy manufacturing process (e.g., direct compression).

Recently, an ocular bioerodible or soluble minitab containing the diagnostic agent sodium fluorescein and exhibiting sustained release properties was developed, in vitro optimized and in vivo evaluated. This formulation was well accepted by healthy volunteers.^{13,14} After application in the fornix, the minitab exhibited a gelling behavior, resulting in an extended residence at the site of action of the drug. Consequently, this concept is interesting for the treatment of corneal ulcers. The purpose of the present study was twofold: to verify the acceptability of a ciprofloxacin-containing minitab by healthy volunteers and to determine how long an in vitro optimized minitab can provide sustained release in vivo in the precorneal area. The value of this ocular minitab was compared with a ciprofloxacin hydrochloride ophthalmic solution (Ciloxan; Alcon Laboratories, Fort Worth, TX), developed and approved for treating mild to moderate bacterial keratitis. Before the in vivo study, the irritation potential of ciprofloxacin, the nonsterilized ciprofloxacin-containing powder mixture, and the corresponding sterilized powder mixture was evaluated with the slug mucosal-irritation test. This test has been validated as an alternative for screening the eye-irritation potential of chemicals and has been used to evaluate the irritation potential of bioadhesive powder formulations.¹⁵⁻¹⁷

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FIGURE 1. Ocular minitabket (6 mg, diameter 2 mm) prepared with drum-dried waxy maize starch/Carbopol 974P/sodium stearyl fumarate/ciprofloxacin hydrochloride (90.5/5/1/3.5, wt/wt/wt/wt).

METHODS

Preparation and Sterilization of Powders and Minitablets

Carbopol 974P was supplied by Noveon (Cleveland, OH) and drum-dried waxy maize starch (DDWM, containing 99.9% amylopectin) was obtained from Eridania Béghin-Say Cerestar (Vilvoorde, Belgium). Sodium stearyl fumarate and ciprofloxacin hydrochloride were a gift of Edward Mendell Co. Inc. (New York, NY) and Dr. Reddy's Laboratories (Hyderabad, India), respectively.

The powder mixture DDWM/Carbopol 974P/sodium stearyl fumarate/ciprofloxacin hydrochloride 90.5/5/1/3.5 (wt/wt/wt/wt) was obtained by homogeneously mixing the different compounds in a laboratory mixer (Turbula T2A; Willy A. Bachoffen-WAB Maschinenfabrik, Basel, Switzerland). The minitables (diameter 2 mm, 6 mg; Fig. 1) were prepared by compressing the powder mixture at a force of 1.000 kN using an eccentric compression machine (Korsch Type EKO; Berlin, Germany) equipped with four concave punches. An irradiation dose of 25 kGy (Gammir-I-Sulzer irradiator unicell; Sterigenics, IBA-Mediris, Fleurus, Belgium) was used to sterilize the minitables. The same irradiation dose was used to prepare the sterilized powder mixture used in the irritation test.

Mucosal-Irritation Test in Slugs

The principle of the mucosal-irritation test in slugs is based on the fact that slugs exposed to irritating substances produce mucus to protect the body wall.¹⁸ The amount of mucus produced by the slugs is a measure of irritation. In addition, membrane damage can be estimated from the release of proteins and enzymes from the body wall of the slugs. The original procedure was modified for the evaluation of the irritation potential of bioadhesive powder mixtures and will be described briefly.^{16,17}

Slugs (*Arion lusitanicus*) weighing between 3 and 6 g were isolated from the culture 2 days before the start of an experiment and were placed in a vented plastic box lined with a paper towel, moistened with phosphate-buffered saline (PBS, pH 7.4) at 18°C to 22°C. The slugs were then placed in a Petri dish on 20 mg of the powder to be examined, for 30 minutes. For each powder, five slugs were used. Slugs treated with DDWM were used as the negative control (NC), whereas slugs treated with DDWM/benzalkonium chloride 95/5 (wt/wt) (DDWM/BAC) were used as the positive control (PC). The amount of mucus produced during the 30-minute contact period was measured by weighing the Petri dishes containing the powder (without the slugs) before and after the contact period. The mucus production (MP) was expressed as a percentage (wt/wt) of the body weight. After the contact period, the slugs were transferred to a fresh Petri dish containing 1 mL PBS. The PBS samples were collected with a micropipette

after 60 minutes. Then, the slugs were placed in a fresh Petri dish, and again 1 mL PBS was added. After 60 minutes, the PBS samples were removed. The PBS samples were analyzed immediately for the presence of the proteins, lactate dehydrogenase [LDH] and alkaline phosphatase [ALP] released from the body wall of the slugs. The slugs were placed in a Petri dish on a membrane filter (cellulose acetate 0.45 μm; Sartorius AG, Goettingen, Germany) moistened with 2 mL PBS until the next contact period. This procedure was repeated during five successive days.

The protein concentration in the samples was determined with a protein-quantitation kit (NanoOrange; Molecular Probes, Leiden, The Netherlands) and is expressed as micrograms per milliliter per gram body weight. The activities of LDH (EC 1.1.1.27) and ALP (EC 3.1.3.1) were measured with an enzyme kit (DG 1340-UV and DG 1245-UV, respectively; Sigma Diagnostics, Bornem, Belgium) and are expressed as units per liter per gram body weight.

For each slug, the total mucus production, the mean protein release (without the data of the first day of treatment), the mean LDH release, and the mean ALP release were calculated, and the data were analyzed with a one-way ANOVA. The data were tested for normal distribution with a Kolmogorov-Smirnov test. The homogeneity of variances was tested with the Levene's test. If the variances were found not to be equal, the data were transformed to their logarithm. To compare further the effects of the different treatments, a multiple comparison among pairs of means was performed with a Scheffé test. $P \leq 0.05$ or less was significant. A computer (SPSS ver. 11.0, SPSS, Chicago, IL) was used for all the statistical analyses.

In Vivo Study

The in vivo study was an open two-way crossover study involving six healthy volunteers of either gender, between 20 and 30 years of age. All procedures used were approved by the local ethics committee. All subjects were informed in accordance with the requirements of the Declaration of Helsinki before study initiation. The volunteers gave written informed consent after receiving a detailed explanation of the investigational nature of the study and the potential benefits and risks. Subjects were excluded if they were pregnant; they wore contact lenses; they had used oral or topical ocular medication within 7 days before the study; they had a history of allergy to ciprofloxacin, fluoroquinolones, or one of the additives of the eye drop solution; or they had a history of eye diseases. As the control, both eyes of each volunteer were evaluated before and after the in vivo study by slit lamp examination and by measuring the secretion of tears (Schirmer test) and visual acuity. Subjects were randomly assigned to receive 1 drop of a 0.3% (wt/vol) ciprofloxacin solution (Ciloxan; Alcon Laboratories, Inc., Fort Worth, TX) or a sterilized minitabket containing 3% (wt/wt) ciprofloxacin. The minitabket was positioned temporally in the fornix of the volunteer with a device that consisted of a small tube leading to a small balloon. After the tube was squeezed, the minitabket fell into the fornix. A 100-mm visual analog scale (VAS), a versatile unidimensional scale, was used to evaluate the acceptability of the minitabket and the eye drop. The volunteers answered a series of questions related to ocular irritation (general and at the puncti), lacrimation, and vision (Table 1). The sensation observed after the application of an eye drop or a minitabket was evaluated by means of a score from 0 (no sensation) to 5 (hurting).

Without instillation of local anesthesia, tear samples were collected on preweighed surgical sponges (Fine Science Tools [FST], Heidelberg, Germany). According to Small et al.,¹⁹ these sponges have advantages rapidity, ease, accuracy, and good precision.¹⁹ Tear samples were taken 5, 15, 30, 60, 120, 180, 240, and 300 minutes after instillation of the eye drop and 30, 60, 120, 300, 390, and 480 minutes after application of the minitabket.²⁰ The concentration of ciprofloxacin in the tear samples was determined by HPLC performed with a commercial system (125 mm, 3 mm; Purosphere RP-C18e column; Merck Eurolab, Leuven, Belgium). A guard column (4 mm, 4 mm, 5 μm; model 100 RP-18e; Lichrosphere; Merck Eurolab) was used to protect the analyt-

TABLE 1. Questions to Evaluate the Acceptability of the Ophthalmic Preparation

Parameter Evaluated	Questions	Pain Visual Analog Scales
Ocular irritation (general)	Does the preparation cause a painful sensation in the eye?	No irritation (0 mm); stinging (100 mm)
Ocular irritation (puncti)	Does the preparation cause a painful sensation in the eye?	No irritation (0 mm); stinging (100 mm)
Lacrimation	Does the preparation increase lacrimation?	No increased lacrimation (0 mm); overflow on the cheek (100 mm)
Vision	Does the preparation cause blurring of vision?	Clear vision (0 mm); blurred vision (100 mm)
Sensation	How does the preparation feel?	No sensation (0), smooth (1), thick (2), sticky (3), sandy (4), hurting (5)

ical column, which was maintained at room temperature. According to the method described by Garcia et al.,²¹ a mobile phase was prepared with 87 volume parts water with orthophosphoric acid (85%; Merck Eurolab), KH_2PO_4 (0.020 M; Merck Eurolab) and tetraethylammonium bromide (0.012 M; Sigma-Aldrich) and 13 volume parts acetonitrile (Biosolve, Valkenswaard, The Netherlands).²¹ The mobile phase was adjusted to pH 3.0, using NaOH (2 M). The flow rate was set at 0.56 mL/min. The ciprofloxacin concentration was measured using a fluorescence detector (L-7480; LaChrom; Merck Eurolab) at an excitation wavelength (λ_{ex}) of 278 nm and an emission wavelength (λ_{em}) of 450 nm. Integration of the peaks obtained was performed with an integrator (L-7000; LaChrom). The calibration curves were established in the range of 5 to 500 ng/mL. The accuracy was determined by comparing seven measured concentrations with their true values. The precision (inter- and intraday) of the method was calculated at two concentrations (i.e., 80 and 200 ng/mL). Recoveries were determined by extracting samples containing 80 and 200 ng/mL ciprofloxacin. The detection and quantification limits of ciprofloxacin were 3.13 and 10.42 ng/mL, respectively. Before the determination of the ciprofloxacin concentration, 5 mL mobile phase was added to the tear samples. After centrifugation at 4000 rpm for 10 minutes, 50 μL of the supernatant was injected. The concentration of the drug in the tear film was calculated by dividing the amount of drug, expressed in micrograms, present in the surgical sponge by the weight of the tear sample to yield an effective concentration, expressed as micrograms of drug per gram tear fluid.

RESULTS

Mucosal-Irritation Test

The irritation potential of ciprofloxacin hydrochloride, the nonsterilized powder mixture DDWM/Carbopol 974P/sodium stearyl fumarate/ciprofloxacin hydrochloride 90.5/5/1/3.5 (wt/wt/wt/wt), and the corresponding sterilized powder mixture was evaluated with the slug mucosal-irritation test. The effects of the repeated treatment with the powders examined on the end points of this irritation test are presented in Table 2. The total mucus production of the slugs treated with the nonsterilized powder mixture or the sterilized powder mixture was

comparable to that of the negative control slugs ($P > 0.05$). Multiple comparison presented the following two homogeneous subsets for the total amount of mucus secreted: (1) negative control, sterilized powder mixture, and nonsterilized powder mixture, and (2) nonsterilized powder mixture, ciprofloxacin, and positive control. The repeated treatment of the slugs with ciprofloxacin, the nonsterilized, and the sterilized powder mixture resulted in a protein release comparable to that of the negative control slugs ($P > 0.05$). The slugs treated with DDWM/BAC 95/5 (wt/wt) released significantly higher concentrations of proteins than did the negative control slugs ($P < 0.05$). An increased release of the cytosolic enzyme LDH and the membrane-bound enzyme ALP from the mucosa of the slugs is an indication of severe membrane damage. For the slugs treated with DDWM, ciprofloxacin, the nonsterilized and the sterilized powder mixture, the release of these enzymes was under the detection limit. Enzyme release was detected only in the positive control slugs. From these results, it was concluded that the powder mixture can be used to prepare sterilized minitables for further evaluation in healthy volunteers.

In Vivo Study

The administration of the minitab in the lower fornix of the eye was well tolerated by the healthy volunteers because of the small dimensions of the tablet, as can be seen in Figure 2. The acceptability scores of the eye drop and minitab as a function of time are shown in Table 3. No irritation or a soft sensation in the eye was reported by the volunteers after application of both formulations in the fornix. Only one volunteer reported a burning sensation after application of the minitab at the 5-hour time point, due to the disintegration of the gelled system. Another volunteer had a burning sensation at 5 minutes after application of 1 eye drop, probably due to the effect of the preservative benzalkonium chloride present in the eye drop on the tear film. A decrease in the score for general irritation and irritation at the puncti was observed after application of the eye drop. The irritation properties of the minitab were higher, but not statistically significant, com-

TABLE 2. Results of the Mucosal Irritation Test Using Slugs

Slug Treatment	Total MP (%)	Mean Protein Release ($\mu\text{g}/\text{ml}/\text{g}$)	Mean LDH Release (U/L/g)	Mean ALP Release (U/L/g)
DDWM (NC)	2.7 \pm 1.7	4 \pm 3	—	—
Ciprofloxacin	10.2 \pm 3.4*	22 \pm 33†	—	—
Nonsterilized powder	3.9 \pm 0.9*†	4 \pm 2†	—	—
Sterilized powder	2.5 \pm 0.7†	8 \pm 7†	—	—
DDWM/BAC 95/5 (PC)	12.3 \pm 8.1	241 \pm 93	2.38 \pm 2.71	0.26 \pm 0.58

Data are presented as the mean \pm SD ($n = 5$); —, below the detection limit. MP, mucus production expressed as % (wt/wt) of the body weight.

* Data are not significantly different from the positive control (PC) slugs ($P > 0.05$, Scheffé test).

† Data are not significantly different from the negative control (NC) slugs ($P > 0.05$, Scheffé test).

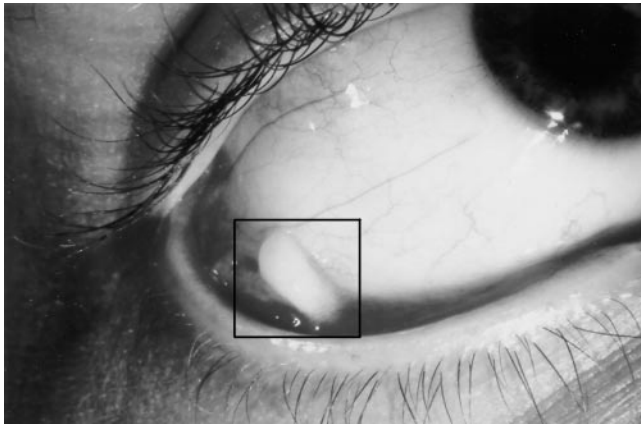


FIGURE 2. Macroscopic examination of the gelling minitabket in the eye, 4 hours after application.

pared with those of the eye drop. As a result of the shear forces exerted on the gelled system during blinking, small parts sometimes eroded from the system and caused blurred vision for a few minutes. This was observed at the 300-minute time point. The vision was more blurred in the case of the minitabket than of the eye drop ($P < 0.05$). Blurred vision occurred in four volunteers after 4.5 hours for a short period, whereas it was reported after 6.5 and 7.5 hours by the other two volunteers. Lacrimation occurred in general immediately after application of the minitabket. Because the lacrimal fluid started to hydrate the minitabket immediately, the sensation was reduced to an acceptable level. However, after 4.5 hours, the gel eroded gently, as mentioned previously, and increased lacrimation.

The mean ciprofloxacin concentrations and corresponding standard deviations in the tear film over an 8-hour period are shown in Figure 3. High tear film levels for more than 8 hours were measured after the application of the ocular minitabket containing ciprofloxacin. The mean tear ciprofloxacin concentration was $33.0 \pm 19.0 \mu\text{g/g}$ at the 30-minute time point, increased to $135.2 \pm 66.0 \mu\text{g/g}$ at the 5-hour time point, and decreased to $33.7 \pm 37.6 \mu\text{g/g}$ after 8 hours. Five minutes after application of one drop of Ciloxan (Alcon Laboratories) the tear film contained $84.7 \pm 32.5 \mu\text{g/g}$ of ciprofloxacin. The concentration decreased rapidly to 45.6 and $8.4 \mu\text{g/g}$ at the 30- and 60-minute time points, respectively.

DISCUSSION

From the slug mucosal-irritation test, it can be concluded that ciprofloxacin hydrochloride caused mild irritation of the slug

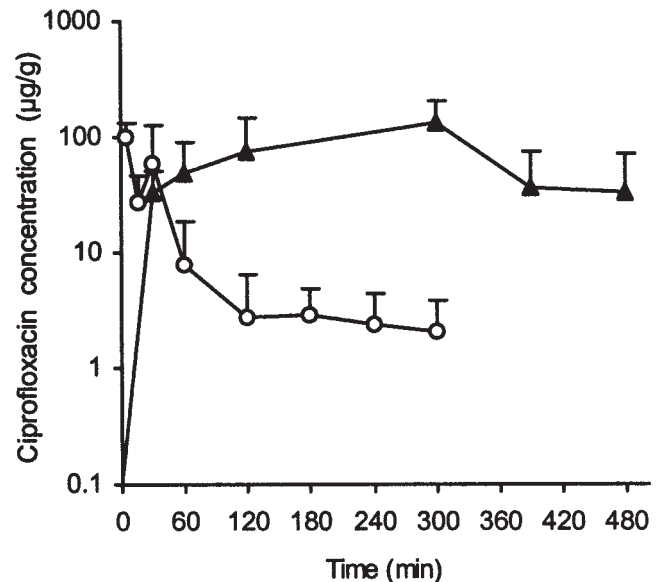


FIGURE 3. Ciprofloxacin concentration in the tear film of healthy volunteers as function of time, after topical application of a single eye drop (○), and an ocular minitabket (▲) ($n = 6$, mean \pm SD).

mucosa, as ciprofloxacin induced an increased mucus production. However, no increased protein and enzyme release were detected. The nonsterilized and the sterilized powder mixtures containing ciprofloxacin did not irritate the slug mucosa. The mucus production and the protein release were low, and no enzyme release was detected. Ceulemans et al.¹³ reported similar irritation data during the evaluation of a nonsterilized bioadhesive powder formulation (DDWM, Carbopol 974P, and sodium stearyl fumarate; 94/5/1, wt/wt/wt) with fluorescein as drug molecule.¹³ The present study showed that sterilization had no influence on the irritating properties of the bioadhesive formulation tested. Both the nonsterilized and the sterilized ciprofloxacin-containing powder mixtures did not irritate the slug mucosa.

The in vivo study demonstrated that the ciprofloxacin-containing sterilized ocular minitabkets were well tolerated by the human volunteers and that high tear film levels were obtained. Other in vivo studies on ciprofloxacin concentrations in human and rabbit tears after application of an eye drop, showed similar tear concentrations of ciprofloxacin to those in the present study.^{20,22,23} The results of the present study are in agreement with the results obtained with ocular drug delivery systems, using gentamicin and fluorescein in an insert or

TABLE 3. Evaluation of the Acceptability of the Preparations at Different time Intervals

	Ocular Irritation (General)	Ocular Irritation (Puncti)	Vision	Lacrimation
Eye drop				
30 min	3.00 (4.83)	3.42 (5.22)	2.28 (4.78)	1.00 (1.52)
60 min	1.42 (1.25)	1.00 (1.49)	0.71 (1.49)	0.57 (1.21)
300 min	0.42 (0.79)	0.28 (0.76)	0.29 (0.76)*	0.29 (0.76)
480 min	—	—	—	—
Minitabket				
30 min	4.42 (5.71)	3.00 (4.35)	1.42 (1.99)	5.29 (9.36)
60 min	5.00 (9.02)	1.86 (1.95)	4.86 (4.18)	1.57 (2.15)
300 min	4.71 (8.69)	1.14 (1.68)	23.86 (19.16)*	3.14 (3.76)
480 min	2.42 (2.99)	1.71 (2.36)	14.57 (17.22)	2.14 (3.67)

Scores from 0 (acceptable) to 100 (unacceptable) mm.

* $P < 0.05$, unpaired t -test ($n = 6$, mean \pm SD).

sodium fluorescein in a minitab. ^{14,24,25} The minimum inhibitory concentrations (MIC₉₀) for ciprofloxacin against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* are 1 µg/g. Higher concentrations of ciprofloxacin measured in the tear film than these MICs were obtained after the applications of both formulations. However, a more important clinical parameter is the time-kill determination. Speciale et al. ²⁶ reported that in the case of moxifloxacin, that the times to 3 log reduction at 1× and 4× MIC for *Staphylococcus aureus* were 12 hours and 4 hours, respectively. ²⁶ Thus, the combination of high concentrations and a long presence of the antibacterial agent in the tear film resulted in the efficient killing of the ocular pathogens. Both conditions were obtained after application of the ocular minitab. To obtain similar ciprofloxacin concentrations in the tear film during 8 hours, Ciloxan eye drops (Alcon) must be instilled at least every 30 minutes. The *in vivo* study demonstrated that the sterilized ocular minitables containing ciprofloxacin can be considered as a promising new erodible drug delivery system to treat ulcerative bacterial keratitis, as it offers sustained drug release in the tear film for a prolonged period. Thus, the administration of one minitab in the fornix of the infected eye in the evening could avoid the frequent instillation of eye drops at night. This increases greatly the comfort of patient and care provider, although there is still a minor irritation issue associated with its use.

No preservatives were present in the bioadhesive formulation tested, which is an important advantage, as preservatives can have adverse effects on corneal wound healing. Studies are in progress to verify whether other antimicrobials can be incorporated into this ocular bioadhesive formulation for the treatment of viral, fungal, and other microbial ocular diseases.

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