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# Solubility characteristics of three fluoroquinolone ophthalmic solutions in an in vitro tear model

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**Abstract**

The solubility characteristics of three commercially available fluoroquinolone ophthalmic solutions CILOXAN™ (Ciprofloxacin 0.3%), CHIBROXIN™ (Norfloxacin 0.3%) and OCUFLOX™ (Ofloxacin 0.3%) were evaluated using an in vitro tear turnover model. The model was used to simulate the effects of ocular dosing of each product on the temporal changes in tear pH and drug concentration in relation to the saturation solubility of the drug in the tear film and the possible formation of precipitates. Results showed that tear pH immediately following fluoroquinolone dosing was dominated by formulation pH with CILOXAN™ decreasing tear pH to 4.7 (formulation pH 4.5), CHIBROXIN™ to pH 5.3 (formulation pH 5.2) and OCUFLOX™ to pH 6.4 (formulation pH 6.4). Tear pH normalized to baseline (pH > 6.8) within 15 min for all formulations due to tear turnover and drainage. Following CILOXAN™ dosing, rapid precipitation of ciprofloxacin was observed in the model beginning at 8 min post-dose (tear pH 6.1) producing turbidity and a significant decline in soluble drug concentration. Precipitation was quantitatively shown to be driven by ciprofloxacin supersaturation in tears. The tear drug concentrations of ofloxacin and norfloxacin remained below solubility at all pH values so that precipitation was neither observed nor predicted in the model. Analysis of ciprofloxacin precipitates by dark field microscopy with image analysis revealed polydisperse crystalline needles of 183 $\mu$m average length (SD = 54 $\mu$m). These findings highlight the impact formulation properties can have on physicochemical changes in the tear film following ocular drug dosing and explain in part the reported clinical occurrences of precorneal deposits associated with the use of CILOXAN™. 

*Keywords:* Fluoroquinolones; Solubility; Corneal deposits; Tear film; Precipitation; Tear model

## 1. Introduction

Ophthalmic formulations of several new potent fluoroquinolone antibiotics including norfloxacin,

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Table 1
Formulation comparison of the commercial fluoroquinolone ophthalmic solutions

| Antibiotic | Ofloxacin 0.3% solution | Ciprofloxacin HCl 0.3% solution | Norfloxacin 0.3% solution |
|---|---|---|---|
| Trade name | OCUFLOX™ (Allergan) | CILOXAN™ (Alcon) | CHIBROXIN™ (Merck) |
| Fluoroquinolone concentration | 0.3% | 0.3% | 0.3% |
| Preservative | BAK 50 ppm | BAK 60 ppm<br>EDTA 0.05% | BAK 25 ppm<br>EDTA |
| Buffer | None | Sodium acetate | Sodium acetate |
| Other | Sodium chloride 0.9%<br>Purified water | Mannitol 4.6%<br>Purified water | Sodium chloride 0.9%<br>Purified water |
| pH | 6.4 | 4.5 | 5.2 |
| Osmolality (mOsm/kg) | 300 | 300 | 285 |

ciprofloxacin and ofloxacin have become available for the treatment of external ocular infection and, more recently, for the treatment of corneal ulcer. As shown in Table 1, these products are similar in that they are all formulated as sterile, isotonic, aqueous solutions, preserved with benzalkonium chloride and contain 0.3% of the respective fluoroquinolone drug. Differences between the formulations are noted in terms of the pH (CILOXAN™ most acidic and OCUFLOX™ least acidic) and the presence of buffering agents in the CILOXAN™ and CHIBROXIN™ formulations.

Crystalline corneal deposits have been reported with the clinical use of CILOXAN™ solution (Leibowitz, 1991; Parks et al., 1993) and ointment (Wilhelmus et al. 1993) with an incidence from 13 (Wilhelmus et al. 1993) to 42% (Parks et al., 1993). Previously published reports of ciprofloxacin corneal deposits did not provide a mechanism for their occurrence. It was unclear why drug precipitation has not been reported for the other commonly used ophthalmic fluoroquinolones such as norfloxacin and ofloxacin. It is known that ciprofloxacin is the least soluble of the commercially available fluoroquinolones, with particularly low solubility near the pH of tears ( $\sim$ pH 7) (Ross and Riley, 1990). The formation of such deposits indicates a decrease in the concentration of drug dissolved in the tear film and, consequently, a potential decrease in drug bioavailability. Additionally, the presence of deposits in the tear film creates safety concerns.

In the present study, an in vitro tear-turnover model was developed and used to simulate the dynamics of ocular tear turnover and drainage. The model was used to study the temporal changes in tear pH and drug concentration (both total and soluble drug concentration) following the instillation of commercially available CILOXAN™ (Ciprofloxacin 0.3%), CHIBROXIN™ (Norfloxacin 0.3%) and OCUFLOX™ (Ofloxacin 0.3%) ophthalmic solutions. The pH-solubility profile of each drug substance was determined in separate experiments in simulated tears which allowed the correlation of the pH-concentration profiles of each drug in the tear-turnover model with the solubility limits under the same conditions.

## 2. Materials and methods

### 2.1. Materials

Sodium phosphate monobasic monohydrate (JT Baker, USP grade), sodium chloride (Mallinckrodt, USP grade), 1 N NaOH (Mallinckrodt, NF grade), 0.05–1 N HCl (JT Baker, NF grade), 5 N HCl (JT Baker, NF grade), dodecyl sodium sulfate (MCB Reagents, reagent grade), glacial acetic acid (Mallinckrodt, USP grade), acetonitrile (American Burdick and Jackson, HPLC grade), ofloxacin (Daiichi), ciprofloxacin HCl (Sigma), norfloxacin (Sigma) were used without further purification. Purified water was sourced from a Milli-Q Water System

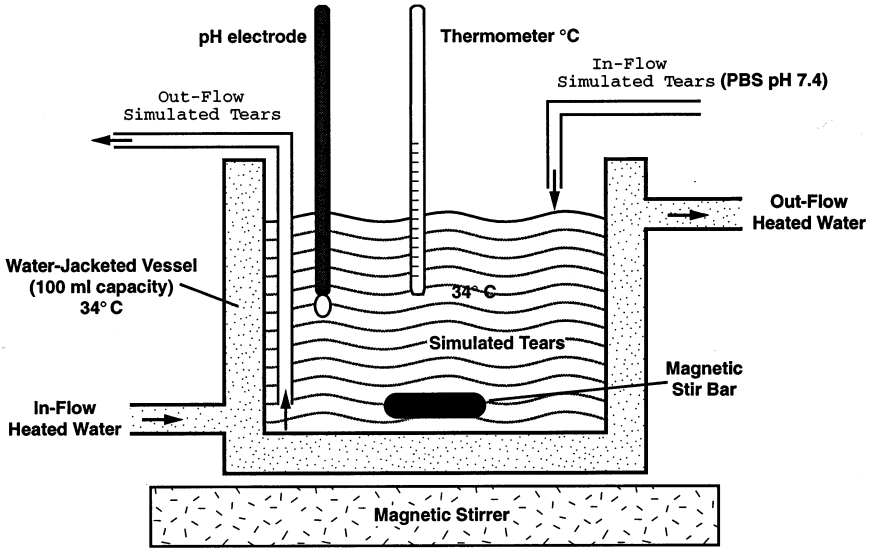


Fig. 1. Schematic diagram of the in vitro tear turnover model.

(Millipore). The drug products CILOXAN™ (ciprofloxacin HCl 0.3% sterile ophthalmic solution, Alcon Laboratories) and CHIBROXIN™ (norfloxacin 0.3% sterile ophthalmic solution, Merck) were sourced from commercial suppliers. OCUFLOX™ (ofloxacin 0.3% sterile ophthalmic solution, Allergan) was sourced from Allergan.

### 2.2. Model tear turnover studies

#### 2.2.1. Model tear system

The pH and drug concentration dynamics in the tear film following a single drop instillation of a commercial ophthalmic drug product was simulated in an in vitro tear turnover model. A schematic diagram of the model is shown in Fig. 1. The system was comprised of a well-stirred, jacketed Pyrex cell of 100 ml capacity. To create the dynamics of tear turnover and drainage, the model cell was provided with: (1) constant rate inflow of fresh tears constituting 5 mM phosphate buffered saline (pH 7.4, 300 mOsm/kg) to simulate tear production, and (2) apparent first-order outflow from the cell to simulate tear drainage. The inflow and outflow of tears was provided by a constant rate positive displacement pump and a variable speed, peristaltic pump, respectively.

The model incorporated an apparent first-order drainage rate in accordance literature values (Chrai et al., 1973). To achieve apparent first-order drainage, the outflow drainage rate (ml/min) was varied proportionately to the instantaneous volume present in the cell. The outflow drainage rate was, therefore, highest, immediately following drug instillation (when cell volume was greatest) and progressively diminished thereafter as total cell volume declined to baseline and net tear drainage rate returned to steady-state (inflow equals outflow).

The cell was temperature-controlled at 34°C with a water jacket to maintain the temperature of the human tear film (Morgan et al., 1995). Well-stirred conditions within the cell were maintained via a magnetic stir bar. Following instillation of drug product to the cell, the following parameters were followed as a function of time: cell pH (Beckman Φ10 pH meter), total drug concentration (HPLC), soluble drug concentration (HPLC after filtration) and presence of crystalline precipitates as determined visually and by light microscopy.

#### 2.2.2. Choice of tear solution

Human tears are well known to be complex, approximately isotonic solutions containing

proteins, lipids, electrolytes and other components with pH values in the range 5.2–8.6 with pH 6.8–7.5 the most typical (Abelson et al., 1981). The principle buffering agent in human tears is considered to be the bicarbonate system with additional contributions from histidine and other ionizable residues on tear proteins (Carney et al., 1989). These components provide buffering capacity in the pH range 7.0–7.7. Bicarbonate systems are, however, unstable over time when used in open systems due to outgasing of carbon dioxide and, for this reason, are not readily utilized for laboratory studies of the present kind. The simulated tear solution used in the present study was 5 mM phoshate buffered saline (pH 7.4, 300 mOsm/kg) which was chosen because of its pH stability, analytical non-interference and relationship to the human tears (pH, tonicity, buffering range and buffer capacity). Furthermore, it was desired to keep the simulated tear solution simple (i.e. lipids and protein components were excluded) so as to prevent complications from possible drug-protein binding and analytical interference.

*2.2.3. Model parameters and scale-up*

To simulate the turnover and drainage dynamics of the tear film, published values for tear film volume, tear production rate and measured drug product drop volumes were scaled-up for the model tear system as listed in Table 2. Due to the fact that the measured drop volumes of the commercial drug products were in the range 35–45 $\mu$l, first-order drainage rate constants of 0.60–0.65 $min^{-1}$ were utilized in accordance with Chrai (Chrai et al., 1973). These rate constants were implemented in the model as a stepwise decrease in outflow rate to achieve a tear volume half-life of approximately 1 min (i.e. tear volume was reduced by approximately 50% during each minute interval due to net drainage) until the basal tear film volume was reestablished.

Table 2
Parameters used for the tear turnover model

| Parameter | Human tear film ($\mu$l) | Model system[a] (ml) |
|---|---|---|
| Tear film volume | 7 | 10 |
| Drug product drop volume | | |
| Ofloxacin 0.3% | 45 | 64 |
| Norfloxacin 0.3% | 38 | 54 |
| Ciprofloxacin HCl 0.3% | 35 | 50 |
| Tear production (inflow) rate | 1 $\mu$l/min | 1.4 ml/min |

[a] Model system parameters scaled-up by a factor of approximately 1430 from the human tear film.

*2.2.4. Tear turnover experiments*

Each experiment was set-up by transferring 10 ml of simulated tears to the cell which represented the basal tear film volume. Magnetic mixing was initiated and the tear temperature was equilibrated to 34°C by continuous warm water circulation through the cell jacket. The inflow rate of fresh simulated tears was initiated at 1.4 ml/min and was left at this rate for the duration of the experiment. Simultaneously, the outflow rate from the cell was initiated at 1.4 ml/min. This set-up prior to drug product instillation represented the steady-state tear turnover model.

At time representing $t = 0$ of the experiment, a volume of one of the commercial fluoroquinolone ophthalmic drug products was rapidly instilled into the cell (see Table 2 for product volumes). The outflow rate was then immediately increased and then decreased in a step-wise fashion according to the product specific profiles listed in Table 3 to simulate apparent first-order drainage. The outflow profiles listed in Table 3 are product specific because of the different drop volumes.

At designated time-points following instillation, the cell pH was recorded and, simultaneously, two aliquots were removed from the cell: (1) a 100 $\mu$l aliquot was diluted for HPLC analysis for total drug content, and (2) a 300 $\mu$l aliquot which was immediately filtered (Gelman LC-13, 0.2 $\mu$, PVDF) and 100 $\mu$l of the filtrate was diluted for HPLC analysis for soluble drug content. For any experiment, the cumulative aliquot volume removed from the cell represented 4% or less of the total cell volume. Experiments were performed in duplicate for each drug product.

Table 3
Outflow rates used to simulate first-order drainage

| Drug product | Time interval after instillation (min) | Outflow rate (ml/min) | Net outflow rate (outflow-inflow[a], ml/min) | Volume remaining at end of time interval (ml) |
|---|---|---|---|---|
| Ofloxacin 0.3% | 0–1 | 35.2 | 33.8 | 40.2 |
| | 1–2 | 17.6 | 16.2 | 24.0 |
| | 2–3 | 15.3 | 13.9 | 10.1 |
| | ≥3 | 1.4 | 0 | ~10 |
| Norfloxacin 0.3% | 0–1 | 30.7 | 29.3 | 34.7 |
| | 1–2 | 15.3 | 13.9 | 20.8 |
| | 2–3 | 12.2 | 10.8 | 10.0 |
| | ≥3 | 1.4 | 0 | ~10 |
| Ciprofloxacin HCl 0.3% | 0–1 | 30.7 | 29.3 | 30.7 |
| | 1–2 | 15.3 | 13.9 | 16.8 |
| | 2–3 | 8.2 | 6.8 | 10.0 |
| | ≥3 | 1.4 | 0 | ~10 |

[a] Inflow rate was maintained at 1.4 ml/min (see Table 2).

*2.2.5. HPLC analysis*

Samples from both the solubility and the model tear turnover studies were analyzed for drug content by isocratic, reversed phase HPLC using UV detection. A Beckman HPLC system equipped with a model 116 pump, a model 506 autosampler and a model 166 UV detector set at 277 nm was utilized. Data acquisition and integration were performed using Beckman System Gold software. The samples were diluted with 0.05 N HCl and 20 $\mu$l were injected onto a Beckman Ultrasphere™ ODS column (4.6 × 250 mm, 5 $\mu$ particle size). The samples were eluted with a mobile phase composed of 5 mM sodium dodecyl sulfate/glacial acetic acid/acetonitrile (58:2:40 by volume) at a flow rate of 1.5 ml/min. Retention times for the three fluoroquinolones were 7.4 min for ciprofloxacin, 7.0 min for norfloxacin and 7.3 min for ofloxacin. No degradation peaks were observed in the chromatograms of any compound for either solubility or tear turnover study samples indicting excellent chemical stability under the present conditions.

*2.2.6. Microscopy*

Crystalline precipitates from the cell were isolated and analyzed using an Olympus SZH stereomicroscope (magnification 64 ×) equipped with dark field contrast. Images were captured and analyzed with a color CCD video camera attached to the microscope and a Videometric 150 image analysis system (Oncor Instrument Systems).

*2.3. Solubility studies*

The equilibrium solubilities of the three fluoroquinolone drug substances (ciprofloxacin HCl, norfloxacin and ofloxacin) were each determined at 34°C in 5 mM phosphate buffered saline over a range of pH values. The tonicity of the buffer solutions was adjusted to approximately 300 mOsm/kg with the addition of sodium chloride. The pH ranges used for the solubility studies were chosen to cover the pH span observed in the model tear turnover experiments (see below) for each drug product. The solubility study pH ranges were: pH 4.5–7.2 for ciprofloxacin HCl, pH 5.0–7.2 for norfloxacin and pH 6.4–7.2 for ofloxacin.

Solubility samples were prepared by adding an excess amount of drug to a 4 ml capacity amber glass vial containing 2 ml of the buffer of interest. The pH of each solution was adjusted, if necessary, to the target value with 5 N NaOH or 1–5 N HCl and the vials were sealed with a septum-lined screw cap. The samples were then placed on a rotating rack and stored in a light occluded temperature controlled oven at 34°C for up to 3

days. Each sample was visually checked periodically to ensure the presence of excess drug. After 3 days, the samples were removed from the oven, immediately filtered through a 0.2 $\mu$ membrane (Gelman LC-13, PVDF) and the filtrate was collected after discarding the initial 2 drops. An aliquot of the filtrate was then taken for HPLC assay and the remaining filtrate was tested for pH. Solubility was determined in duplicate for each drug at each pH.

## 3. Results

### 3.1. Temporal changes in model tear pH

Tear pH profiles following the addition of each drug product are shown in Fig. 2 as functions of time after dosing. Tear pH in all cases immediately decreased to a value dictated by the drug formulation pH. CILOXAN™ decreased tear pH to 4.7 (formulation pH 4.5), CHIBROXIN™ to pH 5.3 (formulation pH 5.2) and OCUFLOX™ to pH 6.4 (formulation pH 6.4). Tear pH normalized to baseline (pH > 6.8) within 15 min for all formulations due to tear turnover and drainage.

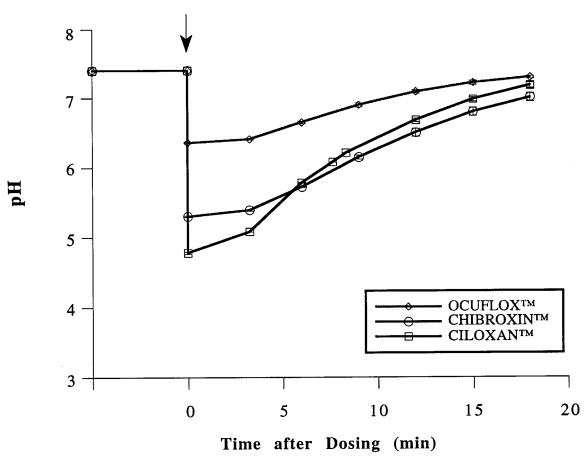


Fig. 2. pH-time profiles following addition of OCUFLOX™, CHIBROXIN™ or CILOXAN™ to the tear turnover model. The arrow indicates point of dosing of each drug product to the model. Data are mean values with error bars representing $\pm$ S.D. ($n = 2$).

### 3.2. Temporal changes in model tear drug concentration

Model tear drug concentration profiles (both soluble and total drug) are shown in Fig. 3 for the three fluoroquinolone drug products as functions of time after dosing. Samples taken for drug assays coincided with tear pH measurements (see above) so that an instantaneous drug concentration could be associated with a known tear pH. The resulting total drug concentrations immediately following drug product addition (approximately 2.5 mg/ml) reflected concentrations in the drug product (0.3% or 3 mg/ml) diluted slightly by the presence of tears in the cell prior to dosing. In all cases, the total drug concentration in the tears continuously decreased with time (half-life 6–8 min) as a result of tear turnover and drainage.

The soluble drug concentration, estimated by assaying a filtered aliquot of tears taken at a given time-point, reflects the concentration of drug fully dissolved in the tears at that time.

Following dosing of OCUFLOX™ and CHIBROXIN™ (Fig. 3A,B), the soluble drug concentration was identical (within experimental error) to the total drug concentration at all time points and the model tear solution remained clear and particulate-free as assessed visually throughout the full course of the experiments. Following CILOXAN™ dosing (Fig. 3C), however, the soluble drug concentration fell significantly below the total drug concentration at approximately 8 min after drug addition (tear pH 6.1) at which point the tear solution became turbid and within minutes appeared milky white indicative of a concentrated precipitate. At approximately 12 min post-dose (tear pH 6.6), the soluble ciprofloxacin concentration was reduced to almost half (54%) of the total drug concentration due to continued precipitation. Analysis of isolated ciprofloxacin precipitates by dark field microscopy (Fig. 4) revealed white polydisperse crystalline needles approximately 183 $\mu$m in average length (S.D. = 54 $\mu$m) as determined by image analysis.

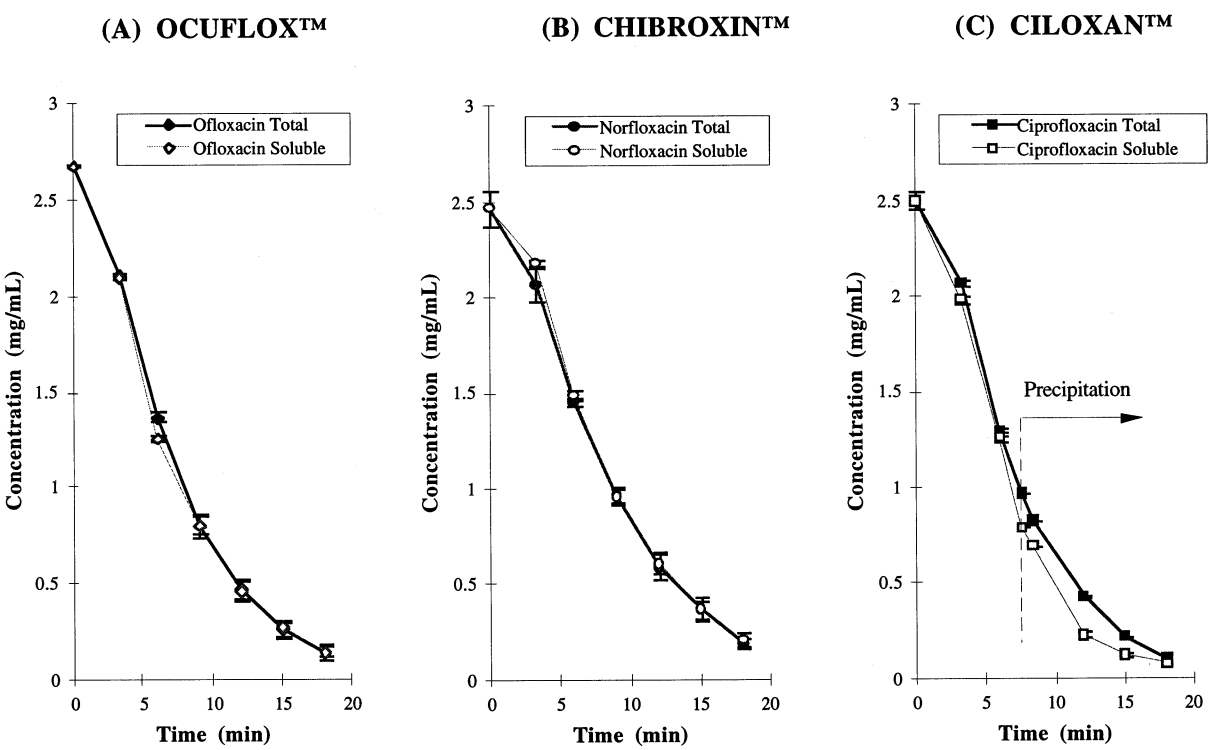


Fig. 3. Timecourse of total (heavy lines) and soluble (light lines) drug concentrations following dosing of (A) OCUFLOX™, (B) CHIBROXIN™ and (C) CILOXAN™ to the tear turnover model. Data are mean values with error bars representing ±S.D. ($n = 2$). Note the onset of precipitation for CILOXAN™ at 7.6 min.

### 3.3. Fluoroquinolone pH-solubility profiles

The pH-solubility profile for each of the three fluoroquinolones drug substances was independently determined at 34°C in 5 mM PBS over the same pH range observed for each individual drug product in the tear model. The resulting pH-solubility profiles, shown in Fig. 5, are typical of the fluoroquinolones previously reported (Ross and Riley, 1990). Solubility of this class of drugs generally exhibits a minimum near physiologic pH corresponding to the zwitterionic form of the drug.

## 4. Discussion

The physical stability of a drug in the tear film exhibiting pH-dependent solubility will depend on the temporal relation between drug concentration and tear film pH following drug instillation. These, in turn, are affected by formulation properties (drug concentration, pH, buffer capacity and drop volume), drug properties (pH-solubility profile) and by the dynamics of tear turnover and drainage. The in vitro model utilized in this work, although producing half lives (for pH and drug concentration normalization) slightly longer than might be expected in vivo (Coles and Jaros, 1984), provides a systematic method of comparing the physical disposition of ophthalmic drugs.

Despite pH-dependent solubility typical of the fluoroquinolones, both norfloxacin and ofloxacin remained fully solubilized in the model tear fluid, following dosing of CHIBROXIN™ and OCUFLOX™, respectively, even at pH values approaching the solubility minimum ( ~ pH 7). This is quantitatively demonstrated by superimposing the pH-solubility profiles for norfloxacin and ofloxacin on their drug concentration-pH profiles

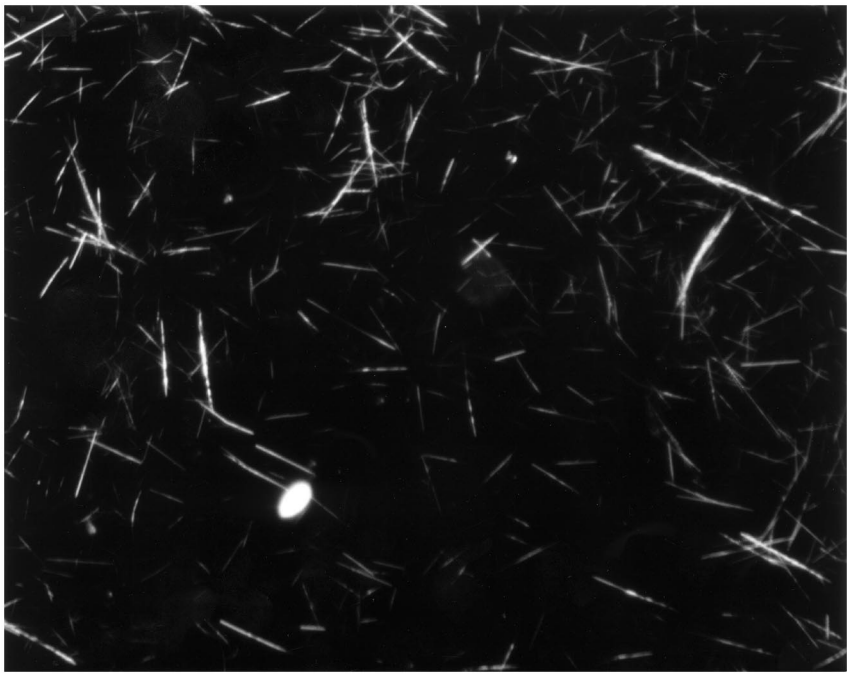

Fig. 4. Dark field contrast photomicrograph of ciprofloxacin crystalline precipitates isolated from the in vitro model following dosing of CILOXAN™ (magnification 64 ×).

determined from the tear turnover model as shown in Fig. 6A,B, respectively. These figures show that drug concentrations for norfloxacin and ofloxacin in the tears remain below their respective solubility limits at all pH values, and, therefore, no drug precipitation was expected nor observed.

Fig. 6C shows the pH-solubility profile superimposed on the drug concentration-pH profile for ciprofloxacin following dosing of CILOXAN™. While the ciprofloxacin concentration immediately after dosing is below the solubility limit at the initial pH (i.e. pH 4.7), the drug concentration in the tears soon thereafter 'crosses over' and exceeds the solubility limit (pH 6.1) as normalization of tear pH due to tear turnover occurs. This produces a supersaturated solution of ciprofloxacin in the tears which drives precipitation, development of turbidity and the decline of the soluble ciprofloxacin tear concentration.

The results of the present study are in good agreement with clinical reports of the use of ophthalmic fluoroquinolone solutions and provide a physicochemical basis (supersaturation) for the observed physical instability of ciprofloxacin in the tear film (Leibowitz, 1991; Parks et al., 1993). A recent study (Essepian et al., 1995) used scanning confocal microscopy to examine ciprofloxacin crystals that formed following instillation of the drug in human and rabbit eyes with corneal defects. In all cases, the crystals primarily formed in the area of the primary corneal defect. It has been suggested that crystalline deposits of ciprofloxacin may interfere with corneal wound healing (Kanellopoulos et al., 1994).

## 5. Conclusions

The solubility characteristics of three commercially available fluoroquinolone ophthalmic solutions (ciprofloxacin, norfloxacin and ofloxacin) have been evaluated in an in vitro tear-turnover model. Comparison of drug concentration/pH data from the model with the pH-solubility profiles of the respective drugs has provided a means

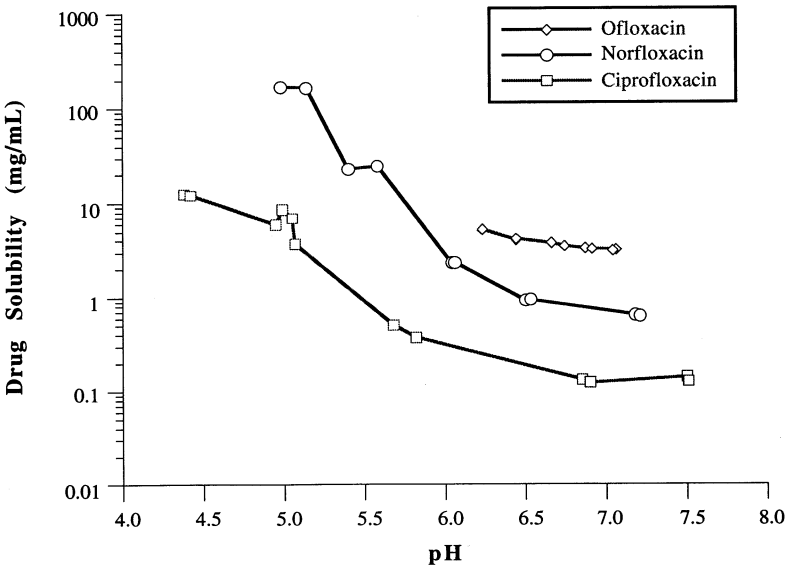


Fig. 5. pH-solubility profiles of ofloxacin, norfloxacin and ciprofloxacin-HCl in 5 mM PBS at 34°C over the pH ranges observed in the tear turnover model for each drug.

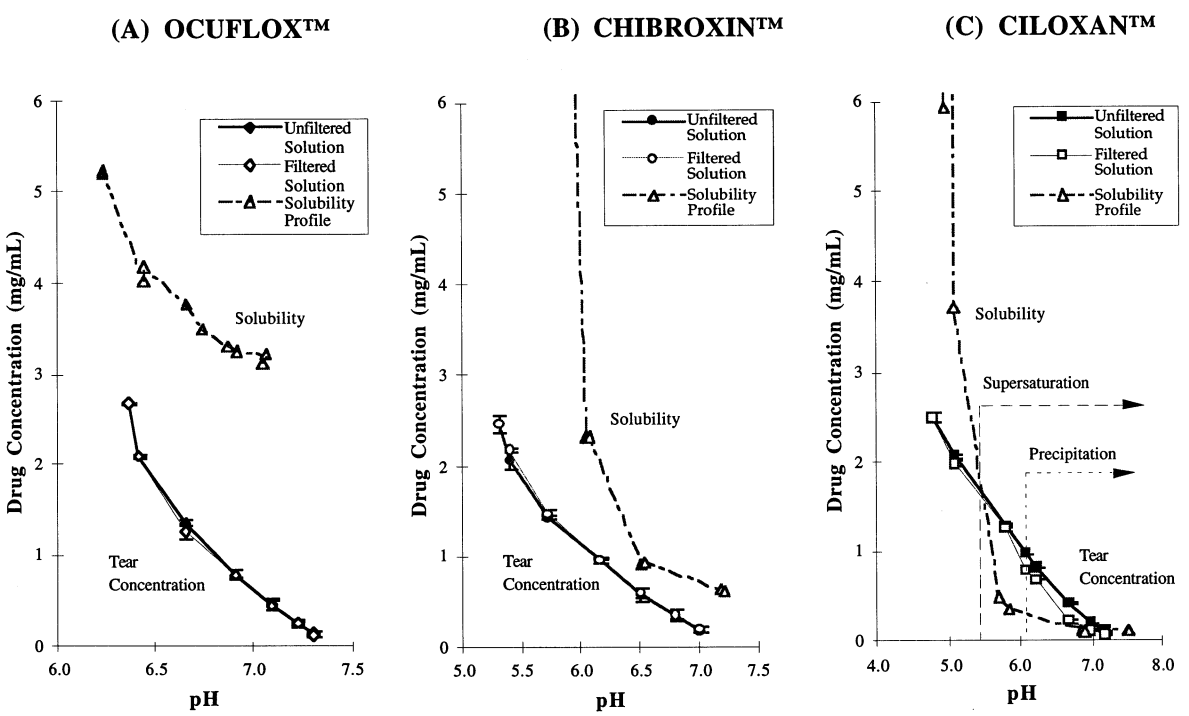


Fig. 6. Relationship between drug concentration in the tear turnover model (solid lines) and drug solubility limits (dashed lines) for (A) OCUFLOX™, (B) CHIBROXIN™ and (C) CILOXAN™. Data for tear concentration are mean values with error bars representing $\pm$S.D. ($n = 2$).

to predict the physical stability of the drugs in the tears. The precipitation of ciprofloxacin has been quantitatively demonstrated to be driven by supersaturation as tear pH and drug concentration normalize following ocular instillation. Drug concentrations for norfloxacin and ofloxacin in the tears remain below their respective solubility limits at all pH values and, therefore, no drug precipitation was expected nor observed. The effects of the demonstrated physical instability of ciprofloxacin in the tears on bioavailability, efficacy and safety warrants further evaluation.

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