International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.3,pp 1750-1763, July-Sept 2011

Design and Evaluation of Ciprofloxacin Hydrochloride Ocular Inserts

Mohamed A. Attia*, Mohamed Al-Azizi and Mohamed S. Hashish

Pharmaceutical Technology Dept., Faculty of Pharmacy and Biotechnology, German University in Cairo, Egypt.

Corres.author: mohamed.shafie@guc.edu.eg

Abstract : Ocular Conjunctivitis is one of the main causes of red eye syndrome. The present work focuses on the treatment of ocular conjunctivitis by using combined mechanisms:
1. Formulation of ocular inserts to provide prolonged and sustained release system of the drug.
2. Use of therapeutic agent, as ciprofloxacin hydrochloride in combination with the polymers used. The selected polymers were methylcellulose (MC), hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), and Eudragit RS100 (ERS 100). The developed ocular inserts were evaluated for physic-chemical, mechanical, drug release, drug permeability, and In-vivo characteristics. The ocular inserts showed desired delivery of the drug to the ocular tissue of the rabbit's eye. In-vivo studies showed that ciprofloxacin hydrochloride had a significant effect on reduction of induced ocular conjunctivitis.
Keywords: Ocular inserts, mechanical properties, release study, permeation, In-vivo study.

Introduction:

Continuous delivery of drugs to the eye offers major advantages over conventional therapies that involve administration of drug solutions suspensions as eye drops

Ophthalmic inserts offer many advantages over conventional dosage forms like increased ocular residence time, possibility of releasing drugs at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf-life[1-3].

Moreover, the use of these devices reduces systemic absorption, which otherwise freely occurs with eye drops. It also ensures better patient compliance due to lower frequency of administration and lower incidence of side effects[4-6].

Soluble bioadhesive ophthalmic drug inserts was developed for the treatment of external ocular diseases such as conjunctivitis, keratoconjunctivitis sicca and superficial corneal ulcers[7].

The broad spectrum activity of fluoroquinolones allow their use in a variety of infections including those affecting the respiratory tract, urinary tract, skin, soft tissues and the eyes.

Studies directly comparing the efficacy of the fluoroquinolones are sparse. However, studies in other literature[8-11] have shown that fluoroquinolones antibiotic group were more effective in the treatment of ocular infections than some other broad spectrum antibiotics for example, Gentamycin, Chloramphenicol, Tobramycin, Erythromycin and Tetracycline.

Staphylococcus aureus is the most causative micro-organism implicated in bacterial conjunctivitis[12]. The micro-organism was most sensitive to ciprofloxacin, followed by ofloxacin, and then norfloxacin. Thus ciprofloxacin is the most effective of the three drugs and it is therefore recommended as the best choice

topical fluoroquinolone antibiotic for the treatment of bacterial conjunctivitis[13].

Materials:

Ciprofloxacin hydrochloride was a gift from Unipharm, Egypt. Hydroxypropylcellulose MF (HPC) was purchased from Kolmar, California, USA. Methylcellulose (MH20) (MC) was obtained from Sanofi-Aventis, Paris, France. Triethyl citrate was obtained from Merck&Co. Hydroxypropylmethylcellulose E4M PREM was purchased from Dow chemical company, USA. Eudragit RS100 PM was obtained from Rohm and Haas G.m.b.H Pharma Darmstadt, Germany. All other materials were of analytical grade.

Preparation of ocular inserts:

MC (5% w/v), HPC (5% w/v) solutions dissolved in water were mixed together in a predetermined ratio and stirred continuously until a clear solution was obtained as shown in formulation composition (**Table 1**). Glycerin or triethyl citrate (5, 10 and 20 % on dry basis as plasticizer) was mixed uniformly to obtain a clear viscous, air bubble free liquid. Eudragit RS100 (5% w/v) was dissolved in a mixture of 70 ml acetone and 30 ml isopropyl alcohol. A predetermined ratio of methylcellulose solution and Eudragit RS100 solution blend were prepared. The solutions were degassed by placing it in a dessicator attached with vacuum pump until a clear, bubble free solutions were obtained. Each formula was prepared by pouring 15 ml of the formula into Polytetrafluorethylene (PTFE) molds, and it was left for an hour to stabilize, then the films were dried at 45°C for 24 hours. The dried films were carefully removed from the mold (41.78 cm^2), checked for any imperfection or air bubbles and punched into circular discs (0.785 cm^2). For the medicated inserts, calculated amount of drug (0.3%) was incorporated in the polymeric solution before the addition of the plasticizer, and then casting was performed the same was as mentioned earlier.

The films were subjected to the following evaluation:

1. Mechanical properties: - Assessment of tensile strength, percentage elongation; strain by means of a tensile-testing machine (Zwick / Roell Z100, Ulm, Germany).
2. Determination of the film thickness by measuring the thickness of the film at five random points using digital micrometer (Kraftixx, Bremen, Germany).
3. Weight variation by weighing five inserts individually using digital balance.
4. Moisture absorption percentage by weighing three inserts individually out of each formulation and then place it in dessicators, which maintained high relative humidity (RH) at about 75±5% RH using excess amount of sodium chloride solution[14], after 3 days the inserts were taken out and reweighed. The percentage moisture absorption was calculated using equation -1 :

$$\% \text{ Moisture Absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100 \quad \ldots\ldots.(1)$$

Table 1: Formulation Composition (%)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Ciprofloxacin HCl	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Methylcellulose	5	5	5	5	5	5	-	-	-	-	-	-	-	-
MC 5% / HPC 5%	-	-	-	-	-	-	1:1	1:2	3:1	2:1	3:1	-	-	-
HPMC	-	-	-	-	-	-	-	-	-	-	-	10	-	-
MC 5% / ERS100 5%	-	-	-	-	-	-	-	-	-	-	-	-	1:1	3:1
Glycerin	5	10	20	-	-	-	10	10	10	-	-	10	-	-
Triethyl citrate	-	-	-	5	10	20	-	-	-	10	10	-	20	20

*MC: Methylcellulose, *HPC: Hydroxyproprlcellulose, *HPMC: Hydroxypropylmethylcellulose
*ERS100: Eudragit RS100

5. Drug content to check the uniformity of the drug in the circular inserts, five inserts were taken out from each film. Each insert was placed in a glass vial containing 5 ml of simulated tear fluid (STF). The inserts were dissolved by the aid of a magnetic stirrer, the solution was then filtered through filter membrane of 0.45 µm. 1 ml from the filtrate was withdrawn and assayed spectrophotometrically after suitable dilution at 275 nm [(15)].
6. Folding endurance was determined by repeatedly folding the film at the same point until it broke. The number of times the film could be folded at the same point without breaking or cracking gave the value of the folding endurance.
7. Drug-polymer interaction, FTIR spectra for the medicated and non medicated inserts using Nicolet Avatar 380 spectrometer.
8. In-vitro drug release studies. The n-vitro drug release studies were carried out using cellophane membrane (MWCO 30/32). The insert was placed on the cellophane membrane and 0.7 ml STF was added to it. The entire surface of the membrane was in contact with the receptor compartment containing 10 ml of STF in 50 ml beaker. The content of the receptor compartment was stirred continuously using magnetic stirrer and its temperature was maintained at 37°C ± 0.5°C. at certain time intervals, 1 ml of the solution in the receptor compartment was withdrawn and replaced with 1 ml of fresh STF. The withdrawn sample was suitably diluted with distilled water, and then it was analyzed using UV spectrophotometer at 275 nm against blank, which was prepared using the same procedure but with non medicated insert.
9. Permeability study to measure the corneal permeability, an apparatus was adopted from an in-vitro system previously developed[(16)]. The glass diffusion cells were constructed from 50 ml Erlenmeyer flasks. Then of the 5 cm sidearm projection on each half-cell it has a ground finish opening. The cross-sectional surface area of this opening was 0.442 cm^2. Rabbits were sacrificed with an overdose of Phenobarbital. The entire globe was surgically removed intact from the animal's eye-socket. The remaining intraocular parts exposing the corneal endothelium were removed. The cornea was positioned on the donor half-cell such that the epithelial surface was centered in the sidearm opening. The receptor, half-cell was positioned symmetrically with respect to the donor cell facing the endothelial surface. After the cornea was securely mounted, 20 ml of isotonic phosphate buffer solution pH 7.4 was added to the receptor cell. Likewise, the ocular insert (F12) was placed in an isotonic phosphate buffer solution pH 7.4 in the donor half-cell. The entire apparatus was placed in a water bath thermostated at 37°C. Aliquots of 1 ml were withdrawn from the receptor solution at 0.5,1,2,3,4,5,6,12 and 24 hours. Each sample was replaced in the receptor solution with 1 ml buffer solution. Samples were analyzed spectrophotometrically at 275 nm. The same procedure was repeated using different ocular inserts (F13 and F14).
10. In-vivo study. The study was conducted in accordance with the ethical procedures and policies approved by animal care and use committee of faculty of pharmacy German University in Cairo. A clinical isolate of *Pseudomonas aeruginosa* was obtained from the central laboratory of the ministry of health, Egypt. Male albino rabbits of 1800-2000 g body weight were randomly divided into five groups, each group composed of three rabbits as shown in (**Table 2**). After one week of acclimatization, experimental *Ps. Aeruginosa* conjunctivitis was achieved by the intraconjunctival injection of 10 µl of tryptic soy broth containing $1.5 \times 10^2 \pm 120$ CFU of *Ps. aeruginosa* via a 30-gauge needle attached to a 100 µl syringe. The bacteria were grown to the early log phase in tryptic soy broth in a shaker water bath at 37°C and the final inoculums size was adjusted. Portions of the suspension were plated in triplicate onto tryptic soy agar, and the number of bacteria in the inoculums was determined. Treatment was initiated 24 hours after the induction of infection (Fabricated insert was placed carefully and gently in the lower cul-de-sac of each eye); sterile swabs were used to take samples from the eyes of the animals on daily basis, the swabs were streaked onto the surface of tryptic soy agar plates. The plates were incubated at 37°C for 24 hours and the growing colonies were counted by using viable count technique. The group 1 serve as a control for formulations F13 and F14 (insert without drug was inserted).

    Visual observation was performed on daily basis for 8 days. The level of anterior segment inflammation was evaluated on a scale of 0-3 (0=normal; 3=worst) according to Peyman scale[(17)].

Peyman Scale

Conjunctiva

0= Normal
1= Mild oedema
2= Oedema, mild hyperaemia, slight exudates
3= Oedema, marked hyperaemia, heavy exudates

Cornea
0= Clear
1= Focal oedema
2= Diffuse oedema
3= Opaque

Table 2: Different Animal Groups Used in the *in vivo* Study.

Group #	Infected	Treated	Formulation
1 (Control to group 2 & 3)	No	No	N/A
2 (Control to group 4)	Yes	Placebo	F-13*
3 (Control to group 5)	Yes	Placebo	F-14*
4	Yes	Yes	F-13
5	Yes	Yes	F- 14

* Formula F-13:- MC (5%)/ERS 100 (5%) ratio (1:1) with triethyl citrate.
* Formula F- 14:- MC (5%)/ERS 100 (5%) ratio (3:1) with triethyl citrate.

Table 3: Mechanical Properties of Ocular Films.

Formulation Code	Tensile Strength* (kg/mm^2)	Elongation at Break* (mm %)	Elastic Modulus* (kg/mm^2)	Strain*
1	6.70(0.077)	5.40(0.53)	429.60(0.019)	0.025(0.026)
2	5.00(0.053)	7.50(0.20)	376.00(0.034)	0.029(0.031)
3	3.00(0.048)	7.80(0.15)	189.70(0.041)	0.042(0.013)
4	6.70(0.092)	2.20(0.19)	494.60(0.014)	0.028(0.038)
5	5.40(0.021)	2.75(0.32)	433.78(0.036)	0.029(0.076)
6	4.03(0.066)	4.00(0.73)	287.31(0.027)	0.034(0.048)
7	2.00(0.059)	4.65(0.52)	83.19(0.031)	0.027(0.024)
8	2.54(0.011)	9.70(0.43)	162.65(0.011)	0.035(0.016)
9	3.19(0.005)	9.10(0.23)	182.02(0.81)	0.030(0.019)
10	2.95(0.024)	3.25(0.14)	187.86(0.62)	0.040(0.034)
11	3.66(0.078)	2.65(0.10)	294.10(0.086)	0.026(0.044)
12	0.51(0.059)	16.60(0.34)	25.00(0.050)	0.060(0.020)
13	0.90(0.064)	18.40(0.35)	45.57(0.020)	0.058(0.029)
14	1.41(0.019)	7.10(0.41)	63.90(0.036)	0.042(0.015)

* Indicates average of five readings. The S.D values are given between parentheses.

Table 4: Physico-chemical Characteristics of Ciprofloxacin Hydrochloride Ocular Inserts.

Formulation Code	Weight of ocular insert*(mg)	Moisture Uptake* (%)	Thickness* (mm)	Drug Content*(%)	Folding Endurance*
1	0.096(0.013)	4.44(0.020)	0.13(0.032)	96.70(0.25)	40(1.51)
2	0.113(0.011)	6.12(0.031)	0.14(0.090)	97.60(0.34)	70(2.32)
3	0.158(0.022)	7.75(0.023)	0.15(0.025)	96.90(0.19)	100(1.27)
4	0.105(0.015)	2.03(0.008)	0.14(0.033)	98.40(0.42)	30(1.19)
5	0.127(0.058)	2.70(0.029)	0.14(0.016)	98.00(0.26)	20(1.64)
6	0.178(0.039)	2.85(0.006)	0.15(0.023)	99.50(0.39)	10(1.33)
7	0.107(0.005)	6.22(0.018)	0.14(0.034)	96.10(0.48)	80(0.28)
8	0.136(0.022)	3.95(0.042)	0.15(0.028)	97.10(0.27)	110(1.15)
9	0.148(0.022)	1.90(0.032)	0.15(0.049)	97.30(0.37)	20(0.69)
10	0.074(0.028)	1.58(0.019)	0.13(0.047)	98.90(0.18)	25(0.37)
11	0.125(0.007)	3.79(0.023)	0.14(0.052)	97.40(0.16)	65(0.20)
12	0.241(0.063)	6.09(0.015)	0.16(0.025)	98.60(0.36)	350(2.18)
13	0.211(0.004)	1.49(0.006)	0.16(0.030)	99.20(0.10)	120(1.22)
14	0.306(0.057)	1.66(0.005)	0.17(0.056)	99.50(0.15)	200(1.10)

* Indicates average of five readings. The S.D values are given between parentheses.

Results and Discussion:

In the present study trials have been made to formulate ocular inserts of ciprofloxacin hydrochloride using different formulation parameters.

Preparation of ocular inserts:

Ocular inserts of ciprofloxacin hydrochloride were prepared using polymers methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, Eudragit RS100 and their combinations. Polymers were chosen according to their biodegradability, film forming properties and retardant to biodegradability to provide sustained release pattern. Glycerin and triethyl citrate were used as plasticizers in different concentrations. The prepared ocular inserts were found to be uniform, transparent and flexible.

Mechanical properties:

Since the mechanical properties represent one of the factors which determine the suitability and acceptability of ocular inserts, it was important to characterize the ophthalmic films under investigation. The tensile strength data (**Table 3)** showed that concentration of plasticizer is the only factor affecting the tensile strength of the film. The type of plasticizer has no effect on tensile strength. The addition of plasticizer (glycerin and triethyl citrate) decreased the tensile strength of the formulated films. This result is consistent with previous findings which suggested that the addition of plasticizer leads to a decrease in intermolecular forces along the polymer chains which produces the observed improvement in flexibility[18]. Also, increasing the plasticizer concentration leads to a considerable decrease in the tensile strength and an increase n the percentage elongation of the films[19]. According to both elongation at break and strain there is a difference in flexibility between the films plasticized with glycerol and the films plasticized with triethyl citrate. The difference in flexibility may be explained according to previous finding[20]; the mechanical properties of plasticized films are influenced mainly by the number of functional hydroxyl groups and the molecular weight of the plasticizer. Glycerol and triethyl citrate used in this study have different structures, however glycerol has more hydroxyl groups than triethyl citrate and hence could be expected to enhance the mechanical properties of the films, in addition to, the effectiveness of glycerol is most likely due to its small molecular weight (size) which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on the films' mechanical properties than larger molecules[20].

Film thickness:

The prepared films were evaluated for their thickness at five random points (**Table 4).** The low standard deviation of the measured thickness of all

formulations indicated uniform thickness of the prepared ocular inserts.

Weight variation:

The weight of the ocular inserts of all formulation was found to be uniform with low standard deviation. The mean weight value varied between 0.096 (±0.013) mg to 0.306 (±0.057) mg (**Table 4)**, as it is affected by the increase or decrease of the film thickness proportionally.

Moisture absorption:

The percentage moisture absorption was calculated for all formulations and the mean values of five replicates were shown in **Table 4.** The results revealed that using glycerin as a plasticizer in formulations; F1-F3 showed higher moisture absorption than formulations F4-F6 which were plasticized with triethyl citrate. Increasing the plasticizer concentration increases the moisture absorption.

Drug content percentage:

All formulations' drug content as shown in **Table 4** were found to be uniform and were in the range of 96.1% (±0.48) to 99.5% (±0.15).

Folding endurance:

Folding endurance of all batches were found between 10 to 350, the large difference in folding endurance of the prepared films is due to the use of different plasticizer types, concentration and polymer type.

Drug-polymer interaction:

The study was performed by IR spectroscopy. The IR charts revealed that ciprofloxacin hydrochloride does not interact in any way with the polymers used.

In-vitro drug release studies:

In-vitro drug release studies were carried out in triplicates, for different time intervals, samples were withdrawn and cumulative percentage drug release was calculated. The drug release from the inserts follows diffusion mechanism. The in-vitro release study was conducted to investigate the effect of: plasticizer type and concentration; the polymer type and polymer ratio on the release time profiles of ciprofloxacin hydrochloride from the ocular inserts.

The effect of plasticizer concentrations on the release of ciprofloxacin HCl from methylcellulose (5%) ocular inserts plasticized with triethyl citrate and glycerin citrate are shown in **Figures 1 and 2**. The release of ciprofloxacin HCl from ocular inserts prepared from methylcellulose (5%) and plasticized with triethyl citrate different concentrations was characterized by two stages: the first stage which includes the initial and the end of the release profile; the second stage which includes the intermediate period (0.5-3 hours).

In the first stage there was no difference in the release profile of ciprofloxacin HCl from the ocular inserts formulated with different concentrations of triethyl citrate. In the second stage the effect of the plasticizer concentration is pronounced especially between the concentration 5% and 20% triethyl citrate as shown in **Figure 1.**

From **Figure 1** formulation F6 shows the highest cumulative percentage drug release (97.3%) at the end of four hours, followed by the formulation F5 (95.3%) and then F4 (93%). Therefore, it is probable that the drug release data from the three above mentioned formulations is due to the variation in the concentration of triethyl citrate used in the formulations. Increasing the concentration of triethyl citrate incorporated into the polymer matrix, leads to an increase in the percentage cumulative release. This could be attributed to the solubilizing effect of triethyl citrate on the polymer chains of methylcellulose used in this study.

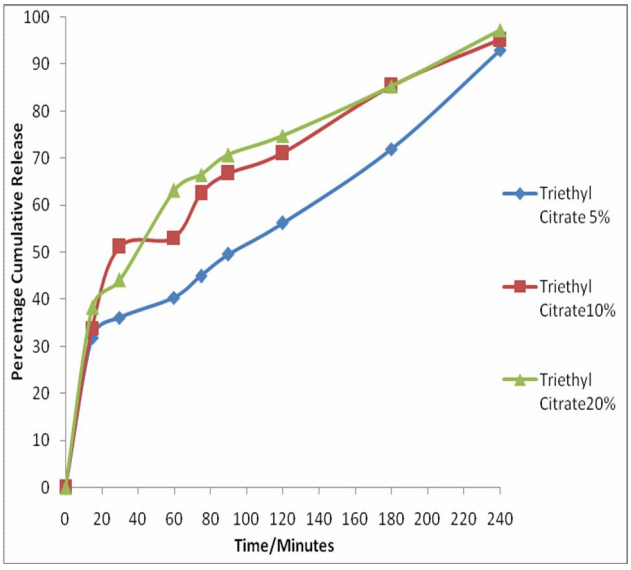


Figure 1: Effect of Triethyl Citrate Concentration on the Release of Ciprofloxacin HCl from MC (5%) Ocular Insert

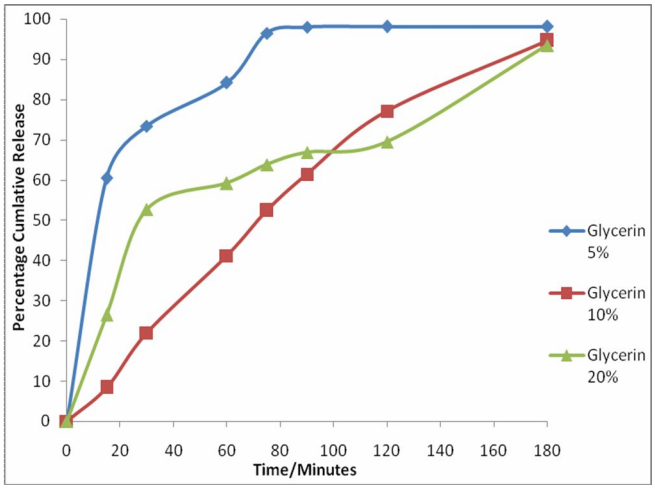


Figure 2: Effect of Glycerin Concentration on the Release of Ciprofloxacin HCl from MC (5%) Ocular Insert.

The release study showed that, at the beginning and at the end of the drug release there was no difference in the percentage cumulative release of ciprofloxacin HCl from formulations F4, F5, and F6, in spite of incorporation of different concentrations of triethyl citrate. This confirms our postulation mentioned above that at four hours (end of the release) the solubilizing effect of triehtyl citrate on the methylcellulose matrix reached to the maximum and consequently no effect of the plasticizer concentration on the percentage cumulative release of ciprofloxacin HCl.

The release profile of ciprofloxacin HCl from the ocular inserts prepared from methylcellulose (5%) plasticized with different concentration of glycerin is shown in **Figure 2 .**

The data revealed that ocular inserts containing 5% glycerin as a plasticizer gave a high release profile, compared to the release profile of other ocular inserts containing 10 and 20 % glycerin.

The increase in the glycerol concentration from 5% to 20% did not result in a significant ($P > 0.05$) increase in the drug release. The effect of glycerin on the release has been attributed to the facilitation of the solvent passage to the polymer chains. Probably, 5% of glycerin is sufficient to reach the majority of these chains. So 5% glycerin is sufficient to produce the plasticizing effect with methylcellulose. Concerning the effect of the type of plasticizer on the release profile of ciprofloxacin HCl there was no difference in the drug release from films plasticized with glycerin and from these plasticized with triethyl citrate.

This finding is similar to that obtained from previous study demonstrated , the effect of glycerin amount on the release of sulfadiazine[21].

The effect of polymer ratios on the release – time profiles of ciprofloxacin HCl from ocular inserts (F13 & F14) was investigated and the data is presented in **Figures 3-5.** It is clearly shown that ciprofloxacin HCl release decreased as the ratio of methylcellulose in the film decreased (**Figure 3)**. This could be explained by the results taken from moisture absorption property of the film **Table 4**.

The methylcellulose ocular inserts has a high moisture uptake compared to others ocular inserts prepared from other polymers. The presence of methylcellulose might have been responsible for this situation because it's hydrophilic property. Increasing the ratio of Eudragit RS100, increasing hydrophobicity of the formulation (F13), which lead to prolong the release of ciprofloxacin HCl from the insert. This finding is in agreement with other study, observed that the drug release was increased with increasing the concentration of HPMC and the release of the drug was inversely proportional to the amount of Eudragit RS100 present [22].

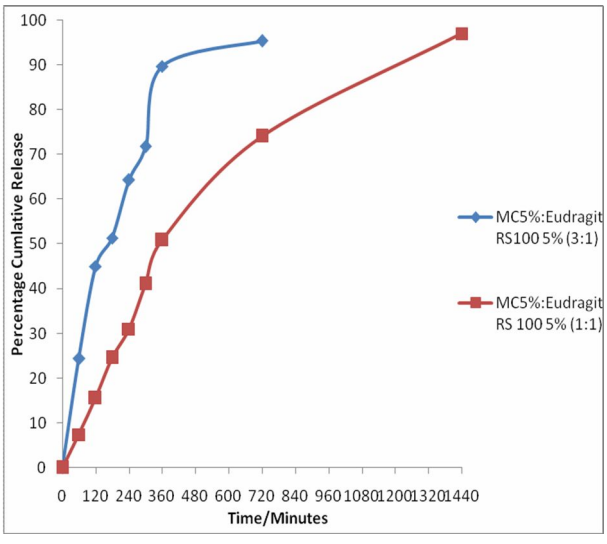


Figure 3: Effect of Polymer Ratio on the Release of Ciprofloxacin HCl from MC and Eudragit RS100 with Triethyl Citrate (20%) Ocular Insert.

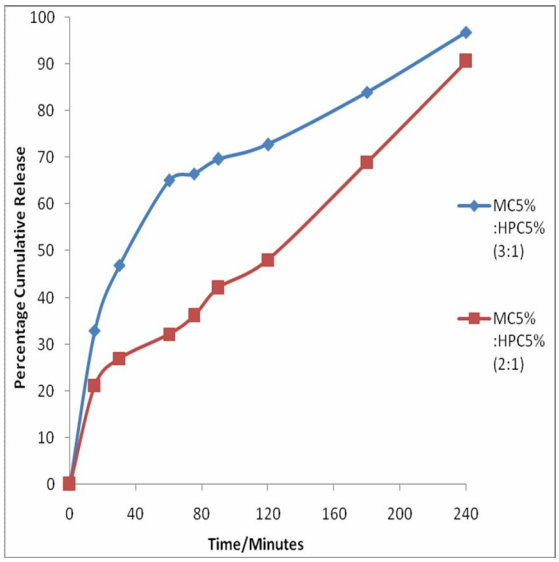


Figure 4: Effect of Polymer Ratio on the Release of Ciprofloxacin HCl from MC and HPC with Triethyl Citrate (10%) Ocular Insert.

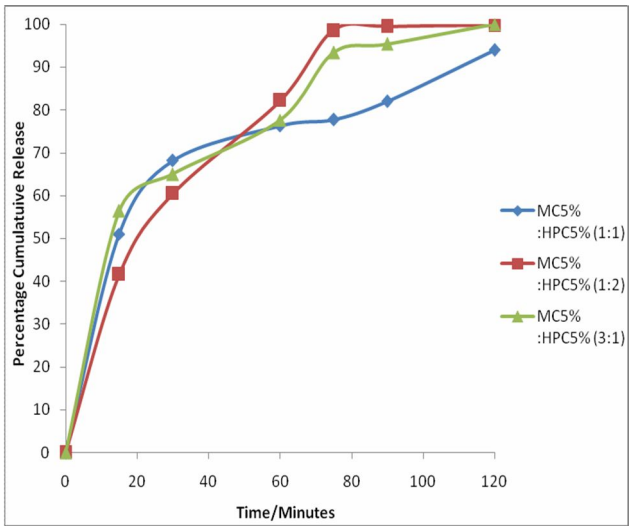


Figure 5: Effect of Polymer Ratio on the Release of Ciprofloxacin HCl from MC, HPC with Glycerin (10%) Ocular Insert.

Figure 4 illustrate the effect of methylcellulose / hydroxypropylcellulose ratios on the release of ciprofloxacin HCl from ocular inserts plasticized with triethyl citrate (10%).

Increasing the ratio of methylcellulose increases the release profile of ciprofloxacin HCl from the ocular insert formulated from methylcellulose / HPC polymers (3:1) and plasticized with triethyl citrate 10% (F11). The percentage cumulative release of the drug can be arranged in descending order: F11 > F10. The reason for higher release of the drug from F11 is maybe due to the higher ratio of the methylcellulose (hydrophilic) polymer in the formulation than the ratio of HPC. The difference in the release profile between F11 and F10 was more pronounced during the release period extended from one hour to three hours.

F11 shows a maximum percentage cumulative release of (96.8%) at the end of four hours, followed by formulation F10 (90.6%).

The effect of methylcellulose / HPC ratio on the release of ciprofloxacin HCl from ocular insert plasticized with glycerin (10%) is shown in **Figure 5.** The results revealed that, at the initial release – time profile (first hour) the difference in the release of ciprofloxacin HCl from different polymer ratios is small, then the difference became bigger beyond this time.

This could be explained on that at the beginning of the release the polymers were in the wetting process then followed by swelling and consequently increasing in the percentage cumulative release of ciprofloxacin HCl. Increasing the ratio of methylcellulose in the polymer combination MC / HPC has small increase in the percentage cumulative release, while increasing the ratio of HPC in the polymer combination from 1:1 to 1:2 (F7 and F8) has a more pronounced increase on the release specially at the second hour of the release period.

On comparison between the effect of glycerin and triethyl citrate as a plasticizer on the release of ciprofloxacin HCl from the ocular insert formulated from a combination of MC / HPC (3:1), we found that glycerin enhanced the release of ciprofloxacin HCl from ocular insert F9 than from the ocular insert plasticized with triethyl citrate F11.

After two hours the ocular inserts plasticized with 10% glycerin released almost twice more than those plasticized with 10% triethyl citrate. Ocular inserts plasticized with 10% glycerin (F9) released 100% of the drug against 48.1% in those plasticized with 10% triethyl citrate. This finding is confirmed by the data[23] which , attributed the effect of glycerol on the release of silver sulfadiazine to two main factors.

Firstly, glycerol is extremely miscible with water (which was the predominant component in the

polymer solutions). In addition, glycerol has low molecular weight, which allows it to fit easily among the polymer chains. In this way, the hydroxyl groups of glycerol develop polymer-plasticizer interactions that can enhance the flexibility and mobility of the polymer chains, leading to an increase in the amount of drug released.

Secondly, the high affinity of glycerol for water allows leakage of a portion of the glycerin to the medium. The mechanism probably involves the opening of channels in the films which facilitate the solvent uptake, leading to an enhancement in the swelling properties of the polymer matrix[24, 25].

The effect of plasticizer concentration on the release of ciprofloxacin HCl from methylcellulose from ocular inserts is illustrated in **Table 5**. The results revealed that, increasing the concentration of glycerol above (5%) reduces the amount released of the drug, while increasing concentration of triethyl citrate increase the amount of the drug released.

This could be explained, that the miscibility of glycerol with water is higher than that of triethyl citrate, so the (5%) glycerol is enough to produce pores and maximum miscibility of the membrane with water. Increasing the concentration of triethyl citrate increases the number of pores in the membrane and the amount released of the drug.

Permeability studies:

The formulations which showed better physico-chemical parameters with prolonged release were selected for permeation studies. Out of fourteen formulations, three formulations were selected. The selected formulations are F12 containing 10% HPMC; F13 containing combination of MC / Eudragit RS100 (1:1); and F14 containing a combination of MC / Eudragit RS100 (3:1). The selected formulations were found to be promising, since it showed prolonged release with almost 100% at the end of 6, 12, 24 hours respectively. Also, the three formulations (F12, F13 & F14) were found to be transparent, flexible and the thickness was fairly uniform.

The flux of ciprofloxacin HCl was higher for the formulation F12, followed by F14 and F13 (**Table 6)**. There was a noticeable slow but steady flux of the drug during permeation experiment.

The permeability coefficient results showed to be dependent on the type of the ocular insert polymer. The more solubility of the polymer is the higher the permeability coefficient, so after six hours the majority of ciprofloxacin HCl was permeated. In the case of F14 the amount of ciprofloxacin HCl permeated through the cornea was almost 100% after twelve hours. The permeation of ciprofloxacin HCl through the cornea was prolonged for twenty four hours in the case of F13.

Table 5: Effect of Plasticizer Concentration on the Release of Ciprofloxacin HCl From Methylcellulose Ocular Inserts.

Polymer	Plasticizer		Cumulative amount released% after 3 hours
	Type	Concentration	
Methylcellulose	Glycerin	5	98.1
		10	94.6
		20	93.4
Methylcellulose	Triethyl Citrate	5	93.0
		10	95.2
		20	97.3

Table 6: Permeability Coefficient and Steady-State Fluxes of Ciprofloxacin HCl through Rabbits' Corneas from Different Ocular Inserts.

Formulation Code	Permeability Coefficient ($cm \cdot hr^{-1}$)	Steady-State Flux ($\mu g \cdot cm^{-2} \cdot h^{-1}$)
12	3.940	0.057
13	1.733	0.016
14	2.214	0.032

These results indicate that the type of the polymer greatly influence the steady-state flux and the permeability of ciprofloxacin HCl. The more hydrophilic the polymer is the more flux and permeation of the drug (F12). Formulation F13 & F14 with Eudragit RS 100 as a rate controlling membrane were found to be better, since constant and about 100% of the drug permeation was observed up to 12 & 24 hours respectively.

Comparison between the permeation of ciprofloxacin HCl through the rabbits' corneas from the formulations F13 and F14 indicates that increasing the amount of methylcellulose in the combination (MC/ E RS100) leads to increase the amount permeated of ciprofloxacin HCl. While increasing the amount of E RS100 in the blend lead to a decrease in the amount of the drug permeated.

This result is in agreement with other finding stated that , the rate-controlling membranes of Eudragit RL100 and Eudragit RS100 with PVP demonstrated sustained and controlled release of carvedilol across guinea pig skin during in-vitro permeation studies[26] .

The kinetic parameters of drug permeation for different formulations are presented. Formulations F12 follow Higuchi diffusion mechanism due to the high polarity of the polymer used (HPMC), while the formulation F13 (MC / E RS100, 1:1) showed first order mechanism. In case of formulation F14 (MC / E RS100, 3:1) the data showed a zero-order mechanism i.e. permeation is independent of time.

The ranking of drug permeation according to permeability coefficient in **Table 6** is in a descending order as follows (F12 > F14 > F13). The lower proportion of ammonium groups in Eudragit RS100 is responsible for prolonged release of ciprofloxacin HCl and also low permeability coefficient (F13).

This result is similar to the finding , that the higher proportion in quaternary ammonium groups in Eudragit RL100 resulted in rapid hydration and drug release, whereas the lower proportion of ammonium groups in Eudragit RS100 is responsible for prolonged release of ofloxacin[27].

The flux was calculated by dividing the cumulative amount of drug permeated per cm^2 of the rabbit's cornea with time. The corresponding flux of the drug was 0.057, 0.032 and 0.016 $\mu g \cdot cm^{-2} \cdot h^{-1}$ for F12, F14 and F13 respectively. So, increasing the ratio of hydrophobic polymer like Eudragit RS100 leads to sustain the drug in the eye as seen in formulation F13 (MC / E RS100, 1:1).

This could be due to that methylcellulose reduces the resistance offered by the Eudragit RS100 film alone, and by increasing pores and / or their diameter as the drug diffuses with less resistance[28].

In Vivo Studies:

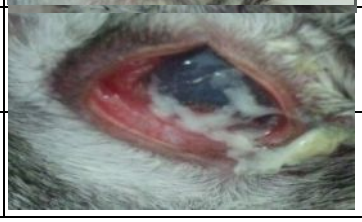
Acute conjunctivitis was noted one day after the initial infection by *Ps. aeruginosa*. Severe conjunctivitis and keratitis were observed in untreated animal groups as shown in **Figure 6.** On the other hand, the symptoms were milder in the groups that received ciprofloxacin HCl ocular inserts. This was demonstrated by the level of bacterial loads in eye tissues. As shown in **Figure 7** and **Table 8,** treatment with ciprofloxacin HCl significantly reduced ($P < 0.0001$) when the control group is compared with either of the treated groups (1 or 2) while there was no significant difference ($P > 0.05$) when both treated groups compared with respect to each other, One-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test were used for comparison of the means of different groups. The bacterial load in treated groups was reduced by two folds compared to control groups; the effect of the drug was prominent after receiving three doses of formulation I and six doses of formulation II.

Based on visual observation using the Peyman scale as shown in **Figures 7** and **8,** symptoms were completely relieved in 16 and 50% of those animals which received formula 13 and 14 respectively**.** The rest of the animals developed redness and keratitis, but the symptoms were mild compared to untreated animals.

Pseudomonas aeruginosa is among the leading cause of bacterial conjunctivitis. The microorganism can cause severe ocular infection, keratitis that can progress rapidly, resulting in intense inflammation, irreversible stromal scarring, and probable loss of vision. The prognosis for patients with *Ps. aeruginosa* corneal disease is often poor. Even if the infected cornea is rendered sterile with antibacterial chemotherapy, the resultant pathologic condition may range from restoration of visual acuity with minimal corneal scarring to extensive tissue destruction and the need for corneal transplantation. Keratitis and endophthalmitis are serious sight-threatening conditions which can result in permanent loss of vision if appropriate treatment is not instituted promptly.

Figure 6: Effect of Ocular Insert (F 14) on Rabbit Eye Visually and Using Peyman Scale: (A) Placebo. (B) Treated Animal.

(A)

Day #		Peyman score		Treatment
		Conjunctiva	Cornea	
1		2	1	Placebo
2		3	2	Placebo
3		3	3	Placebo
6		3	3	Placebo

(B)

Day #		Peyman score		Treatment
		Conjunctiva	Cornea	
1		2	1	Yes
2		2	1	Yes
3		1	0	Yes
6		0	0	Yes

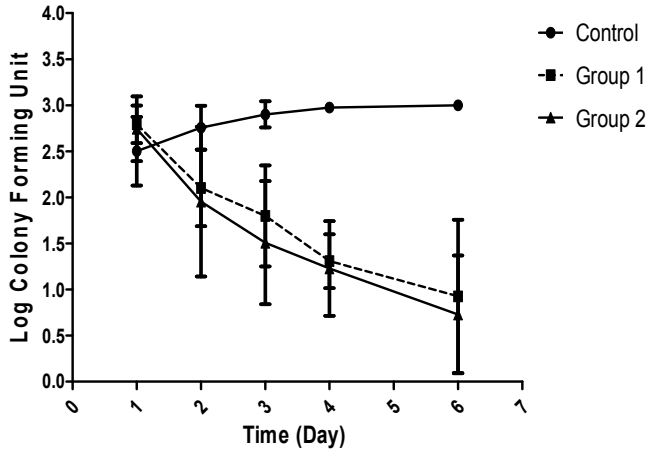
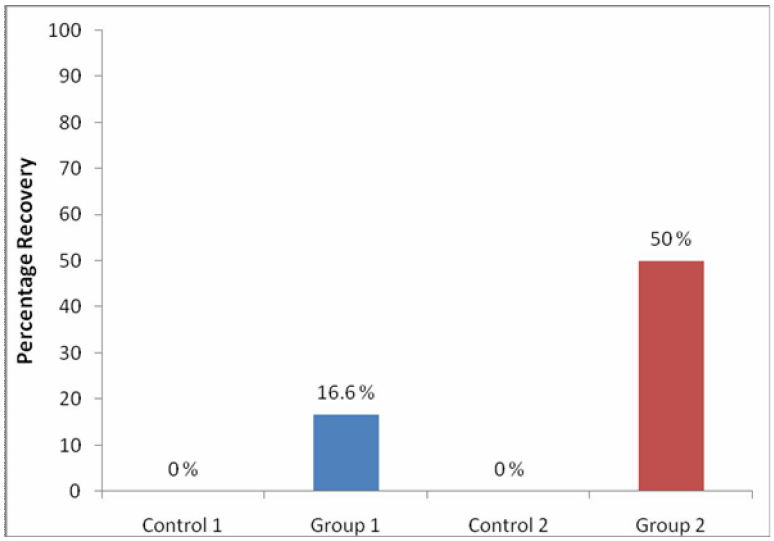


Figure 7: Effect of Ciprofloxacin HCl Ocular Inserts on In Vivo Experimental Conjunctivitis in Rabbits. Group 1 (F-13) Group 2 (F-14).

Figure 8: Percentage Recovery of Different Groups.



* Group 1 using Formula F-13 : - MC (5%)/ERS 100 (5%) ratio (1:1) with triethyl citrate.
* Group 2 using Formula F-14 : - MC (5%)/ERS 100 (5%) ratio (3:1) with triethyl citrate.
* Control 1 & 2 is the placebo for F-13 & F-14 respectively .

The fluoroquinolones were introduced for the treatment of corneal and conjunctival infections and in the prophylaxis of postoperative endophthalmitis[29]. Currently, the cornerstone for the successful treatment of infective keratitis is effective topical medication with ciprofloxacin[30]. Ciprofloxacin, a second generation fluoroquinolone, is considered a potent drug and has virtually replaced the use of combination therapy[31].

In this study two formulations of ciprofloxacin were tested against Pseudomonas ocular infection in rabbit model. The drug was investigated based on its effectiveness to relief the symptoms of conjunctivitis and to minimize the chance of development of keratitis. Rabbit was selected in this study because of its big eye ball. The data demonstrated that ciprofloxacin was effective in treatment of conjunctivitis caused by *Ps. aeruginosa* and in minimizing the risk of development of keratitis. Previous studies showed that ciprofloxacin is an effective antibiotic in treatment conjunctivitis[32], corneal ulcers[33], keratitis[34] and endophthalmitis[35] caused by Pseudomonas.

Both formula exerted similar effect on the bacterial load of the eye tissues. They needed 3 days to show significant killing of the bacteria even though they couldn't completely eradicate the microorganism after 6 days of treatment. Based on visual observation of the conjunctivitis symptoms, it is clear that formula II was significantly more effective than formula I where the former was capable completely relief of the symptoms in 50% of the infected animals compared to 16% by the other formula. This could be attributed to the difference in the number of doses given to the animals.

It is obvious from our data that using topical ciprofloxacin is an efficient mean of treatment of bacteria conjunctivitis caused by *Ps. aeruginosa*, and minimizing the risk of development of severe keratitis.

References:

1. Attia, M. A., Kassem, M. A. and Safwat, S. M.. "In vivo performance of [3H]dexamethasone ophthalmic film delivery systems in the rabbit eye." International Journal of Pharmaceutics (1988) 47(1-3): 21-30.
2. Hume, L. R., H. K. Lee, L. Benedetti, Y. D. Sanzgiri, E. M. Topp and V. J. Stella . "Ocular sustained delivery of prednisolone using hyaluronic acid benzyl ester films." International Journal of Pharmaceutics (1994) 111(3): 295-298.
3. Bharath, S. and S. R. Hiremath . "Ocular delivery systems of pefloxacin mesylate." Pharmazie (1999) 54(1): 55-8.
4. Maichuk, Y. F. . "Editorial: Ophthalmic drug inserts." Invest Ophthalmol (1975)14(2): 87-90.
5. Ozawa, H., S. Hosaka, T. Kunitomo and H. Tanzawa . "Ocular inserts for controlled release of antibiotics. " Biomaterials(1983) 4(3): 170-4.
6. Grass, G. M., J. Cobby and M. C. Makoid . "Ocular delivery of pilocarpine from erodible matrices." J Pharm Sci (1984) 73(5): 618-21.
7. Baeyens, V., O. Felt-Baeyens, S. Rougier, S. Pheulpin, B. Boisramé and R. Gurny . "Clinical evaluation of bioadhesive ophthalmic drug inserts (BODI®) for the treatment of external ocular infections in dogs." Journal of Controlled Release (2002) 85(1-3): 163-168.
8. O'Brien, T. P., M. G. Maguire, N. E. Fink, E. Alfonso and P. McDonnell . "Efficacy of ofloxacin vs cefazolin and tobramycin in the therapy for bacterial keratitis. Report from the Bacterial Keratitis Study Research Group." Arch Ophthalmol (1995) 113(10): 1257-65.
9. Bower, K. S., R. P. Kowalski and Y. J. Gordon . "Fluoroquinolones in the treatment of bacterial keratitis." Am J Ophthalmol (1996) 121(6): 712-5.
10. Jauch, A., M. Fsadni and G. Gamba . "Meta-analysis of six clinical phase III studies comparing lomefloxacin 0.3% eye drops twice daily to five standard antibiotics in patients with acute bacterial conjunctivitis." Graefes Arch Clin Exp Ophthalmol (1999) 237(9): 705-13.
11. Thibodeaux, B. A., J. J. Dajcs, A. R. Caballero, M. E. Marquart, D. O. Girgis and R. J. O'Callaghan . "Quantitative comparison of fluoroquinolone therapies of experimental gram-negative bacterial keratitis." Curr Eye Res (2004) 28(5): 337-42.
12. Chalita, M. R., A. L. Hofling-Lima, A. Paranhos, Jr., P. Schor and R. Belfort, Jr. . "Shifting trends in in vitro antibiotic susceptibilities for common ocular isolates during a period of 15 years".Am J Ophthalmol (2004) 137(1): 43-51.
13. Whiteley, H. E., R. L. Peiffer, M. H. Wanda, G. R. Colin and A. W. Matthew . The Eye. Handbook of Toxicologic Pathology (Second Edition). San Diego, Academic Press: (2002) 539-584.
14. Gorle, A. P. and S. G. Gattani . "Design and evaluation of polymeric ocular drug delivery system." Chem Pharm Bull (Tokyo) (2009) 57(9): 914-9.
15. Gilhotra, R. M. and D. N. Mishra . "Alginate-chitosan film for ocular drug delivery: effect of surface cross-linking on film properties and characterization." Pharmazie (2008) 63(8): 576-9.
16. Mosher, G. L. and T. J. Mikkelson . "Permeability of the n-alkyl p-aminobenzoate esters across the isolated corneal membrane of the rabbit." International Journal of Pharmaceutics (1979) 2(3-4): 239-243.
17. Peyman, G. A., J. T. Paque, H. I. Meisels and T. O. Bennett . "Postoperative endophthalmitis: a comparison of methods for treatment and prophlaxis with gentamicin." Ophthalmic Surg (1975) 6(1): 45-55.
18. Yang, L. and A. T. Paulson . "Mechanical and water vapour barrier properties of edible gellan films." Food Research International (2000) 33: 563-570.
19. Arvanitoyannis, I. S., A. Nakayama and S. i. Aiba . "Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties." Carbohydrate Polymers (1998)37: 371-382.
20. Sothornvit, R. and J. M. Krochta . "Plasticizer effect on oxygen permeability of beta-lactoglobulin films." J Agric Food Chem (2000) 48(12): 6298-302.
21. Eduardo, P. A., D. P. S. Tásia, V. M. N. Marco, C. M. Aldo, F. G. Marconi and N. R. Fernanda . "Mechanical properties and release studies of chitosan films impregnated with silver sulfadiazine." Journal of Applied Polymer Science (2006) 102(4): 3462-3470.
22. Kevin, C. G., J. S. Anil and H. S. Pratik . "Formulation and in - vitro characterization of

monolithic matrix transdermal systems using HPMC/Eudragit S 100 polymer blends." International Journal of Pharmacy and Pharmaceutical Sciences (2009) 1(1): 108-120.

23. Okor, R. S. . "Influence of hydrophilic character of plasticizer and polymer on certain film properties." International Journal of Pharmaceutics (1982)11(1): 1-9.
24. Remuñán-López, C. and R. Bodmeier . "Mechanical, water uptake and permeability properties of crosslinked chitosan glutamate and alginate films." Journal of Controlled Release (1997) 44(2-3): 215-225.
25. Myllärinen, P., R. Partanen, J. Seppälä and P. Forssell . "Effect of glycerol on behaviour of amylose and amylopectin films." Carbohydrate Polymers (2002) 50(4): 355-361.
26. Tanwar, Y. S., C. S. Chauhan and A. Sharma . "Development and evaluation of carvedilol transdermal patches." Acta Pharm (2007) 57(2): 151-9.
27. Sahoo, S. K., A. A. Mallick, B. Barik and P. C. Senapati . "Formulation and in vitro Evaluation of Eudragit® Microspheres of Stavudine." Tropical Journal of Pharmaceutical Research (2005)4(1): 369-75.
28. Rao, V. and S. Shyale. "Preparation and Evaluation of Ocular Inserts Containing Norfloxacin." Turkish Journal of Medical Sciences (2004) 34(4): 239-46.
29. Ciulla, T. A., M. B. Starr and S. Masket . "Bacterial endophthalmitis prophylaxis for cataract surgery : An evidence-based update." Ophthalmology (2002) 109(1): 13-24.
30. Leibowitz, H. M. "Clinical evaluation of ciprofloxacin 0.3% ophthalmic solution for treatment of bacterial keratitis." Am J Ophthalmol (1991) 112(4 Suppl): 34S-47S.
31. Hyndiuk, R. A., R. A. Eiferman, D. R. Caldwell, G. O. Rosenwasser, C. I. Santos, H. R. Katz, S. S. Badrinath, M. K. Reddy, J. P. Adenis and V. Klauss . "Comparison of ciprofloxacin ophthalmic solution 0.3% to fortified tobramycin-cefazolin in treating bacterial corneal ulcers. Ciprofloxacin Bacterial Keratitis Study Group." Ophthalmology (1996) 103(11): 1854-62; discussion 1862-3.
32. Adebayo, A., J. Parikh, S. McCormick, M. Shah, R. Huerto, G. Yu and T. Milman . "Shifting trends in in vitro antibiotic susceptibilities for common bacterial conjunctival isolates in the last decade at the New York Eye and Ear Infirmary." Graefe's Archive for Clinical and Experimental Ophthalmology: (2010)1-9.
33. Darrell, R. W., S. M. Modak and C. L. Fox, Jr. "Norfloxacin and silver norfloxacin in the treatment of Pseudomonas corneal ulcer in the rabbit." Trans Am Ophthalmol Soc . (1984) 82: 75-91.
34. Hobden, J. A., J. J. Reidy, R. J. O'Callaghan, M. S. Insler and J. M. Hill . "Quinolones in collagen shields to treat aminoglycoside-resistant pseudomonal keratitis." Invest Ophthalmol Vis Sci (1990) 31(11): 2241-3.
35. Kim, I. T., K. H. Chung and B. S. Koo . "Efficacy of ciprofloxacin and dexamethasone in experimental pseudomonas endophthalmitis." Korean J Ophthalmol (1996) 10(1): 8-17.

.

*****