In-vitro Investigation of Ocular E-Coli Infection via Norfloxacin

Peter Obidoa; Ugwo Ugueche *
Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Cross-River State University of Technology, Calabar, Nigeria

Received: 04 October 2017 Accepted: 11 November 2017 Published: 19 November 2017

Abstract
The purpose of the present research was to formulate ocular inserts of norfloxacin along with bioadhesive polymers to sustain drug release and assess its antimicrobial potential against E. coli, in vitro. Ocular inserts of norfloxacin were prepared by film casting method using bioadhesive polymers HPMC/ SCMC/ sodium alginate/ chitosan/carbopol 934 and PVP K30 as film forming polymer. The formulated bioadhesive norfloxacin ocular inserts were screened for various pharmacotechnical parameters and in vitro drug release. The optimized formulation was subjected to HET CAM test and antimicrobial efficacy against E. coli. Of the screened formulations, maximum bioadhesive strength (36.5 ± 0.10 x 10⁻³ dynes/cm²) was observed for chitosan based ocular insert. Kinetic modeling of the in vitro drug release data revealed matrix based release was governed by both diffusion and swelling phenomena. Ocular insert consisting formulated with chitosan was found promising, as it showed acceptable pH, good bioadhesive strength, sustained drug release (98.33% at 6 h) and active against selected microorganism.

Keywords: Norfloxacin, bioadhesive ocular insert; physicochemical characterization; sustained release;

1. Introduction
Inflammation of conjunctiva (conjunctivitis), observed as red eye or pink eye can be caused by microorganisms, potential irritants and allergens [1]. Various strains of E. coli have been associated with eye infections such as conjunctivitis, keratitis, cellulitis and endophthalmitis, however, E. coli is an uncommon cause of these types of infections. Individuals with fecal contamination, those who have suffered penetrating eye injuries, or those with weakened immune systems may be vulnerable to E. coli infections of the eye. Early recognition and appropriate treatment is crucial. These infections most commonly occur in patients who are debilitated, immunocompromised, or diabetic or in corneas with an underlying pathologic condition [2]. Fluoquinolone antimicrobial agents have been effectively used in treatment of conjunctivitis and have been researched for a variety of ocular drug delivery systems [3-11]. Norfloxacin has in vitro activity against a broad spectrum of gram-positive and gram-negative aerobic bacteria. The fluorine atom at the 6 position provides increased potency against gram negative organisms and the piperazine moiety at the 7 position is responsible for anti-pseudomonal activity. Norfloxacin inhibits bacterial deoxyribonucleic acid synthesis and is bactericidal. At the molecular level three specific events are attributed to norfloxacin in E. coli cells: (i) Inhibition of the ATPdependent DNA super coiling reaction catalyzed by DNA gyrase, (ii) Inhibition of the relaxation of super coiled DNA, and (iii) Promotion of double-stranded DNA breakage. Hence it provides relief from inflammation in bacterial conjunctivitis [12]. Commercially available as ophthalmic solution Chibroxin® [13] has the major drawbacks pertinent to eye drops prominently requiring frequent instillation, rapid and extensive precorneal loss of drug caused by drainage and high tear fluid turnover [14-16]. These drawbacks may be minimized by use various ocular drug delivery systems, such as nanosuspension [17], liposomes, in situ gel and ocular inserts[18]. Ocular inserts increase contact time between drug and tissue,
prolong drug release, reduce systemic side effects and afford better patient compliance [19]. Sustained topical delivery of norfloxacin has been investigated by various controlled drug delivery systems namely microspheres [20], ocular minitablet [21], in situ gel [22] and ocular insert [7]. The reported ocular inserts of norfloxacin were formulated as hydrophilic monolithic reservoir system. However, the adhesivity of the ocular insert is questionable. The aim of this study was to formulate and evaluate bioadhesive ocular insert to develop a sustained release drug delivery system of norfloxacin. Bioadhesive characteristic of ocular insert will not allow ocular insert to move in eye, and hence adherence to conjunctiva [23].

2. Materials and Methods

Materials

Norbloxacin was obtained as a gift sample from Crown Pharmaceuticals, Alwar, India. Polyvinyl pyrrolidone K 30 was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Hydroxy propyl methyl cellulose K 15M was obtained as a gift sample from Colorcon Asia, Goa, India. Sodium carboxy methyl cellulose was purchased from Marine Chemicals Cochin, Kerala, India. Sodium alginate and carbopol 934 were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Polyethylene glycol 400 was purchased from Merck Specialties Pvt. Ltd. Mumbai, India.

Table 1: Composition of norfloxacin bioadhesive ocular inserts

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation 1</th>
<th>Formulation 2</th>
<th>Formulation 3</th>
<th>Formulation 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug % w/w</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>PVP K 30 (g)</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>1% w/w HPMC K 15M (ml)</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1% w/w SCMC (ml)</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1% w/w Sodium alginate (ml)</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>1% w/w Carboxol 934 (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>PEG 400 (w/v of polymer)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethanol (ml) q.s.</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Bioadhesive polymeric hydrogel was prepared by dispersing the polymer (1% w/v) in distilled water using a mechanical stirrer (600 rpm) fitted with a four-bladed paddle at room temperature. PVP K-30 solution and polymeric hydrogel were mixed with each other under the same constant stirring. Triethanolamine was added to neutralize the carbopol hydrogel to pH 6.9-7.2. 1% w/v chitosan hydrogel was prepared by dispersing the polymer in acetic acid solution [25]. The samples were stored for 24 h at 4–8°C before casting to ensure total hydration of the polymers and to exclude entrapped air. The resulting polymeric gels were brought back to room temperature. Norfloxacin and poly ethylene glycol 400 were added under constant stirring (600 rpm) in polymeric gel. The aqueous (hydro alcoholic) polymeric hydrogels were poured onto mercury surface-containing glass rings (6 cm diameter and 10 ml volume) placed over mercury in the glass petri dish and dried at 38°C in an oven for 24 h. The films were stored in a dessicator at room temperature after wrapping in sealed plastic sheets. The prepared formulations were evaluated for pharmacotechnical characteristics namely appearance, uniformity of weight, uniformity of thickness, drug content, percentage swelling index, folding endurance, surface pH determination, tensile strength, percent elongation at break, ex vivo bioadhesive strength, in vitro drug release studies to select the optimized formulation.

2.2 Evaluation

Physical characterization

The ocular inserts were evaluated for their physical characters such as color, texture, and appearance visually.

2.3 Uniformity of weight

The weight variation test was carried out using electronic balance. Mean weight of inserts (n = 10) of each formulation was recorded and standard deviations of weight variation were computed from the mean value.

2.4 Thickness and endurance

The thickness of inserts was determined using a vernier caliper (digital vernier caliper, Aerospace, Mumbai) and recorded as the mean of ten measurements. The standard deviations in thickness were computed from the mean value. Folding endurance was determined by repeatedly folding the inserts at the same place till it broke.

2.5 Surface pH

The inserts were allowed to swell on an agar plate at room temperature for 1 h. The agar plate was prepared by dissolving 2 % w/v agar in warm simulated tear fluid (composition of STF; sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride (2H₂O): 0.008 g, and purified water q.s. 100 ml) having pH 7.4 [26] under stirring and then pouring the solution into a petri dish till gelling at room temperature. The pH of the wet surface was measured by means of pH paper placed on the surface of the swollen insert.

MedBioTech J. 2017; 1(2): 96-103
2.6 Drug content

It was determined by assaying the individual insert. Ocular insert of each formulation (n=3) was dissolved in suitable quantity of simulated tear fluid, pH=7.4 and the solution was filtered and content was analyzed spectrophotometrically at 276.60 nm (UV spectrophotometer, Shimadzu 1800, Kyoto, Japan).

2.7 Mechanical strength

An ocular insert with good tensile strength and percent elongation at break would resist tearing due to stress generated by the blinking action of the eye. The insert was cut into strips (10 mm × 10 mm). Tensile strength and elongation at break was determined by modifying the reported method [27]. The design of apparatus consisted of a base plate with a pulley aligned on it. One aluminium clip was fixed on one end of the base plate, to which the insert (n = 3) was clipped. The other end of the insert was clipped to a movable aluminium clip. A thread was tied to the movable clip and passed over the pulley, to which a small pan was attached to hold weights. A small pointer was attached to the thread that travels over the scale affixed on the base plate. The weights were gradually added to the pan till the insert (that was affixed between two clips) was broken. The weight required to break the insert was recorded as break force and the simulation of swelling index is:

\[
\text{Swelling index} = \left( \frac{W_t - W_0}{W_0} \right) \times 100
\]

Where Wo was initial weight of the insert and Wt was weight of the swollen insert after time t.

2.8 Swelling test

In this test initial weight of insert (n = 3) was taken, and then placed in an agar gel plate (2% w/v agar in STF, pH 7.4) and incubated at 37 ± 1°C for 30 min. The insert was removed from the plate at the interval of 5 min, surface water was removed with the help of filter paper, and it was reweighed. The equation used for calculation of swelling index is:

\[
\text{Swelling index} = \left( \frac{W_t - W_0}{W_0} \right) \times 100
\]

2.9 Ex vivo bioadhesive strength

For the measurement of bioadhesive strength, freshly excised conjunctiva of an adult goat was used as model membrane. The conjunctiva was placed in an aerated saline at 4°C and later washed with isotonic phosphate buffer, pH 7.4 before use. Bioadhesive strength of ocular insert (n = 3) was measured on a modified two-arm physical balance. The pan at the left arm of the balance was detached and a vertical thread was hung to the lever of the left arm which had a rubber stopper tied to its end, hanging downward. The ocular insert to be tested was adhered to the downward facing side of the rubber stopper. Conjunctival membrane was tied onto the open mouth of a glass vial which was filled with isotonic phosphate buffer. The vial was fitted in the centre of a glass beaker filled with STF (pH 7.4, 37°C). The apparatus was set such that the vial (conjunctival membrane tied on it, facing upward) lies exactly below the rubber stopper (insert tied on it, facing downward). The rubber stopper was lowered so as to make the insert come in contact with the membrane. After facilitating the contact between the two, weight was put on the right limb of balance (gram force) required to detach the insert from the conjunctival surface [28]. The detachment stress (dynes/cm²) was calculated by using eq. 4.

\[
\text{Detachment stress} = \frac{\text{Weight required for the detachment of insert x Acceleration due to gravity}}{\text{Area of tissue exposed}}
\]

2.10 In vitro drug release

The donor receiver compartment model, designed using commercial semi-permeable membrane cellophane membrane (Sigma-Aldrich Corporation, USA) was used to carry out the in vitro drug release studies. Semipermeable membrane was used to mimic in vivo conditions like corneal epithelial barrier. It was pre-soaked overnight in the freshly prepared dissolution medium that is STF of pH 7.4. The insert (n = 3) was put inside the donor compartment in contact with the semipermeable membrane. The entire surface of the membrane was in contact with the reservoir compartment that contained 25 ml of STF with pH 7.4, which was stirred continuously using a magnetic stirrer at 20 rpm to simulate blinking action. A sample of 2 ml was withdrawn from the sampling port at periodic intervals and it was replaced with equal volume of STF with pH 7.4. Drug content in each sample was analyzed using STF pH 7.4 as blank on UV-VIS Shimadzu 1800 spectrophotometer. The in vitro drug release data was analyzed using different kinetic models like zero-order, first-order, Higuchi diffusion model and Korsmeyer-peppas model to check the mechanism of drug release from the prepared ocular inserts [29].

2.11 Drug-excipient (s) compatibility

The FTIR absorption spectra of pure drug and drug loaded ocular inserts were recorded in the wavenumber range of 4000-500 cm⁻¹ by KBr disc method using FTIR spectrophotometer (Shimadzu IR-AFFINT, Kyoto, Japan).
2.12 Selection of optimized formulation

Based on physicochemical tests and in vitro release study optimized formulation was selected and subjected to HET CAM test for eye irritancy potential and microbiological challenge against *E. coli*.

2.13 HET CAM test

Hen’s egg test-chorioallantoic membrane (HET-CAM) test was carried out on fertilized hen’s eggs. Three eggs (weight 50-60 g) were selected and candled in order to discard the defective ones. The eggs were incubated for 3 days in humidification chamber at a temperature of 37 ± 0.5°C. After every 12 h, the trays containing eggs were rotated manually in a gentle manner. On day 3rd, egg albumin (3 ml) was removed by using sterile techniques from the pointed end of the egg. The hole was sealed by 70% alcohol-sterilized parafilm with the help of heated spatula. The eggs were kept in the equatorial position to develop CAM away from the shell. The eggs were candled on the 5th day of incubation and every day, thereafter, nonviable embryos were removed. On the 10th day, a window (2 x 2 cm) was made on the equator of the eggs through which formulations (0.5 ml) were instilled. Effects were measured by the onset of: (1) hemorrhage; (2) coagulation; and (3) vessel lysis. A 0.9% w/v NaCl solution was used as a control. The scores were recorded according to the scoring schemes [29] as listed in Table 2.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Score</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No visible hemorrhage</td>
<td>0</td>
<td>Non irritant</td>
</tr>
<tr>
<td>Just visible membrane discoloration</td>
<td>1</td>
<td>Mild irritant</td>
</tr>
<tr>
<td>Structures are covered partially due to membrane discoloration or hemorrhage</td>
<td>2</td>
<td>Moderately irritant</td>
</tr>
<tr>
<td>Structures are covered totally due to membrane discoloration or hemorrhage</td>
<td>3</td>
<td>Severely irritant</td>
</tr>
</tbody>
</table>

2.14 Microbiological testing

The optimized ocular inserts of norfloxacin were evaluated for microbiological study in *vitro*. Nutrient agar seeded with the test organism *E. coli*. was allowed to solidify in the petri dish. Ocular insert was placed over the agar layer. The plates were then incubated at 37 ± 0.5°C for 24 h. After incubation the zone of inhibition around the ocular insert was measured.

3. Result and Discussion

In the present study, efforts have been made to prepare ocular inserts of norfloxacin using polymers such as HPMC, SCMC, sodium alginate, chitosan and carbopol. The drug delivery system was designed as a matrix. Formulations were developed using film casting method. Various researchers have studied the mechanism of film formation from polymer dispersions. The three stages of film formation are (i) evaporation of casting solvent and subsequent concentration of polymer particles; (ii) deformation and coalescence of polymer particles; (iii) further fusion by interdiffusion of polymeric molecules of adjacent polymer particles [30]. The formulated inserts were translucent, light yellow coloured, smooth in texture, uniform in appearance and showed no visible crack. The ocular inserts were evaluated for physicochemical characteristics and in *vitro* drug release. The physicochemical data presented in Table 3 shows that the weight of ocular inserts was found to be in the range of 17.43 ± 0.35 to 19.76 ± 0.12 mg. The data suggested uniformity in weight as indicated by low values of standard deviation within the batch. The ocular inserts had a thickness that ranged from 0.25 ± 0.01 mm to 0.41 ± 0.01 mm. The low standard deviation of the measured thickness of all formulations ensured uniformity of thickness. It was observed that both, the weight and thickness of the inserts increased with the increasing total polymer concentration. The surface pH of ocular inserts varied between 5.80 ± 0.00 to 6.40 ± 0.05. It indicates that the inserts will not cause irritation on application as the pH is within the accepted ocular range [26]. The average drug content was consistent in all batches and ranged from 96.66 ± 0.29 to 98.50 ± 0.86%. The folding endurance was determined for all formulations manually and the films did not show any crack for more than 300 folds and it revealed the good film forming property for all the polymers [27]. The strength of ocular inserts is a significant factor with respect to damage during handling and long term.

<table>
<thead>
<tr>
<th>Table 2: Scoring chart for HET-CAM test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
</tr>
<tr>
<td>No visible hemorrhage</td>
</tr>
<tr>
<td>Just visible membrane discoloration</td>
</tr>
<tr>
<td>Structures are covered partially due to membrane discoloration or hemorrhage</td>
</tr>
<tr>
<td>Structures are covered totally due to membrane discoloration or hemorrhage</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3: Physicochemical parameters of bioadhesive ocular inserts of norfloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
</tr>
<tr>
<td>F1</td>
</tr>
<tr>
<td>F2</td>
</tr>
<tr>
<td>F3</td>
</tr>
<tr>
<td>F4</td>
</tr>
<tr>
<td>F5</td>
</tr>
</tbody>
</table>

All values are mean ± SD (n = 3); *Value as mean ± SD (n = 10).
The strength and flexibility of inserts is expressed by the tensile strength and elongation to break [30]. It was concluded from the satisfactory elongation at break parameters for all inserts that addition of PEG 400 as a plasticizer formed inserts of good mechanical properties [31]. The tensile strength of the ocular inserts ranged from 1.20 ± 0.03 to 2.40 ± 0.01 g/mm². Formulation F5 showed minimum tensile strength of 1.20 ± 0.03 g/mm². Maximum tensile strength of 2.40 ± 0.01 g/mm² was observed with formulation F4. The values for percent elongation at break ranged from 10.36 ± 0.21 to 30.46 ± 0.45. It was observed that formulation F4 showed least percent elongation at break of 10.36 ± 0.21 and formulation F5 exhibited maximum value of 30.46 ± 0.45.

3.1 Swelling Index
Swelling test was conducted to measure the bulk hydrophilicity and hydration of polymers as it affects drug release from polymeric matrix. The minimum swelling index value 185.50 ± 0.50% was observed with formulation F1 which swelled rapidly and expanded in its size. Formulation F4 showed maximum swelling index value of 1250.00 ± 2% with great expansion in its size though it maintained its integrity throughout the study (Fig. 1). It is reported that the high swelling capacity of F4 is attributed to the extremely hydrophilic nature of chitosan due to the presence of hydroxyl and amino groups in its structure that have the ability to interact with water molecules [11]. The formulation F2 got swelled in comparatively shorter time. Its swelling index value was 198.66 ± 1.52%. The films of formulation F3 maintained their integrity throughout the swelling study and swelling index value ranges from 61.66 ± 2.08% to 451.00 ± 1%. The formulation F5 was soft and sticky. The swollen ocular insert (F5) failed to preserve its integrity and was easily fragmented when removed from the swelling medium. Its swelling index value was 600.66 ± 0.57%. The swelling of the polymer is required for initiating its bioadhesive character that starts shortly after the beginning of swelling by weak bonds. Following that, the adhesion increases with the increase in polymer hydration leads to a sudden drop in adhesive strength as a result of distentanglement at the polymer tissue interface [24]. Added to that, the rate and extent of insert hydration and swelling affect the drug release from the insert. So swelling index property plays a major role in bioadhesion of ocular insert as well as on drug release from ocular insert. In order to be a good bioadhesive polymer it must make intimate contact with the membrane. All the ocular inserts showed appreciable bioadhesive detachment force that varied from 20.4 ± 0.26 x 10⁻³ to 36.5 ± 0.10 x 10⁻³ dynes/cm².

The bioadhesive values show considerable potential of sustaining the residence and enhancing contact with ocular tissue. Various factors affect the bioadhesion of ocular delivery systems because of the composition, physicochemical properties and structure of the tear film. Different theories like electronic, adsorption, wetting, diffusion or interpenetration were proposed to describe bioadhesion [31]. Formulation F1 showed least bioadhesive detachment force of 20.4 ± 0.26 x 10⁻³ dynes/cm². The highest bioadhesive detachment force (36.5 ± 0.10 x 10⁻³ dynes/cm²) of formulation F4 could be attributed to the fact that at neutral and alkaline pH, chitosan has numerous amine and hydroxyl groups as well as a number of amino groups that might increase the interaction with the negatively charged group in biological membrane, resulting in effective bioadhesion. An anionic polymer carbopol is a polyacrylic acid derivative. Its mucoadhesive property is due to hydrogen bonding with mucin. The adhesive behaviour of sodium alginate was due to the low surface tension of the alginate, which was lower than the critical surface tension of the mucin-coated cornea, resulting in good adhesion [32].

3.2 In vitro drug release
In vitro release study revealed that formulations F2 and F5 showed sustained drug release for a period of 4 h.
Formulations F1, F3 and F4 sustained the drug release for a period of 6 h (Fig. 2). Formulation F1 released 99.00% drug in 6 h and maintained its integrity throughout the release period. F2 made with SCMC released 98.83% drug in 4 h. Though the cumulative drug release (CDR) was comparable to F1 however, the rate of drug release was faster. In the case of SCMC composition, excessive hydration could lead to a decrease in formulation consistency and hence weaken the bioadhesive bond thereby resulting in comparatively less sustainment of drug release than others. On the other hand F3 (made with sodium alginate) sustained the drug release for 6 h with a CDR of 97.66%. Its integrity was maintained throughout the release period.
Formulation F4 made with chitosan released 98.36% drug in 6 h. The ocular insert made with carbopol was very soft and sticky and the swollen ocular insert failed to maintain its integrity and because of that 98.30% drug released in 4 h.

The data obtained from in vitro release studies of all the five formulations was subjected to kinetic modeling, in order to determine the mechanism of drug release. The preference of a certain release mechanism was based on the correlation coefficient value for the parameters studied, where the highest correlation coefficient is preferred for the selection of the mechanism of drug release. It was observed that the release data from films were near to 0.997 for Higuchi model (Table 4). It indicated that the release of norfloxacin from the films followed diffusion controlled release mechanism.

Further to confirm the exact mechanism of drug release from these films, the data was fitted according to Korsmeyer equation which is a simple empirical equation to describe general solute release behaviour from controlled release polymer release matrices [33].

\[ \frac{M_t}{M_{\infty}} = k \cdot t^n \]  

Eq. 5

Where \( \frac{M_t}{M_{\infty}} \) was fraction of drug released, \( k \) was kinetic constant, \( t \) was release time and \( n \) was the diffusion exponent for drug release. In this model, the value of \( n \) characterizes the release mechanism of drug.

When \( n = 0.5 \) corresponds to a Fickian diffusion mechanism, 0.5 < \( n \) < 1 to non-Fickian transport, \( n = 1 \), the release is zero order [34]. The portion of the release curve where \( \frac{M_t}{M_{\infty}} < 0.6 \), should be used to find the exponent of \( n \) [35]. Hence in this case the drug release from the matrix is controlled by both the phenomenon diffusion as well as swelling as value of \( n \) for all the formulation ranges between 0.5 - 1.

3.3 Drug Excipient Compatibility

Compatibility studies were carried out to ascertain any kind of interaction of the drug with the excipients used in the formulations of ocular insert. The FTIR spectra (Figure 3A) shows a peak at 3511 nm for the –NH stretching, at 1845 nm for –C=O stretching and at 932 nm for –C-C stretching. All the above peaks were retained drug loaded ocular inserts (Figure 2 B, C, D, E and F). As there were no shifting, disappearance and broadening of the peak observed in the spectrum, it can be concluded that no chemical interaction had occurred.

Table 4: Kinetic modelling of the release data of bioadhesive ocular inserts of norfloxacin

<table>
<thead>
<tr>
<th>Regression coefficient value</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-order release</td>
<td>0.987</td>
<td>0.983</td>
<td>0.981</td>
<td>0.987</td>
<td>0.96</td>
</tr>
<tr>
<td>First-order release</td>
<td>0.822</td>
<td>0.875</td>
<td>0.853</td>
<td>0.929</td>
<td>0.955</td>
</tr>
<tr>
<td>Higuchi model</td>
<td>0.996</td>
<td>0.997</td>
<td>0.997</td>
<td>0.992</td>
<td>0.988</td>
</tr>
<tr>
<td>Korsmeyer-peppas model</td>
<td>0.995</td>
<td>0.998</td>
<td>0.996</td>
<td>0.984</td>
<td>0.999</td>
</tr>
<tr>
<td>n value for Korsmeyer-peppas model</td>
<td>0.600</td>
<td>0.544</td>
<td>0.565</td>
<td>0.534</td>
<td>0.523</td>
</tr>
</tbody>
</table>

On the basis of physicochemical characterization and in-vitro drug release study formulation F4 (optimized formulation) was selected for HET-CAM test. The chick embryo chorioallantoic membrane is an extraembryonic membrane. Because of its extensive vascularization and easy accessibility, the CAM has been widely used to study the eye irritancy test. Testing with incubated eggs is a borderline case between in vivo and in vitro systems so it does not conflict with the ethical and legal obligations. The obtained result from formulation F4 was compared with those obtained using normal saline, which was used as the control that is supposed to be practically non-irritant. The formulation F4 did not produce any injury in the part of chorioallantoic membrane so it was found to be non-irritant and well tolerated. The selected formulation F4 showed antimicrobial activity against E. coli when tested microbiologically on solidified agar. The obtained diameter of clear zones of inhibition was 25.66 ± 0.57 mm against test organism. Thus inserts of norfloxacin with chitosan
were found to be active, as indicated by clear zone of inhibition.

4. Conclusion
Norfloxacin bioadhesive ocular inserts were prepared using bioadhesive polymers HPMC, SCMC, sodium alginate, chitosan and carbopol934 with the aim of sustaining drug release. Chitosan based ocular insert not only had adequate bioadhesive strength as well as had sustained drug release for up to 6 h. Results of HET-CAM test showed that the formulation is non irritant and well tolerated. In vitro microbiological challenge concluded that norfloxacin bioadhesive ocular insert can be successfully administered for the treatment of bacterial conjunctivitis associated with E. coli infection.

References