Research Article

Preparation and Evaluation of Bioadhesive Ocular Inserts of Aceclofenac

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ABSTRACT

In the present work, bioadhesive ocular inserts of aceclofenac were developed to increase the contact time between the preparation and the conjunctival tissue to ensure a sustained release. Bioadhesive ocular inserts of aceclofenac were prepared using polymers HPMC, SCMC, sodium alginate, chitosan and carbopol 934 and PVP K30 as film forming polymer. Solvent casting method was used to prepare ocular inserts. Bioadhesive ocular inserts were evaluated for various parameters such as 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, HET CAM test for eye irritancy. *In vitro* drug release studies were performed using donor-receptor compartment model. To access the mechanism of drug release, in vitro drug release data was treated according to zero order, first order, Korsemeyer Peppas and Higuchi kinetics. It was concluded from kinetic studies that drug release from matrix was governed by both diffusion and swelling phenomena. Chitosan based ocular insert showed maximum bioadhesive strength. Ocular insert consisting of sodium alginate was found to be best film as it showed acceptable pH, good bioadhesive strength and gave a sustained drug release (98.33% at 6 h).

Keywords: Aceclofenac, conjunctivitis, HPMC K15M, SCMC, Sodium alginate, chitosan, carbopol 934.

INTRODUCTION

A Fine transparent membrane that lines the eyelids and the front of the eyeball is known as Conjunctiva. Where it lines the eyelids it consists of highly vascular columner epithelium. Corneal conjunctiva consists of avascular stratified epithelium. When the eyelids are closed the conjunctiva forms a closed sac. It protects the delicate cornea and the front of eye.[1]

Conjunctivitis is defined as inflammation of the conjunctiva due to various infectious agents like bacteria, viruses, or fungi and noninfectious causes like allergic, chemical and mechanical. In bacterial conjunctivitis, mild to severe purulent discharge persists throughout the day. Bacterial conjunctivitis is commonly classified according to its clinical manifestations: hyper acute, acute, or chronic. Viral conjunctivitis typically presents as an itchy red eye with mild watery discharge. Allergic conjunctivitis is also common. Patients typically report itching and redness of both eyes in response to an allergen exposure.[2]

Most ocular diseases are cured with topical application of solutions administered as eye-drops.[3] Poor bioavailability of drugs from ophthalmic dosage forms is mainly due to the tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium.[4]

Different drug delivery systems have been used to deliver drugs to the eye. An effective approach to improve the ocular bioavailability of topically applied drugs and diminishing the adverse effects is the use of polymer vehicles, liposomes, nano particles or polymeric inserts which resist drug drainage from pre corneal area.[5]

The advantages of ocular inserts in comparison with liquid formulations are numerous. Because of the prolonged retention of the device and a controlled release, the effective drug concentration in eye can be maintained over extended time period. Dosing of the drugs can also be more accurate and the risk of systemic side-effects can be decreased. Inserts without appropriate bioadhesive properties can move around on the ocular surface, causing further irritation and might be easily lost.[6]

In the present study, a bioadhesive ocular insert of non-steroidal anti-inflammatory drug aceclofenac is developed. Aceclofenac will provide relief from ocular irritation. The rationale of preparing formulation is to increase the contact of drug with ocular tissue and provide relief from conjunctivitis.

MATERIALS AND METHODS

Materials

Aceclofenac was obtained as a gift sample from Intas Pharmaceutical Limited, Ahmedabad, and India. Polyvinyl pyrrolidone K 30 was purchased from HiMedia Laboratories Private Limited, Mumbai, India. Hydroxy propyl methyl cellulose K 15M was obtained as a gift sample from Colorcon Asia Private Limited, Goa, India. Sodium carboxy methyl cellulose was purchased from

Ases Chemical Works, Jodhpur, India. Chitosan was purchased from Marine Chemicals Cochin, Kerala, India. Sodium alginate and carbopol 934 were purchased from Loba Chemie Private Limited, Mumbai, India. Polyethylene glycol 400 was purchased from Merck Specialities Private Limited, Mumbai, India. All the chemicals used were of analytical grade.

Formulation of ocular insert

Bioadhesive ocular inserts of aceclofenac were prepared using film casting method[7,8] [Table 1]. Hydoxy propyl methyl cellulose K 15M, sodium carboxy methyl cellulose, chitosan, carbopol 934, and sodium alginate were employed as bioadhesive materials. PVP K-30 was used as a film-forming polymer. Poly ethylene glycol 400 was used as plasticizer. PVP K-30 solution (6% w/v) was prepared in ethanol using mechanical stirrer (600 rpm) which was fitted with four bladed paddle at room temperature. It was mixed with bioa dhesive polymeric hydrogel that 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. Carbopol hydrogel was neutralized to pH 6.9-7.2 using triethanolamine. Chitosan hydrogel was prepared by dispersing the polymer in 1% w/v acetic acid solution. 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 (25°C). Aceclofenac and poly ethylene glycol 400 were added under constant stirring (600 rpm) in polymeric gel. The aqueous (hydroalcoholic) polymeric hydrogels were poured onto mercury surface-containing glass rings (6 cm diameter and 10 ml volume) placed over mercury in the glass petridishes and dried at 38°C in an oven for 24 h. The films were stored in a desiccator at room temperature after wrapping in sealed plastic sheets. The prepared formulations were evaluated for 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. The formulation F3 was subjected to HET CAM test for eye irritancy.

Table 1: Composition of aceclofenac bioadhesive ocular inserts

Ingredients	Formulations				
	F1	F2	F3	F4	F5
Drug % w/v	2.0	2.0	2.0	2.0	2.0
PVP K30 (gm)	6.0	6.0	6.0	6.0	6.0
1% w/v HPMC K 15M (ml)	1.0	-	-	-	-
1% w/v SCMC (ml)	-	1.0	-	-	-
1% w/v Sodium alginate (ml)	-	-	0.5	-	-
1% w/v Chitosan (ml)	-	-	-	0.5	-
1% w/v Carbopol 934 (ml)	-	-	-	-	1.0
PEG 400 (w/w of polymer)	1.0	1.0	1.0	1.0	1.0
Ethanol (ml) q.s.	100.0	100.0	100.0	100.0	100.0

EVALUATION OF BIOADHESIVE OCULAR INSERTS

Physical characterization

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

Uniformity of weight

Inserts from each formulation were randomly selected and weighed individually on electronic balance. Mean weight of inserts (n = 10) of each formulation was recorded.

Uniformity of thickness

Thickness of inserts was determined using a vernier caliper (digital vernier caliper, Aerospace, Mumbai) and recorded as the mean of ten measurements.

Surface pH determination

Inserts were left to swell for 1 h on an agar plate. 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 ($2H_2O$): 0.008 g, and purified water q. s. 100 ml) having pH 7.4 under stirring and then pouring the solution into a petridish 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.[9]

Folding endurance value

It is expressed as the number of folds (number of times the insert is folded at the same place) either to break the insert or to develop visible cracks. The insert (n=3) was folded in the centre, between the fingers and the thumb, and then opened. This was termed as one folding. The process was repeated till the insert showed breakage or cracks in centre of insert. The total folding operations were named as folding endurance value.[9]

Drug content uniformity

It was determined by assaying the individual insert. Ocular insert of each formulation was dissolved in suitable quantity of simulated tear fluid (pH=7.4) and the solution was filtered and content was analyzed spectrophotometrically at 273.20 nm (UV-Shimadzu 1800, Kyoto, Japan). This test was performed on three ocular inserts for each formulation.

Mechanical strength

An ocular insert with good tensile strength and percent elongation 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 method used.

The design of apparatus consisted of a base plate with a pulley aligned on it. One aluminum 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 aluminum 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 slowly increased 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 simultaneous distance travelled by the pointer on the scale indicated the elongation at break.[10] The following parameters were calculated as per equations:

Tensile strength (g/mm^2) =

$$\frac{\text{Break Force (gm)}}{\text{Cross sectional area of the sample (mm2)}} \qquad \text{Eq. 1}$$

Elongation at break (%) =

$$\left\{\frac{\text{Increase in length at break point(mm)}}{\text{Original length (mm)}}\right\} \times 100 \qquad \text{Eq. 2}$$

Swelling test

Swelling test was conducted to measure the bulk hydrophilicity and hydration of polymers as it affects drug release from polymeric matrix. 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°C ± 1^{0}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.[10]

The formula used for calculation of swelling index is:

$$\text{Swelling index} = \left[\frac{wt - w0}{w0}\right] \times 100 \qquad \text{Eq. 3}$$

Where W_0 and Wt represent initial weight of the sample and weight of the sample at t time respectively.

***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, 37°C) 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.[8, 11] The detachment stress was calculated by using formula:

$$\text{Detachment stress (dyne/cm}^2\text{)} = \frac{\text{Weight required for the detachment of insert} \times \text{Acceleration due to gravity}}{\text{Area of tissue exposed}}$$

Eq. 4

Figure 1: Modified physical balance for determination of bioadhesive strength of ocular insert

***In vitro* drug release studies**

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. Semi-permeable 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 semi permeable cellophane 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 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.[12,13]

HET CAM test for eye irritancy

Hen's egg test-chorioallantoic membrane (HET-CAM) test was carried out on fertilized hen's eggs. Three eggs for each formulation (weight 50-60 g) were selected and candled in order to discard the defective ones. The eggs were incubated in humidification chamber at a temperature of 37 ± 0.5°C for 3 days. After every 12 h, the trays containing eggs were rotated manually in a gentle manner. On day 3, 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. For the development of CAM away from the shell, the eggs were kept in the equatorial position. The eggs were candled on the fifth day of incubation and every day, thereafter, nonviable embryos were removed. On the tenth 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% NaCl solution was used as a control because it had been reported to be practically non irritant. The scores were recorded according to the scoring schemes[14] as listed in table 2.

Table 2: Scoring chart for HET-CAM test

Effect	Scores	Inference
No visible hemorrhage	0	Non irritant
Just visible membrane discoloration	1	Mild irritant
Structures are covered partially due to membrane discoloration or hemorrhage	2	Moderately irritant
Structures are covered totally due to membrane discoloration or hemorrhages	3	Severe irritant

RESULTS AND DISCUSSION

The present investigation was undertaken with the objective of preparing a bioadhesive ocular insert of aceclofenac using PVP K-30 as the matrix former and hydoxy propyl methyl cellulose K 15M, sodium carboxy methyl cellulose, chitosan, carbopol 934, and sodium alginate were employed as bioadhesive materials. Poly ethylene glycol 400 was used as plasticizer in the preparation to get inserts with good elasticity.[15] Film casting procedure was followed to prepare formulations that resulted in the preparation of uniform aceclofenac bioadhesive ocular inserts. Various researchers have studied the mechanism of film formation from polymer dispersions.[12] 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 inter diffusion of polymeric molecules of adjacent polymer particles. The prepared inserts were translucent, colour less, and smooth in texture, uniform in appearance and showed no visible crack. Ocular inserts were evaluated on the basis of physico-chemical characteristics and in vitro release studies.

Table 3: Physicochemical parameters of ocular inserts of aceclofenac

Formulation code	Weight* (mg)	Thickness* (mm)	pH*	% Drug Content#
F1	19.47 ± 0.04	0.37 ± 0.01	6.20 ± 0.06	96.50 ± 0.50
F2	19.84 ± 0.05	0.42 ± 0.01	6.40 ± 0.00	98.33 ± 0.28
F3	18.18 ± 0.04	0.31 ± 0.01	6.10 ± 0.00	98.83 ± 0.28
F4	17.27 ± 0.04	0.24 ± 0.01	5.70 ± 0.06	97.83 ± 0.28
F5	18.51 ± 0.07	0.35 ± 0.01	6.00 ± 0.10	97.16 ± 0.57

*Value as mean ± SD (n = 10). #Value as mean ± SD (n = 3)

Physicochemical data presented in Table 3 gives information of weight, thickness, surface pH and drug content. The ocular inserts had a weight varying from 17.27 ± 0.04 mg to 19.84 ± 0.05 mg. The low standard deviation of the measured weight of all formulations ensured uniformity of weight. The ocular inserts had a thickness that ranges from 0.24 ± 0.005 mg to 0.42 ± 0.005 mm. It was found that low standard deviation value indicates the uniformity of thickness. It was observed that the thickness and weight of the inserts increased with the increasing total polymer concentration. pH of ocular inserts varied from 5.7 to 6.4. It indicates that the inserts did not have an irritation potential as the pH is within the accepted ocular range. The average percent drug content was consistent in all batches and ranges from 96.50 ± 0.50 to 98.83 ± 0.28.

The values of folding endurance, tensile strength, percentage elongation and percentage equilibrium swelling and detachment force of ocular inserts of aceclofenac are shown in table 4. The recorded values of folding endurance for all batches was greater than 300, which was considered satisfactory and reveals good film properties.[10] The strength of ocular inserts is an important consideration with respect to damage during handling and long term durability, and may govern some aspects of the insert performance. The strength and flexibility of inserts is expressed by the tensile strength and elongation to break. Addition of PEG 400 as a plasticizer gave inserts of good mechanical properties as evident from the satisfactory elongation at break parameters for all inserts. The tensile strength of the ocular inserts ranged from 1.20 ± 0.02 g/mm^2 to 2.39 ± 0.01 g/mm^2. Formulation F5 showed minimum tensile strength of 1.20 ± 0.03 g/mm^2. Maximum tensile strength of 2.39 ± 0.01 g/mm^2 was observed with F4. The values for percent elongation at break ranged from 10.46 ± 0.15 to 30.6 ± 0.26.

Table 4: Folding endurance, tensile strength, percentage elongation and percentage equilibrium swelling and detachment force of ocular inserts of aceclofenac

Formulation code	Folding endurance value	Tensile strength (g/mm^2)	(%) Elongation	(%)equilibrium swelling	Detachment force (dyne/cm^2 x 10^{-3})
F1	> 300	1.22 ± 0.01	20.63 ± 0.15	180.30 ± 0.28	20.33 ± 0.28
F2	> 300	1.81 ± 0.01	10.56 ± 0.15	200.40 ± 0.35	25.40 ± 0.36
F3	> 300	2.02 ± 0.14	10.50 ± 0.20	400.16 ± 0.28	27.66 ± 0.21
F4	> 300	2.39 ± 0.01	10.46 ± 0.15	1200.00 ± 0.57	36.30 ± 0.26
F5	> 300	1.20 ± 0.03	30.60 ± 0.26	600.00 ± 0.15	29.50 ± 0.43

Value as mean ± SD (n = 3)

It was observed that formulation F4 showed least percent elongation at break of 10.46 ± 0.15 and formulation F5 exhibited maximum value of 30.6 ± 0.26.[16]

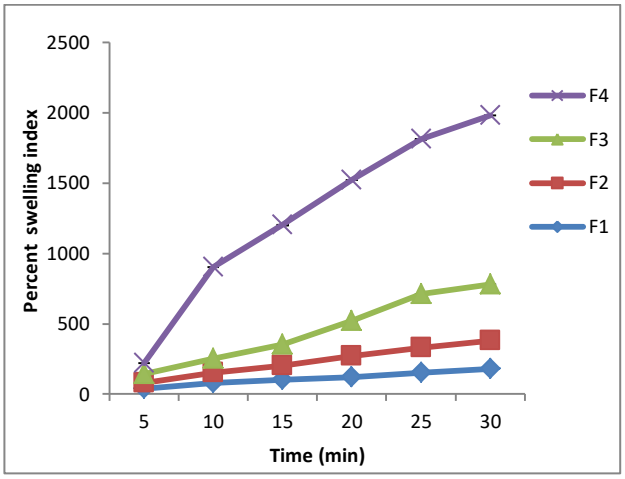

Figure 2: swelling index of ocular inserts from batch F1 to F5

Swelling test was investigated to measure the bulk hydrophilicity and hydration of polymers as it affects drug release from polymeric matrix. The HPMC-based ocular insert belonging to formulation F1 swelled rapidly and expanded in its size. Its swelling index value was 180.30%. The formulation F2 took the shortest time for swelling. Its swelling index value was 200.4%. The films of formulation F3 maintained their integrity throughout the swelling study and swelling index values ranges from 60% to 400%. It was also evidenced by swelling study that highest swelling capacity was observed for formulation F4 where rapid increase in its swelling index accompanied by great expansion in swelling index occurred. The formulation F4 maintained their integrity throughout the swelling study and swelling index values ranges from 80.46% to 1200.00%. It is reported that the high swelling capacity of F4 is attributed to the extremely hydrophilic nature of chitosan as a consequence of the presence of hydroxyl and amino groups in its structure that have the ability to interact with water molecules.[7] The formulation F5 was very soft and sticky. The swollen ocular inserts failed to preserve its integrity and was easily fragmented upon removal from the swelling medium. Its swelling index value was 600.00%. The swelling of the polymer is essential 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 distent anglement at the polymer tissue interface. [7] 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 bio adhesion of ocular insert as well as on drug release from ocular insert.

All inserts showed appreciable bioadhesive detachment force, which varied from 20.33 ± 0.28 dyne/cm^2 x 10^{-3} to 36.3 ± 0.26 dyne/cm^2 x 10^{-3} . It shows a potential of sustaining the residence and enhancing contact with ocular tissue. Various factors will influence the bio adhesion 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.[17] In order to be a good bioadhesive polymer it must make intimate contact with the membrane. Formulation F1 showed least bioadhesive detachment force of 20.33 ± 0.28 dyne/cm^2 x 10^{-3}. The highest bioadhesive detachment force (36.3 ± 0.264 dyne/cm^2 x 10^{-3}) 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 bio adhesion. Carbopol is an anionic polymer and is a polyacrylic acid derivative. Its muco adhesive property is due to hydrogen bonding with mucin. The adhesive behavior of sodium alginate was because of the low surface tension of the alginate, which was lower than the critical surface tension of the mucin-coated cornea, resulting in good adhesion.[17]

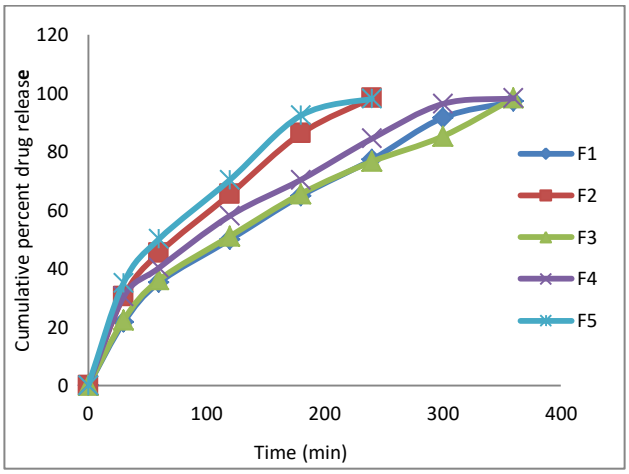

Figure 3: cumulative percent drug release versus time

Figure 3 shows the graph of cumulative percentage of drug released as a function of time for all the five formulations. In vitro release study revealed that F1, F3 and F4 formulations showed sustained drug release for a period of 6 h. F2 and F5 formulations showed sustained drug release for a period of 4 h. From F1 formulation 97.2% drug released in 6 h. They maintained their integrity throughout the release period.

In the case of SCMC, excessive hydration could lead to a decrease in formulation consistency and hence weaken the bioadhesive bond and results in comparatively less sustained drug release than others. From F2 formulation 98.37% drug released in 4 h. From F3 formulation 98.33% drug released in 6 h. They maintained their integrity throughout the release period. The films formed with chitosan maintained their integrity throughout the swelling study. F4 formulation released 96.40% drug in 5 h. The films formed with carbopol were very soft and sticky. The swollen ocular inserts failed to preserve its integrity and because of that 98.16% drug released in 4 h.

In order to determine the release mechanism that provides the best description to the pattern of drug release, the in vitro release data were fitted to zero-order, first-order, diffusion-controlled release mechanism according to the higuchi model. 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 revealed that the release data from films were near to 0.99 for higuchi model. It indicated that the release of aceclofenac from the films followed diffusion controlled release mechanism.

In order to confirm the exact mechanism of drug release from these films, the data were fitted according to Korsmeyer et al equation which is a simple empirical equation to describe general solute release behaviour from controlled release polymer release matrices:

$M_t/M_\infty = k.t^n$

Where M_t/M_∞ 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. To find out the exponent of n the portion of the release curve, where $M_t/M_\infty < 0.6$ should only be used.[18, 19]

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.

Table 5: Kinetic modeling for the formulations

	F1	F2	F3	F4	F5
Regression coefficient value for Zero-order release	0.9840	0.9840	0.9810	0.9680	0.9610
Regression coefficient value for First-order release	0.8790	0.8950	0.8180	0.9010	0.9560
Regression coefficient value for Higuchi model	0.9930	0.9970	0.9970	0.9920	0.9880
Regression coefficient value for Korsmeyer-peppas model	0.9920	0.9990	0.9960	0.9990	0.9990
n value for Korsmeyer-peppas model	0.546	0.551	0.58	0.531	0.5

On the basis of *in-vitro* drug release study formulation F3 was selected for HET-CAM test. The chick embryo chorioallantoic membrane (CAM) is an extra embryonic 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 F3 was compared with those obtained using normal saline, which was used as the control that supposed to be practically non-irritant. The formulation F3 did not

produce any injury in the part of chorioallantoic membrane so it was found to be non-irritant.

CONCLUSION

Ocular inserts of aceclofenac were prepared using bioadhesive polymers HPMC, SCMC, sodium alginate, chitosan and carbopol 934 with the aim of sustaining drug release. Sodium alginate 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 for sodium alginate based bioadhesive film showed that the formulation is non-irritant. It may be concluded that bioadhesive ocular insert can be a promising drug delivery system for aceclofenac in providing relief from inflammation in conjunctivitis.

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