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Research paper

FORMULATION DEVELOPMENT AND EVALUATION OF OCULAR INSERTS OF DICLOFENAC SODIUM FOR CONTROLLED DRUG DELIVERY

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We envisaged that the problem of poor ocular bioavailability could be solved by increasing contact time of the drug on ocular surface by using mucoadhesive agents/polymers. Initially the objective of study was to screen various polymers for their mucoadhesive properties on goat, sheep & buffalo corneal surface and the reason for choosing goat, sheep & buffalo corneal is based on the earlier report. which suggest that due to the great morphological uniformity of mammalian cornea like goat, sheep & buffalo corneal they are suitable for *in-vitro* ocular permeation studies. The main object of present work has been to overcome the existing problems of poor ocular bioavailability arising out of different ocular barriers and pre-corneal factors. Conventional dosage forms for topical drug delivery are associated with their inherent limitations that make less drug availability and hence require frequent dosing to attain desired therapeutic concentration. Utilization of the principles of controlled release by the means of ocular formulation development seems attractive approach to enhance drug availability at the desired site. The polymers showing best mucoadhesive properties, residence time and minimal damage to cornea has been selected for the enhancement of ocular bioavailability of non-steroidal anti-inflammatory drugs, namely diclofenac In addition to evaluation of role of polymers in increasing ocular bioavailability, the effect of presence and absence of other additives like preservatives, chelating agents, tonicity modifiers in formulations containing, diclofenac.

Keywords: Ocusert Pilo-20 , Dibutylphthalate, PEG600, PVA, HPMC

INTRODUCTION

A significant challenge to (bypass) the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy.[1] A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time.[2] Consequently it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of in situ gel or colloidal suspension or using ocular insert to prolong the precorneal drug retention.[3] Typically topical ocular drug administration is accomplished by eye drops, but they have only a short contact time on the eye surface. The contact, and thereby duration of drug action, can be prolonged by formulation design. (e.g. gels, gelifying formulations, ointments and inserts). [4]The progress in materials sciences and pharmaceutical formulation have provided new

exciting possibilities to develop controlled release formulations to deliver drugs to the posterior segment and to guide the healing process after surgery.[5] Even through the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia. It is advantageous for corneal penetration to adjust the pH of the solution to increase the proportion of unionized drug in the instilled dose.[6] Drugs, which are highly water insoluble, do not readily permeate the cornea. It is throught that tears are largely absorbed by the mucous membrane that lines the ducts and the lachrymal sac; only a small amount reaches the nasal passage. Typical physical parameters include pH, osmolality, viscosity, color and appearance of the product. Chemical parameters include assays for the active and degradation product and preservative content.[7] Microbiological parameters include sterility and antimicrobial preservative efficacy of the product and bioburden of all components.[8] There are several bioadhesive polymers now available with varying degree of mucoadhesive performance Carboxymethyl cellulose, Carbopol, Carbopol and hydroxypropyl cellulose. Osmotically controlled inserts have also been described, where release is by diffusion and osmotically controlled. Due to difficulty with self-insertion, foreign body sensation, only few insert products are listed and pharmaceutical manu-facturers are not actively developing inserts for commercialization.

Disadvantages of ocuserts- Sometimes the insert twists to form 'a figure eight', which diminishes the delivery rate.

Advantages of ocuserts- Reduction of the number of administrations and thus better patient compliance, comfort.[9]

MATERIAL AND METHODS

The diclofenac sodium ocular inserts were prepared by solvent casting method. Twelve batches (F1 to F12) of formulation were prepared using drug and polymers as shown in table 13. The polymer was dissolved in ethanol (8 ml) under stirring condition. The weighed amount of diclofenac sodium (106 mg, passed throughsieve# 400) was added to prepared solution and stirred for 12 h to get uniform dispersion. Diclofenac sodium(M.P. Biomedical, Ltd; Mumbai), Poly vinyl alcohol, Sodium hydroxide,Chloroform, PEG400, Potassium dichromate, Methyl cellulose, Ethanol& Dibutylphthalat(E.Merck (India) Ltd; Bombay), Sodium chloride (New India chemical, Cochi), Ethylcelloulose 15csp(p)(Genuine chemical co. Bombay), Calorimeter (DSC 60) & FTIR, U.V Spectrophotometer(Shimadzu Japan), were provided by Rajasthan college Pharmacy, Udaipur. Spectral Analysis (IR, NMR & Mass) was done at NIPER Mohali.

Method

Pre-formulation studies of diclofenac sodium Identification Tests

Identification tests were carried out as per the official methods (British Pharmacopoeia, 2005).[54]

50.0 mg of drug was dissolved in methanol and diluted to 100.0 ml with the same solvent. 2.0 ml of the solution was diluted to 50.0 ml with methanol. This was scanned between 220 nm and 370 nm. About 10 mg of drug was dissolved in 10 ml of alcohol. To 1 ml of this solution, 0.2 ml of freshly prepared mixture containing 6g/L solution of potassium ferricyanide and a 9g/L solution of ferric chloride were added. The solution was allowed to stand and protected from light for 5 min. To this 3 ml of a 10.0g/L solution of hydrochloric acid was added. Then again it was allowed to stand and protected from light for 15 min. A blue colour should develop and a precipitate is to be formed.[10]

Description/Appearance It was confirmed with naked eye to check whether it complies with the specifications of the Pharmacopoeia.

Solubility profile of Diclofenac sodium

Solubility of diclofenac sodium in common solvent

The solubility of diclofenac sodium was tested in various common solvent. A definite quantity (10mg) of drug was dissolved in 10 ml of each investigated solvent at room temperature . The solubility was observed only via visual inspection. Solubility of diclofenac sodium in various solvents was determined and compared with data given in British Pharmacopoeia [54] as shown in table 1.

Saturation solubility

For the determination of saturation solubility, excess quantity of diclofenac sodium was taken in 10 ml of different solvents and shaken in a shaking water bath (100 agitations/ min) for 24 h at room temperature. This solution was passed througha whatman no.41 filter and the amount of the drug dissolved was analyzed spectrophotometrically at 275 nm.

Table 1: Solubility of diclofenac sodium in different solvents

Solvent
Acetone
Methanol
Ethanol
Alcohol
Dichloromethane

Analytical method for estimation of the drug (UV method)

Analytical methods were employed for analysis of diclofenac sodium obtained from transcorneal permeability determination studies. Care was taken to avoid potential interference from vehicles or other chemical agents used.[11]

Method

The drug solution was scanned in between the wavelength of 200-400nm. The wavelength of 275nm was selected and used for further quantitative analysis.

Preparation of phosphate buffer, pH 7.4 (Indian Pharmacopoeia, 1996)[55]

50.0 ml of 0.2 M potassium dihydrogen phosphate was taken in a 200 ml volumetric flask followed by addition of 24.4 ml of 0.2 M sodium hydroxide and

the volume was made up to 200 ml with distilled water.

Standard plot of diclofenac sodium in methanol

Weighed quantity of diclofenac sodium (10 mg) was dissolved in methanol and the volume was made up to 100 ml with methanol to give a concentration of 100 µg/ml. From this stock solution, different volumes 0.2, 0.4, 0.6,0.8, 1.0, 1.2 and 1.4 ml were transferred into 10 ml volumetric flasks and volumes were made up to 10 ml with distilled water to get different concentrations of 2, 4, 6, 8, 10, 12 and 14 µg/ml. The absorbance was measured at 275 nm against a blank using UV spectrophotometer. The experiment was repeated in triplicate and the average of three readings was taken to plot the standard curve.[12]

Standard plot of diclofenac sodium in phosphate buffer, pH 7.4

Weighed quantity of diclofenac sodium (10 mg) was dissolved in methanol and the volume was made up to 100 ml with methanol to give a concentration of 100 µg/ml. From this stock solution different volumes were transferred into 10 ml volumetric flasks and volume were made upto 10 ml with phosphate buffer, pH 7.4 to get different concentrations ranging from 2 to 14 µg/ml concentrations. The absorbance was measured at 275 nm against a blank using UV spectrophotometer. The experiment was repeated in triplicate and the average of three readings was taken to plot the standard curve.[13]

Preparation & evaluation of diclofenac sodium ocular insert

Preparation of drug reservoir

The diclofenac sodium ocular inserts were prepared by solvent casting method. Twelve batches (F1 to F12) of formulation were prepared using drug and polymers as shown in table 13. The polymer was dissolved in ethanol (8 ml) under stirring condition. The weighed amount of diclofenac sodium (106 mg, passed throughsieve# 400) was added to prepared solution and stirred for 12 h to get uniform dispersion. After proper mixing the casting solution (3 ml) was poured in clean glass petridish (area 12.571cm^2) and covered with an inverted funnel to allow slow and uniform evaporation at room temperature for 48 h. The dried films thus obtained were cut by cork borer into circular pieces of definite size (6 mm diameter) containing 106 mg of drug. The ocular inserts were then stored in an airtight container (desiccators) under ambient condition.[14-15.]

Preparation of rate controlling membrane

The rate controlling membrane was prepared using different concentration of polymer (3%, 4% and 5%) and employing polyethylene glycol (PEG) and glycerin as a plasticizer. polyethylene glycol (PEG) was used in the concentration of 30% w/w based on the weight of dry polymer and glycerin was used in concentration 40% w/w based on the dry polymer. Films were prepared by solvent casting method using acetone as a casting solvent.[16] After drying at room temperature circular rings of 8 mm diameter were cut using cork borer and the drug reservoir was sandwiched in between the two rate controlling membrane. Sealing was done by applying chloroform on the edges of rate controlling

membrane the ocular inserts were stored in an airtight container (desiccator) under ambient condition. The prepared diclofenac sodium ocular inserts are depicted in figure 16.

Fig. 1: Diclofenac sodium ocular insert

Uniformity of weight

From each batch (n=3), inserts were taken out and weighed individually using digital balance (Asco, India). The mean weights of the ocular inserts were noted.

Uniformity of thickness

The thicknesses of the inserts were determined using a Vernier caliper (Mitotoyo, Japan) at five separate points of each insert. For each formulation n=3 inserts were taken.

Drug content

Ocular inserts were taken from each batch and dissolved / crushed in 10 ml of isotonic phosphate buffer pH 7.4 in a beaker and were filtered into 25 ml volumetric flask and the volume was made up to the mark with buffer. One ml of the above solution was withdrawn and the absorbance was measured by UV-VIS spectrophotometer (Systronics -2202, India) at 275 nm after suitable dilutions.[17]

% Moisture absorption

The percentage moisture absorption test was carried out to check physical stability / integrity of the film at humid condition. The films were weighed and placed in desiccators containing saturated solution of aluminum chloride and 84% humidity was maintained. After three days, the films were taken out and weighed. The % moisture absorption was calculated using the formulae. [18]

$$\%\ \boldsymbol{Moisture\ absorption} = \frac{\text{Final weight} - \mathit{Initial\ weight}}{\mathit{Initial\ weight}} \times 100$$

Surface pH

The Diclofenac sodium inserts were allowed to swell in closed Petri-dish at room temperature for 30 minutes in 0.1 ml of distilled water. The swollen device was removed and placed under digital pH meter (Elico, India) to determine the surface pH.

Folding endurance

Folding endurance was determined by repeatedly folding the film at the same place till breaking or first sign of breaking. The number of time the film could be folded at the same place without breaking gives the folding endurance value.[19]

***In-vitro* transcorneal permeation studies**

Whole eye ball of goat was transported from local butcher shop to the laboratory in cold (4°C) normal saline within 1 *h* of slaughtering the animal. The cornea was carefully excised along with 2 to 4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from proteins. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments of modified glass Franz diffusion cell in such way that

its epithelial surface faced the donor compartment. The receptor compartment was filled with 15 ml of freshly prepared buffer solution. One square cm of ocular insert was placed on the cornea and opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained 35°C with constant stirring, using Teflon coated magnetic stir bead. One ml sample was withdrawn from receptor compartment at various time intervals up to 24 *h*s and was analyzed spectrophotometrically at 275 nm. Each sample withdrawn was replaced with equal volume of buffer.[20,21]

Stability study

Storage at ambient condition test must also be conducted under condition which accelerates any changes occurring at ambient temperature and humidity. Changes to stability testing requirements at an international level have resulted in the following different stability long-term study conditions for hot and humid climates:

- 30°C/65%RH (e.g. WHO, ICH, SADC, GCC , Brazil)
- 30°C/70%RH (e.g. WHO previous, Cuba, Brazil previous) 30°C/75%RH (e.g. ASEAN) countries which have hot and very humid areas, such as Brazil, Cuba, China, India and all of the asian countries.

Calculations based on meteorological data have demonstrated that the existing long-term stability conditions in WHO guidelines for Zone IV (30°C/65%RH) do not reflect climatic conditions in many. [22,23.24]

All regulatory bodies accept only real time data for any drug or pharmaceutical for purpose of assessing the shelf life. Only accelerated stability studies might serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature. The accelerated stability studies were carried out in accordance with the ICH guidelines. A sufficient number of ocular inserts (packed in aluminum foil) were stored in humidity chamber, with relative humidity of 75 % and at temperature of 40 ± 0.5°C and long term testing 25°C±2°C, 60% RH. The samples were tested for drug content after 0, 3, 6 and 9 months respectively.[25]

RESULTS

Preformulation studies

Description / Appearance

The appearance of diclofenac sodium was white or almost white crystalline powder which is in

Table 2: Definition and storage condition for four climatic zones:

Climatic zones	Definition	Storage condition
I	Temperate climate	21ºC/45% RH
II	Subtropical and mediterranean climate	25ºC/60% RH
III	Hot, dry climate	30ºC/45% RH
IV	Hot, humid climate	30ºC/70%RH

compliance with British Pharmacopoeia., 2005.

Solubility

The solubility of the drug was checked and the results are shown in table 3.

Table 3: Solubility profile of diclofenac sodium in different solvents

Solvent	Solubility
Methanol	Soluble
Ethanol	Soluble
Alcohol	Soluble
Chloroform	Slightly soluble
PBS (pH 7.4)	Soluble
Water	Insoluble

Detection of absorbance

The ultraviolet spectrophotometric method was used to analyze diclofenac sodium at a wavelength of 275 nm. The standard plots of diclofenac sodium were prepared in phosphate buffer pH 7.4 and methanol (table 4).

Table 4: Absorbance data of diclofenac sodium at different concentration

Concentration (µg/ml)	Absorbance at 275 nm*	
	Phosphate buffer saline pH 7.4	Methanol
0	0	0
2	0.044	0.05
4	0.112	0.123
6	0.162	0.176
8	0.217	0.230
10	0.274	0.294
12	0.310	0.344

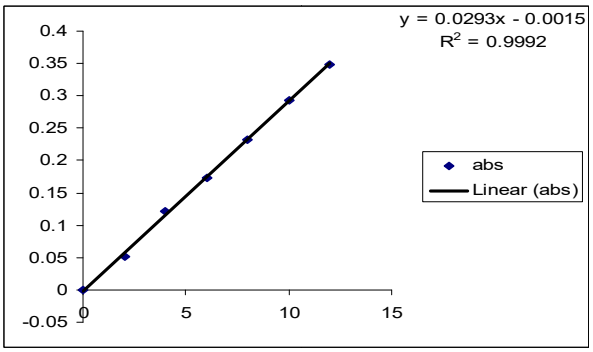

Fig. 2: Standard plot of Diclofenac sodium in methanol

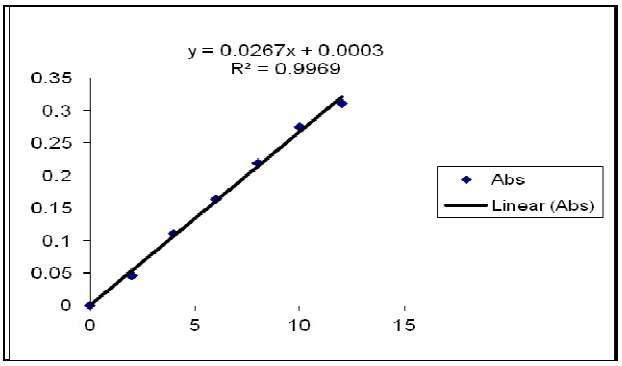

Fig. 3: Standard plot of Diclofenac sodiumin phosphate buffer pH7.4

Interaction studies

Fourier transform infrared spectroscopy

The FTIR spectra of pure drug diclofenac sodium, placebo formulations (without drug) and drug loaded ocular inserts were recorded. The results are shown in the figure 19 to 28 C=O stretching of COOH and CH bending of CH3 group respectively indicates the presence of drug in the polymer without any interaction and the peaks at 1857.95 nm, 2553.01 nm, 3027.69 and 963.53 nm confirms the presence of drug. All the above peaks were also present in drug loaded ocular inserts. From the result it is evident that peaks alone and in combination are coinciding which indicate that the drug do not seems to have interaction with the excipients of ocular insert in physical mixture.

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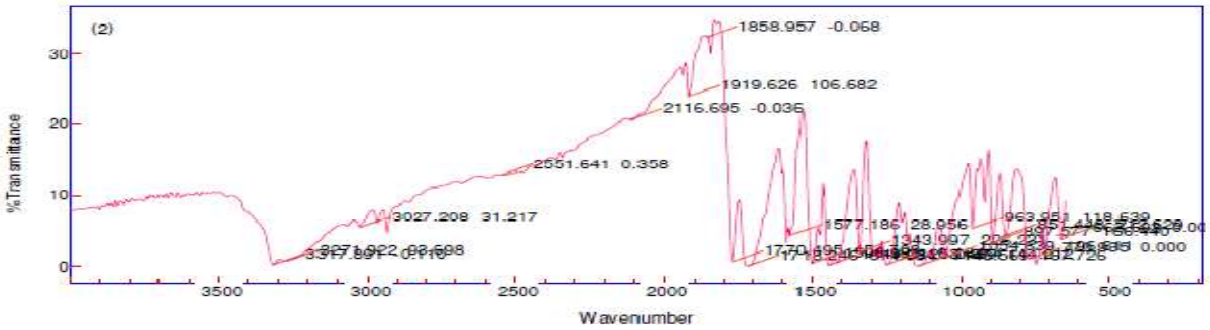

Fig. 4: FTIR spectra of pure Diclofenac sodium

Fig.5: FTIR spectra of HPMC

Fig. 6: FTIR spectra of EC

Fig.7: FTIR spectra of Chitosan

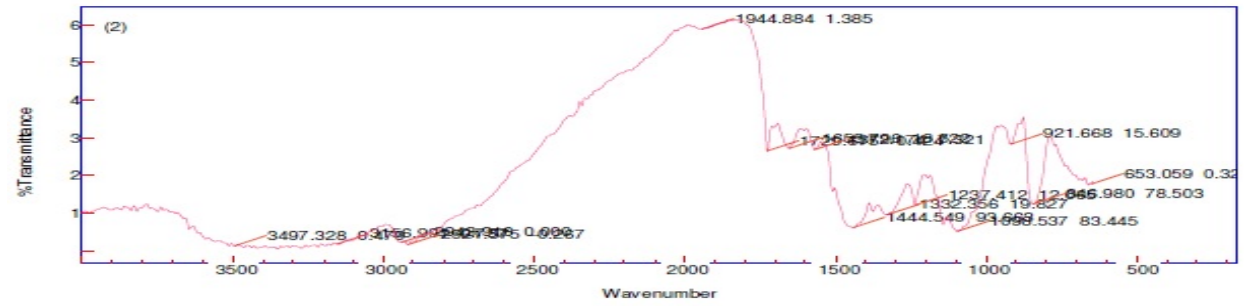

Fig.8: FTIR spectra of PVA

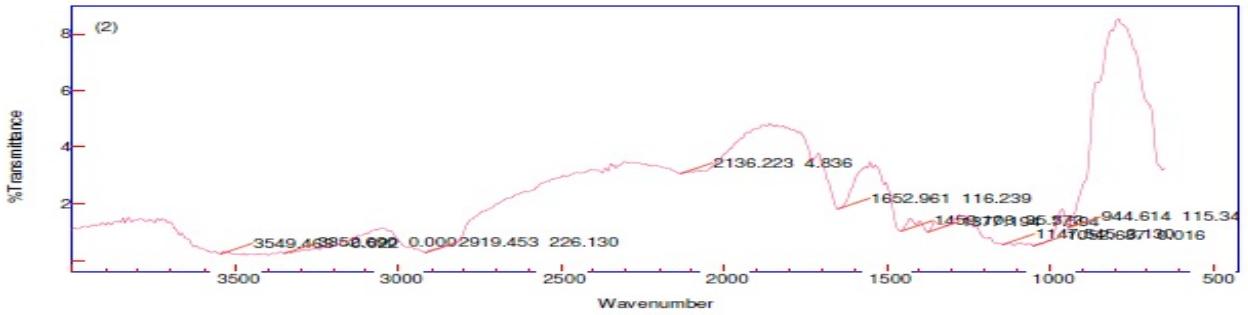

Fig. 9: FTIR spectra of MC

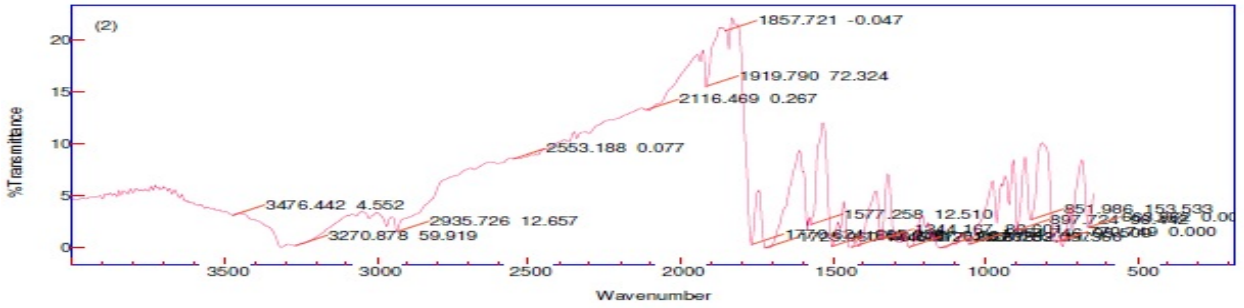

Fig.10: FTIR spectra of formulation F2

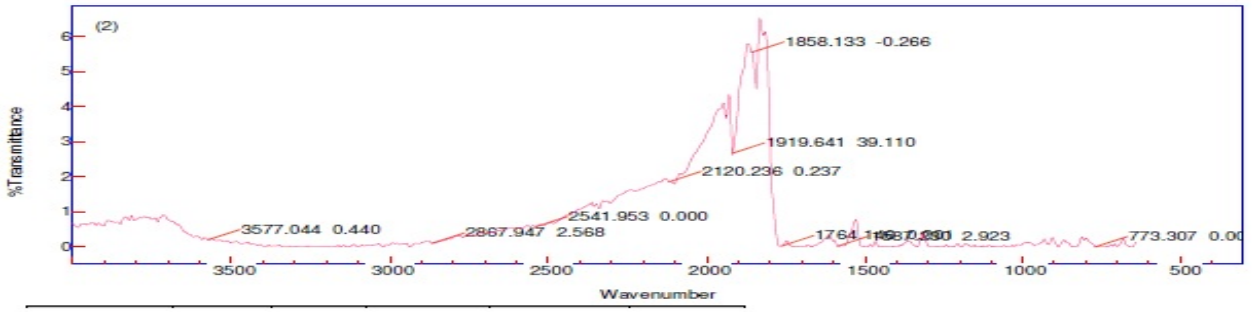

Fig.11: FTIR spectra of formulation F5

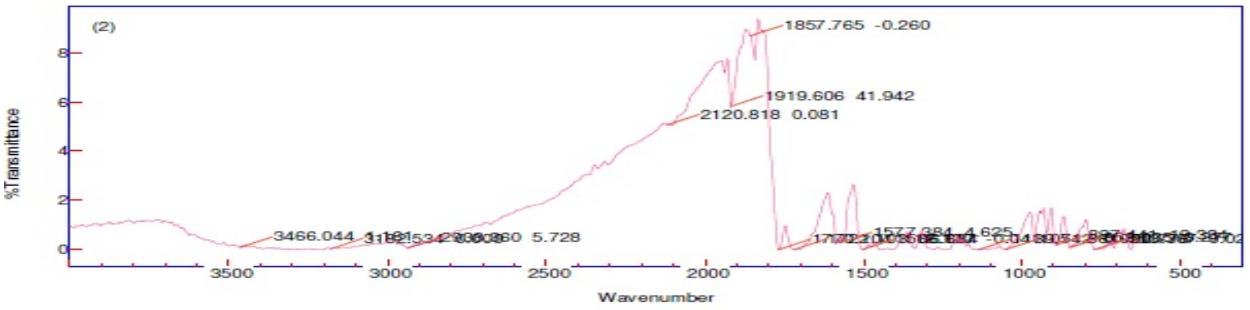

Fig. 12: 27 FTIR spectra of formulation F8

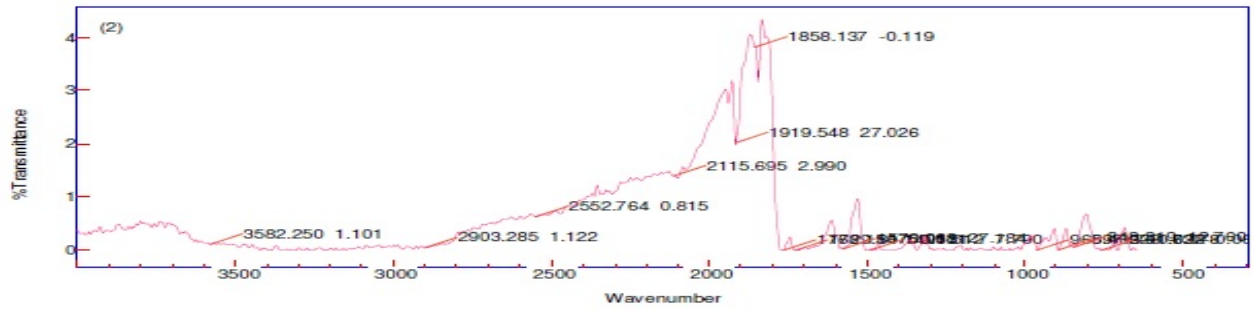

Fig. 13: FTIR spectra of formulation F11

Evaluation of Ocusert

Tensile strength and % elongation at break

Diclofenac sodium ocular insert having 5 cm of length and 0.95 cm of width were cut and held between two pair of acrylic slides with the help of clamps. One pair of acrylic slides grips upper end of ocular insert stripes, while other pair to another end by hanging a flat pan (for adding weight) with the help of metal wire. Tensile strength and % elongation at break can be conveniently observed with the help of traveling microscope. The rate of change of stress kept constant by increasing the load of flat pan at rate of 10g/2 min because stress strain relationship change with the rate of changes in stress. The tensile strength , % elongation at break and stress were calculated by using formula and tensile strength of diclofenac sodium ocular insert was found to be 0.044(0.039) to 0.198(0.024) $\frac{N}{mm^2}$. Tensile strength of ocular insert were found to be in order of F2> F8>F5> F3>F6>F4>F7> F1>F9>F10>F11>F10. However, % elongation and stress at break showed flexibility of ocular insert and it was found to be 20.86(0.61) to 33.13(0.35) % and 0.012(0.1) to 0.8(0.02) mm.

Uniformity of weight

The weights of the diclofenac sodium ocular inserts were found to be in the range of 45±0.3 mg to 74±0.7 mg (table 10). The uniformity of the weights of the films indicates good distribution of the drug, in polymer and plasticizer.

Uniformity of thickness

The thickness of the diclofenac sodium ocular insert varied between 0.31±0.017mm to 0.61±0.061mm which was found to be directly

related to concentration of polymer. (table 10) The formulations did not produce any irritation when placed in the cul de sac since they were not thick enough to produce irritation.

Drug content

For the various formulations (F1 to F12) of diclofenac sodium ocular insert drug content was found to vary between 0.610±0.001 to 0.740±0.064mg (table 10). Hence there was no significant variation among the all formulation, which indicates that the method used for ocular insert was steadfast.

% Moisture absorption

The % moisture absorption study revealed that formulation F3 & F10 showed high and low moisture. The high moisture absorption (16.3±0.12) may be due to presence of more hydrophilic polymer (HPMC) & (PVA) which are hydrophilic in nature and readily absorb moisture when exposed to atmosphere. While low moisture absorption (5.2±0.65) was found due to presence of (MC) & Chitosan which are hydrophobic in nature. The results % moisture absorption studies are shown in the (table 10).

Surface pH

The surface pH of prepared inserts was found in range of 6.4±0.056 to 7.2±0.021 (table 10) which clearly indicates that the prepared inserts would not alter the pH of the tear fluid in the eye.

Table 10. Evaluation of diclofenac sodium ocular insert of different batches

FC	Weight (mg)	Thickness (mm)	Drug content (mg)	Surface pH	% moisture absorption	Folding endurance	Tensile strength $\frac{N}{mm^2}$	% elongation	Strain mm
F1	50±0.3	0.31±0.017	0.612±0.041	6.9±0.018	8.2±0.12	55.25±1.54	0.096(0.031)	23.53(0.28)	0.4(0.07)
F2	53±0.4	0.42±0.020	0.610±0.001	7.2±0.021	10.5±0.24	58.21±1.67	0.198(0.024)	33.13(0.35)	0.8(0.02)
F3	66±0.4	0.46±0.037	0.646±0.025	7.18±0.05	16.3±0.12	62.2±1.64	0.129(0.058)	29.45(0.51)	0.32(0.08)
F4	63±0.2	0.44±0.052	0.639±0.056	7.26±0.041	5.75±0.23	52.85±2.64	0.115(0.081)	30.32(0.21)	0.2(0.06)
F5	45±0.3	0.52±0.023	0.640±0.058	6.68±0.025	6.1±0.10	45.24±1.54	0.136(0.065)	28.35(0.71)	0.1(0.05)
F6	54±0.4	0.52±0.015	0.665±0.061	7.15±0.065	5.8±0.24	50.28±1.61	0.127(0.084)	25.82(0.32)	0.23(0.2)
F7	70±0.5	0.32±0.048	0.622±0.054	7.23±0.054	8.9±0.12	55.34±1.64	0.114(0.073)	25.52(0.91)	0.19(0.02)
F8	47±0.2	0.42±0.035	0.642±0.090	6.99±0.089	9.69±0.16	54.45±2.44	0.144(0.054)	27.25(0.61)	0.18(0.01)
F9	49±0.5	0.48±0.045	0.650±0.002	6.9±0.024	11.7±0.85	54.56±1.25	0.091(0.021)	26.31(0.15)	0.22(0.03)
F10	55±0.4	0.49±0.056	0.695±0.098	6.4±0.056	5.2±0.65	41.52±1.15	0.044(0.039)	20.86(0.61)	0.035(0.02)
F11	67±0.6	0.52±0.059	0.711±0.045	7.19±0.068	7.7±0.54	45.12±1.54	0.069(0.094)	23.61(051)	0.012(0.1)
F12	74±0.7	0.61±0.061	0.740±0.064	6.9±0.054	6.2±0.45	51.45±2.54	0.85(0.064)	28.31(0.69)	0.015(0.02)

All value replicate of three observation, Figure inside the parenthesis indicate the Standard Deviation (S.D) value, FC indicate Formulation Code

Table 11 *In-vitro* drug release profile

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	0	0	0	0	0
2	10.25	17.25	8.5	6.5	7.63	5.25	8.25	15.25	8.5	4.89	4.29	4
4	22.24	29.21	18.52	14.54	12.32	10.23	20.29	28.25	15.33	10.25	8.32	7.54
6	31.25	41.21	26.15	22.21	19.34	16.85	28.39	38.35	24.12	16.45	14.21	11.56
8	41.18	53.12	36.25	30.54	26.24	22.21	36.91	50.24	33.45	25.2	22.4	20.12
10	52.55	62.15	45.25	38.55	32.21	28.12	46.23	59.01	41.54	32.8	30.01	26.25
12	60.25	72.31	52.21	46.69	42.24	36.89	55.29	67.91	50.21	40.89	36.69	34.55
18	76.45	87	65.65	60.25	55.14	47.23	70.22	84.11	63.94	55.32	48.23	50.21
24	90.32	99.54	83.25	76.89	66.25	62.23	83.32	97.24	80.28	66.45	60.1	56.11

Value as means ± SD (n=3), RMS indicate (Root mean square)

5.3.7 Folding endurance

Folding endurance of diclofenac sodium ocular insert was measured by evaluating breaking strength and endurance. This is the number of time the film may be folded at one place until it breaks or sign of breakage appears. Folding endurance various formulations (F1 to F12) of diclofenac sodium ocular insert were found to be 41.52±1.15 to 62.2±1.64. This result shows enough strength of ocular insert to withstand handling shock.

In-vitro transcorneal permeation studies

The release profile of the formulations is depicted in the figure 41 and table 20. The formulation containing HPMC, and polyvinyl alcohol (PVA) showed complete release (up to 99%) in 24 *h*. The release of the drug form the formulation F2 and F8 containing 4 % polymer ratio (HPMC and PVA) were found to be 99.54 % and 97.24 % at the end of 24 *h* respectively. On the other hand the release of the drug from the formulation F5 and F11 containing 4 % polymer ratio of chitoson and methyl cellulose respectively were found to be 66.25 % and 60.1 % at the end of 24 *h*. The extended and prolonged period of diclofenac sodium release (up to 24 *h*) observed in present study could be due to slow diffusion of drug from combined polymer and plasticizer and probably due to the formation of hydrogen bond between drug and polymer which have helped in controlled rate of drug release. The release of the drug from the formulation F1, F4, F7 and F10 with 3% polymer ration of HPMC, chitoson, Polyvinyl alcohol (PVA) and methyl cellulose MC were found to be 90.32%, 76.89%, 83.32% and 66.45% at the end of 24 *h* respectively and the release of the drug from the formulation F3, F6, F9 and F12 with 5% polymer ratio of HPMC, chitoson, Polyvinyl alcohol (PVA) and methyl cellulose MC were found to be 83.25%, 62.23, 80.28 and 56.11% . The *in - vitro* drug diffusion

data was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equations, Higuchi and Korsmeyer models to ascertain the mechanism of drug diffusion. The results of linear regression analysis of data including regression coefficient are summarized in table 21. When the regression coefficient 'r' value of zero order and first order plots were compared, it was observed that the 'r' values of zero order were in the range of 0.96 to 0.99 whereas the 'r' values of first order plots were found to be in the range of 0.57 to 0.97 indicating that drug release from all the formulations follow zero order kinetic.

Table 12. Drug release kinetics

Formulation code	Zero order	First order	Higuchi	Krosmeyerpeppas	
	RMS Values				n
F1	0.9787	0.5738	0.9789	0.9788	0.76
F2	0.9835	0.6785	0.9933	0.9642	0.57
F3	0.9862	0.6865	0.9796	0.9847	0.74
F4	0.9888	0.6954	0.9888	0.9911	0.73
F5	0.9968	0.9768	0.9654	0.9657	0.58
F6	0.9874	0.9665	0.9681	0.9698	0.54
F7	0.9889	0.9625	0.9592	0.9609	0.58
F8	0.9665	0.9742	0.9569	0.9799	0.71
F9	0.9865	0.9733	0.9689	0.9632	0.56
F10	0.9878	0.9714	0.9659	0.9663	0.57
F11	0.9789	0.9784	096468	0.9723	0.77
F12	0.9784	0.9684	0.9782	0.9821	0.86

Value as means ± SD (n=3), RMS indicate (Root mean square)

Stability study

Two formulations were selected based on their evaluation parameters as well as prolonged drug release study. The results showed in table 24 reveals that there was no change in physical appearance of ocular insert. The drug content showed no marked change after nine months and formulation F2 and F8 passed the stability test. Results clearly show that ocular insert F2 and F8 are chemically, physically and microbiologically stable at room temperature for 9 months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life. In conclusion both the formulations were stable and no major degradation was found and a shelf life of 9 month can be safely assigned to the ocular insert F2 and F8.

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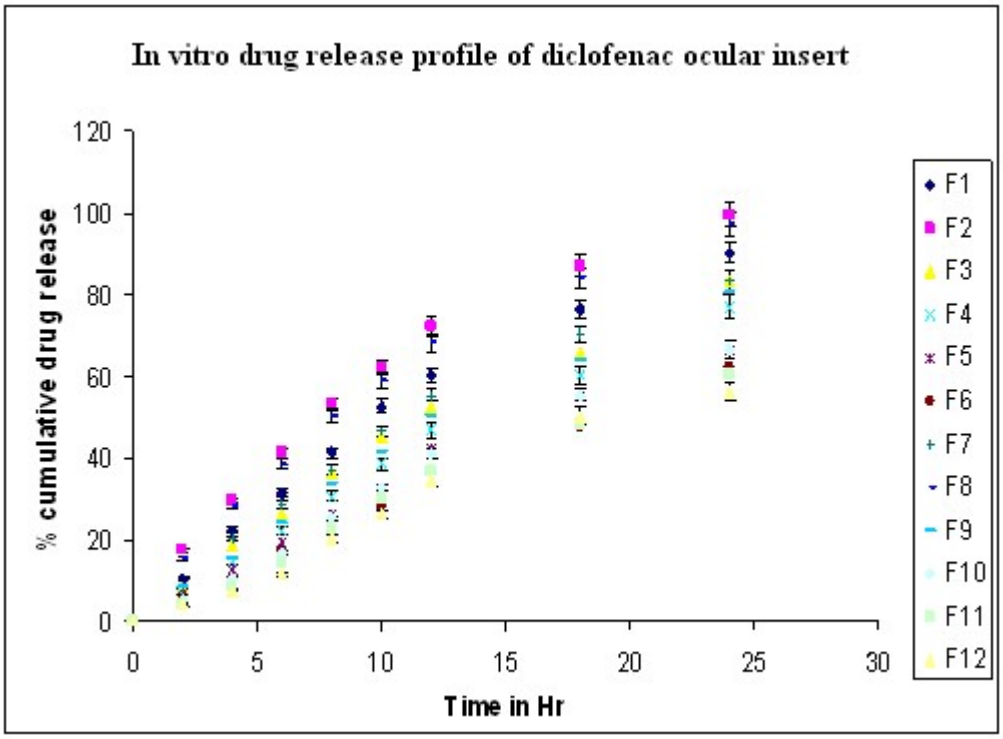

Fig.14: *In-vitro* drug release data of diclofenac sodium ocular insert containing different polymer

Table 13: Stability study of diclofenac sodium ocular insert.

Formulation code	25°C±2°C ± 60 %RH					40°C±2°C ± 75 %RH				
	Physical appearance	% Drug content				Physical appearance	% Drug content			
Months		0	3	6	9		0	3	6	9
F2	+++	98±0.8	97±0.2	94±0.9	92±77	+++	98±0.2	96±0.7	94±0.5	89±44
F8	+++	98±0.1	95±51	90±12	89±81	+++	98±0.3	94±23	92±28	85±66

(Mean±SD, n=3), +++ Good physical appearance (ocular inserts were thin, transparent and visually smooth surfaced)

DISCUSSION

We envisaged that the problem of poor ocular bioavailability could be solved by increasing contact time of the drug on ocular surface by using mucoadhesive agents/polymers. Initially the objective of study was to screen various polymers for their mucoadhesive properties on goat, sheep & buffalo corneal surface and the reason for choosing goat, sheep & buffalo corneal is based on the earlier report. The release of the drug form the formulation F2 and F8 containing 4 % polymer ratio (HPMC and PVA) were found to be 99.54 % and 97.24 % at the end of 24 *h* respectively. On the other hand the release of the drug from the formulation F5 and F11 containing 4 % polymer ratio of chitoson and methyl cellulose respectively

were found to be 66.25 % and 60.1 % at the end of 24 *h*. The extended and prolonged period of diclofenac sodium release (up to 24 *h*) observed in present study could be due to slow diffusion of drug from combined polymer and plasticizer and probably due to the formation of hydrogen bond between drug and polymer which have helped in controlled rate of drug release. The release of the drug from the formulation F1, F4, F7 and F10 with 3% polymer ration of HPMC, chitoson, Polyvinyl alcohol (PVA) and methyl cellulose MC were found to be 90.32%, 76.89%, 83.32% and 66.45% at the end of 24 *h* respectively and the release of the drug from the formulation F3, F6, F9 and F12 with 5% polymer ratio of HPMC, chitoson, Polyvinyl alcohol (PVA) and methyl cellulose MC were found to be 83.25%, 62.23, 80.28 and 56.11% . The *in - vitro* drug diffusion data was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equations, Higuchi and Korsmeyer models to ascertain the mechanism of drug diffusion. The results of linear regression analysis of data including regression coefficient are summarized in table 21. When the regression coefficient 'r' value of zero order and first order plots were compared, it was observed that the 'r' values of zero order were in the range of 0.96 to 0.99 whereas the 'r' values of first order plots were found to be in the range of 0.57 to 0.97 indicating that drug release from all the formulations follow zero order kinetic.

CONCLUSION

Two formulations were selected based on their evaluation parameters as well as prolonged drug release study. The results showed in table 24 reveals that there was no change in physical appearance of ocular insert. The drug content showed no marked change after nine months and formulation F2 and F8 passed the stability test. Results clearly show that ocular insert F2 and F8 are chemically, physically and microbiologically stable at room temperature for 9 months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life. In conclusion both the formulations were stable and no major degradation was found and a shelf life of 9 month can be safely assigned to the ocular insert F2 and F8. Folding endurance of diclofenac sodium ocular insert was measured by evaluating breaking strength and endurance. This is the number of time the film may be folded at one place until it breaks or sign of breakage appears. Folding endurance various formulations (F1 to F12) of diclofenac sodium ocular insert were found to be 41.52±1.15 to 62.2±1.64. This result shows enough strength of ocular insert to withstand handling shock. The % moisture absorption study revealed that formulation F3 & F10 showed high and low moisture. The high moisture absorption (16.3±0.12) may be due to presence of more hydrophilic polymer (HPMC) & (PVA) which are hydrophilic in nature and readily absorb moisture when exposed to atmosphere. While low moisture absorption (5.2±0.65) was found due to presence of (MC) & Chitosan which are hydrophobic in nature. The results % moisture absorption studies

are shown in the (table 10). For the various formulations (F1 to F12) of diclofenac sodium ocular insert drug content was found to vary between 0.610±0.001 to 0.740±0.064mg (table 10). Hence there was no significant variation among the all formulation, which indicates that the method used for ocular insert was steadfast.

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