Original Article

Studies on Formulation and Evaluation of Eudragit RS PO Based Nanoparticulate System of Aceclofenac for Ocular Delivery

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ABSTRACT

Aim: Ocular drug delivery is the most challenging and interesting goal in front of the pharmaceutical scientist. Ocular inflammation is the most commonly affecting disease of the eye. The objectives of present investigation were to formulate and evaluate eudragit RS PO (ERS PO) based Aceclofenac (ACF) Nano suspension for ophthalmic application. **Materials and Methods:** The ACF Nano suspensions were prepared by Nano precipitation method and the optimized formulation was lyophilized and further characterized for, particle size, polydispersity index, zeta potential, drug entrapment efficiency, *in-vitro* drug release study, pH study, *ex-vivo* trans corneal study, corneal hydration (%) study, Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Colorimetry (DSC), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). **Results:** The optimized ACF Nano suspension was compared with standard ACF solution prepared according to USP. The Nano suspensions were prepared successfully by Nano precipitation method. The FTIR, DSC and XRD studies confirmed absence of drug-polymer interactions. DSC and XRD results reflected the amorphization of drug in formulation. The SEM images revealed that there will be no irritation cause after ocular administration due to spherical and smooth surface. The results of *in vitro* drug release study indicated that higher % entrapment efficiency of drug in Nano suspension delays the drug release and increase the corneal residence time. **Conclusion:** From *ex vivo* trans corneal study it was found that optimized formulation showed higher % permeation of drug as compared with 0.1 % Aceclofenac solution with no signs of corneal damage and eye friendly behavior.

Key words: Aceclofenac, Nanosuspension, Occular, Eudragit, Permeation.

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INTRODUCTION

Ocular drug delivery is today's one of the most challenging and interesting goal in front of the pharmaceutical scientist. The delivery of drugs to the certain areas of the eye is relatively inaccessible by systemic route and it has been a difficult task because of the complex anatomy and physiological barriers of the eye.[1] Ocular inflammation is the most commonly affecting disease of the eye. Inflammation is caused by a cellular and vascular response to the injury, ischemia, infection and excessive or inappropriate operation of immune mechanism. The response is raised by release of some chemical mediators such as acidic lipids e.g. prostaglandins, leukotriene's, vasoactive amines, thromboxane's, cytokines etc and activation of inflammatory cells.[2,3] Medications administered via topical route offers advantages like rapid action, avoidance of hepatic first-pass metabolism, avoidance of systemic side effects, patient compliance etc. Moreover, certain areas of an eye are relatively inaccessible to drugs delivered by systemic route because of different physiological barriers of eye such as the epithelial, blood-retinal barrier and blood-aqueous barrier.[1] The use of

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topical therapy of corticosteroids is quite common in the treating ocular inflammatory disorders, allergic diseases and dry eye syndrome but the corticosteroid's use is often limited and associated with severe adverse effects such as increase in intraocular pressure, cataract formation, glaucoma progression and risk of infection.[4] To devoid these severe side effects, non-steroidal anti-inflammatory drugs (NSAIDs) have been found to be safer alternatives to steroids in treating inflammation at ocular site.[5] Aceclofenac, BCS class II, is a NSAID which is structurally similar to diclofenac. Aceclofenac acts by preferential selective inhibition of cyclooxygenase-2 (COX-2) after conversion into an active metabolite which could be excessively beneficient in ocular inflammation.[6] Aceclofenac also have inhibitory effect on the tumor necrosis factor-α and interleukin-1 secretion. Furthermore, Aceclofenac have good anti-inflammatory, analgesic activities and have better gastric tolerability as compared with some other NSAIDs i. e. diclofenac and indomethacin.

Mostly, all ocular medications has been given to the patient as aqueous solution but 90% of the topically applied dose from such aqueous solutions is lost because of the rapid tear turnover and pre-corneal losses i. e. lacrimation and drainage that lead to less ocular bioavailability.[7] Hence, there is a grateful need for an ideal ocular drug delivery system which could increase the contact time of the drug molecule with the eye surface and promote the transport of drug molecules into the eye tissue.

Various approaches that have been used earlier for enhancement of ocular bioavailability include use of viscosity and penetration enhancers, ocular inserts, pro drug approach and *in situ* gel. These approaches are suitable to solve the problems of less permeable drugs but could not resolve the issue of less water soluble drugs. Furthermore, these formulations are non-compliant which lead to blurring of the vision due to increasing viscosity, using gels, inserts etc.[8]

Nanotechnology and particle-engineering is a rapidly developing field in which Nano precipitation have been used to improve stability of poorly soluble drugs. Along with improving stability, Nano precipitation method is used to enhance aqueous dissolution rate and ocular availability of less water soluble drugs.[9]

There are various colloidal drug delivery approaches such as polymeric micelles, liposomes, nanocapsules/ nanoparticles; dendrimers possess all the important characteristics for improved ocular bioavailability. Nanoparticles have well tolerability and deposits in the cul-de-sac of an eye for prolonged time period because of their small size. Nanoparticles also prolong the drug residence time and ensure the optimal contact between the eye mucosa and the formulation resulting in sufficient drug concentration in ocular tissues.[10]

Nano particulate drug delivery approach has been widely used to avoid the limitations of the conventional ocular formulations like eye drops, suspensions such as overcoming various disadvantages of aqueous drops like rapid tear turnover, lacrimation, drainage, blinking reflex etc. Other advantages of nanoparticles include, prevention of any sight impairments of eye and is well tolerated which helps to increase patient compliance. Moreover, self-administration as compared to the implants and other devices is possible.[11]

Due to the above mentioned reasons, an ophthalmic drug delivery system approach such as Nano suspension which is used to overcome the various disadvantages of conventional ocular dosage forms, possesses controlled or sustained delivery of ophthalmic drugs with increase in corneal residence time and enhances aqueous solubility of BCS class II drugs would be beneficial.[12] Nano suspensions are defined as biphasic system and colloidal dispersions composed of drug particles which are dispersed and suspended in an aqueous solvent in which the diameter of the particles is less than 1000 nm in size.[13] Eudragit RS PO is a copolymer of poly(ethyl-acrylate, methyl-methacrylate and chlorotrimethyl-ammonio ethyl methacrylate) which contains 4.5–6.8 % of quaternary ammonium groups, hence due to its positively charged surface that interacts with negatively charged drugs or with the cellular surface of a target tissue such as anionic cornea. Therefore positive charge on polymer is responsible for its mucoadhesive property.[14,15] It has been previously stated, in treating ocular inflammatory disorders, NSAIDs are superiorly preferred instead of steroid like drugs because of less ocular adverse effects. Among the NSAIDs, one could present therapeutic benefit by selectively inhibiting COX-2 enzyme, as COX-2 produces prostaglandin at the site of inflammation. Thus, Aceclofenac is a COX-2 inhibitor and seems to be an ideal candidate for treating ocular inflammatory conditions.

The objective of the present research was to formulate and evaluate eudragit RS PO (ERS PO) based Aceclofenac (ACF) Nano suspension for ophthalmic application and to improve ocular bioavailability of Aceclofenac.

MATERIALS AND METHODS

Materials

ACF was received as gift sample from Ranbaxy Research Lab-oratories (Gurgaon, India). Eudragit RS PO was

procured from Roehm Pharma polymer, Degussa, Mumbai. All other chemicals utilized were of analytical grade agents and fresh eyeballs of goat were purchased from local butcher's shop (Satara, India) within one hour of animal slaughtering.

Methods

Formulation of ACF Nano suspensions using 3^2 factorial design

The ACF Nano suspensions were prepared by a Nano precipitation method with a slight modification to the previously reported process.[5] To prepare the Nano suspension, drug (40mg) and specific quantity of ERS PO were dissolved in 10 ml acetone. Then the solution of the drug and polymer was poured into distilled water (40 ml) containing specific quantity of hydrophilic surfactant PVA under constant stirring by magnetic stirrer (Remi Equipment Ltd. Mumbai, India.) at 500 rpm. The above solution was stirred continuously for 2 hr to evaporate the solvent and the final volume of the Nano suspension was collected. The different Nano suspension formulations were prepared using a range of concentrations of surfactant (0.1 % w/v to 0.3 %w/v), polymer (360mg to 440mg) as shown in the Tables 1-3.

Freeze drying of nanosuspension

The optimized formulation of ACF Nano suspension was lyophilized by deep freezing using Quick freezer (Remi, Model RQFV-170) for 48 hr at 33°C and then dried by using Freeze dryer (Delvac, mini lyodel) at -80°C and pressure 0.100 mbar for 48 hr. After 48 hr, sample was completely dried. Mannitol (5 % w/v) was used as a cryoprotectant in formulation before freeze drying. Lyophilized sample was collected and stored in desiccators until further study.

Table 1: Design layout of Aceclofenac Nanosuspensions (ANS) by using 3^2 factorial design.

Formulation code	Variable X_1	Variable X_2
ANS 1	-1	-1
ANS 2	-1	0
ANS 3	-1	+1
ANS 4	0	-1
ANS 5	0	0
ANS 6	0	+1
ANS 7	+1	-1
ANS 8	+1	0
ANS 9	+1	+1

Where +1 is higher level, 0 is mid-level and -1 is lower level for independent variable

Table 2: Different factors with coded value.

Level	X_1 (Drug: Polymer ratio)	X_2 (Surfactant conc. (%w/v)
-1	1: 9	0.1
0	1: 10	0.2
+1	1: 11	0.3

Table 3: Composition of final batches of ACF Nano suspension by using 3^2 factorial design.

Formulation Code	Drug (ACF) mg	Polymer (ERS PO) mg	Surfactant (PVA) mg
ANS 1	40	360	40
ANS 2	40	360	80
ANS 3	40	360	120
ANS 4	40	400	40
ANS 5	40	400	80
ANS 6	40	400	120
ANS 7	40	440	40
ANS 8	40	440	80
ANS 9	40	440	120

Evaluation of ACF nanosuspension

The ACF Nano suspensions were further characterized for, particle size, polydispersity index, zeta potential, drug entrapment efficiency, *in-vitro* drug release study, pH study, *ex-vivo* trans corneal study, corneal hydration (%) study, Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Colorimetry (DSC), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM).

Particle size, Polydispersity index and Zeta potential measurements

The mean particle size and polydispersity index for the ACF Nano suspensions were determined by Nanoparticle Analyzer SZ-100 (Horiba Scientific, Japan). The zeta potential was determined by a laser Doppler anemometer coupled with Nanoparticle Analyzer SZ-100 (Horiba Scientific, Japan). The dispersed formulations were measured after dilution (1:100) i.e. 1 ml formulation was dispersed in 100 ml HPLC water to produce the required count rate (50-200) to enable accurate measurement. All experiments were done in triplicate.[16]

Determination of drug entrapment efficiency (%)

Entrapment efficiency of ACF Nano suspensions was determined by determining free or entrapped drug concentration in formulation by centrifugation method.[17] The ACF Nano suspension (10 ml) was centrifuged at

9000 rpm at 4°C using the cooling centrifuge instrument (Remi C-30 Equipment's Ltd. Mumbai, India) for 45 min. The supernatant was separated out; the absorbance was recorded for the free drug content using a UV/ Visible spectrophotometer (Shimadzu 1800, Japan) at 273 nm. The % entrapment efficiency (EE %) of all batches were calculated by using following equation.

$$\text{Entrapment efficienty (\%)} = \frac{\text{Initial drug} - \text{free drug}}{\text{Initial drug}} \times 100$$

pH study

The pH of ophthalmic Nano suspension formulations should be in range of 6.5 to 7.6 as at this pH range formulations do not cause any irritation to the patient upon administration. The pH of the prepared formulations was checked by using pH meter (Hanna instruments).[18]

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy

The infrared spectra of pure drug, polymer, physical mixture (ACF+ERS PO) in 1:1 proportion and freeze dried Nano suspension (ANS 8) was obtained using ATR-FTIR spectrophotometer (Shimadzu, IRAFFINITY-1 E. Japan). The spectra were obtained for the range of 500-4000 cm^{-1}.[19]

X-ray diffraction (XRD)

The XRD studies were performed to study crystalline state characteristics of samples. The x-ray diffraction pattern of ACF, ERS PO, physical mixture (ACF+ERS PO) in the 1:1 drug polymer ratio and freeze dried Nano suspension (ANS 8) were recorded with XPERT-PRO X-ray diffractometer (Seifert and Co. D 2070, Ahrensburg) using PRS measurement program using Ni-filtered, Cu Ka radiation with a voltage of 45 Kv and a current of 40 Ma. The instrument was operated in the continuous scanning speed over 2θ range of 5° to 40°.[17]

Differential Scanning Calorimetry (DSC)

The DSC spectra's of pure ACF, ERS PO, physical mixture (ACF+ERS PO) in 1:1 drug to polymer ratio and freeze dried Nano suspension (ANS 8) were obtained using differential scanning calorimeter (Shimadzu DSC 60, Japan). About 5 mg of sample was heated in a hermetically sealed aluminum pans in the temperature range of 30°C to 300°C at a heating rate of 10°C/ min. and nitrogen purge at 50ml/ min. through cooling unit.[20]

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM) was performed for determining the particle surface morphology and shape of Nano suspension. SEM micrographs of optimized freeze dried Nano suspension were obtained using a Hitachi scanning electron microscope (model S-2600 N, Tokyo, Japan) operating in the high-vacuum mode and an acceleration voltage of 20 Kv.[21]

In vitro drug release study

The *in-vitro* drug release study of 0.1 % ACF solution was performed for 3 hr and all formulations of ACF Nano suspension were performed for 8 hr in the modified USP dissolution apparatus-1 composed of a two sided open glass cylinder. A previously soaked dialysis membrane was properly attached to the terminal part of the glass cylinder. The 2 ml of Nano suspension was added accurately into the glass cylinder and then this glass cylinder was fixed on the stirrer of apparatus. The stirrer was dipped in 200 ml dissolution medium of Sorenson's phosphate buffer (pH 7.4) where temperature is maintained at 37°C± 0.5°C at 25 rpm so that the dialysis membrane of cylinder end just touched the dissolution medium surface. Aliquots of 4 ml sample were removed at different predetermined time intervals with volume replacement. The withdrawn samples were properly diluted with Sorenson's phosphate buffer (pH 7.4) and analyzed for drug content, by measuring absorbance at 273 nm in the UV/Visible spectrophotometer (Shimadzu 1800, Japan). All the experiments were properly performed in triplicate.[10,17]

Ex-vivo trans corneal permeation study

Ex-vivo trans corneal permeation studies were performed using freshly excised goat corneas. The cornea was attached between receptor and donor compartments of Franz diffusion cell. The cornea was fixed in such manner that epithelial surface of cornea faced the donor cell. The area of cornea for diffusion was 0.50 cm^2. Freshly prepared 20 ml simulated tear fluid (pH 7.4) was filled in the receptor compartment. All air bubbles were removed out from the compartment. An aliquot (1 ml) of 0.1 % ACF solution and optimized Nano suspension (ANS 8) separately were placed on the cornea and cover slip was kept over the opening of the donor cell to seal it; receptor medium was kept at 37°C temperature and stirred constantly by use of a Teflon-coated magnetic bead. This study was performed for 2 hr. After specific time interval samples were removed from receptor, diluted properly and analyzed accurately for drug content by recording absorbance at 273 nm in the UV/ Visible spectrophotometer (Shimadzu 1800, Japan). Results were expressed as % permeation and amount of drug permeated which is directly related with *in vitro* ocular availability.[5,17] The % permeation or *in vitro* ocular availability was calculated by following formula:

$$\text{Permeation (\%)} = \frac{\text{Amount of drug permeated in receptor}}{\text{Initial amount of drug in donor}} \times 100$$

Corneal hydration (%)

The effect of the 0.1 % ACF solution and optimized ACF Nano suspension (ANS 8) on corneal hydration was determined separately. The corneal hydration study is one of the important parameter to assess any damage to corneal tissue (epithelium and/or endothelium). The tissue of sclera was removed from corneal surface at the end of experiment and its epithelial surface was cleaned and wiped with help of filter paper and weighed. The weight is considered as initial weight. Then the cornea was soaked in 1 ml of methanol, properly dried overnight at temperature of 90°C and reweighed. Reweighed weight was considered as final weight. From the difference between initial and final weight, % corneal hydration was calculated by following formula.[5]

$$\text{\% Corneal hydration} = \frac{(\text{Initial weight} - \text{Final weight})}{(\text{initial weight})} \times 100$$

Drug release kinetics modeling

The kinetics of ACF release from Nano suspension was determined by using various release kinetics and the drug release mechanism as like zero order kinetics, first order kinetics, Higuchi model, Hixson-crowell model and Korsmeyer-Peppas model. The release data were obtained by calculating various parameters. The parameters exponent coefficient (n) and regression coefficient (R^2) were determined by equation of Korsmeyer-Peppas model to understand the drug release mechanism. The model which best fits the drug release data was choosed based on the R^2 value in different models. The model that gives high R value was considered as best fit model.[16,22]

Comparative study of optimized ACF nanosuspension with standard ACF solution

The optimized ACF nanosuspension was compared with standard ACF solution prepared according to USP and was further evaluated for *in vitro* drug release, *ex-vivo* trans corneal permeation study and corneal hydration (%).

Preparation of 0.1 % ACF solution pH (7.2)

ACF solution of 0.1 % (w/v pH 7.2) concentration was made into isotonic phosphate buffer according to USP. The required quantity of drug was properly dissolved in 100 ml isotonic phosphate buffer pH 7.2 to have 0.1 % (w/v) concentration.[23]

RESULTS AND DISCUSSION

Particle size, Zeta potential measurements and Polydispersity index of ACF Nano suspension

Particle size

The appearance of bluish opalescence color in Nano suspension revealed that the formulation was in nanometers range. The particle size of ANS formulations was observed in between 74.9±9.6 nm to 88.9±14.7nm (Table 4). The smallest particle size was observed with ANS 7 whereas ANS 3 showed largest particle size. The results showed that drug to polymer ratio does not have significant impact on mean particle size but it was observed that increase in the Concentration of PVA increased mean particle size of all Nano suspensions except ANS 4. The role of stabilizer in the nanoparticles preparation is long-term physical stabilization of nanoparticles. It attaches to the surface of particles and prevents aggregation of particles. The positive effect of PVA concentration on particle size was observed. The reason behind this is, the increased concentration of PVA affects the viscosity of formulations. Increased viscosity of formulations due to the increase in PVA concentration may result in formation of larger nanoparticles. The mean particle size of optimized batch (ANS 8) was 77.1±13.9 nm. All batches showed a small mean size (below 500nm), suitable for possible ocular application. In trial studies, particle size increases with the polymer concentration due to the steep difference of polymer concentration among batches, but in final batches the negative effect of drug polymer- ratio on particle size observed due to the less difference of polymer concentration among batches and may be due to the presence of interaction of stabilizer in formulation.

Effect of independent variables on particle size using 3^2 factorial design

The factorial design suggested a linear model for the effect of particle size. The relationship between the coded factors and particle size was studied using equation 1 and 3D plots (Figure 1).

$$\text{Particle size} = 80.08 - 3.02A + 2.38B \quad (1)$$

As per the ANOVA results (Table 5) for optimization process, the model F-value of 7.84 was obtained, which suggested that model is significant. There is only a 2.12 % chance that an *F*-value this large could occur because of noise. *P*-value is less than 0.0500 for A and B which

Table 4: Results of Physicochemical characterization of ACF nanosuspensions.

Batch code	Formulation variables		Formulation response				
	Drug: Polymer ratio	Conc. of PVA (% w/v)	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Entrapment efficiency (%)	pH
ANS 1	1:9	0.1	79.4+11.3	34+3.30	0.351+0.01	72.62+0.91	6.43+0.04
ANS 2	1:9	0.2	82.1+9.2	41.6+4.82	0.391+0.04	70.50+0.44	6.66+0.04
ANS 3	1:9	0.3	88.9+14.7	35.5+2.96	0.322+0.06	74.74+0.83	6.50+0.00
ANS 4	1:10	0.1	80.3+19.6	29.9+4.18	0.337+0.08	80.25+0.36	7.06+0.04
ANS 5	1:10	0.2	78+6.8	35.1+6.03	0.358+0.05	75.59+0.42	6.80+0.04
ANS 6	1:10	0.3	79.7+21.5	27+1.50	0.303+0.02	77.71+0.17	6.90+0.00
ANS 7	1:11	0.1	74.9+9.6	31+4.68	0.351+0.01	86.18+0.81	7.23+0.04
ANS 8	1:11	0.2	77.1+13.9	34.3+1.70	0.312+0.09	91.27+0.11	7.30+0.00
ANS 9	1:11	0.3	80.3+17.8	30.3+3.12	0.350+0.04	89.57+0.76	7.20+0.00

(Mean ±SE, n=3).

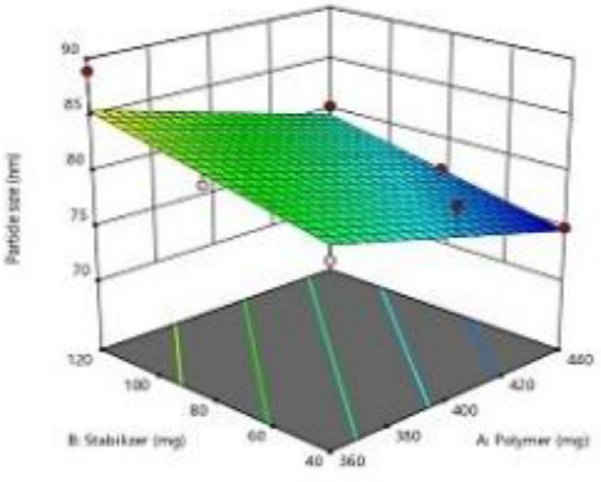

Figure 1: Response surface plot of the effect of independent variables on particle size.

indicated that model terms are significant. Based on the equation, it can be found that polymer (A) had negative coefficient whereas the value of coefficient for stabilizer (B) was positive. The negative sign for the coefficient of polymer indicated that the particle size decreases with increase in polymer concentration. The positive sign for the coefficient of stabilizer indicated that the particle size increases as the concentration of stabilizer increases. The graph (Figure 1) also revealed that as polymer concentration increases particle size decreases and as stabilizer concentration increases particle size also increases.

Zeta potential

The zeta potential is an important surface characterization method which gives information related to the surface charge of nanoparticles. The magnitude of zeta potential indicates the potential stability of

Table 5: Results of ANOVA for particle size.

Variables	*F*-value	*p*-value	R^2	R^2 adj.	
Model	7.84	0.0212	0.7233	0.6310	Significant
A	9.65	0.0209			
B	6.03	0.0495			

Nano particulate system. The zeta potential values of Nano suspension containing ERS PO was found in between 27.5±1.50 mV to 41.6±4.82 mV as shown in Table 4. Zeta potential of optimized batch (ANS 8) was found to be 34.3±1.70 mV, positive zeta potential values were obtained which might be due to the positive surface charge of the Eudragit RS PO. All other ANS formulations showed positive zeta potential (Figure 2). As corneal surface possesses negative charge, the positive charge on nanoparticles could help in efficient adhesion to the corneal surface which in turn would enhance bioavailability of ocular drugs.

Effect of independent variables on zeta potential using 3^2 factorial design

The factorial design suggested a quadratic model for the effect of zeta potential. The relationship between the coded factors and zeta potential was studied using equation 2 and 3D plots (Figure 3).

$$\text{Zeta potential} = 34.48 - 2.58A - 0.3500B - 0.5500AB + 3.78A^2 - 5.72B^2 \quad (2)$$

As per the ANOVA results (Table 6) for optimization process, the model *F*-value of 10.31 was obtained, which suggested that model is significant. There is only a 4.17 % chance that an *F*-value this large could occur because of noise. *P*-value is less than 0.0500 which indicated that model terms are significant. From an equation, the main effects (A-polymer and B-stabilizer),

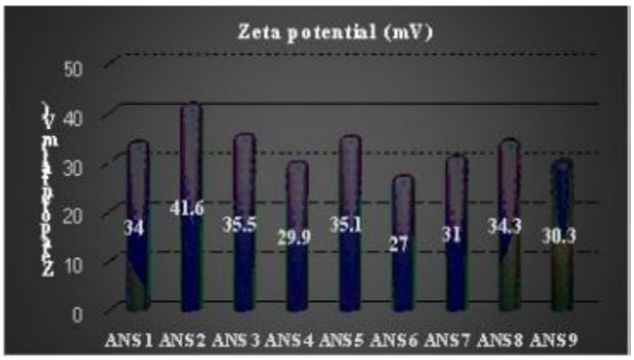

Figure 2: Graphical representation of Zeta potential (mV) of ACF nanosuspension.

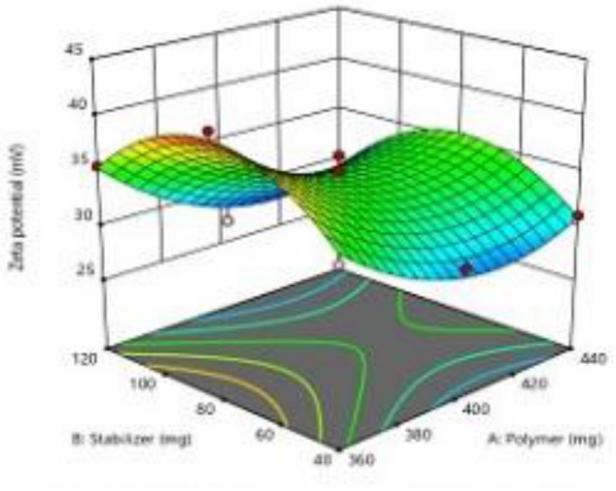

Figure 3: Response surface plot of the effect of independent variables on zeta potential.

interactive effect (AB) and quadratic effects (A^2 and B^2) were observed. From these effects, only A, A^2 and B^2 are significant ($P<0.0500$) which played an important key role in influencing zeta potential of formulations. In the main effects both A (polymer) and B (stabilizer) individually showed negative effect on zeta potential, i.e. the increase in concentration of both polymer and stabilizer lead to decrease in the zeta potential values amongst which polymer had strong effect on zeta potential as compared to stabilizer, also the polymer and stabilizer combinely showed negative impact on zeta potential, the graph and higher order equation reflected an interaction/ combined effect of A and B on zeta potential of Nano suspension.

Polydispersity index

The polydispersity index (PDI) values indicate the particle size distribution. It also suggests the stabilization of formulation. PDI values were obtained in the range of 0.01 to 0.5 as shown in Table 4, which demonstrates narrow size distribution, while PDI above 0.7 indicates very broad size distribution. The polydispersity index of all formulations containing ERS PO varies from 0.303±0.02 to 0.391±0.04. The optimized Nano suspension (ANS 8) provides 0.312±0.09 PDI. Particle size distribution of all the formulations were found to be of narrow size range (Figure 4) and well suited for ocular use.

Table 6: Result of ANOVA for zeta potential.

Variables	F-value	*p*-value	R^2	R^2 adj.	
Model	10.31	0.0417	0.9450	0.8533	Significant
A	15.18	0.0300			
B	0.2786	0.6342			
AB	0.4587	0.5468			
A^2	10.85	0.0459			
B^2	24.78	0.0156			

Drug Entrapment Efficiency (%)

Drug entrapment efficiency (%) of the ERS PO based ACF Nano suspensions were found to be in between 70.50±0.44 % to 91.27±0.11 % as shown in Table 4. As the drug-polymer ratio increases the % drug entrapment efficiency (%) also get increased. The optimized formulation (ANS 8) showed 91.27±0.11% highest drug entrapment efficiency. (Figure 5)

Effect of independent variables on entrapment efficiency (%) using 3^2 factorial design

The factorial design suggested a linear model for the effect of entrapment efficiency (%). The relationship between the coded factors and % entrapment efficiency was studied using equation 3 and 3D plots (Figure 6).

$$\text{Entrapment efficiency (\%)} = 79.83 + 8.19A + 0.4950B \quad (3)$$

As per the ANOVA results (Table 7) for optimization process, the model *F*-value of 24.55 was obtained, which suggested that model is significant. There is only a 0.13 % chance that an *F*-value this large could occur

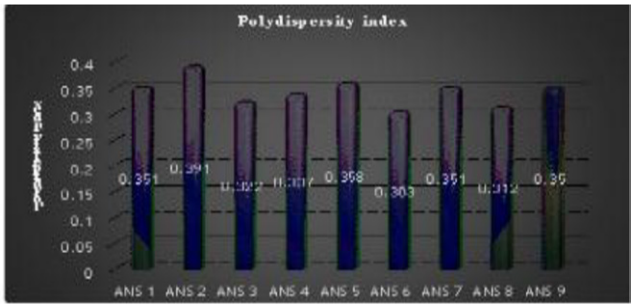

Figure 4: Graphical representation of PDI of ACF nanosuspension.

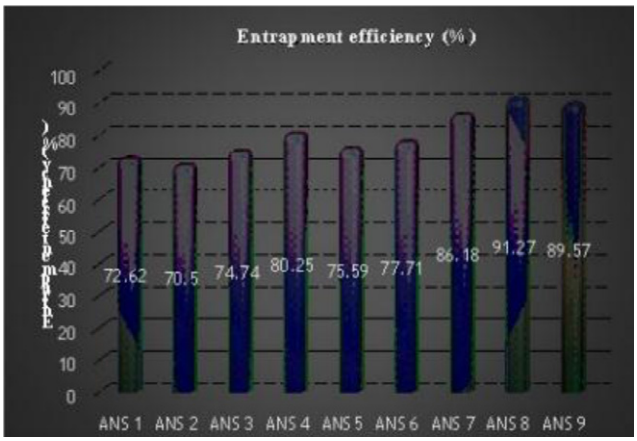

Figure 5: Graphical representation of Entrapment efficiency (%) of ACF nanosuspensions.

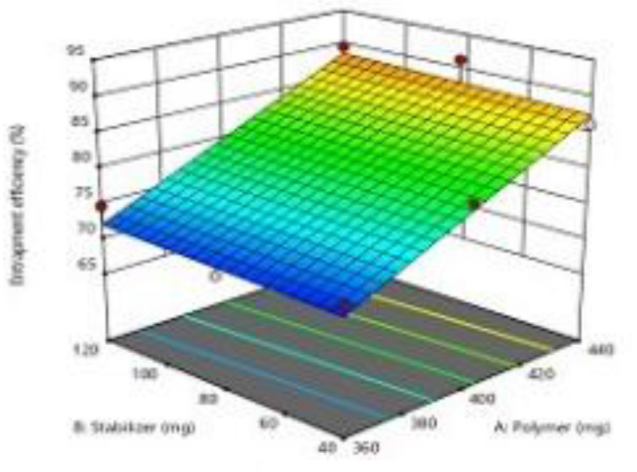

Figure 6: Response surface plot of the effect of independent variables on % entrapment efficiency.

because of noise. *P*-value is less than 0.0500 which indicated that model terms are significant. Based on the equation, it can be found that polymer (A) and stabilizer (B) had positive coefficient but only A is significant ($P<0.0500$). The positive sign for the coefficient of a (polymer) indicated that the entrapment efficiency increases with increase in concentration of polymer. From graph (Figure 6) it is revealed that stabilizer has constant effect on entrapment efficiency while entrapment efficiency increases with increase in polymer concentration.

pH

pH is the most important parameter considered in the formulation process. The pH value of ocular Nano suspension should be such that the Nano suspension formulation will be stable at that pH value and it would not cause irritation to the patient upon administration.

Table 7: Result of ANOVA for % entrapment efficiency.

Variables	F-value	*p*-value	R^2	R^2 adj.	Significant
Model	24.55	0.0013	0.8911	0.8548	
A	48.92	0.0004			
B	0.1786	0.6873			

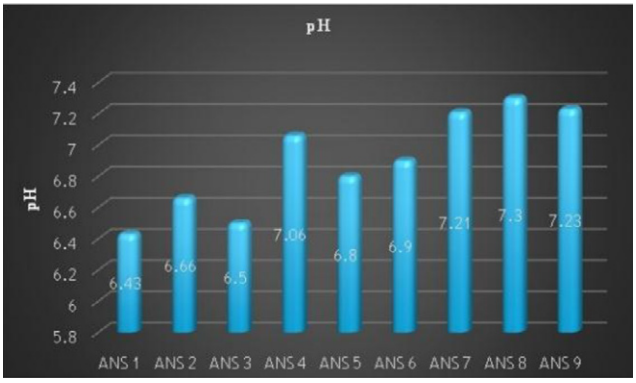

Figure 7: Graphical representation of pH of ACF nanosuspensions.

The optimized batch showed pH 7.3±0.00. The acceptable pH range 6.43±0.04 to 7.3±0.00 was observed (Figure 7 and Table 4) and hence it is predicted that formulation would not cause irritation to the eye upon administration. It was also observed that increase in Eudragit RS PO polymer causes a slight increase in pH for formulations.

ATR-FTIR spectra

IR spectras of ACF, Eudragit RS PO, Physical mixture, ANS 8 were obtained as shown in Figure 8. IR spectrum of optimized formulation (ANS 8) showed the characteristic peak of Eudragit RS PO at 3385.07 cm^{-1} (O-H stretching), 1726.29 cm^{-1} (C=O stretching for ester), 1238.30 cm^{-1} (C-O stretching) and peaks of mannitol at 3277.06 cm^{-1} (O-H stretching) and 2985.81, 2941.44 cm^{-1} (C-H stretching). There is no peak observed for ACF which might be due to drug is present in amorphous form in formulation.

X-ray diffraction (XRD)

In order to study the physical nature of pure drug, polymer, physical mixture and encapsulated drug, the X-ray diffraction (XRD) was used. The XRD pattern of the ACF was showed in (Figure 9). It was observed that the pattern of the ACF exhibited intense crystalline peaks at 11.53°, 18.56°, 22.33°, 24.54°, 25.61° and 32.23° 2θ, which proved that the ACF was in crystalline form.

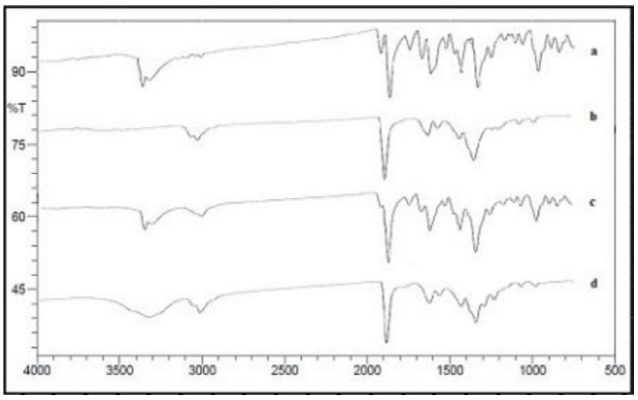

Figure 8: ATR-FTIR layout of a) ACF, b) Eudragit RS PO, c) Physical mixture (ACF+ ERS PO), d) ANS 8.

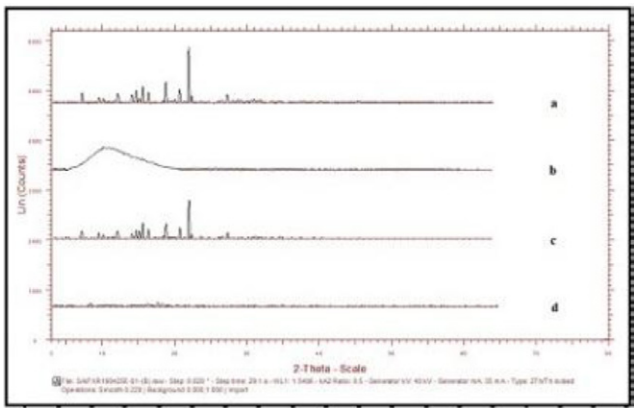

Figure 9: XRD layout of a) ACF b) Eudragit RS PO c) Physical mixture (ACF + ERS PO), d) ANS 8.

From the XRD patterns of Eudragit RS PO, it is clear that it is completely amorphous in nature as there are no sharp peaks observed which was showed in (Figure 9). The XRD patterns of physical mixture (ACF+ERS PO) exhibited characteristics peaks of ACF at 11.50°, 18.55°, 22.32°, 24.53° and 32.18° 2θ. This result indicates that physical blending did not affect the drug diffraction (as shown in Figure 9). The XRD pattern of eudragit RS PO based ACF Nano suspension showed peaks of mannitol, no sharp intense peaks of ACF observed, which suggests that drug is changed from its crystalline form to amorphous form in the formulation which is indication of enhanced solubility of ACF (as shown in Figure 9).

Differential Scanning Calorimetry (DSC)

From DSC study, it has been observed that ACF is crystalline in nature. It exhibit sharp melting endotherm at temperature of 153.51°C. (Figure 10). The thermal characteristics peak of Eudragit RS PO is observed as a broad melting endotherm at 82.42°C. (Figure 10). The physical mixture of ACF and Eudragit RS PO showed characteristic peak at 96.77°C and depressed

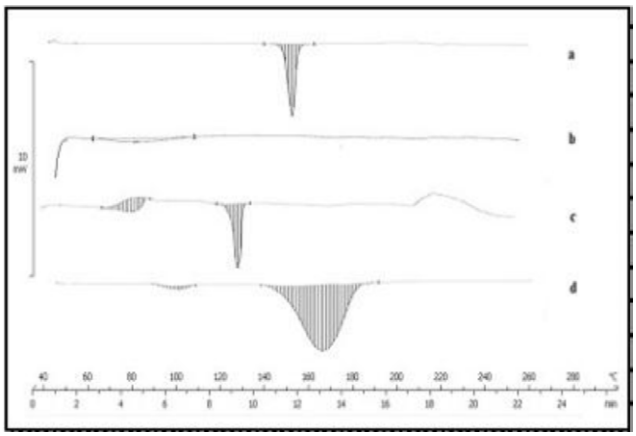

Figure 10: DSC layout of a) ACF, b) Eudragit RS PO, c) Physical mixture (ACF+ERS PO), d) ANS 8.

endotherm of drug at 130.57°C. (Figure 10). The DSC spectra of lyophilized formulation of ANS 8 showed endothermic peak at 166.26°C and 101.89°C (Figure 10) may be due to presence of mannitol and eudragit RS PO respectively. There is no peak observed for ACF in DSC spectra of formulation which might be due to drug is present in amorphous form in the formulation.

Scanning Electron Microscopy (SEM)

Morphological evaluation of optimized ACF Nano suspension formulation was performed using Scanning Electron Microscopy (SEM). The SEM study of lyophilized powder was carried out. SEM images of optimized Nano suspension (ANS 8) lyophilized powder of optimized batch was taken at different magnification power i.e. X500, X1500, X3500 and X7000 and all images was showed in Figure 11. The rough and granular surface was appeared at low magnification power is of fluffy lyophilized powder. The SEM images suggests that the particles of ACF Nano suspension were found to be small in size (75.90 nm, 72.16 nm) and has a spherical shape with smooth surface of powdered particles that suggesting possible stabilization of nanoparticles. Nano suspensions will possibly does not cause any irritation into eye after administration, as it is already stated that isometric particles of thick edges and angles cause less irritation than particles of sharp edges and angles.

In vitro drug release study

In vitro drug release study was carried out for all formulation by using dialysis membrane in Sorenson's phosphate buffer solution (pH 7.4) as the release medium. The release profile of all formulation and the percentage drug release versus time profile are depicted in Table 8.

In vitro drug release kinetics

The drug release data which is obtained from *in vitro* drug dissolution experiment was subjected to various kinetic equations to evaluate the drug release mechanism and kinetics. The *in vitro* drug release data were fitted into various kinetic equations; zero order release kinetics, first order release kinetics, Higuchi model, Hixson-crowell model and Korsmeyer-Peppas equation. The *in vitro* drug release data fitting values are showed in Table 9. *In vitro* drug release is best explained by Korsmeyer-Peppas equation. The R^2 values of Korsmeyer-Peppas equation were close to 1. The diffusion coefficient values of Korsmeyer-Peppas equation of all formulation batches ranged from 0.5811 to 0.8973. Thus it was concluded that the values of *n* (slope) for all formulation batches as per Korsmeyer-Peppas equation were found to be in between 0.5 to 1 which indicate that the drug release from the Nano suspensions follows Anomalous (non-Fickian) diffusion mechanism, i.e. contributed by combination of dissolution and diffusion. According to the correlation coefficients (R^2) of all formulations at 8 hr measurements, the release patterns were best fitted to the Higuchi-square-root release kinetics model.

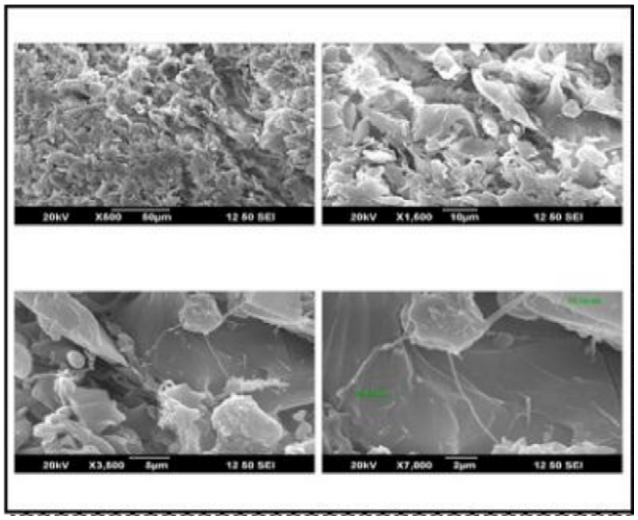

Figure 11: SEM images of lyophilized nanosuspension.

Table 8: *In vitro* drug release study of ACF nanosuspensions.

Time (hrs)	% Cumulative drug release.								
	ANS 1	ANS 2	ANS 3	ANS 4	ANS 5	ANS 6	ANS 7	ANS 8	ANS 9
0.5	10.42+0.70	13.81+1.02	9.57+0.51	7.03+0.53	11.69+0.33	8.30+0.83	5.33+0.79	7.45+0.21	7.03+0.39
1	19.74+0.31	25.25+0.51	22.28+0.43	18.89+0.45	21.01+0.54	14.23+0.23	12.96+0.11	15.08+0.21	12.11+0.12
2	26.94+0.20	35.84+1.01	34.57+0.48	28.64+0.68	37.54+0.17	24.40+0.87	20.59+0.45	25.67+0.43	19.32+0.41
3	35.42+0.37	42.62+0.10	44.32+0.70	38.38+0.55	41.35+0.53	33.30+0.64	29.91+0.51	34.57+0.35	28.22+0.66
4	45.16+0.88	50.67+0.57	52.79+0.66	46.44+0.47	53.64+0.51	42.62+0.28	39.23+0.49	42.20+0.23	35.00+0.75
5	51.10+0.58	56.61+0.20	58.72+0.38	53.64+0.25	57.45+0.79	49.83+0.13	50.67+0.15	47.28+0.37	44.74+0.71
6	58.30+0.40	64.23+0.36	62.11+0.30	58.30+0.42	60.42+0.27	57.45+0.67	59.15+0.35	54.91+0.40	52.79+0.22
7	63.38+1.26	70.59+0.13	67.22+0.50	61.27+0.85	64.23+0.38	62.11+0.48	60.00+0.64	57.88+0.83	59.57+0.45
8	69.74+0.73	74.40+0.41	71.86+0.71	65.50+0.47	70.59+0.31	67.20+0.37	66.77+0.47	61.27+0.54	63.38+0.18

(Mean ±SE, *n*=3).

Table 9: Kinetic profiles of *in vitro* drug release data of ACF nanosuspensions.

Batch code	Zero order R^2	First order R^2	Higuchi model R^2	Hixson-crowell model R^2	Koresmeyer-Peppas		Diffusion mechanism
					R^2	N (slope)	
ANS 1	0.9856	0.9961	0.9943	0.9976	0.9936	0.6640	Anomalous (non-Fickian) diffusion
ANS 2	0.9718	0.9951	0.9967	0.9941	0.9900	0.5811	Anomalous (non-Fickian) diffusion
ANS 3	0.9346	0.9893	0.9906	0.9765	0.9669	0.6865	Anomalous (non-Fickian) diffusion
ANS 4	0.9442	0.9876	0.9929	0.9767	0.9637	0.7588	Anomalous (non-Fickian) diffusion
ANS 5	0.9252	0.9769	0.9826	0.9644	0.9764	0.6281	Anomalous (non-Fickian) diffusion
ANS 6	0.9847	0.9992	0.9955	0.9983	0.9990	0.7651	Anomalous (non-Fickian) diffusion
ANS 7	0.9792	0.9886	0.9848	0.9889	0.9905	0.8973	Anomalous (non-Fickian) diffusion
ANS 8	0.9626	0.9925	0.9975	0.9854	0.9892	0.7508	Anomalous (non-Fickian) diffusion
ANS 9	0.9946	0.9916	0.9807	0.9958	0.9971	0.8088	Anomalous (non-Fickian) diffusion

Table 10: *In vitro* drug release study of ANS 8 and 0.1 % (w/v) ACF solution pH 7.2.

Time (hours)	ANS 8	0.1 % (w/v) ACF solution
0.5	7.45+0.21	47.71±0.42
1	15.08+0.21	77.79±0.21
2	25.67+0.43	91.35±0.65
3	34.57+0.35	98.55±0.28
4	42.20+0.23	-
5	47.28+0.37	-
6	54.91+0.40	-
7	57.88+0.83	-
8	61.27+0.54	-

(Mean ±SE, *n*=3).

Table 11: *Ex-vivo* transcorneal study of ANS 8 and 0.1 % (w/v) ACF solution pH 7.2.

Formulation	% corneal permeation (2 hrs)	% corneal hydration
0.1 % ACF solution	3.91	72.97
ANS 8	7.39	76.24

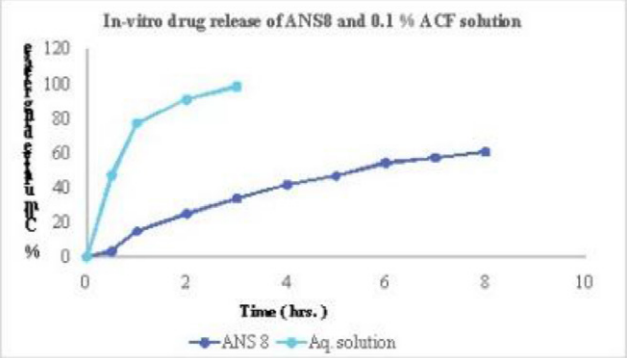

Figure 12: *In vitro* drug release study of ANS 8 and 0.1 % ACF solution.

Evalutionary Comparison of 0.1 % (w/v) ACF solution pH 7.2 with optimized ACF Nano suspension (ANS 8)

In-vitro drug release study

From the *in-vitro* drug release study, it is observed that the optimized ACF Nano suspension (ANS 8) showed 7.45±0.21 % drug release in 0.5 hrs and 61.27±0.54 % release in 8 hr while 0.1 % ACF solution showed 47.71±0.42 % drug release in 0.5 hr and 98.55±0.28 % release in 3 hr From results obtained, it is indicated that ACF Nano suspension showed sustained drug release profile as compare to 0.1 % ACF solution. The release profile of formulations and the percentage drug release versus time profile are depicted in Table 10 and showed in Figure 12 respectively. By studying literature, it is found that drug release studies for ophthalmic forms was carried out even if it is present in the solution form for comparison with the prepared formulation. Here drug release from 0.1 % ACF solution showed drug release extended for 3 hr. Aceclofenac belongs to BCS class-II that may be one of the reasons for the same. Even if drug is already dissolved it is possible because it first diffuses through cellophane membrane (accepted dissolution method in literature) and then comes in contact with the dissolution medium. The time required for solution to diffuse through cellophane membrane and its interaction with the dissolution media are considered as 3 hr extended release time from 0.1% ACF solution.

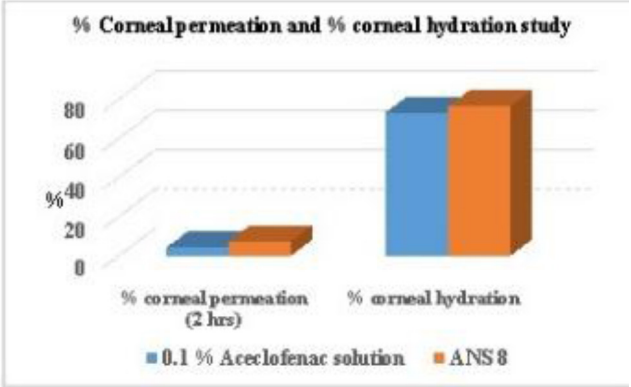

Figure 13: Graphical representation of *ex vivo* transcorneal permeation study.

Ex- vivo transcorneal permeation and % corneal hydration study

From *ex-vivo* trans corneal study, a 2-fold increase in permeation of ACF from ANS 8 was observed when compared to the 0.1 % ACF solution across goat cornea as shown in Table 11 and Figure 13. ACF showed 7.39 % *in vitro* ocular availability as % permeation is directly related with *in vitro* ocular availability. The corneal hydration (%) remained in normal range that is 75 to 80 % which indicated the eye friendly behavior of optimized formulation. The positively charged nanoparticles due to eudragit RS PO polymer interacts with the negatively charged cornea which is responsible for mucoadhesion and increase in the retention time of nanoparticles. The higher permeation from the nanoparticles as compared to the solution may be because of retention and depot of nanoparticles on the corneal surface due to mucoadhesion.

CONCLUSION

The present investigation highlighted the successful formulation and characterization of ocular delivery of Aceclofenac through Eudragit RS PO based Nano

suspension. The Nano suspensions were prepared successfully by using Nano precipitation method which is easiest and most reproducible method to prepare Nano suspension without need of any sophisticated instruments. The FTIR, DSC and XRD studies confirmed that there was no drug-polymer interaction. From DSC and XRD study, the amorphization of drug in formulation was observed from which it can be predicted that solubility of drug could be enhanced. The small particle size, narrow size distribution and pH within normal range for all prepared Nano suspensions were observed which is acceptable for ocular administration. All formulations showed positive zeta potential from which it is be expected that formulation could interact with anionic cornea and increases residence time. The SEM images revealed that there will be no irritation cause after ocular administration due to spherical and smooth surface. The results of *in vitro* drug release study indicated that higher% entrapment efficiency of drug in Nano suspension delays the drug release and increase the corneal residence time. From *ex vivo* trans corneal study it was found that optimized formulation showed higher % permeation of drug as compared with 0.1 % Aceclofenac solution with no signs of corneal damage and eye friendly behavior.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

ACF: Aceclofenac; **FTIR:** Fourier Transform Infrared spectroscopy; **DSC:** Differential Scanning Colorimetry; **XRD:** X-ray Diffraction; **SEM:** Scanning Electron Microscopy; **NSAIDs:** Non-steroidal anti-inflammatory drugs; **COX-2:** Cyclooxygenase-2; **ERS PO:** EudragitRS PO; **PDI:** Polydispersity index.

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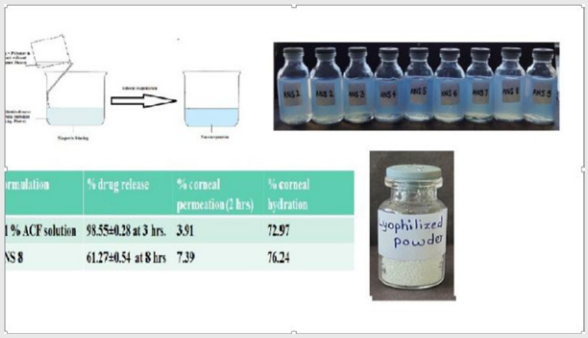
PICTORIAL ABSTRACT



SUMMARY

Aceclofenac Nano suspensions were successfully prepared by using Eudragit RS PO polymer using Nano precipitation technique. Amongst the prepared formulations, ANS 8 formulation was selected as the optimized formulation based on particle size, zeta potential measurements, Polydispersity index, pH and drug entrapment efficiency. From *ex vivo* transcorneal study it was found that optimized formulation showed higher % permeation of drug as compared with 0.1 % Aceclofenac solution with no signs of corneal damage and eye friendly behavior. Hence it can be concluded that Aceclofenac Nano suspension prepared by using Eudragit RS PO polymer using Nano precipitation technique can be a choice for ocular inflammation.

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