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Characterization, Stability And Anti-Inflammatory Efficacy Of Aceclofenac Eye Drops

Osama A. Soliman, Marwa S. El-Dahan And Sara N. Maria*
Pharmaceutical Department, Pharmacy College, Mansura University, Mansoura, 35516, Egypt.
Corresponding Author: Sara N. Maria

Abstract: *Non steroidal anti-inflammatory drugs (NSAIDs) used to be the mainstay of topical therapy in the management of ocular inflammations. Aceclofenac (ACF) possesses better analgesic, antipyretic and anti-inflammatory efficacy than other NSAIDs. Aceclofenac eye drop is not available in the market so this study is considered as a novel approach to formulate ACF eye drops to reduce eye inflammation. The goal of the present study was to formulate ACF eye drop using different polymers such as hydroxypropyl methylcellulose (HPMC), capobol 934 (CP 934) and sodium alginate (S. Alg). These formulae were evaluted with respect to drug content, pH, viscosity, in-vitro release, and stability for 6 months. Kinetic analysis of the release data was done. The efficacy of the selected formulation (FI) against prostaglandin E_1 (PGE_1) induced ocular inflammation in rabbit's eye was determined by counting the polymorphonuclear leukocytes (PMN) migrated in tear fluid. Collectively, all formulations exhibited accepted drug content, pH, and viscosity values. The drug release was varied with polymer type in the order of HPMC > CP 934 > S. Alg. Also, HPMC eye drops (FI) exhibited the greatest stability after 6 months. Considering the anti-inflmmatory efficacy, HPMC eye drops (FI) showed a significant inhibition of PGE_1-induced (PMN) migration as compared to control formula.*
Keywords: *Aceclofenac, eye drops, stability study, anti-inflammatory efficacy.*

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I. Introduction

Topical ophthalmic NSAIDs are used to treat ocular surface and anterior segment inflammation, post operative management of pain and inflammation as well as treatment of seasonal allergic conjunctivitis. As a class, they have proven to be an effective, useful and a safe alternative to corticosteroids in the topical management of ocular inflammations, as corticosteroids cause serious side effects such as elevation of intraocular pressure and progression of cataracts **[1].**

Topical application of ocular anti-inflammatory drugs is the most advantageous route to treat eye inflammation. It is affecting the anterior segment because it is easy to apply, can give a high drug concentration, and avoids systemic absorption. As a result of the physiological and anatomical constraints of the eye only few amount of the drug is ocularly absorbed **[2].** The continuous secretion of the tear fluid decreases the contact time with the eye surface. So, the viscosity increasing agents have been used to increase the contact time with ocular surface **[3].**

ACF is a very potent NSAIDs, antipyretic and analgesic drug as compared with other NSAIDs which are usually used for treatment of arthritis, pain and inflammation. It is preferred to use for the management of various ocular inflammatory conditions due to its relatively better tolerance. Also, it has a lipophilic nature allows it to be better absorbed by the cornea and ocular tissue ensuring relief of pain in cataract, refractive surgery and corneal abrasion **[4].**

Aceclofenac is chemically named (2-[2-[2-(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy acetic acid) **[2].** It is mainly act by inhibition of prostaglandins (PGs) synthesis. It inhibits the cyclooxygenase (COX) enzyme, which is involved in the synthesis of PGs that are the main reason causing pain, swelling and inflammation **[5].** It also inhibits the synthesis of the inflammatory cytokines, interleukins, and tumor necrosis factors, so ACF is considered as an effective pain killer.

Formulation of ACF eye drops as well *in-vitro* and *in-vivo* assessments have not been accomplished in the earlier studies. This study designed to formulate ACF eye drop, to reduce eye inflammation, using different polymers. The different ACF eye drops were subjected to various physical evalution and *in-vitro* release study. Also, the stability studies for the prepared eye drops were investigated at different temperatures (4, 30, 40 ^{o}C). Moreover, the selected eye drops (F1) was assessed for its anti-inflammatory efficacy in rabbit's eyes.

II. Materials And Methods

1- Materials

Aceclofenac (ACF) was obtained from Amoun company for Pharmaceuticals & Chemical Industries, Cairo, Egypt. Hydroxypropyl methylcellulose (HPMC 4000 cPs), capobol 934 (CP 934) and sodium alginate (S. Alg) were provided by Epico company for Pharmaceuticals & Chemical Industries. Disodium hydrogen phosphate, potassium dihydrogen ortho phosphate were purchased from Adwic, El Nasr, Pharmaceutical Chemicals Co, Egypt. Triethanolamine (TEA) was provided by LoBa Chemie, India. Prostaglandin E_1, Alfoprostadil, Alexandria Company for pharmaceuticals and chemical industries. Spectra/Pore® Dialysis membrane (12000–14000 Mw cutoff) was provided from Spectrum Laboratories Inc., Rancho Dominguez, Canada. All other chemicals were of analytical grade; freshly double-distilled water was utilized during the study.

2-Methodology

2.1. Preparation of ACF Eye Drops

ACF eye drops (F1–F3) were prepared using different polymers namely HPMC, CP 934 and S. Alg (Table I). The polymer was dispersed in phosphate buffer solution PBS, pH 7.4 in which the preservatives (methyl- and propylparabens) were previously dissolved **[6].** ACF 0.1% (w/v) was dissolved in the previous polymeric solutions under constant stirring until uniform and clear solution was obtained. In case of CP 934, 5 µl of TEA was added to enhance viscosity since CP 934 (polyacrylic acid) undergoes a sol–gel transition in aqueous solution at pH above its pKa 5.5 **[7].** All formula completed to 100 ml with distilled water.

Table I. Composition of ACF Eye Drops

Formulae code	Polymer (%w/v)
FI	0.4 % HPMC
FII	0.1 % CP 934
FIII	0.5 % S. Alg

All formulae contain 0.1%w/v ACF, 0.02 %w/v methylparaben and 0.01%w/v propylparaben. HPMC hydroxypropyl methylcellulose, CP 934 Carbopol 934 and S. Alg sodium alginate.

2. 2. Physicochemical Characterization of ACF Eye Drops

2.2.1. Drug Content

One ml from each formulation was dissolved in 100 ml PBS, pH 7.4, shaken and heated in thermostatically controlled water bath (Grant Instrument Cambridge Ltd., Barrington Cambridge (B2, 5002, England) at 37 ± 0.5°C for 15 min. Then, the solution was filtered using 0.45 µm millipore filter, properly diluted and measured spectrophotometrically (Ultra violet-visible spectrophotometer, JASCO, V-530, Japan) at 273 nm for drug content.

2.2.2. pH Measurements

The pH of each formulation was measured using pH meter (Beckman Instrument Fullerton, CA 92634, Germany).

2.2.3. Viscosity Determination

The viscosity of ACF eye drops was measured using a cone and plate rotary viscometer (Haake Inc., Germany). From each formulation, 1 ml was placed on the stationary plate of viscometer and allowed to equilibrate for 5 min. to attain the running temperature. The rotary viscometer was thermostatically controlled at 37 ± 0.5°C **[8].** Then, the viscosity values were calculated according to Eq. (1):

$$\eta = G.S/N \qquad (1)$$

Where; η = Viscosity in mPa.s (mPa.s = 1 centipoise, cP), G is the instrumental factor = 14200 (mPa.s/scalagrad.min), S is the torque (scale grad) and N is the speed (rpm).

2.2.4. In-vitro Release Study

The drug release from different ACF eye drops in PBS, pH 7.4 through cellophane membrane that was soaked previousely in the buffer was carried out according to the method previously represented by Levy and Benita **[9].** The membrane was spread over the open- end of a glass tube with a diameter of 3 cm, and was wrapped by rubber bands. Two ml of each formulation were accurately measured and thoroughly spreaded on the membrane. To each tube, 0.5 ml of the buffer solution was added. Then, the tubes were immersed upside-down in a beaker and each one was adjusted, so that the membrane was just below the release medium surface (150 ml PBS, pH 7.4) which is maintained at 37 ± 0.5°C using GFL Germany shaking incubator: type 3033 with

orbital motion (50 rpm). Three ml were taken at predetermined time intervals up to 8 h and replaced by fresh buffer to maintain sink condition. Each sample was filtered using millipore filter, and assayed spectrophotometrically at 273 nm to determine the percent released of the drug at each time interval. The measurements were done against a blank of the corresponding plain formula to abolish interference of components other than the drug, if any, with UV measurements. ACF suspension (drug in water 0.1% w/v) was used as control (Ct). The experiments were done in triplicate and the mean values were calculated.

2.2.5. Kinetics of Release Data

The different release kinetics is supposed to reveal various release mechanisms. The *in-vitro* release data were analyzed according to three kinetic models: zero-order, first-order, and Higuchi model **[10],** and the kinetic modeling of drug release was determined. The model with the highest correlation coefficient (r^2) was considered to describe ACF release from the prepared formulations. The Korsmeyer–Peppas model was used for more analysis, where the value of the release exponent (n) is governed by the release mechanism, so could be used to describe it **[11]**.

2.3. Stability Study

The stability of different eye drops containing ACF drug was investigated by storage in air tight amber glass jars and stored at different temperatures of 4 ± 1 (in the refregirator), 30 ± 1 and 40 ± 1°C in thermostatically controlled hot air ovens (Gering model SPA-GELMAN Instrument No. 16414, Germany) for 6 months. The relative humidity was maintained at 75 ± 5% using saturated solution of sodium chloride **[12]**. Measurements of drug content, pH and viscosity were done monthly according to the previously illustrated procedures. As well, any changes in color and odor were recorded. Accelerated stability testing was demonstrated in different literature **[13]**. Some functions of drug concentration in each formula monthly determined at each temperature were plotted against time and analyzed according to zero-order and first-order kinetics. The rate constant (K) values at each temperature were calculated using the slope of the linear plot of the fitting kinetic model with the highest correlation coefficient (r^2). The slope of Arrhenius plot of log k against the reciprocals of the absolute temperature was used to estimate the activation energy (Ea) employing the relation slope = −Ea/2.303R. The values of K_{25} were calculated using the relation Log (k_2/k_1) = Ea (T_2-T_1)/2.303RT_2T_1 provided that K_1 =K_{25} and K_2 =K_4, K_{30}, or K_{40}. The value of K_{25} was then used to obtain a measure of the stability of the drug under ordinary shelf storage conditions (shelf life, $t_{90\%}$) and (half-life , $t_{50\%}$) through the relations ($t_{90\%}$ = 0.105/K_{25}) and ($t_{50\%}$ =0.693/k_{25}) **[14]**.

Arrhenius equation:

$$\textbf{Log k = Log A- Ea / 2.303 RT} \qquad \textbf{(2)}$$

Where;

K = The specific reaction rate constant at temperature (t).
A = The frequency factor
Ea = Activation energy (Cal/mole).
R = The gas constant (1.987 Cal/de.mole).
T = The absolute temperature (^oC + 273).

2.4 . Ocular Anti-inflammatory Study

As discussed below, the results of *in-vitro* release and stability studies have clarified that the selected formula for screening of anti-inflammatory efficacy of ACF was HPMC eye drops (FI). The PGE_1-induced ocular inflammation in rabbit model was used to examine the effectiveness of HPMC eye drops containing ACF **[15].** The animal experimental protocol conform to the ethical principles of the scientific committee of the Faculty of Pharmacy, Mansoura University, Egypt.

Two groups, each of six white albino rabbits weighing (1–1.5 kg) were selected for this study. Animals were housed in an institutional animal household under customary environment offering unrestricted access to diet and water **[16].** For each rabbit, left eye served as the control and received 50 µl of isotonic phosphate buffer solution (PBS, pH 7.4), while the right eye of the rabbit received 50 µl of (FI) eye drop (Group I) or 50 µl of ACF (0.1%) suspended in water (Ct) (Group II). After 30 min of administration of PBS solution, ACF eye drops and ACF suspended in water (Ct) in respective eyes, 50 µl of PGE_1 (50 ng/ml in normal saline) was applied in both eyes of all the rabbits. All eyes were then evaluated for the inflammation by counting (PMN) migrated in tear fluid **[17**].

PMN migration was assessed by recording its count in tear fluid. The normal saline (100 µl) was applied onto lower cul-de-sac of the rabbit eye and after moderate blending, 50 µl of the tear fluid was withdrawn by a micropipette at different time intervals following PGE_1 application. Following adequate diluting by Turke's fluid (1.5% Glacial acetic acid, 1% gention violet and distilled water up to 100 ml), the count of PMN in tear fluid was calculated using a Neubauer's hemocytometer (PRECICOLOR, HBG, Germany) **fig. 1 [18].**

Figure. 1. Neubauer's hemocytometer

2.5. StatisticalAnalysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Statistical calculations were carried out using GraphPad prism-5 software (GraphPad software Inc.,San Diego, CA, USA) **[19].**

III. Results And Discussion

3.1. Physicochemical Characterization of ACF Eye Drops

3.1.1. Drug Content

Table II represents the actual ACF content of the formulated eye drops. It was found that, the percentage of drug content ranged from 98.54 ± 0.06 to 98.65 ± 0.09 % which complies with the pharmacopeial limits ranging from of 90 to 110% of the label claim **[20].**

3.1.2. Formulations pH

The eye can tolerate the ophthalmic formulations with a wide pH range (3.5–10.5) without any discomfort or irritation **[14].** Because the ideal ophthalmic dose is only one drop, the tear film can be rapidly restored neutral pH **[21].** The prepared formulations had an acceptable pH values ranged from 7.15 ± 0.05 to 7.21 ± 0.03 that could be tolerated easily by the eye without any irritation **(TableII).**

3.1.3. Eye Drops Viscosity

Viscosity values of the prepared eye drops were measured and the values were ranged from 562 ± 28 to 582 ± 24 cP . The viscosity of ACF eye drops can be arranged in the following order: S. Alg > CP 934 > HPMC **(Table II).** These outcomes can be clarified that the higher viscosity of eye drops led to an increase in their contact time with the eye surface and decrease the rapid drainage of the formulations from the eye which in turn improved their bioavailability **[22].**

Table II. Physicochemical Characterization ACF Eye Drops

Formulae	Drug content (%w/v)	pH	Viscosity (mPa.s)
FI	98.65 ± 0.09	7.21 ± 0.03	562 ± 28
FII	98.54 ± 0.06	7.15 ± 0.05	573 ± 32
FIII	98.56 ± 0.07	7.19 ± 0.04	582 ± 24

All values are expressed as means ± SD (n=3)

3.1.4. *In-vitro* Drug Release Study

Fig. 2. illustrates the *in-vitro* release behavior of ACF from the formulated eye drops. The *in vitro* release results of ACF in PBS, pH 7.4 from eye drops showed a significant decrease in drug release as compared with the control. This may be referred to the free solubility of ACF in isotonic PBS, pH 7.4 and the rapid dissolution of drug from control. The hydrophilic polymers nature affected the release of ACF from the prepared eye drops. Also, it is obvious that, the release of ACF from HPMC eye drops was significantly ($p < 0.05$) higher compared with other drops after 8 h. This could be due to the dissimilarity in their viscosities when exposure to the release conditions, as the greater the viscosity, the delaying drug release rate **[23].** The release of ACF from eye drops was in the following order; HPMC > CP 934 > S. Alg. Generally, it is believed that the incorporation of a viscosity increasing agent in an ophthalmic solution will improve the ocular bioavailability by increasing contact time of the drug with the ocular surface **[4].**

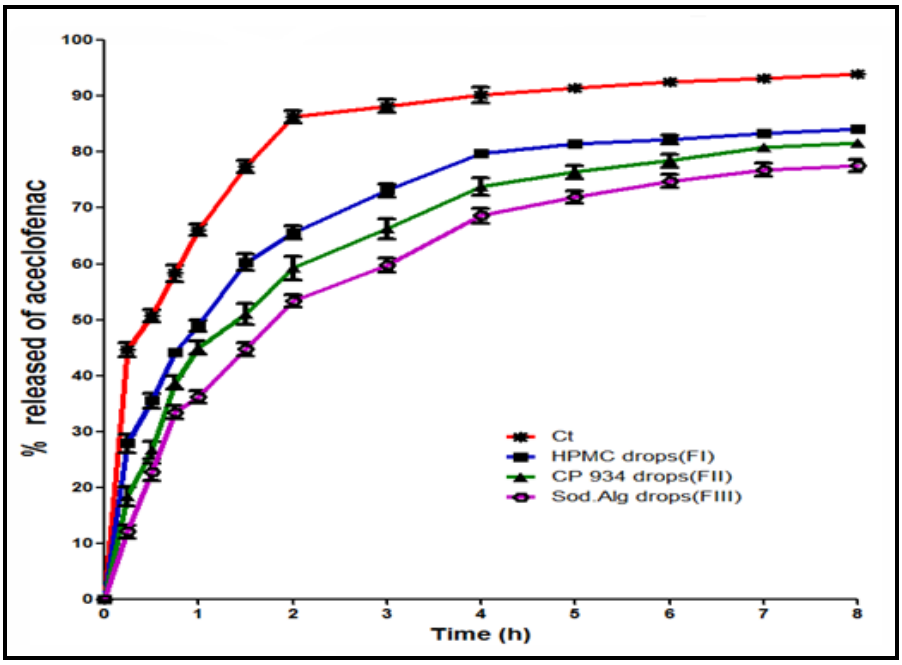

Figure 2: *In-vitro* dissolution profile of aceclofenac from different eye drops

3.1.5. Kinetics of Drug Release

The kinetic analysis of the release data **(Table III)** showed that ACF released from eye drops followed the Higuchi model, suggesting that the release mechanism of the drug was most fitted to diffusion controlled mechanism, while the control drops followed first-order model. Further examination of the release data using the Korsmeyer–Peppas equation showed that (n) values for control was below 0.45, which indicated Fickian diffusion mechanism suggesting that the release depends only on dissolution of ACF, while (n) values for all eye drops were between 0.45 and 0.89 that indicated exhibition of non-Fickian (anomalous) diffusion. This suggested that, all prepared eye drops released ACF via diffusion accompanied by erosion of polymers.

Table III: Kinetic Analysis of the Drug Release Data

Formulae	Correlation Coefficient (r^2)			Release order	Korsemeyer-Peppas		Main transport mechanism
	Zero	First	Higuchi		r^2	n	
Ct	0.5637	0.8381	0.7956	First	0.9257	0.2388	Fickian
FI	0.7156	0.8793	0.9081	Diffusion	0.9515	0.4633	Non- Fickian
FII	0.7419	0.8904	0.9200	Diffusion	0.9725	0.6535	Non- Fickian
FIII	0.8024	0.9309	0.9558	Diffusion	0.9563	0.5482	Non- Fickian

Where; (n) is release exponent.

3.2. Stability Study

At different temperatures, physical stability was indicated by the absence of color and odor changes. The results showed slightly lowered drug content, pH and viscosity than those initially determined. However, percentage drug content values were still complying with pharmacopeial limits **[20],** and pH range still could be tolerated by the natural buffering system of the eye **[14]** and maintain the drug stability. Storage at 30^{O}C and 40^{O}C might affect the integrity of the polymer and lowering the viscosity to some extent more than at 4^{O}C. The kinetic analysis data used to determine the mechanism of degradation, $t_{50\%}$ and $t_{90\%}$ was represented in **Table IV**. After the analysis of the data, the degradation rate of ACF followed the first-order model. HPMC eye drops (FI) exhibited the highest $t_{90\%}$, thus it was selected for further investigation of ACF anti-inflammatory efficacy in rabbit's eyes.

Table IV: Stability Data at Different Temperatures

Storage Temp. (°C)	Parameters	Formulae		
		FI	FII	FIII
4	Drug content	95.79 ± 0.18	95.36 ± 0.15	95.08 ± 0.19
	pH	7.11 ± 0.55	7.05 ± 0.51	7.00 ± 0.19
	Viscosity	560 ± 17	571 ± 27	580 ± 31
30	Drug content	95.09 ± 0.26	94.91 ± 0.19	94.69 ± 0.09
	pH	7.00 ± 0.51	6.97 ± 0.40	6.95 ± 0.15
	Viscosity	553 ± 51	565 ± 34	573 ± 19
40	Drug content	94.71 ± 0.26	93.96 ± 0.04	93.85 ± 0.15
	pH	6.90 ± 0.38	6.86 ± 0.22	6.82 ± 0.33
	Viscosity	540 ± 36	550 ± 11	558 ± 25
$K_{25}x10^{-3}$ mon^{-1}		5.754	6.209	6.561
Half-life, $t_{50\%}$ (month)		120.44	111.61	105.62
Shelf-life, $t_{90\%}$ (month)		18.25	16.91	16.00

3.3. Ocular Anti-inflammatory Study

On the basis of the release rate data, FI eye drops was selected for the ocular anti-inflammatory study. Topical application of PGE_1 resulted in polymorphonuclear leukocyte (PMN) migration in tears that was the parameter of induced inflammation **[15].** Therefore, PMN count in tear fluid was used to evaluate the anti-inflammatory efficacy of ACF for both the control (Ct) and the chosen formula (FI)

Fig. 3. illustrates the PMN migration in tear fluid of rabbit's eye. It was found that, the inhibitory effect of (FI) was higher than that of control drops, furthermore, HPMC eye drops of ACF significantly ($p < 0.05$) increased the anti-inflammatory efficacy compared to Ct drops (drug suspended in water) and those of PBS solution.

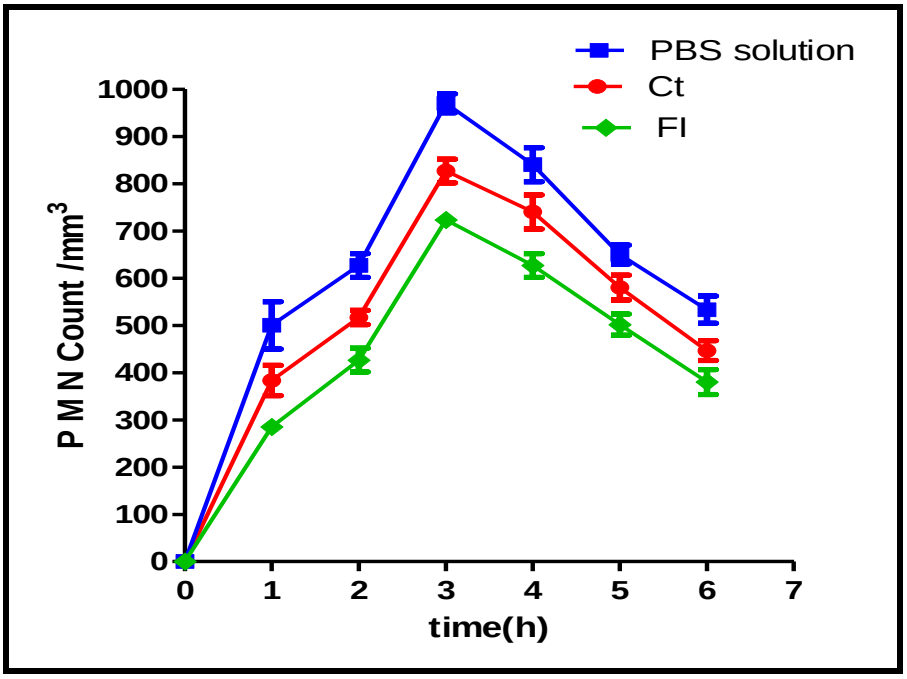

Figure 3: Effect of ACF eye drop on PGE_1 induced PMN migration in tears of rabbit's eye

IV. Conclusion

Aceclofenac eye drops were successfully formulated as a novel approach. ACF release was affected by the nature of polymer used. HPMC formula (FI) showed the highest drug release as compared with other polymers. All ACF eye drops have pH and viscosity values that are compatible with the eye. HPMC eye drops (FI) exhibited the highest physical and chemical stability up to 6 months of storage at different temperatures compared to other eye drops. Furthermore, HPMC eye drops containing ACF possessed superior anti-inflammatory activity compared to ACF suspended in water (Ct). Based on these results, ACF eye drops containing HPMC may be represented as potential ophthalmic drops for enhanced the ocular delivery of ACF.

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