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IN-VITRO AND *IN-VIVO* EVALUATION OF OCULAR DELIVERY OF DICLOFENAC SODIUM FROM OPHTHALMIC SOLUTIONS AND GEL

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ديكلوفيناك الصوديوم عقار يستخدم بنجاح فى علاج الأمراض الرمدية. تم تحضير ديكلوفيناك الصوديوم (٠,١٪ وزن/حجم) فى محاليل لزجة وهلامية لدراسة انطلاق العقار للوصول إلى مدى التحكم فى الكمية المنطلقة والتى لها تأثيرا موضعيا على العين.

البوليمر الذى تم استخدامه فى تحضير المحاليل اللزجة والهلامية هو بولوكسامير ٤٠٧ ، وقد أجريت تجارب معملية لقياس لزوجة المستحضرات كما أجريت سلسلة من التجارب لدراسة انطلاق العقار من المحاليل اللزجة والهلامية وأثبتت الدراسة أن كمية العقار المنطلقة تقل كلما زاد تركيز البوليمر فى المستحضر وأن معدل انطلاق العقار يتبع ميكانيكية الانتشار لهوجوشى.

وقد أجريت تجارب بالأشعة تحت الحمراء والتحليل السعرى التفاضلى (DSC) لمعرفة إن كان هناك تفاعلات بين العقار والبوليمر وأثبتت النتائج أنه لا يوجد أى تفاعلات عند عمل مخلوط طبيعى بنسبة ١:١ عقار إلى بوليمر.

وقد أجريت التجارب الحية على عيون الأرانب المتقرحة حراريا (والتى تم إحداثها فى قرنية عيون الأرانب) لمعرفة مدى تأثير هذه المستحضرات على سرعة التئام هذه القرح ، وقد أثبتت الدراسة والتحاليل الإحصائية لها أن للعقار تأثير فعال على التئام هذه القرح ، وأن هذا التأثير يكون ذو دلالة إحصائية فى المستحضرات التى لها لزوجة بمدى من ٥-١٥ سنتيبواز وقد وضح من الدراسة أن للعقار تأثير واضح وفاعل على التئام القرح عند صواغته مع بوليمر البولوكسامير والذى ثبت علميا أن له تأثير فاعل على التئام القرح والجروح. وأيضا لما له من تأثير على زيادة لزوجة الوسط مما يؤدى إلى زيادة فترة الملامسة مع القرنية ولما له من تأثير سطحى نشط يزيد من امتصاص العقار خلال القرنية.

Diclofenac sodium is successfully used in the treatment of ophthalmic disorders. Diclofenac sodium (0.1% w/v), was formulated in various viscous solutions and gel in order to modulate the release of the drug and thereby, achieve controlled drug delivery for topical ophthalmic application.

The polymer utilized to prepare the viscous solutions and gel was pluronic F 127 (poloxamer 407). The rheological behaviour of the preparations was studied and showed pseudoplastic flow characteristics at high polymer concentrations.

The IR spectra and DSC analysis for the drug, poloxamer and physical mixture (1:1, drug:polymer ratio) were investigated and the results obtained revealed that, there is no interaction between the drug and the utilized polymer.

The in-vitro release of the drug, from the prepared formulations into simulating tears fluid, were carried out adopting the dialysis method. The study depicted that as the concentration of the polymer increases the amount of drug released was progressively decreased and the release profile followed Higuchi diffusion mechanism.

The effect of diclofenac sodium formulations (viscous solutions, gel and voltaren eye drops) on the healing rate of ulcerative corneal rabbits were studied. The statistical analysis of the in-vivo data revealed that, the viscosity of the system dictates the in-vivo performance of the drug only within narrow and low range i.e. 5-15 cp. The in-vivo study also, revealed that poloxamer enhances, drastically, the drug healing rate of ulcerative rabbits cornea. This is due to that, poloxamer recovered the lethally heat-shocked fibroblast and acted as a non

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ionic surfactant offering means of enhancing drug permeation through the cornea. Also, poloxamer acts as viscosity imparting agent which lead to increase in drug contact time with the ulcerative cornea.

INTRODUCTION

Limited absorption of the drug through the lipophilic corneal barrier is mainly due to short precorneal residence time related to tear turn-over, rapid nasolacrimal drainage of instilled drugs from the tear fluid and non-productive absorption through the conjunctiva. Only a small proportion (1-3%) of the instilled drug penetrates the cornea and reaches intraocular tissues.[1]

Inflammations of the eye are induced and maintained by series of mediators. Metabolites of arachidonic acid are liberated in the tissues and cell membrane, the so called "arachidonic-cascade" is induced simultaneously by two enzymes, viz. the cyclo-oxygenase and the lipo-oxygenase. The former produces endoperoxides which are transformed to prostaglandin which induces inflammation. Diclofenac sodium is a potent non-steroidal anti-inflammatory drug. It owes its principle effect to inhibition of cyclooxygenase enzyme which transforms arachidonic acid into prostaglandines.[2-5]

It was reported that diclofenac sodium is successfully used as anti-inflammatory agent after strabismus surgery in place of corticosteroids.[5] The treatment of epidemic kerato conjunctivitis with topical diclofenac sodium was found to be effective and safer alternative than topical steroids, since steroids elevate the intraocular pressure with harmless side effect.[6] Diclofenac sodium (0.1%) eye drops is reported to be effective and safe for the control of postoperative inflammation after cataract surgery.[7] Tauber *et al.*[8] reported that 0.1% diclofenac sodium eye drops reduces the ocular signs and symptoms associated with acute seasonal allergic conjunctivitis.

Pluronic F127 (Poloxamer 407) is non toxic copolymer of polyoxyethylene-polyoxypropylene units. It is commonly used for pharmaceutical and medical purposes. Poloxamer 407 gels containing various drugs have been used for treating patients with ocular conditions such as red eye, corneal edema and dry eye syndrome[9] and glaucoma.[10,11]

One main characteristic of this copolymer is its ability to undergo a reverse thermal gelation. Concentrated solutions (20-30% w/v) of the polymer are fluid at 4-5°C, but turn to soft gels at the body temperature. In addition, low toxicity, mucomimetic properties and optical clarity make poloxamer 407 particularly suitable for ophthalmic formulation.[12] The pluronic F127 gels have been employed for topical delivery of lidocaine and benzocaine,[13] the anti-cancer drugs 5-fluorouracil and adriamycin,[14] and for healing of burn wounds.[15,16]

In the present study, diclofenac sodium (0.1% w/v) is formulated in various viscous solutions and gel of poloxamer 407 in order to modulate the release of the drug and thereby achieve controlled drug delivery for topical ophthalmic application. In addition, the effect of these formulations on the healing rate of the induced ulcers in the rabbits eye have been also studied.

EXPERIMENTAL

Materials

* Diclofenac sodium, DIC (Sigma Chemical Co., St. Louis, USA).
* Pluronic F127 (Poloxamer 407, BASF, Germany).
* All other chemicals were of reagent grade.

Methods

Preparation of DIC ophthalmic solutions and gel

Isotonic phosphate buffer solution (pH 6.8) containing benzalkonium chloride (0.01% w/v) as preservative and sodium metabisulfite (0.1% w.v) as stabilizer was prepared. An appropriate amount of DIC (0.1% w/v) was dissolved in the buffer solution. The drug solution was cooled to 4°C and the amounts of poloxamer were added

with gentle stirring. The mixtures were left overnight in a refrigerator to affect complete desolvation of the polymer. The concentrations of polymer added were 5, 10, 15 and 20% w/v. Warming the mixtures to room temperatures, the latter concentration transformed to clear viscous gel while the former ones (5, 10 and 15% w/v) transformed to viscous solutions. The solutions and gel were sterilized by keeping in water bath for 1 hour, then packed after cooling in neutral glass vials and collapsible tubes respectively. The formulations were then stored in refrigerator until ready for use.

Investigation of the rheology of the ophthalmic preparations

The viscosity of solutions and gel were determined using Brookfield viscometer (Massachusetts U.S.A.). The viscosity was calculated at the lower level of shear rate, dictated by the physiology of blinking in the eye of rabbit (4 times/hr.).[17] The viscosity for non Newtonian flow, at any rate of shear, was calculated utilizing steiger trippi's equation.[18]

Infrared spectroscopy (IR)

Studies of the infrared spectra of DIC, poloxamer 407 and physical mixture of drug and polymer (1:1 ratio) were conducted with an infrared spectrophotometer (IR-470, Shimadzu, Japan) using the KBr disc method.

Differential scanning calorimetry (DSC)

DSC analysis for DIC, poloxamer and the mixture of drug and the polymer (1:1 ratio) was carried out using Shimadzu DSC-50 (Kyoto) connected with TA-501. Nitrogen was used as burge gas (40-50 ml/min). A scanning speed of 10°C/min was employed. The sample size in the aluminium sample pan was in the range of 3-5 mg.

***In-vitro* release of diclofenac sodium**

The release of the drug from solutions and gel was carried out. One gram from the prepared solutions or gel was placed on a semipermeable fisher cellulose membrane (5x5 cm, Fisher Type 30/32, Fischer Scientific Co., England) stretched over an open end of glass tube (2.5 cm diameter) and tightly fixed by rubber band. The tube was suspended, so that the membrane was just below the surface of 25 ml isotonic phosphate buffer (pH 6.8) at 35 ± 0.2°C contained in 50 ml beaker. All cells were fixed in a shaker water bath (35 ± 0.2°C) and agitated at 50 shakes/min. The drug contents, in the withdrawn samples at time intervals, were determined spectrophotometrically at 276 nm (Shimadzu, Japan). Each experiment was performed in triplicate.

Induction of inflammation (ulcers) to the eye of rabbits

A group of six male rabbits (1.5-2 kg) was considered, in each eye of each rabbit, two drops of 0.4% solution of benzoxinate HCl were instilled onto rabbit's eye as local anaesthetic. One to two minutes post instillation, eight inflammatory areas (ulcers) were induced in the epithelium of the cornea of each eye, away from the pupil, using a thermal technique.[19] The ulcers had a circular shape (2 mm diameter) and reached in depth of the corneal epithelium. This was ascertained by the instillation of fluorescein sodium solution (0.2%). Since, fluoresceine does not stain tissue unless the epithelium is disrupted, the inflammatory areas develop a green fluorescence. The absence of green color was thus taken as a criterion of ulcers healing. The data of corneal inflammation healing time were subjected to statistical analysis according to student t-test.[20]

***In-vivo* performance of diclofenac sodium formulations on the inflammed eye of rabbits**

One drop ($\simeq 50$ μl) of sterile chloramphenicol solution (0.5%) was instilled into each eye. For each rabbit one eye was considered as a test and the other eye as a control. The control treatment involved instillation of one drop of chloramphenicol solution every morning throughout the observation period. The test treatment involved the instillation of one drop of chloramphenicol followed by one drop of the drug solutions (voltaren eye drops or viscous solutions) or an amount equivalent to 50 μl of the gel formulation every morning throughout the observation period.[19]

RESULTS AND DISCUSSION

Rheological behaviour of aqueous solutions and gel of poloxamer

It has been known that, poloxamer exists in solution state at temperature of ≃4°C, and in the gel state on warming to room temperature. It was found that, the viscosity of 5 and 10% (8 and 11 cp respectively) are not affected by increasing the temperature. While, 15% solution of poloxamer exhibits a marked increase in viscosity with increase in temperature, but, it still in the liquid form at room temperature (14 and ≃20 cp at 4 and 25°C respectively). On the other hand, the viscosity at 20% w/v poloxamer is ≃52.3 P at shear rate of 0.37 sec^{-1} viz. ≃0 (simulating that of rabbit's eye blinking) at 25°C (Table 1). Figure (1) showes the rheogram of poloxamer gel form. From the down curve, it has been shown that the viscosity of the gel decreased with increasing the shear rate at 25°C. It corresponded to pseudoplastic (shear thining) flow indicating structural breakdown of the existed intermolecular interactions between polymeric chains.[21] It was reported that the polymer solution had the Newtonian behaviour at 4°C but at higher polymer concentration (≃20%) the gel had non-Newtonian behaviour (pseudoplastic) at room temperature.[22] It was also, reported that, aqueous solutions and gel of poloxamer 407 appeared to withstand terminal autoclaving without undergoing major changes in their rheological properties.[23]

Infrared spectroscopy (IR) and differential scanning calorimetry (DSC)

Figure (2) shows the IR sepctra of poloxamer, diclofenac sodium and 1:1 drug: polymer physical mixture system. There was no absorption band for polymer in the region of carbonyl stretching vibration (1850-1450 cm^{-1}). Therefore, the discussion will be focused on the region of carbonyl stretching bands near 1600 cm^{-1}. The spectrum of diclofenac sodium (Curve b) shows one absorption band at 1571 cm^{-1} due to carboxyl carbonyl stretching. This band was not affected by the presence of polymer in the physical mixture (Curve c) which emphasized that, there is no interaction between the drug and the polymer at normal condition. Figure (3) shows DSC curves of poloxamer, diclofenac sodium and 1:1 drug:polymer physical mixture. Two endothermic peaks were observed in the physical mixture (Curve c). One of them was around 55.4°C due to the fusion of the polymer, and the other was around 263°C due to fusion of the drug-polymer dispersion which was produced during heating process. Melting point of drug was around 280.5°C (Curve b). In conclusion the extent of any type of complexation or interaction between the poloxamer and diclofenac sodium was absent in the normal condition as indicated by IR spectra and DSC.

In-vitro release of diclofenac sodium from ophthalmic solutions and gel of poloxamer 407

The effect of poloxamer concentration on the release of DIC was evaluated using the dialysis method. The cellulose membrane was permeable to diclofenac sodium and impermeable to poloxamer micells. The rate of release across the membrane was measured at pH 6.8.[24] As shown in Fig. (4) the presence of 15 and 20% poloxamer in the doner phase, 15.3 and 12.4% of the drug were released, respectively, within 5 hours. On the other hand, 52.1 and 27.3% of the drug were released during the same period for 5 and 10% poloxamer respectively, viz. as the concentration of the polymer increased the amount of drug released progressively decreased. This may be attributed to the increase in vehicle viscosity which alter the migration of the drug molecules through it into the release media. For voltaren eye drops the amount of diclofenac released was 30.7% after 5 hours, i.e. a little more than that released from viscous solution containing 10% w/v poloxamer.

Drug release kinetics

The release data of diclofenac sodium from solutions (5, 10 and 15%), gel (20% w/v) poloxamer and voltaren eye drops were treated mathematically according to zero, first order[25] and Higuchi diffusion model mechanisms.[26] The data are summarized in Table (1) which depict

Table 1: Mathematical treatment of the release data according to zero, first order and Higuchi diffusion mechanisms for diclofenac sodium from ophthalmic vehicles containing different concentrations of poloxamer and voltaren eye drops.

Polymer conc.	5%		10%		15%		20%		Voltaren eye drops	
Type of mechanism	r	k	r	k	r	k	r	k	r	k
Zero-order	0.896	0.1304	0.897	0.0780	0.877	0.0419	0.789	0.0320	0.882	0.0776
First-order	0.934	0.0008	0.916	0.00041	0.886	0.00020	0.798	0.00015	0.903	0.00041
Higuchi model	0.988	2.6751	0.987	1.5984	0.976	0.8684	0.936	0.7069	0.980	1.6047

r= Correlation coefficient

k= Release rate constant; $mg.min^{-1}$, min^{-1} and $mg.min^{-½}$ for zero, first and Higuchi model mechanisms, respectively.

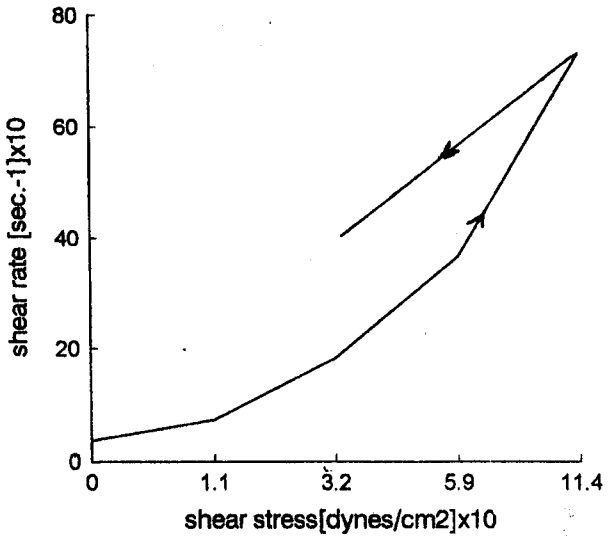


Fig. 1: Flow curve of 20% w/w poloxamer solution.

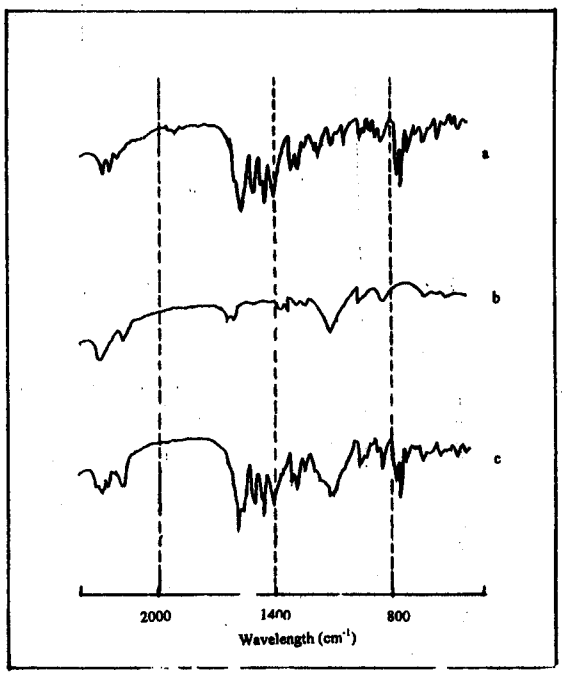


Fig. 2: Infrared spectra of diclofenac sodium systems. a) diclofenac sodium alone; b) poloxamer alone; c) diclofenac sodium-poloxamer physical mixture (1:1 molar ratio).

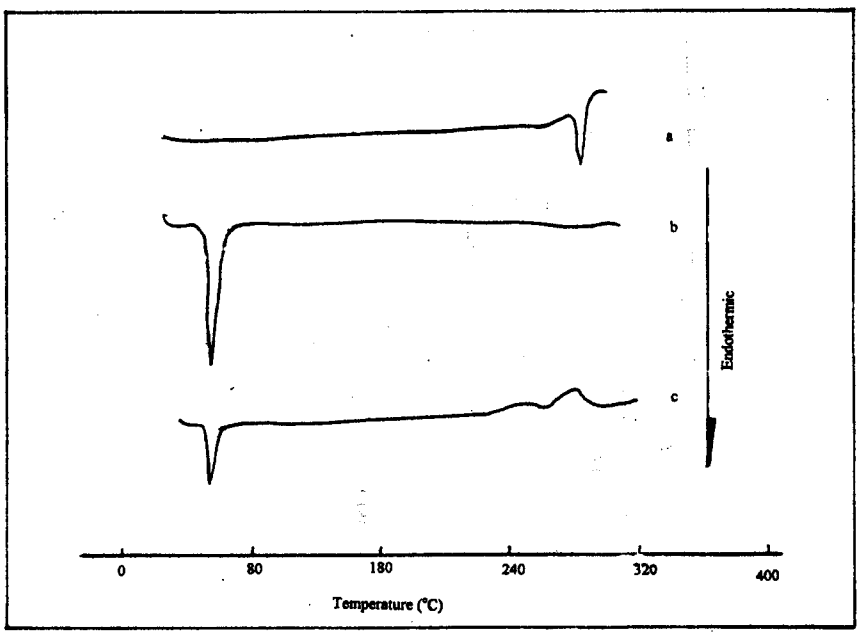


Fig. 3: Differential scanning calorimetry patterns of diclofenac sodium systems.
a) diclofenac sodium alone; b) poloxamer alone; c) diclofenac sodium-poloxamer physical mixture (1:1 molar ratio).

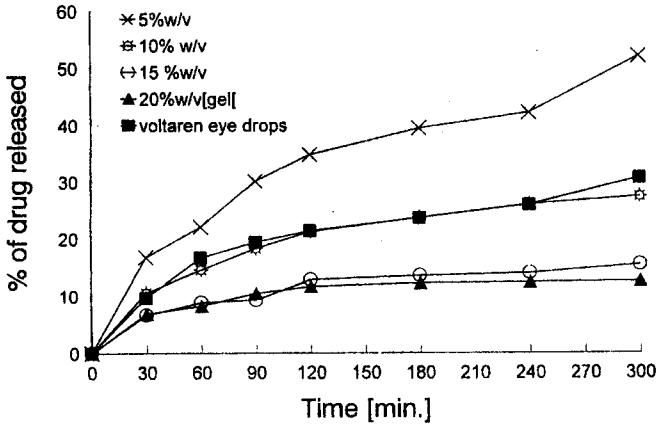


Fig. 4: Release profile of diclofenac sodium from ophthalmic solutions and gel containnig different concentrations of poloxamer.

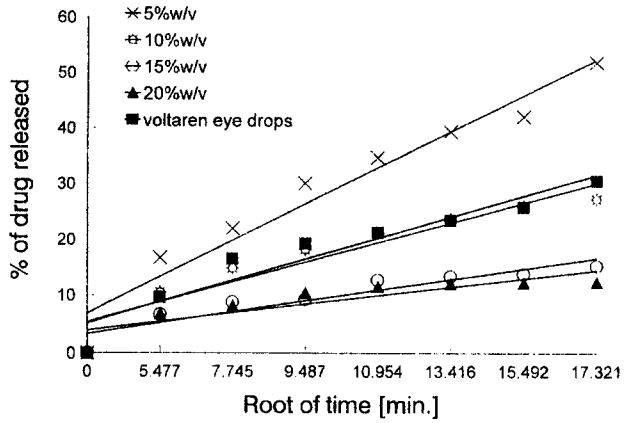


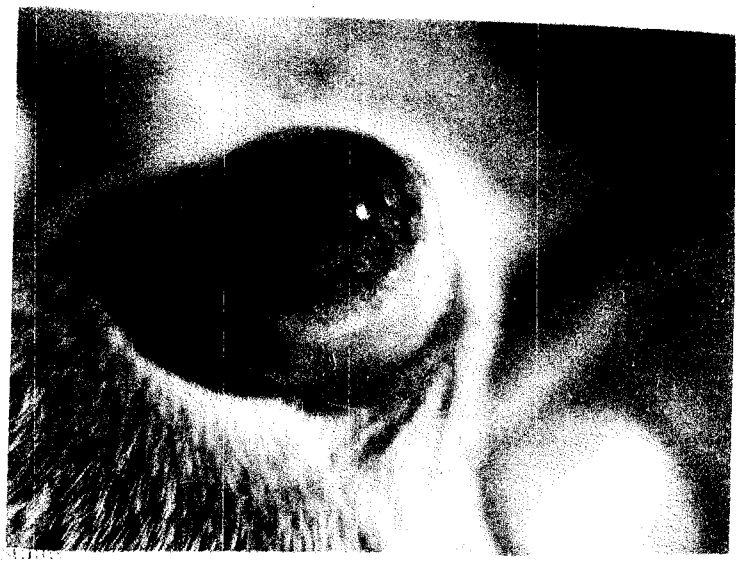
Fig. 5: Plot of the rlease data of diclofenac sodium according to Higuchi mechanism for ophthalmic solutions and gel containing different concentrations of poloxamer.

that, the release kinetics is diffusion controlled. In Fig. (5) the cummulative amount of diclofenac sodium released from different concentrations of poloxamer as well as, voltaren drops were plotted as a function of square-root of time. Linear responses ($r > 0.930$) for all formulations were produced, indicating that, the release mechanism is in compliance with the Higuchi equation. The release rate constant calculated, however, were decreased from 2.675 to 1.5984 to 0.8684 and 0.7069 $mg.min^{-½}$ as poloxamer concentration were increased from 5% to 10% to 15% and 20% respectively. The marketable eye drops (voltaren) with 0.1% w/v diclofenac sodium have an intermediate release rate constant between 5 and 10% poloxamer solutions. The lower release rate of drug with the higher poloxamer concentration in the present study was in agreement with that reported by Suh and Jun[27] and Lauffer's[28] diffusion theory in gels, which stated that the diffusion coefficient is inversely proportional to the volume fraction occupied by the gel forming agent.

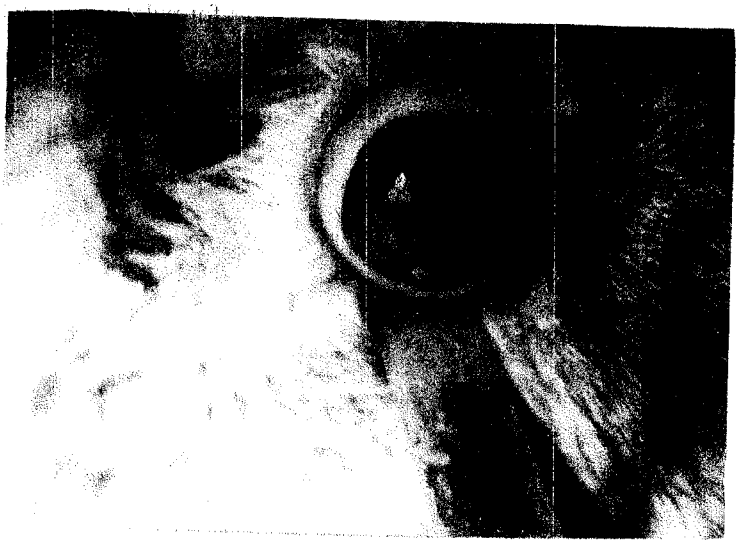
Performance of diclofenac sodium on the healing of ulcerative cornea of rabbits eye

The anti-inflammatory effect was assessed through the rate and extent of healing of inflammed tissues in the ulcerative areas in the rabbit's cornea. The vehicles used were placebo, voltaren eye drops, viscous solutions (10 and 15% w/v) and gel (20% w/v) of poloxamer. All these formulations contain 0.1% w/v diclofenac sodium except that of placebo which was 0.5% chloramphenicol eye drops. The period of observation for placebo, 10% w/v poloxamer and voltaren was 14 days. At the end of this period the recovery rates were 30, 60 and 66% for placebo, 10% and voltaren, respectively. On the other hand, the recovery rate for 15 and 20% were 80 and 90% respectively after elapse of 10 days treatment time (Photographs I & II).

Figure (6) and table (2) depict the healing rate of the corneal ulcers for placebo and the test treatments. It is obvious that the healing process follows first order kinetic. In the absence of diclofenac sodium (placebo), the healing process proceeds very slowly with ulcer half life time of about 64 days. Treatment with diclofenac sodium enahnces drastically the healing process and the ulcer half life were lowered to about 6.5, 8.67 and 11.11 days for 20, 15 and 10% w/v poloxamer, respectively. On the other hand, voltaren eye drops lowered the half life to 12 days when compared to placebo.



Photograph I: Ulcerative rabbit's cornea before treatment by diclofenac sodium preparations (gel form).



Photograph II: Ulcerative rabbit'scornea after treatment by diclofenac sodium preparations (gel form).

Table 2: Mathematical treatment of the healing effect of diclofenac sodium on the ulcerative rabbit's eye according to first order kinetic.

First-order parameters	Placebo	Test formulations			
		10% w/v	15% w/v	20% w/v	Voltaren
r	0.942	0.974	0.954	0.973	0.978
k	0.0107	0.0624	0.0799	0.0985	0.0581
t½	64.8	11.11	8.67	6.48	11.13

r = Correlation coefficient
k = Healing rate constant (day^{-1})
t½ = Half-life time (day)

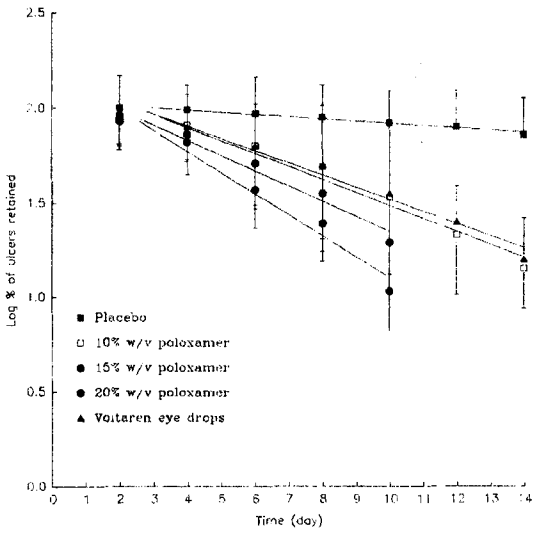


Fig. 6: Effect of poloxamer concentrations on the healing rate of diclofenac sodium for ulcerative rabbits eye.

Statistical analysis of the differences between placebo group, on one hand and any of the test groups, on the other hand, is depecticted in Table (3) with regard to the number of residual ulcers. It is evident that the treatment involving diclofenac and for all formulations brings about highly to very highly significant after elapse of 10 days treatment time. When 10% w/v poloxamer and voltaren eye drops were compared with 15% bring about insignificant differences and from significant to highly significant when both compared with 20% respectively. The statistical analysis of the differences between 15 and 20% w/v poloxamer does not show any significant difference with regard to the residual ulcers by the end of 10 days of treatment (Table 3).

The statistical analysis of the data revealed that, the viscosity of the system dictates the *in-vivo* performance of the drug only within narrow and low viscosity ranges extending up to about 5-15 cp. Throughout this range, increasing the viscosity provides a powerful tool to promot drug effect beyond this range, increasing the viscosity several times does not bring about any significant drug effect with regard to the healing of the ulcerative cornea. These findings are in agreement with that reported by Kassem *et al.*,[29,36] Abd-Elmaged *et al.*,[37] Chrai and Robinson[30] and Adler *et al.*,[31] and demonstrated that the healing of ulcerative rabbit's cornea produced by liquid ophthalmic preparations of low viscosity (≃ 5-15 cp) containing poloxamer polymer are equivalent to that of the gel form.

Conclusion

This study depicts that, treatment with diclofenac sodium enhances drastically the healing process and the ulcer half life time is lowered to significant value when compared with placebo. Also, it was clearly observed that, the presence of poloxamer in different proportions affected the healing of the ulcers to a marked extent, viz. as the concentration of the polymer increases the ulcer healing hlaf life time progressively decreases. These finding

Table 3: Statistical significance of differences between placebo and test groups and between test groups with each other (with regard to the number of residual ulcers) according to t-test analysis.

Days	Placebo with 10%		Placebo with voltaren		Placebo with 15%		Placebo with 20%		10% with voltaren		10% with 15%		10% with 20%		Voltaren with 15%		Voltaren with 20%		15% with 20%	
	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p	t	p
2	2.220	0.05	2.54	0.05	3.87	0.01	5.07	0.001	0.62	0.1	0.337	0.1	1.16	0.1	0.56	0.1	1.72	0.1	0.91	0.1
4	3.093	0.01	3.21	0.01	6.06	0.001	6.799	0.001	2.1	0.1	1.77	0.1	2.886	0.05	1.99	0.1	2.41	0.05	1.54	0.1
6	3.933	0.01	4.11	0.01	6.60	0.001	9.807	0.001	1.65	0.1	1.49	0.1	3.41	0.01	1.77	0.1	4.02	0.01	2.15	0.1
8	4.050	0.01	3.85	0.01	8.83	0.001	11.786	0.001	1.73	0.1	1.41	0.1	2.41	0.05	1.56	0.1	3.93	0.01	1.6	0.1
10	3.842	0.01	4.06	0.01	9.35	0.001	11.673	0.001	1.61	0.1	1.03	0.1	2.72	0.05	1.34	0.1	3.42	0.01	1.16	0.1

The values 0.001, 0.01, 0.05 and 0.1 represent the values of P and they are: very highly significant, highly significant, significant and insignificant, respectively.

t = t-test value.

demonstrates that, poloxamer can be utilized in concomitant with diclofenac sodium, for healing the ulcerative cornea of rabbits eye, and has a considerable influence in enhancing diclofenac healing process. The enhancing effect of poloxamer on ulcer healing was attributed to:

1- Poloxamer enhances, solely, the functional recovery of lethally heat-shocked fibroplasts, as it was reported, poloxamer 188 possesses the ability to self-aggregate into membrane-like structure in aqueous solutions acting as synthetic barrier[33] and restoring membrane integrity.[32]
2- Poloxamer as nonionic surfactants, may offer means of enhancing drug permeation through the cornea, i.e. enhancing diclofenac skin penetration[34] due to changes in the skin barrier properties and in the vehicle-structure corneum partition coefficient.
3- Poloxamer acts as viscolizer, consequently the contact time increased with increasing polymer concentration, which in turn drastically enhances drug absorption, bioavailability[35,36] and consequently ulcer healing.

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