International Journal of Drug Delivery 3 (2011) 535-545

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Ketorolac Tromethamine *In-situ* Ocular Hydro Gel; Preparation, Characterization, and *In-vivo* Evaluation

Randa Zaki[1], Khaled M. Hosny[1], Ahmed Khames[1,2]*, Ahmed Abd-elbary[3]

***Corresponding author:**

Ahmed Khames

1 Department of Pharmaceutics,
Beni Suief University,
Beni Suief,
Egypt
2 Department of pharmaceutics,
Taif University,
Taif, KSA
3 Departments of Pharmaceutics,
Cairo University,
Egypt
*Tel. +966598119986
Email: dr_akhames@yahoo.com

Abstract

The aim of this work is to formulate Ketorolac tromethamine in a new ocular in situ hydro gel delivery system. Two polymers; Chitosan and Carbopol 940 were used in different concentrations for the preparation of the in situ hydro gels, all formulations exposed to visual examination, pH measurement, in-vitro release, rheological study, stability study, and in-vivo anti-inflammatory effect study on the inflamed eye of rabbits. Results showed that all formulations were clear and showed pH within the acceptable range, Chitosan 0.5%w/v gives the highest release rate, all formulae exhibited pseudoplastic flow with a thixotropic behavior, stability study showed that rate of drug degradation followed first order kinetics and 0.5% Chitosan based formula showed longer shelf life (2.532 year), the percent of unhealed ulcers of the inflamed eye of rabbits was 17.5% for 0.5% Chitosan in situ hydro gel compared to 55% for Acular eye drop (positive control). Statistical analysis of the data revealed significance difference between the tested formula and control solution at $p < 0.05$. So this system that combines the advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the latter, also enhances the healing rate of Ketorolac tromethamine in ulcerative rabbit's eye compared to ocular eye drops.

Keywords: Ketorolac Tromethamine; In situ gel; Chitosan; Corneal ulcer; Hydro gel

Introduction

Drugs are commonly applied to the eye for a localized action on the surface or in the interior of the eye [1]. A major problem in ocular therapy is to attain an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage form is mainly due to the precorneal loss factors which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane [2-3]. Due to these physiological and anatomical constraints, only a small fraction of the administered drug, (effectively 1% or even less of the instilled dose) is ocularly absorbed. [4-5].

In order to overcome the problems of conventional ocular therapy, newer delivery systems are being explored. Various approaches, like viscosity enhancement, use of mucoadhesives, particulate drug delivery, vesicular drug delivery, prodrugs, and other controlled systems, like ocuserts, are being explored [2-3, 6-7].

Ketorolac is a non-steroidal agent with a powerful analgesic and moderate anti-inflammatory activity, widely used in the management of both moderate and severe pain [8, 9], Ketorolac is applied topically in the management of seasonal allergic conjunctivitis, postoperative ocular pain and inflammation [10]. It is nonirritating to the eye at 0.5% w/v concentration [11].

The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery or as tear substitutes, they do not allow accurate and reproducible administration of quantities of drugs and, after administration; they often produce blurred vision, crusting of eyelids, and lachrymation. A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the latter, thus in situ hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye [12-13].

Chitosan shows sol to gel transformation when the pH exceeds 6.2 due to the neutralization of chitosan amine groups is obtained by alkaline deacetylation of chitin, which is one of the most abundant polysaccharides in nature found in various organisms including the cell walls of fungi and exoskeletons of arthropods such as crabs, shrimps and insects [14]. It is cationic, nontoxic, biocompatible and biodegradable. In addition, chitosan has mucoadhesive properties [15].
Carbopol is a polyacrylic acid (PAA) polymer, which shows a sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5 [16], is widely used in ophthalmology to enhance precorneal retention to the eye [12]. Carbopol offers the advantage of exhibiting excellent mucoadhesive properties when compared with other polymers.

The formulation of a stable dosage form is essential for the patients' safety and drug efficacy. The physicochemical parameters, such as the presence of additives as well as the storage conditions, which may affect the stability of drugs, have received considerable attention in the field of pharmaceutics [17].

The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under the influence of various environmental recommended storage conditions, retest periods and shelf-lives to be reestablished [18].

So, the aim of this work is to formulate Ketorolac tromethamine in a new ocular in situ hydro gel delivery system using Chitosan and Carbopol 940 at different concentrations. The prepared in situ hydro gel formulations will be fully evaluated; also In vivo anti-inflammatory effect on the inflamed eye of rabbits for the prepared formulations will be studied.

Material and Methods

Materials

Ketorolac Tromethamine [ELAMRIA Company, Alexandria, Egypt]. Carbopol 940 [Goodrich Chemical Company, Ohio, USA], Cellulose nitrate membrane filter, diameter pore: 0.45 μm [Albet Company, Spain], Chitosan [Nantong, China]. Triethanolamine [Merck, Germany]

Methods

Preparation of Ketorolac Tromethamine in-situ forming hydro gels:

Using Chitosan

Chitosan solution was obtained by dissolving the required amount of chitosan in 10 mls of 0.1 N HCl under mechanical stirring at room temperature. This solution was cooled down to 5ºC using an ice bath and 2 g of Glycerol (at 5 º C) was added as gelling agent. The calculated amounts of Ketorolac Tromethamine (KT) and NaCl were added to prepare isotonic 5mg/ml

drug solution and the pH was then adjusted to 6.2 by dropwise addition of diluted NaOH (at 5 º C). Finally, cold water was added to obtain a total mass of 20 gm. The final preparations contained 10% (w/w) of a gelling agent [19]. Chitosan Solutions were prepared at concentrations of 0.25, 0.5 and 1% (w/w) before pH adjustment.

Using Carbopol:

The weighed amount of carbopol 940 was gradually sprinkled on 100ml of 0.5% drug solution and stirred with mechanical stirrer at high speed until no lumps was observed. Stirring speed was then reduced to break the foam. Finally pH of the product was adjusted to 5.5 using triethanolamine. Carbopol Solutions were prepared at concentrations of 0.1 and 0.2% (w/v).

Evaluation of the prepared Ketorolac Tromethamine in-situ forming hydro gels:

The prepared formulations were evaluated as follows:

Visual Examination:

The general appearance (clarity) of the formulations was examined visually.

pH Measurement:

The pH of the prepared formulations was checked by using pH-meter.

Determination of Drug Loading Capacity:

The drug loading capacity (drug content) was determined by diluting 1gm of the prepared formulation to 100 ml with simulated tear fluid [STF] pH 7.4 then stirring with magnet until dissolved. Aliquots of 5 ml were withdrawn and further diluted to 25ml with STF. Ketorolac tromethamine concentration was determined spectrophotometrically at λmax 322 nm.

Determination of Gelling Capacity:

In order to identify the compositions suitable for use as in situ gelling systems, gelling capacity of the prepared formulations was evaluated. The gelling capacity was determined as follows: the prepared in situ gelling systems were mixed with freshly prepared STF in the ratio of 25: 7 respectively (application volume 25 µm, normal volume of tear fluid in the eye is 7 µl) at 35ºC± 0.5. Gelation was assessed by visual examination [20]. The time for gelation & the time taken for the formed gel to re- dissolve were recorded. The composition of artificial tear fluid was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride·2H2O 0.008 g, and purified water q.s.100 g [21].

In-vitro release of Ketorolac Tromethamine from the prepared in-situ hydro gel formulations:

A glass cylindrical tube (2.5 cm in diameter and 6 cm in length) containing the formulae to be tested was attached instead of the basket of the dissolution apparatus and tightly covered with a semi permeable membrane (0.45 µm pore size), The cylindrical tube was dipped in a 500 ml simulated tear fluid (PH 7.4), the release study was carried out at 34ºC ± 0.5, [the temperature of the eye] [22], according to predetermined time regimen, aliquots of 5 ml were withdrawn and further diluted to 25 ml with STF. Ketorolac tromethamine concentration was determined spectrophotometrically at λmax 322 nm; the results were the mean values of three runs.

Kinetic analysis of the release data of Ketorolac Tromethamine from the prepared in-situ hydro gel formulations:

The release data were analyzed using the linear regression according to:

Zero Order Kinetics: $\mathbf{Ct = C0 - Kt}$

First Order Kinetics: $\mathbf{Log\ Ct = Log\ C0 - kt/2303}$

And also according to the simplified Higuchi diffusion model [23]

$$\mathbf{Q/A = 2C_0 (A/2n)^{1/2} t^{1/2}}$$

The correlation coefficient (r) was determined in each case.

Measurement of viscosity of the prepared in-situ hydro gel formulations:

The viscosity determination of prepared formulations was carried out using Brookfield Viscometer with spindle 52. Viscosity of samples was measured at different angular velocities. A typical run comprised changing angular velocity from (10 to 100) rpm with equal wait for each rpm. The angular velocity was reversed (100 to 10 rpm). The rheological parameters of different formulations were studied.

Stability study of Ketorolac Tromethamine in the prepared in-situ hydro gelling systems:

On the basis of the previous evaluation studies of the prepared Ketorolac Tromethamine formulations; F1b (0.5%Chitosan) was selected to undergo further stability studies. The selected formulation was stored in dark place at controlled room temperature (25ºC ± 2) for one year to detect any change in its characteristics which may affect efficacy or suitability for use over their shelf life.

At predetermined time intervals, samples were withdrawn and examined physically for any changes and chemically for its Ketorolac Tromethamine content using the HPLC stability indicating assay. The stability study includes both physical and chemical stability.

Physical stability of the selected Ketorolac Tromethamine formulation which include:

Visual Examination - pH Measurements - Determination of The Gelling Capacity - In-vitro Release - Kinetic Analysis of the Release Data of Ketorolac Tromethamine from its Different Formulations after Storage for 12 Month. - Rheological Evaluation.

Chemical stability of the selected Ketorolac Tromethamine formulation.

Samples were analyzed for the percent remaining of Ketorolac tromethamine using HPLC stability indicating assay at time intervals of 0, 2,4, 6,8,10 and 12 months.

In-Vivo anti-inflammatory effect of Ketorolac Tromethamine on the inflamed eye of rabbit:

A group of five male rabbits (1.5-2 kg) was considered in this work, two drops of 0.4% solution of Oxybuprocaine HCl were instilled onto each rabbit's eye as a local anesthetic. 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. The ulcers had a circular shape (2mm diameter) and reached in depth of corneal epithelium. This was ascertained by the instillation of fluroescein sodium solution (0.2%). Since, Fluroescein does not stain tissue unless the epithelium is disrupted; the inflamed areas develop a green fluorescence. The absence of green color was thus taken as a criterion of ulcer healing. One drop of a sterile ciprofloxacin eye drops (0.3%) 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 ciprofloxacin solution every morning throughout the observation period. The test treatment involved the instillation of one drop of ciprofloxacin solution followed by one drop of the Ketorolac tromethamine solution (Acular eye drop) as a positive control or the formula F1b (0.5% Chitosan) every morning throughout the observation period which extended for 12 days. The data of corneal inflammation healing time were subjected to statistical analysis according to student t-test.

Results and Discussion

Evaluation of the prepared Ketorolac Tromethamine in-situ hydro gels:

According to the results listed in table (1), clarity of all prepared formulations was found to be satisfactory. The pH was within acceptable range (from 5.5 to 6.5) and hence would not cause any irritation upon administration on to the eye. Ideally, the ophthalmic preparations should have possessed the pH in the range of 4.5 – 11 [24].

Table 1: Results of different evaluations studies applied on the prepared KT in-situ hydro gel formulations at different polymer concentrations

Formula Number	Composition		Appearance (clarity)	pH Mean ± SD	Drug Content (mg/ml) Mean ± SD
	Polymer type	Polymer Conc.			
F1a	Chitosan	0.25	Clear	6.2±0.006	98.45±0.014
F1b	Chitosan	0.50	Clear	6.2±0.010	99.43±0.025
F1c	Chitosan	1.00	Clear	6.2±0.008	100.5±0.015
F2a	Carbopol	0.10	Clear	5.5±0.000	101.3±0.006
F2b	Carbopol	0.20	Clear	5.5±0.014	99.87±0.020

Percent drug content in all formulations was found to be within the acceptable pharmacopeial range of 98.35 to 101.32% indicating uniform distribution of drug [25].

Determination of gelling capacity:

Results listed in table 2 showed that formulae F1a and F2a, gelled after a few minutes, dissolved rapidly (unacceptable formulations). But Formulae F1b and F2b, gelled instantaneously (less than a minute), and retained their consistency for a few hours on contact with STF (acceptable formulations). F1c, gelled instantaneously (less than a minute) and remained for an extended period (unacceptable formulations) as this may cause irritation to the eye upon application. These results can be easily correlated with the polymer concentration into the prepared in situ hydrogel formulations as follows, formulations of low polymer concentration (low viscosity) showed low gelling capacity (sign+) and formulations of moderate polymer concentration (moderate viscosity) showed acceptable gelling capacity (sign++),While the formulations of high polymer concentration (high viscosity) showed high gelling capacity (sign+++).

Table 2: Results of gelling capacity study applied on the prepared KT in-situ hydro gel formulations at different polymer concentrations.

Formula Number	Gelling Capacity
F1a	+
F1b	++
F1c	+++
F2a	+
F2b	++

Notice: +: Gels after few minutes and rapidly dissolves.
++: Immediately gels and remains for few hours.
+++: Immediately gels and remains for an extended time period.

In-vitro release of Ketorolac Tromethamine from different formulations:

Figure 1 shows that the percentage of drug released after 320 min from F1a, F1b, and F1c was 82.9%, 77.66%, and 66.33% respectively. Figure (2) shows that the percentage of drug released after 320 min from F2a, and F2b were 81.02%, and 74.87% respectively.

It is clear from these results that all formulations showed initial burst release due to the hydrophilic nature of the prepared in situ gel formulations, slowing the release rate later on can be explained on the basis of the diffusion process of the drug

through the formula that markedly retarded due to increase of the its viscosity in contact with the release media. In other words; the release of drug from the prepared in situ hydro gels is inversely proportional to the gel strength. The results of the drug release study can also be inversely related to the polymer concentrations in the prepared formulae.

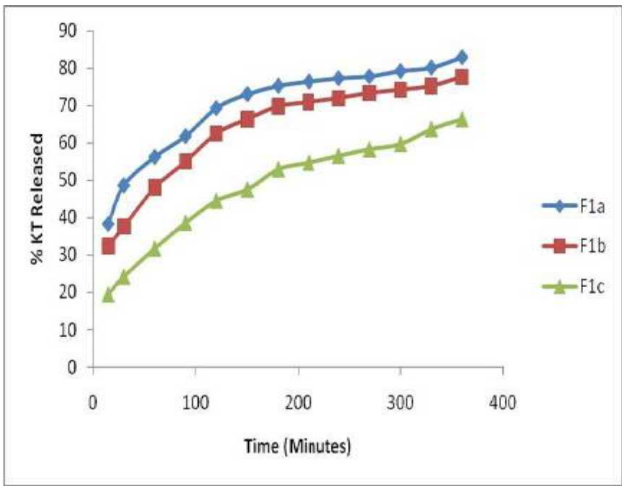

Fig 1: In-vitro release of KT from Chitosan based formulations in simulated tear fluid at 34º C.

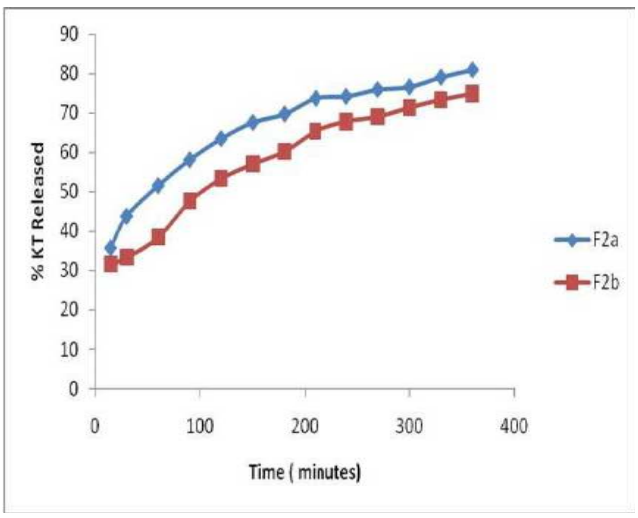

Fig 2: In-vitro release of KT from Carbopol based formulations in simulated tear fluid at 34º C

Kinetic analysis of the release data of Ketorolac Tromethamine from the prepared in-situ hydro gel formulations:

The release data of KT from its different formulations was treated mathematically according to zero, first order and Higuchi diffusion model mechanisms. The data is illustrated in table (3). It is clear that the release of KT follows diffusion controlled mechanism. These results are related to the change in consistency of the in situ hydro gel formulae in contact with the release media.

Measurement of viscosity of the prepared formulations:

The flow behavior of the two formulations which showed acceptable gelling capacity [F1b, F2b] was described in table (4). From the data of the table, all formulations exhibited pseudoplastic flow with a thixotropic behavior. Generally viscosity values in the range of 15-50 cps significantly improve the contact time in the eye. Higher viscosity values are not required as it does not offer significant advantage and have a tendency to leave a detectable residue on the lid margin. The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the pre-corneal film. Since the ocular shear rate is very high, ranging from 0.03 s^{-1} during interblinking periods to 4250 - 28,500 s^{-1} during blinking [26], viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often preferred.

Physical stability of the selected Ketorolac Tromethamine formulation after storage for 12 months:

Formula F1b showed no change in clarity upon storage for one year. The pH showed slightly change but still within the acceptable range,

Table 3: Kinetic analysis of release data of KT from the prepared in-situ hydro gel formulations

Formula Number	Linear Regression Analysis Using Correlation Coefficient (r^2) According to:		
	Zero Order	First Order	Diffusion Model
F1a	0.7338	0.8898	0.9322
F1b	0.8059	0.9213	0.9677
F1c	0.8702	0.9295	0.9911
F2a	0.7397	0.8774	0.9361
F2b	0.7749	0.8761	0.9404

Table 4: Results of the rheological study applied on the selected KT in-situ hydro gel formulations.

Formula Number	Minimum Viscosity (c.p.)	Maximum Viscosity (c.p.)	Furrow's Constant (N)	Flow Behavior
F1b	75.4	30.2	1.633	Pseudoplastic
F2b	250.5	84.06	1.749	Pseudoplastic

Table 5:Results of the stability study applied on KT in-situ hydro gel formula F1b for one year.

Formula Number	Percentage of Ketorolac Tromethamine remained after storage for the following time intervals (Month)						
	0	2	4	6	8	10	12
Fb1	100	99.42	98.72	97.95	97.42	96.61	95.84

Table 6:Results of kinetic analysis of the stability data of KT in-situ hydro gel formula F1b.

Formula Number	Corr. Coeff. (r)		Slope	Intercept	K ($month^{-1}$)	$t_{50\%}$ (month)	$t_{90\%}$ (month)
Fb1	Zero	0.9974	-0.0015	2.0004	0.003455	200.6079	30.395
	First	0.9978					

Table 7:Number and percentage of unhealed ulcers in rabbit's eyes after application of 0.3% Ciprofloxacin eye drops (negative control), Acular eye drops (positive control), and Test formula F1b after 12 days.

Tested Formula	Mean Number of Unhealed Ulcers	Percentage of Ulcer Healing
Ciprofloxacin eye drops (0.3%) (negative control)	7	87.5
Acular eye drops (positive control)	4.4	55
Test formula F1b	1.4	17.5

Table 8: Kinetic analysis of the results of the healing effect of Ketorolac Tromethamine on ulcerative rabbit's eye

Tested Formula	Linear Regression Analysis Using Corr. Coeff(r) According to:		Kinetic Order	First Order Parameters		
	Zero Order	First Order		Slope	Healing Rate Constant [K (day^{-1})]	Half Life $t_{1/2}$ (day)
Ciprofloxacin eye drops (0.3%) (negative control)	0.8689	0.8767	First	-0.00465	0.0107	64.712
Acular eye drops (positive control)	0.9613	0.9658	First	-0.02595	0.0597	11.596
Test formula F1b	0.9248	0.9925	First	-0.06526	0.1502	4.611

Table 9: Statistical analysis of the difference between Control and Acular and between Test Formula (0.5% Chitosan solution) and Acular eye drops {With Regard to the Number of Residual Ulcers} According to t-test Analysis

Days	Acular with control		0.5%Chitosan with Acular	
	t	p	t	p
3	0.632456	N.S	5.879747	0.001
5	0.2886751	0.02	6	0.001
7	6.957011	0.001	5.879747	0.001
10	6.5	0.001	6	0.001
12	6.5	0.001	8.660254	0.001

N.S.: Non significant P: level of significance

also the gelling capacity showed no change. Regarding drug release studies, Fig (3) shows that there storage of the prepared in situ hydro gel formulae did not affect the release of the drug. The release profile of Ketorolac tromethamine from different formulations still follows Higuchi diffusion mechanism.

Chemical stability of the selected Ketorolac Tromethamine formulation

The results of the stability study of the drug in the prepared formulations were presented in table (5) which illustrated that percentage of KT remained in formula F1b after storage for 12 month was found to be 95.84%. Kinetic analysis of the stability data revealed that the degradation followed first-order kinetics, as presented in Figure (4).The predictive shelf life was calculated according to the order equation, which states that: t_{90} = 0.105/ K_{25} and results showed that, F1b possessed a predictive shelf life of 2.532 years as shown in table (6).

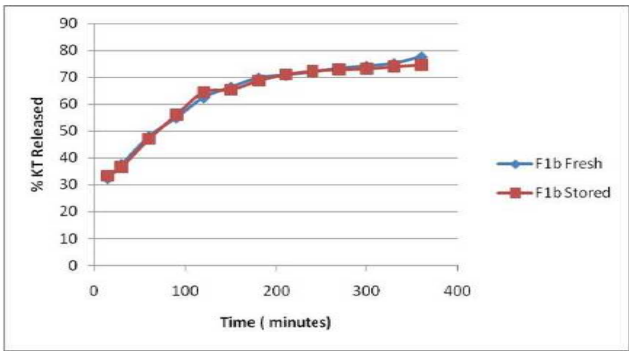

Fig 3: In-vitro release of KT from fresh and stored in-situ hydro gel Formula (F1b) in simulated tear fluid at 34ºC

VIII-Performance of Ketorolac Tromethamine on the healing of ulcerative cornea of rabbit eye:

The anti-inflammatory effect of Ketorolac tromethamine was determined through the rate and extent of healing of inflamed tissue in the ulcerative areas in the cornea of rabbits. The vehicles used were negative control (ciprofloxacin solution), positive control (Acular eye drops) and the test formula F1b (0.5% Chitosan). The period of observation was 12 days. At the end of the observation period the percent of unhealed ulcers were 87.5%, 55% and 17.5% for negative control, positive control and test in situ hydro gel formula F1b respectively as shown in table (7).

Table (7) also shows the healing rate of the corneal ulcers for negative control, positive control and test formula F1b. According to the results of kinetic analysis of the healing data listed in table (8), it is obvious that the healing process follows first order kinetics. In the absence of Ketorolac tromethamine (negative control), the healing process proceeds very slowly with ulcer half life time 64.71days. Treatment with the test formula F1b drastically enhanced the healing process and the ulcer half life was lowered to about 4.61 days. On the other hand Acular eye drops (positive control) lowered the half life to 11.59 days when compared to negative control.
Results of the statistical analysis of the healing data are shown in table (9), it is obvious that, there is a significant difference between test formula F1b and positive control (Acular eye drops) at the tested probability levels.

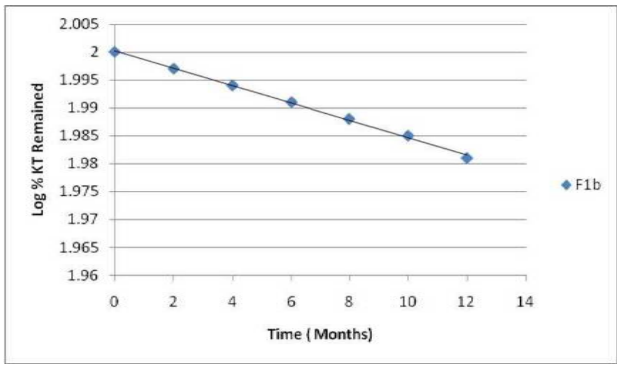

Fig 4: A plot of log percent of Ketorolac Tromethamine remained in-situ hydro gel Formula (F1b) versus time according to first-order kinetics.

Conclusion

Formulation of Ketorolac Tromethamine in chitosan based in situ hydro gel form was able to

reach the target area, to sustain drug release in high therapeutic levels, to decrease frequency of drug administration to prolong contact time, and to enhances the healing rate of Ketorolac tromethamine in ulcerative rabbits eye compared to commercially available eye drops.

REFERENCES

1. Davies NM. Biopharmaceutical considerations in topical ocular drug delivery. Clin. Exp. Pharmacol. Physiol. 2000; 27: 558– 562.

2. Le Bourlais CL, Acar L, Zia H, Sado PA, Needham T and Leverge R.. Ophthalmic drug delivery systems—recent advances. Prog. Retin. Eye Res.1998; 17: 35.

3. Kaur. IP and Kanwar M. Ocular preparations: the formulation approach. Drug Dev. Ind. Pharm. 2002; 28: 473–493.

4. Shell JW. Ophthalmic drug delivery systems. Surv. Ophthalmol.1984; 29:117.

5. Burstein NL and Anderson JA. Corneal penetration and ocular availability of drugs. J. Ocul. Pharmacol. 1985; 1: 309– 326.

6. Sirbat D, Marchal-Heussler L, Hoffman, M, and Maincent P. Ways to improve ocular bioavailability for topical applications. Ophthalmology. 2000; 23: 505–509.

7. Kaur IP and Smitha R. Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. Drug Dev. Ind. Pharm. 2002; 28: 353–369.

8. Schalnus R. Topical nonsteroidal anti-inflammatory therapy in ophthalmology. Ophthalmologiaca. 2003; 217:89–98.

9. Kokki H. Nonsteroidal anti-inflammatory drugs for postoperative pain: a focus on children. Paediatr. Drugs. 2003; 5:103–123.

10. Sweetman CS.; Martindale: The Complete Drug Reference. 34th ed. Pharmaceutical Press, London. 2005.

11. Malhotra M and Mujumdar DK. In vivo ocular availability of Ketorolac following ocular instillations of aqueous, oil, and ointment formulations to normal corneas of rabbits: A technical Note. AAPS Pharma sci tech. 2006; E1-E6.

12. Felt O, Baeyens V, Zignani M, Buri P and Gurny R. Mucosal drug delivery— ocular— Encyclopedia of controlled drug delivery. 1999; 2: 605–622.

13. Saettone MF, Giannaccini B, Guiducci A and Savigni P. Semisolid ophthalmic vehicles. III. An evaluation of four organic hydrogels containing pilocarpine. Int. J. Pharm. 1986; 31:261–270.

14. Ceulemans J, Vinckier I and Ludwig A. The use of xanthan gum in an ophthalmic liquid dosage form: rheological characterization of the interaction with muci. J. Pharm. Sci. 2002; 91: 1117–1127.

15. Yannic B, Schuetz l, Gurny R and Jordan O. A novel thermoresponsive hydrogel based on chitosan. Eur. J. Pharm. Biopharm. 2008; 68:19–25.

16. Carlfors J, Edsman K, Peterson R and Jornving K. Rheological evaluation of Gelrite in in situ gels for ophthalmic use. Eur. J. Pharm. Sci. 1998; 6: 113-119.

17. Chun IK, Kang JH and Gwak HS. Determination of ketorolac in human serum by high-performance liquid chromatography. Arch. Pharm. Res. 1996; 19: 529-534.

18. Ibrahim AA, Bosela AA, Ahmed SM and Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of Ketorolac. Eur. J. Pharm. Biopharm. 2005; 59:485–490.

19. Bourlais CL, Acar L, Zia H, Sado PA., Needham T and Leverge R. Ophthalmic drug delivery systems—recent advances. Prog. Retin. Eye Res. 1998; 17:33–58.

20. Carsentensen JT. Drug stability, 2nd ed. Marcel Dekker. Inc. NewYork,

Basel, Hong Kong. 1995; 6-56.

21. Pharmacopoeia of the United States of America, 23rd ed. Mack Publishing Co. Pennsylvania. 1995; 1360-1364.

22. Le Bourlais CA, Treupel-Acar L, Rhodes CT, Sado PA and Leverge R. New ophthalmic drug delivery systems. Drug Dev. Ind. Pharm. 1995; 21:19.

23. Higushi WI. Analysis of data on medicament release from ointments. J. Pharm. Sci. 1962; 51: 802.

24. Doijad RC, Manvi FV and Malleswara Rao VS. Sustained ophthalmic delivery of gatifloxacin from In situ gelling system. Ind. Pharm. Sci. 2006; 68: 814-818.

25. Edsman K, Carlfors J and Harju K. Rheological evaluation and ocular contact time of some carbomers gels for ophthalmic use. Int. J. Pharm. 1996; 137: 233.

26. Padval MV and Bhargava HN. Liquid chromatographic determination of indapamide in the presence of its degradation products. J. Pharm. Biomed. Anal. 1993; 11:1033-1036.