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Formulation and Evaluation of Insitu gelling System for Ocular delivery of Timolol Maleate

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Abstract
Poor bioavailability of ophthalmic solutions caused by dilution and drainage from the eye can be overcome by using *insitu* forming ophthalmic drug delivery system prepared from polymers that exhibit reversible phase transitions from liquid to gel. This results in better ocular availability of drug. The purpose of this work was to develop an ophthalmic drug delivery system on the concept of ion activated *insitu* gelation for Timolol maleate, an antiglaucoma agent. sodium alginate which gels in the presence of divalent cations present in lacrimal fluid was used as gelling agent HPMC, HEC, HPC Incorporated as the viscosity enhancing agent. The promising formulation F6 showed viscosity 44.2cps at 12rpm.99.72% drug release at the end of 8hrs.The developed formulations were therapeutically effective, stable, non annoyance and provided sustained release of drug over an 8 hrs period. The system is thus a viable alternative to conventional eye drops.
Keywords: Timolol maleate, *insitu* gelation, ophthalmic drug delivery, Sustained release

Introduction
Poor bioavailability of ophthalmic solutions is caused partly by the rapid decrease of Drug concentration in precorneal tear fluid.The rapid precornial elimination of Drugs given in eye drops is mainly due to conjuctival absorption and drainage of drug induced by lachrymation and normal tear turn over[1]. Another reason for low availability is the slow diffusion of water soluble drugs through the cornea [2]. A large proportion of topically applied drug is immediately diluted in tear film and excess of fluid spills over the lid margin and the remainder is rapidly drained in to the nasolachrymal duct. A proportion of Drug is not available for therapeutic action since it binds to extra orbital tissues. These processes lead to corneal contact time of about 1-2 min in humans and because of that ocular bioavailability is less than 10%[3]. Many ophthalmic drugs are applied in higher concentrations due to poor ocular bioavailability. This causes both ocular and systemic side effects [4]. Several new preparations have been developed for ophthalmic use not only to prolong the contact time of the vehicle at ocular surface, also to slow down the elimination of drug at the same time[5]. Successful results were obtained with inserts and collagen shields [6]. Although these preparations present some disadvantages, such as non compliance, especially by elderly people. This problem can be overcome by using insitu forming gel ophthalmic drug delivery system prepared from polymers that exhibit reversible phase transitions and pseudoplastic behaviour to minimize interference with blinking. Such a system can be formulated as drug containing liquid suitable for administration by instillation into the eye as it will shift to the gel phase, which upon exposure to physiological conditions, thus increasing the precorneal residence of the delivery system and enhancing ocular bioavailability. Timolol maleate (TM) is the most commonly used drug that treats the open-angle glaucoma. Systemic absorption of TM may cause respiratory and cardiovascular side effects, so it is important to minimize the systemic absorption and enhance ocular bioavailability of TM. The overall objective of this study was to develop and evaluate Timolol maleate ophthalmic insitu gel forming solution to improve the ocular bioavailability and hence decrease the systemic absorption and side effects of TM.

Materials and Methods
Timolol maleate was kindly provided by Aurobindo Pharmaceutical Industries. (Hyderabad).Sodiumalginate, were purchased from Loba chemicals, Hydroxypropylmethylcellulose (HPMC E15M),were purchased from loba chemicals Hydroxy propyl cellulose, Hydroxy ethyl cellulose, and calcium chloride dihydrate, sodium chloride were

purchased from Fs fine. Instruments used in this study is Electronic weighing balance(LC-T-1202), pH meter(ELICO), UV Visible spectrophotometer(SYSTRONICS), Viscometer(DV-11).

Preparation of Occular Insitu gelling Solution Of Timolol Maleate:

The insitu galling solution was prepared by first dispersing the required amount of sodium alginate in 75ml distilled deionized water in which sodium chloride (0.9%) was dissolved with continuous stirring until completely dissolved. Then the required amount of HPMC was added to alginate solution with continuous stirring until completely dissolved. The drug solution (0.5%) in distilled water was added to the polymer solution under constant stirring until uniform, clear solution was obtained. Distilled, deionized water was then added to make the volume up to 100ml. Timolol maleate is antiglaucoma drug . Sodium alginate was used as gelling agent in combination with hydroxyl propyl methyl cellulose (E15LV) as viscosifying agent. Benzalkonium chloride in suitable concentration was used as preservative. Sodium chloride (0.9%) was used as tonicity adjusting agent.

Table 1: Formulation of Timolol maleate occular *in situ* gel

Ingredients(gms)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	2	1	1.5	2	1	1.5	2	1	1.5
HPMC (E 15)	0.3	0.3	0.3	-	-	-	-	-	-
HPC	-	-	-	0.3	0.3	0.3	-	-	-
HEC	-	-	-	-	-	-	0.3	0.3	0.3
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9

Evaluation of Ophthalmic Gel:

Visual clearance [7]:

The appearance was checked visually. The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds. Results were shown in Table 2.

pH [8] :

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. pH was checked using pH meter. Results were shown in Table 2.

Viscosity [9]:

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. In particular, the feasibility of the *in situ* gelling system as an ocular drug delivery should be a free flowing liquid with low viscosity at non-physiological condition to allow reproducible administration into the eye as drops.The developed formulations were poured into the small sample adaptor of the Brookfield DV II viscometer fitted with cone and plate spindle and the angular velocity increased gradually from 0.5 to 50 rpm. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity. The viscosity of the prepared systems in centipoise (cps) was measured at various shear rates. Results were shown in Table 4.

***Invitro* gelation study [10]:**

All prepared formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as insitu gelling systems. In particular, the feasibility of the *in situ* gelling system as an ocular drug delivery should undergo *in situ* sol-to-gel phase transition at physiological condition to form gel capable of enduring shear forces expected in the eye during and between blinking and facilitate sustained drug release.The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of Artificial tear fluid freshly prepared and equilibrated at 37^0 C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. Results were shown in Table 3.

Drug content [9]:

The drug content was determined by taking 1 ml sample of *in situ* gel into 100 ml volumetric flask and diluting with 100 ml of Artificial tear fluid from this further dilution was done by taking 1 ml of sample and diluting with 10 ml of Artificial tear fluid. The absorbance was measured at 294.52 nm by UV-Spectrometer to calculate percentage of drug content. Results were shown in Table 2.

Sterility test [8]:

The test for sterility is an important aspect for ophthalmic preparations. It is intended for detecting the presence of viable forms of bacteria, fungi, and yeast in or on sterilized preparations. The test must be carried out under conditions designed to avoid accidental contamination of the product during test. IP method was followed for the sterility testing. Sterility testing was carried out by incubating formulations at 30 to 35ºc in the agar media to find the growth of bacteria. Results were shown in fig: 4.

Isotonicity[11]:

The tonicity of the prepared *in situ* gels was evaluated. On two separate slides, few drops of *in situ* gel and marketed eye drops were placed separately. few drops of freshly drawn blood was added to both slides, mixed and then observed under microscope at 45x magnification. The results were compared for any changes in RBCs in two slide. Results were shown in fig: 2, 3.

***Invitro* drug release study [12]:**

The *in vitro* release of Timolol maleate from the formulations was studied through semi permeable egg membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). Membrane, was tied to one end of a specifically designed glass cylinder (open at both ends and of 1 cm diameter). A 2-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic shaft and suspended in 100ml of dissolution medium maintained at 37□□1□C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 20 rpm using magnetic stirrer. Aliquots, each of 2-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 294.52 nm. Results were shown in Table5 and fig 1.

***Ex vivo* corneal permeation studies using goat cornea [12]:**

Goat cornea was used for the present investigation to study permeation across the corneal membrane. Whole eyeballs of goat were procured from a slaughter house and transported to laboratory in cold condition in normal saline maintained at 4ºc. The cornea were carefully removed along with a 5-6mm of surrounding sclera tissue and washed with cold saline. The washed cornea was kept in cold freshly prepared solution of artificial tear buffer of pH7.4. The study was carried out by using modified diffusion cell in such a way that corneum side is continuosly remained in an intimate contact with formulation in the donar compartment.The receptor compartment was filled with ATF pH 7.4 at 34ºc±0.5ºc.The receptor medium was stirred on magnetic stirrer. The samples were withdrawn at different time interval and analysed for drug content. Receptor phase were replenished with an equal volume of ATF (pH7.4) at each time interval. The percent drug released was plotted against time to get dissolution rate curve. Results were shown in Table 7 and fig 5.

6.2.11 Stability studies [13]:

The accelerated stability studies were carried out according to ICH guidelines. optimised formulations F6 were seal in amber colored bottles which cap covered by aluminium foil and these packed formulation was stored in ICH certified stability chamber maintained at 40ºc±2ºc and 75%RH±5% for 3 months. The formulations were evaluated before and after periodic interval for change in appearance, drug content, gelling capacity and *in vitro* drug release.

Results and Discussions

Table 2: Visual Appearance, Clarity, pH, Drug Content for F1-F9 Formulations

Formulations	Tests			
	Visual Appearance	**Clarity**	**pH**	**Drug Content**
F1	Transparent	Clear	6.9±0.05	86.2
F2	Transparent	Clear	6.8±0.02	94.5
F3	Transparent	Clear	6.8±0	99.1
F4	Transparent	Clear	6.8±0.05	101.2
F5	Transparent	Clear	6.9±0	98.4
F6	Transparent	Clear	6.9±0	96.5
F7	Transparent	Clear	6.9±0.05	98.3
F8	Transparent	Clear	6.5±0.05	96.3
F9	Transparent	Clear	6.8±0	98.6

Table 3: Gelling Capacity for F1-F9 Formulations

Formulation	Gelling capacity
F1	+++
F2	+
F3	++
F4	+++
F5	+
F6	+++
F7	+++
F8	+
F9	++

Table 4: Reological Studies of F1-F9 Formulations

Formulation	Angular velocity(rpm)	Viscosity(cps)
F1	5	110.5
F2	40	13.5
F3	12	48.4
F4	12	41
F5	14	32.6
F6	12	44.2
F7	4	121.8
F8	15	37.6
F9	12	48.8

Table 5: In Vitro Drug Release Studies of F1-F9 Formulation

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	14.0±3.51	16.82±1.66	21.5±4.90	15.93± 1.38	29.93± 3.53	16.8± 1.90	14.0±1.66	16.99±2.60	17± 1.21
1	22.5±2.11	24.03± 2.53	33.88± 1.38	17.09± 2.38	41.87± 2.23	25.18 ± 2.23	20.5± 2.53	29.73± 1.65	19.8± 4.32
2	34.36±2.52	36.99± 1.82	57.39± 1.60	24.67± 1.66	55.09± 2.26	44.93 ± 4.80	33.0± 1.60	35.7± 2.44	37.47± 3.23
3	45.90± 3.40	41.92± 0.65	68.87± 2.57	35.06± 1.17	69.32± 2.62	54.45 ± 4.90	42.62± 4.20	43.18± 1.80	43.04±2.23
4	52.72± 2.80	76.40± 1.17	80.34± 1.73	41.81±2.65	71.16± 3.92	62.5± 3.50	65.86± 3.62	53.97± 3.01	69.57± 0.21
5	60.90±1.44	83.17±2.57	81.72± 3.34	42.25± 3.23	81.26± 1.92	75.15 ± 2.44	64.47± 1.60	68.65± 2.44	74.21± 3.75
6	66.81± 2.33	88.00± 2.39	85.39± 3.40	67.53± 0.65	86.31± 3.86	81.43 ± 2.62	69.10± 3.00	82.37± 3.82	77.45± 2.59
7	70.0±1.49	90.42± 4.30	88.15± 3.21	74.02± 4.30	99.67± 4.05	91.85 ± 3.86	74.21± 2.65	99.8± 3.21	82.09± 3.90
8	71.81± 2.60	90.90± 4.80	89.53± 1.66	75.75±2.38		99.72 ± 4.41	76.99± 1.69		85..34± 2.31

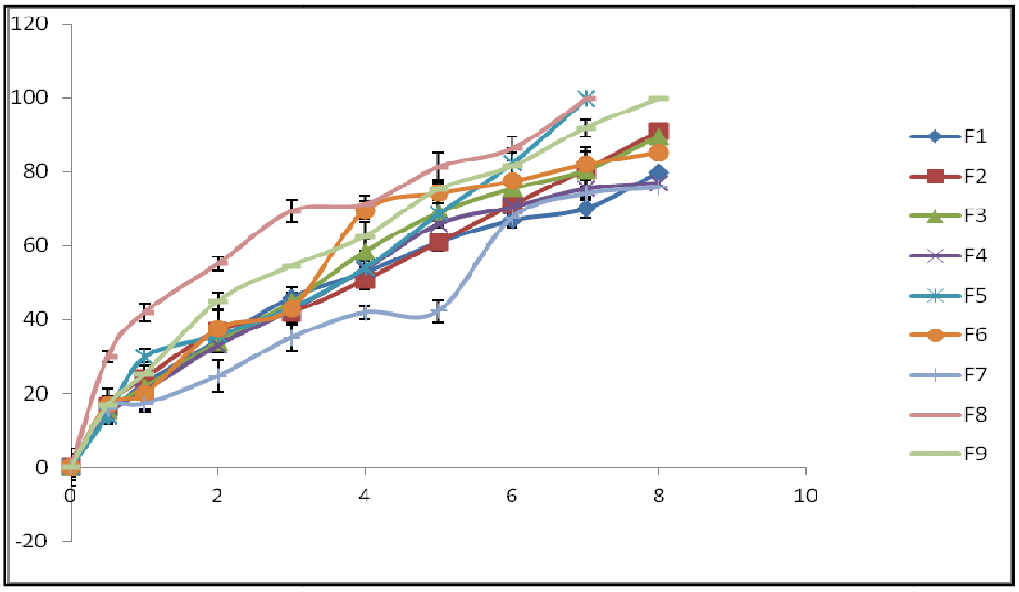

Figure 1: Drug Release Profile

Table 6: In Vitro Drug Release Kinetics

S. No	Formulations	Zero order	First order	Higuchi	Hixon-crowell	Korsemeyer peppas
1	F1	0.9521	0.985	0.9931	0.9914	1.022
2	F2	0.9785	0.901	0.9721	0.9551	0.984
3	F3	0.9722	0.966	0.9818	0.992	0.984
4	F4	0.9621	0.914	0.9202	0.924	0.9694
5	F5	0.8899	0.666	0.9899	0.8644	0.9072
6	F6	0.9636	0.71	0.9893	0.912	1.0432
7	F7	0.9523	0.988	0.9835	0.984	1.022
8	F8	0.9761	0.588	0.9371	0.7881	1.075
9	F9	0.9268	0.981	0.9835	0.9683	1.04

Table 7: Ex Vivo Drug Release Profile

Time	F6
0.5	14.0
1	26.5
2	35.36
3	45.90
4	52.72
5	60.10
6	74.81
7	83.35
8	98.37

Table 8: Evaluation Of Best Formulation F-6 Before And After Stability Studies

Evaluations	Before storage	After storage
Visual appearence	Transparent	Transparent
Clarity	Clear	Clear
pH	6.9±0	6.8±0
Drug content	96.5	95.42
Gelling capacity	+++	+++
Rheological studies	44.2	44.2

Table 9: In Vitro Diffusion Profile for Optimised Formulation before and After Storage

Time	Before storage	After storage
0.5	16.8±1.90	13.82±2.19
1	25.18±2.23	20.62±4.52
2	44.93±4.80	43.86±3.49
3	54.45±4.90	52.63±1.55
4	62.5±03.50	59.32±2.06
5	75.15±2.44	70.12±1.8
6	81.43±2.62	80.45±3.9
7	91.85±3.86	89.85±3.89
8	99.72±4.41	97.65±1.99

Isotonicity Test

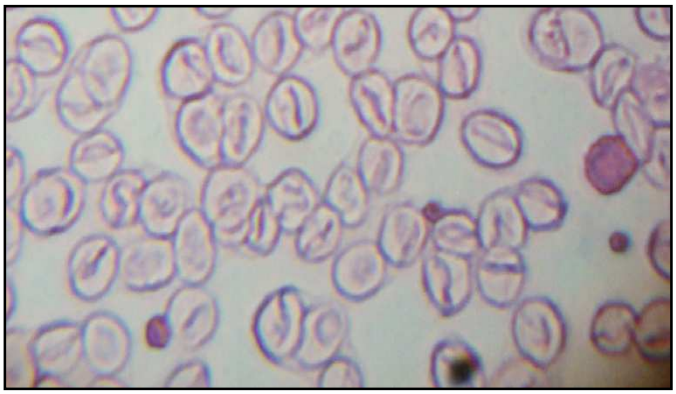

Figure 2: Optimised Formulation F6

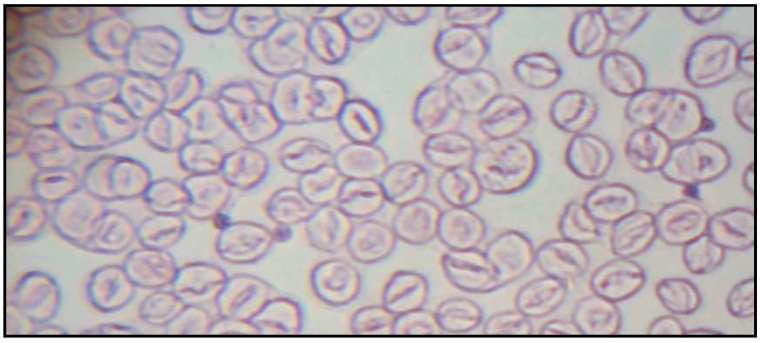

Figure 3: Standard Formulation of Timolol Maleate Eye Drops

Figure 4: Sterility Test for F6 Formulation

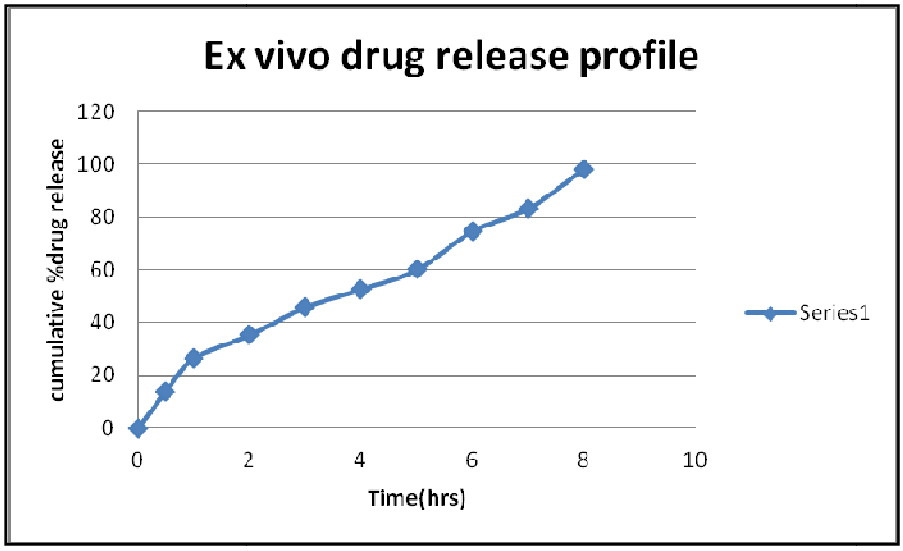

Figure 5: Ex Vivo Drug Release Profile of F6 Formulation

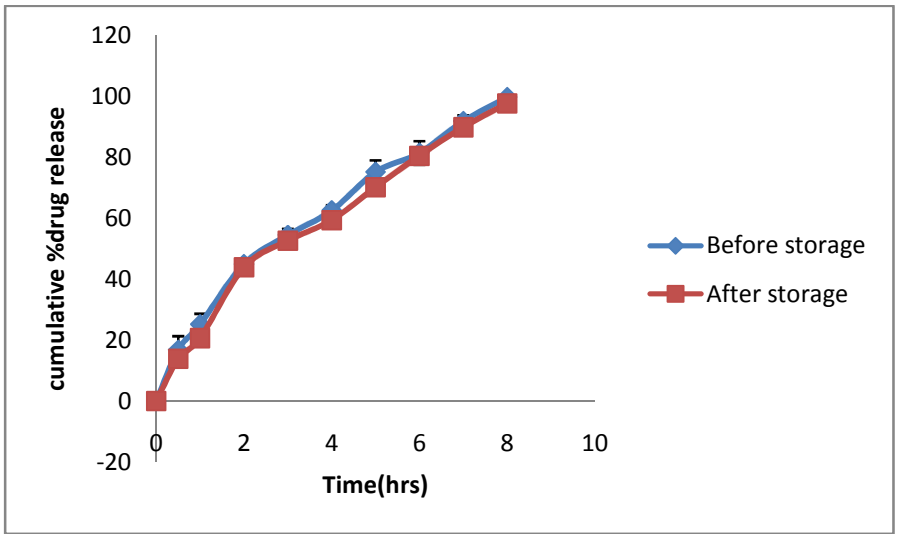

Figure 6: Stability Studies of Optimised F6 Formulation before and After Stability Studies

Conclusion

- Ophthalmic *in situ* gelling system can be formulated using sodium alginate along with HPMC E15, HEC, HPC as viscosity enhancing agent.
- pH of all formulations was found to be satisfactory.
- The drug content was with in acceptable range which ensured dose uniformity in the formulation.
- Gelation studies revealed that, the *in situ* gelling system formed gels instantaneously when contacted with Artificial Tear Fluid. The formed gels would enhance ocular contact time of Timolol maleate in eye.
- From the rheological studies, it was concluded that, formulations exhibited pseudoplastic rheology. The viscosity was maximum for F7 formulation because of the higher concentration of sodium alginate along with HEC. The ocular residence time of drug would be prolonged because of higher viscosity.
- The invitro Drug release studies revealed that, the drug release was 99.72% from F6 formulation after 8hrs.
- The results of kinetic data treatment suggested that drug release from F6 Formulations follows zero order, diffusion controlled release and case ii transport.
- Results of test for sterility confirmed that all the formulations were sterile
- Ex vivo studies conducted for f6 formulation, it shows 81.35% drug release in 8hrs.
- On the basis of drug release and viscosity studies and other parameters it can be concluded F6 was the optimum formulation.
- The results of stability studies indicated that, the most suitable storage condition for *in situ* gelling system of Timolol maleate was 40±1°c and 75% RH
- The *in situ* formed gel preserved its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drug locally in eye for 8hrs.
- Thus it is concluded that Timolol maleate insitu gelling system can be considered as alternative for conventional ophthalmic preparations.

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