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A STUDY ON STABILITY AND *IN VIVO* DRUG RELEASE OF NAPHAZOLINE AND ANTAZOLINE *IN SITU* GELLING SYSTEMS FOR OCULAR DELIVERY

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ABSTRACT

This work describes the stability of the selected *in situ* solutions for ophthalmic delivery of naphazoline hydrochloride (FF17, FF18) and antazoline phosphate (GG17 and GG18) based on the pH triggered concept using Carbopol 940 and HPMC K4M. The formulations were evaluated for their pH, isotonicity, gelling capacity, rheological characteristics, *in vitro* drug release , sterility and *in vivo* studies in New Zealand rabbits' eyes. All the formulations showed satisfactory results at ocular pH environment that remains in contact with the eyes for few hours. The formulations were very stable throughout, at room temperature and at 40 °C. Higher amount of both the drugs were retained in the aqueous humour area over 8 hrs following instillation FF17, FF18, GG17& GG18. Therefore, *in situ* gelling system can be used to enhance the ocular retention time thereby increasing ocular bioavailability and reducing the frequency of dosing .

KEYWORDS : Stability, Naphazoline hydrochloride, Antazoline phosphate, Carbopol 940, HPMC K4M, In situ gelling solutions,

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INTRODUCTION

Naphazoline and antazoline are antihistamines indicated for the treatment of ocular allergy. Presently available as drops and has to be instilled 4-5 times a day[1,2]. Draw back of these solutions are elimination of the drug from the pre-corneal area in a few minutes because of the lacrimal secretions and naso lacrimal drainage. An attempt was made to improve all these drawbacks by formulating *in situ* gelling solutions of these drugs. These formulations containing 0.1 % of naphazoline hydrochloride (FF17, FF18) and antazoline phosphate (GG17 and GG18) are intended to be instilled into the eyes in the form of a solution and are capable of forming a gel *in situ.* The earlier study reveled that the prepared formulations displayed satisfactory results. Hence the selected formulations of naphazoline hydrochloride and antazoline phosphate were subjected to stability studies at 28°C± 2 °C & 60% ± 5% RH and 40°C± 2 °C & 75% ± 5% RH to determine its characteristic qualities at the end of the storage period. *In vivo* studies were carried out for all the four formulations. They were evaluated and compared to prove the *in situ* formulation advantages over the conventional form of naphazoline hydrochloride and antazoline phosphate.

MATERIALS AND METHODS

1. Stability studies of optimized *in situ* gelling formulations of naphazoline hydrochloride (FF17, FF18) and antazoline phosphate (GG17, GG18):

Table 1
Composition of in situ gelling solution of ocular formulations.

Ingredients	Formulations			
	FF17	FF18	GG17	GG18
Naphazoline Hydrochloride (%w/v)	0.1	0.1	-	-
Antazoline Phosphate)%w/w)	-	-	0.1	0.1
Carbopol 940 (% w/v)	0.5	0.6	0.5	0.6
HPMC K_4M (%w/v)	1.0	1.0	1.0	1.0
NaCl (% w/v)	0.9	0.9	0.9	0.9
Benzalkonium Chloride(% w/v)	0.02	0.02	0.02	0.02
Distilled water(ml) to	100	100	100	100

Stability studies were carried out on optimized formulations as per the ICH guidelines. Each formulation was placed in four glass vials separately and sealed aseptically after sterilization with micropore ultra filters and kept for the short term accelerated storage stability study at 28 °C± 2 °C & 60% ± 5% RH and 40± 2 °C & 75 ± 5% RH as per ICH Guidelines. Samples were analyzed at an interval of 15, 30 and 60 days of time for clarity, pH, gelling capacity, drug content, rheological evaluation and *in vitro* release studies. Vials of naphazoline hydrochloride containing formulations FF17 and FF18, and antazoline phosphate containing formulations GG17 and GG18 were withdrawn and evaluated as follows:

i. Determination of pH, visual appearance and clarity[3,4]

The pH of the gel forming ophthalmic solutions was measured using pH meter. The stored formulations for stability testing were then evaluated for visual inspection by placing them under fluorescent light against a white and black background in well-lit cabinet for appearance and clarity.

ii. Determination of gelling capacity

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated tear fluid freshly prepared and equilibrated at 37 ^{o}C and visually assessing the gel formation and noting the time for gelation and the time taken for the formed gel to dissolve. The following grades are given based on gelling capacity. These grading helps in optimization of polymer concentration.

- \- No gelation
- \+ Gels after a few minutes, dissolves rapidly
- ++ Gelation immediate, remains for few hours
- +++ Gelation immediate, remains for extended period
- ++++ Stiff gel

iii. Rheological properties[5]

All the prepared formulations were subjected to rheological studies. Viscosities of these formulations were determined by using Brookefield synchronic viscometer using spindle N.2 at 10rpm.. Procedure was carried in the similar manner as done during the preliminary evaluation of the gelling solution. Both solution as well as the gel was measured for viscosity.

iv. Morphological analysis SEM characterization[5]

The gelling ability of the prepared formulations was determined visually but in the present study the gel formation was also confirmed by studying the surface morphology of the formulations at pH 5.5 (remaining as solutions) and pH 7.4 (becoming a gel) by using scanning electron microscopy.

v. Estimation of drug content [4,5]

The assay was carried out by diluting 1 ml of the formulation (0.1% w/v) in 100 ml of distilled water. From this stock solution 2 ml which corresponds to 20 μg/ml was taken and the drug content was estimated by UV visible spectrophotometer (Jasco V-530) for naphazoline hydrochloride and antazoline phosphate using distilled water as blank.

vi. In Vitro drug release study [4,5, 6]

The *in vitro* release of the drug from these formulations were studied through cellophane membrane using a modified *in vitro* permeation apparatus. The dissolution medium used was STF (freshly prepared) (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 3.4 cm diameter). A 2 ml volume of the formulations FF17, FF18, and GG17, GG18, were separately studied by accurately pipetting into the assembly. The cylinder was attached to the metallic shaft and suspended in 50 ml of dissolution medium so that the membrane just touched the receptor medium surface and maintained at 37 $\pm$ 2 ^{o}C at 50 RPM using magnetic stirrer. Aliquots of 1ml volume, were withdrawn at an hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV spectrophotometer at 276 nm for naphazoline hydrochloride and 242 nm for antazoline phosphate hydrochloride using STF as blank.

vii. Drug release kinetics

Investigation for the drug release from the pH triggered *in situ* gels was done by studying the release data with zero order, first order kinetics and Higuchi equation using PCB Dissolution version 2.08 software. The release mechanism was understood by fitting the data to Korsemeyer Peppas Model.

viii. Isotonicity evaluation [8]

Isotonicity is an important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. Formulations FF17, FF18, GG17 and GG18 were subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the required viscosity. The prepared formulations were mixed with few drops of blood and diluting fluid and observed under microscope

at 45X magnification and compared with standard marketed ophthalmic formulations containing naphazoline hydrochloride and antazoline phosphate. The shape of blood cell was compared with standard marketed ophthalmic formulations.

2. *In vivo studies*[8]

Four rabbits were quarantined for 2 weeks upon arrival. The rabbits were individually housed with food and water provided ad libitum. The rabbits having clinically normal eyes (i.e, free from signs of ocular inflammation) were used in the study. Rabbits were 1.5 to 2 kg in weight. A rabbit for each formulations FF17, FF18 and GG17 and GG18 were used for the study. Left eye was used as control to compare the right eye which was treated with the formulations. Rabbits were coded A to D for treatment with formulations FF17(A), FF18(B), GG17(C)and GG18(D) and again a crosover study was carried out. After the first round of study on these four experimental animals, it was found that the results were very similar in both the series of treatment. [6,8].

I. Test for irritation [8,9]

Four rabbits were chosen with weights varying from 1.5 to 2 kg. They were treated with eye drops on their right eyes and the left eyes served as control. All the rabbits' eyes were observed for any redness or irritation after the instillation of the *in situ* gelling solutions of naphazoline hydrochloride (formulation FF17 and FF18 on the right eyes of Rabbit A and Rabbit B respectively) and *in situ* gelling solutions of antazoline phosphate (formulation GG17 and BB18 on the right eyes of Rabbit C and Rabbit D respectively). The observations were made visually to study whether the components in the formulations caused any redness or irritation and noted down as S - Severe, M - Mild and C -Clear .

II. *Antiallergy activity of the prepared formulations of naphazolin hydrochloride and antazoline phosphate8*

A washout period of 1 week was given to subject the animals for the next study. On the first day, four rabbits were tested on the right eye for the antiallergic effect of naphazoline hydrochloride and antazoline phosphate in formulations FF17(A), FF18(B), GG17(C) and GG18(D) and the left was kept as control to compare.

Initially the normal IOP of both the eyes of the rabbits was noted using standardized shiotz tonometer (Riester, Germany). The allergy was induced in rabbits' eyes using mild natural irritant mustard pollen which turned the rabbits' eyes red and itchy. The itching in the rabbits' eyes was understood by the movements of the rabbits' paws on the eye region. IOP was again checked before and after instillation of the drug solutions. The eyes were treated with 2 drops of the formulations. Observations were made once in an hour till the disappearance of redness and itching in the eyes. The animals were allowed for a wash out period of 1 week and were used for further tests.

III. In vivo drug release [8,9,10]

The developed formulations FF17, FF18, GG17 and GG18 were instilled with 2 drops (each drop containing 30 μg of the drug) to the right eyes in the lower cul-de-sac of coded rabbits A, B, C and D respectively and the upper and lower eyelids were gently held closed for 10 sec. to maximize drug cornea contact. At 0.5, 1, 2, 4, 6, and 8 hours postdose, eyes were anesthetized using 4% xylocaine solution topically and aqueous humor was sampled from the eyes using a 28-gauge needle. Aqueous humour samples (50 μL) were collected and centrifuged at 5000 rpm for 10 min and was suitably diluted and the drug release percentage in aqueous humour were estimated at 276 nm for naphazoline hydrochloride and 242 nm for anatazoline phosphate.

RESULTS AND DISCUSSIONS

1. *Stability Studies*

The *in situ* gel forming solution of naphazoline hydrochloride and antazoline phosphate developed successfully to achieve the drug release in a sustained manner. The optimized *in situ* formulations of naphazoline hydrochloride (FF17 and FF18) and antazoline

phosphate (GG17 and GG18) were studied for stability at various temperature conditions. The details of results and discussions are as follows:

Table 2

Evaluation of stability of in situ gel forming ophthalmic solution of naphazoline hydrochloride and antazoline phosphate on 15th day of storage .

At 28°C± 2 °C & 60% ± 5% RH						
Formulations	**Visual appearance**	**Clarity**	**pH**	**Gelling Capacity**	**Viscosity in cps**	
					Sol	**Gel**
FF17	Transparent	Clear	5.35	+++	10.25	55.04
FF18	Transparent	Clear	5.51	+++	10.75	56.00
GG17	Transparent	Clear	6.12	+++	10.86	56.39
GG18	Transparent	Clear	6.15	+++	10.92	56.88
At 40°C± 2 °C & 75% ± 5% RH						
Formulations	**Visual appearance**	**Clarity**	**pH**	**Gelling Capacity**	**Viscosity**	
					Sol	**Gel**
FF17	Transparent	Clear	5.34	+++	10.22	55..00
FF18	Transparent	Clear	5.53	+++	10.78	56.44
GG17	Transparent	Clear	6.13	+++	10.87	56.42
GG18	Transparent	Clear	6.15	+++	10.92	56.44

Table 3

Evaluation of stability of in situ gel forming ophthalmic solution of naphazoline hydrochloride and antazoline phosphate on 30th day of storage.

At 28°C± 2 °C & 60% ± 5% RH						
Formulations	**Visual appearance**	**Clarity**	**pH**	**Gelling Capacity**	**Viscosity in cps**	
					Sol	**Gel**
FF17	Transparent	Clear	5.35	+++	10.20	55.00
FF18	Transparent	Clear	5.50	+++	10.73	56.00
GG17	Transparent	Clear	6.13	+++	10.84	56.35
GG18	Transparent	Clear	6.15	+++	10.90	56.84
At 40°C± 2 °C & 75% ± 5% RH						
Formulations	**Visual appearance**	**Clarity**	**pH**	**Gelling Capacity**	**Viscosity**	
					Sol	**Gel**
FF17	Transparent	Clear	5.32	+++	10.19	54.98
FF18	Transparent	Clear	5.49	+++	10.71	55.97
GG17	Transparent	Clear	6.11	+++	10.80	56.31
GG18	Transparent	Clear	6.13	+++	10.89	56.40

Table 4

Evaluation of stability of in situ gel forming ophthalmic solution of naphazoline hydrochloride and antazoline phosphate on 60th day of storage .

At 28°C± 2 °C & 60% ± 5% RH						
Formulations	**Visual appearance**	**Clarity**	**pH**	**Gelling Capacity**	**Viscosity in cps**	
					Sol	**Gel**
FF17	Transparent	Clear	5.34	+++	10.20	54.92
FF18	Transparent	Clear	5.50	+++	10.73	55.95
GG17	Transparent	Clear	6.13	+++	10.78	56.11
GG18	Transparent	Clear	6.15	+++	10.80	56.10
At 40°C± 2 °C & 75% ± 5% RH						
Formulations	**Visual appearance**	**Clarity**	**pH**	**Gelling Capacity**	**Viscosity**	
					Sol	**Gel**
FF17	Transparent	Clear	5.32	+++	10.19	54.98
FF18	Transparent	Clear	5.49	+++	10.71	55.97
GG17	Transparent	Clear	6.11	+++	10.85	56.29
GG18	Transparent	Clear	6.13	+++	10.92	56.40

It was observed that all the four prepared formulations were stable on the 15th, 30th and 60th day when evaluated for the physical properties (as reported in Table 1, 2 and 3respectively) at temperatures 28°C± 2 °C & 60% ± 5% RH and at 40°C± 2 °C & 75% ± 5% RH).

Morphological analysis (SEM)

The SEM images of the *in situ* gelling formulation at pH 5.5 and 7.4 are shown in Fig. A, and B. All the formulation had similar morphological features as shown in the figures. The SEM image of the *in situ* gelling formulation at pH 5.5 showed a highly compact surface morphology, whereas at pH 7.4 it became loose and kept spreading and were transparent as shown in Fig. B and this indicates gel formation at pH 7.4.

Sterility Testing

Sterility test was carried out as per the method mentioned in the earlier report and was found satisfactory.

Drug content uniformity

The formulated solutions were subjected to initial drug content estimation before evaluating the drug release from the gel form

Table 5

Drug content of the formulations at 28 °C± 2 °C & 60 % ± 5% RH storage conditions

Formulations	**Percentage Drug content**			
	Initial	15th day	30th day	60th day
FF17	96.05	96.04	96.04	96.03
FF18	97.00	97.00	97.00	96.99
GG17	96.89	96.89	96.87	96.87
GG18	97.21	97.20	97.19	97.17

The initial drug of the marketed solutions N1 (naphazoline hydrochloride) at all storage temperature was approximately 98 % and A1 (antazoline phosphate) was about 97.8 %.

Table 6
Drug content of the formulations at 40 °C± 2 °C & 75% ± 5% RH storage conditions

Formulations	Percentage Drug content			
	Initial	15th day	30th day	60th day
FF17	96.05	96.04	96.03	96.03
FF18	97.00	97.00	97.00	96.99
GG17	96.89	96.89	96.88	96.88
GG18	97.21	97.20	97.18	97.16

Table 6a
Percentage drug release at 28 °C± 2 °C & 60 % ± 5% RH

Time in hours	15th Day				30th day				60th day			
	Formulations				Formulations				Formulations			
	FF17	FF18	GG17	GG18	FF17	FF18	GG17	GG18	FF17	FF18	GG17	GG18
0.25	8.50	5.96	9.69	5.44	8.80	6.09	9.84	5.44	8.53	6.03	9.78	5.38
0.5	15.21	15.18	13.16	12.81	15.25	15.25	13.28	12.91	15.19	15.22	13.28	12.91
1	29.88	21.56	28.06	20.72	29.81	21.88	28.16	20.81	29.78	21.56	28.13	20.75
2	49.63	31.18	31.63	33.66	49.59	31.19	31.72	33.72	49.53	31.19	31.69	33.63
3	60.09	47.03	61.88	48.94	60.10	47.16	63.13	49.03	60.10	47.16	62.5	48.97
4	71.50	57.00	70.91	54.75	71.53	57.13	70.97	54.88	71.47	57.06	70.97	54.84
5	75.53	67.78	79.63	69.94	75.59	67.91	79.66	69.97	75.59	67.88	79.66	69.91
6	81.15	69.15	83.47	75.56	81.50	69.31	83.50	75.63	81.50	69.25	83.5	75.38
7	85.28	79.71	87.25	79.28	85.34	79.84	87.31	79.31	85.31	79.78	87.25	79.28
8	89.46	86.90	90.59	84.22	89.56	87.03	90.63	84.28	89.50	87.00	84.38	84.25

Table 6b
Percentage drug release at 40°C± 2 °C & 75% ± 5% RH

Time in hours	15th day				30th day				60th day			
	Formulations				Formulations				Formulations			
	FF17	FF18	GG17	GG18	FF17	FF18	GG17	GG18	FF17	FF18	GG17	GG18
0.25	8.60	6.10	9.98	5.85	8.60	6.09	9.95	5.83	8.61	6.07	9.95	5.83
0.5	15.22	15.24	13.58	13.59	15.23	15.25	13.55	13.58	15.23	15.22	13.56	13.58
1	29.89	21.59	28.23	20.92	29.89	21.59	28.20	20.90	29.89	21.56	28.20	20.9
2	49.51	31.19	31.75	33.89	49.61	31.19	31.73	33.88	49.60	31.19	31.73	33.88
3	60.07	47.03	63.90	49.50	60.07	47.16	63.87	49.50	60.06	47.16	63.87	49.5
4	71.47	57.11	72.90	58.12	71.46	57.10	72.85	58.11	71.46	57.10	72.85	58.11
5	75.59	67.92	79.73	68.02	75.58	67.92	79.70	68.02	75.58	67.92	79.70	68.00
6	81.52	69.25	83.65	75.63	81.51	69.24	83.64	75.63	81.50	69.24	83.64	75.6
7	85.34	79.88	87.88	79.30	85.34	79.84	87.86	79.29	85.33	79.84	87.86	79.29
8	89.59	87.00	90.10	84.20	89.59	87.03	90.09	84.18	89.60	87.03	90.09	84.18

The in vitro drug release from the marketed solution at all storage conditions were almost the same and was found to be about 94.5 % at the end of the 3rd h. Hence it is clear that the solution form of these drugs does not display any sustained effect likethe prepared insitu gelling solutions (FF17,FF18,GG17,GG18) which released about 90 % of the drug for an extended period of 8 hrs.

Release kinetics/ mechanism

Formulation FF17 followed first order as the best fit model and fickian diffusion formulation FF 18 followed Koresemeyer Peppas model as the best fit model and fickian diffusion. Formulation GG17 followed first order as the best fit model and fickian diffusion. Formulation GG18 followed follows Korsemeyer- Peppas model as the best fit model as fickian diffusion.

Isotonicity Testing

The optimized formulations were subjected to isotonicity as per the methodology, Isotonicity testing of FF17, FF18, GG17 and GG18 formulations exhibited no change in the shape of blood cells (bulging or shrinkage), which reveal the isotonic nature of the formulations FF17, FF18, GG17, GG18 and compared with that of standard marketed ophthalmic of naphazoline hydrochloride and antazoline phosphate N1 and A1 respectively. Since there was no change in the shape of blood cells (bulging or shrinkage), it reveals the isotonic nature of the prepared formulations of naphazoline hydrochloride and antazoline phosphate. The results of this test proved that all the formulations were isotonic in nature.

***IN VIVO* STUDIES**

Test for irritation

Table 7

***Eye Irritation test*.**

Formul-ations	Rabbit No A						Rabbit No B						Rabbit No C						Rabbit No D					
	1 h	2 h	3 h	4 h	5 h	6 h	1 h	2 h	3 h	4 h	5 h	6 h	1 h	2 h	3 h	4 h	5 h	6 h	1 h	2 h	3 h	4 h	5 h	6 h
FF17	N	N	N	N	N	N	-	-					-	-	-	-	-	-	-	-	-	-	-	-
Control	N	N	N	N	N	N							-	-	-	-	-	-	-	-	-	-	-	-
FF18	-	-	-	-	-	-	N	N	N	N	N	N	-	-	-	-	-	-	-	-	--	-	-	-
Control	-	-	-	-	-	-	N	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-	-
GG17	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N	-	-	-	-	-	-
GG18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	N	N	N	N

S- Severe itching / irritation, M-Mild itching / irritation, N- No itching / irritation

It was observed that through IOP was normal before and after the test. No itching was reported.

Determination of anti allergic activity

The study was carried out as per the methodology. The animals were observed for the movements of the paw over the eyes and their frequency. More frequent movements of paw over the eyes indicate severe itching, occasional frequency of paw movements is considered to be mild itching and absence of paw movements over the eyes is considered as absence of any itching /irritation in the eyes. Extent of irritation were also recorded (data not shown) .Visually the severity of redness in the test eyes were compared with the control and observations were made as: R- Very red , M-Mild, C- No redness . It was observed that there was no ocular damage or

abnormal clinical signs to cornea, iris and conjunctiva. It was observed that the IOP was normal throughout the experiment. It is evident that the only mild irritation persisted upto 3 hours. All the rabbits' eyes were normal by the end of 5th hour without any irritation/redness. This proves that all the formulations of *in situ* gels were very effective against common allergic conditions. There were no other adverse affects on the eyes throughout 8 hours study.

Determination of drug bioavailability.

The bioavailability was estimated *in vivo* as per the methodology. In addition, two more rabbits coded E and F were administered with marketed products N1 and A1 on right eyes in a similar manner. as in the case of the rabbits A,B,C, & D. Percentage release of drug was calculated at different intervals of time. Ocular drug release of *in situ* gelling system was evaluated pharmacokinetically. The *in vivo* drug release was assessed from the area under curve (AUC) values by trapezoidal rule. Relative magnitude of biological response was expressed using following equation. The data were statistically processed to determine and were found to be statistically significant.

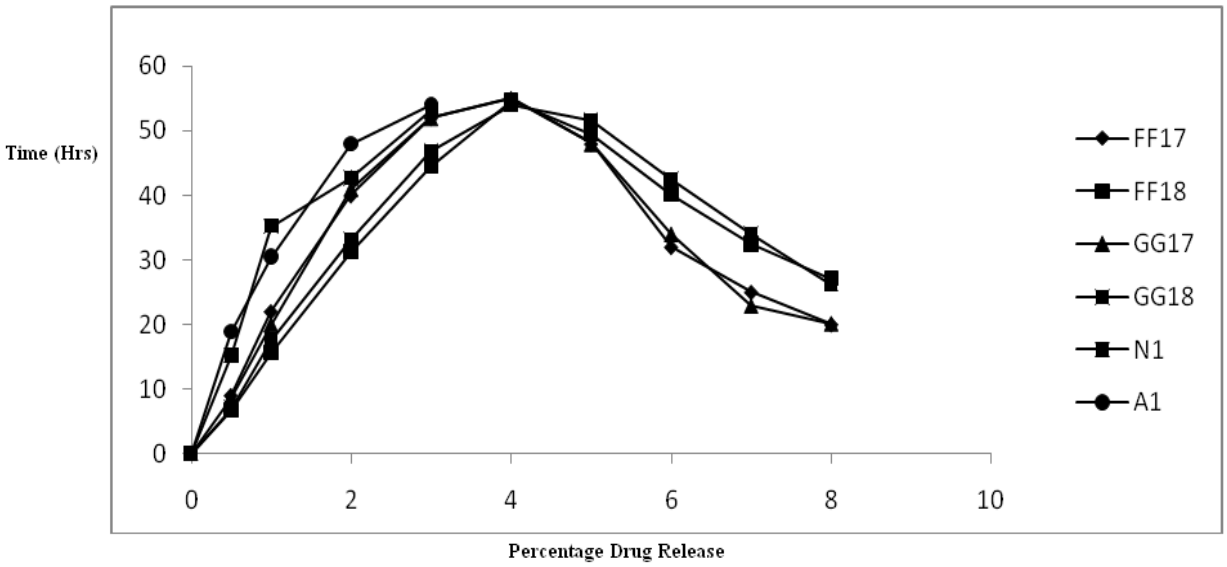

Figure 1
In vivo drug availability from naphazoline hydrochloride (FF17 and FF18) and antazoline phosphate (GG17 and GG18) in situ gelling solutions. compared with marketed products N1 and A1.

The T_{max} for all the *in situ* gelling formulations was found to be 4 hours and C_{max} for FF17, FF18, GG17 and GG18 were 55, 54. 72, 55 and 54 respectively. The area under the curve (AUC) is for FF18 and GG18 were slightly greater than the formulations FF17 and GG17. This may be due to the increase in the percentage concentration of the polymer/ in the FF18 and GG18. It was observed that the marketed solutions of N1 and A1 (naphazoline hydrochloride and antazoline phosphate respectively) reached the highest concentration immediately after instillation and were not bioavailable after 3 hours of instillation. Whereas all the prepared formulation of *in situ* gelling solutions were bioavailable for more than 8 hours(Table 28). Also this study indicates that the tested formulations were capable of retaining a longer duration upto 8 hours in the eye, in the form of *in situ* gelling solutions.

A study of drug tear loss also was carried out(Data not included in this report). It was found that the loss of drug from each

formulations FF17, FF18, GG17 and GG18 was very less, compared to that of the marketed conventional solutions N1 and A1. This indicated that the polymers incorporated in the *in situ* gelling formulations are capable of preventing the drug from being drained off by virtue of its viscous nature to hold the drug within the gelled structure.

The in vitro in vivo correlation was not done as the study was not carried using iv blood samples. The purpose of the study was only to study if the prepared formulations were bio available for an extended period of time in the ocular cavity.

CONCLUSIONS

All the Gel forming ophthalmic solutions released drug to a maximum of 85 to 90 % upto 8 hours. The marketed products of naphazoline hydrochloride solution and antazoline phosphate solutions for ocular use has released the drug about 66 % and 52 % respectively only upto 3 hours. This indicates that there is a drug loss due to lacrimal drainage in conventional form and is not bioavailable to an extent as that of the formulated *in situ* gelling solutions of naphazoline hydrochloride and antazoline phosphate. This means that the frequency of dosing of *in situ gelling* of the drug can be reduced to once in a day (in the FF17, FF18, GG17and GG18) whereas, in conventional marketed solution the frequency of dose administration requires four to five times a day to be effective in treating allergy in the eyes.

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