

## FORMULATION AND EVALUATION OF NIOSOMAL *IN SITU* GEL OCULAR DELIVERY SYSTEM OF BRIMONIDINE TARTRATE

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### ABSTRACT

The aim of present study was to develop Brimonidine tartrate niosomal *insitu* gels for glaucoma treatment. Poor bioavailability of drugs from ocular dosage form is mainly due to tear production, nonproductive absorption, transient residence time, impermeability of corneal epithelium. These problems can be minimized by the use of niosomal vesicular system. Niosomes were formulated by using different ratios of span series and cholesterol. Span 60 (S/C 2:1) niosomes had highest entrapment efficiency and showed prolonged drug release. Small unilamellar vesicles were observed and had the size of about 50-100 nm. *In situ* gelling of niosomal drops was formulated by using HPMC K 15 M and carbopol 940 to maintain the drug localization for extended period of time. The niosomal formulation was transformed into gel when it instilled into the eye. All the gel formulations exhibited pseudo plastic rheological behavior and slow drug release pattern. Antiglaucoma activity of the prepared gel formulations showed more significant and sustained effect in reducing intra ocular pressure than marketed and niosomal drops. Hence niosomal *insitu* gelling may have its potential applications than the conventional ocular therapy and to improve the ocular bioavailability with minimal loss of drug.

**Keywords:** Brimonidine tartrate, niosomes, thin film hydration, *insitu* gels, glaucoma.

### INTRODUCTION

Ophthalmic drug delivery is one of the most interesting challenges faced by pharmaceutical scientists. The primitive ophthalmic solution, suspension and ointment dosage forms are clearly no longer sufficient to combat some present virulent diseases (Saettone, 2002). Successful delivery of drugs into the eye is extremely complicated because the eye is protected by a series of complex defense mechanisms, which make it difficult to achieve an effective concentration of the drug within the target area of the eye. Traditional ophthalmic dosage forms

include solutions, suspensions; ointments are still acceptable, such dosage forms are no longer sufficient to overcome the various ocular diseases like glaucoma due to poor bioavailability (Gokul Gandhi et al, 2007; Jane Burrows et al, 2002; Mohd et al, 2005).

Drug delivery through niosomes is one of the approaches to achieve localized drug action since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the

site of administration. It results in enhancement of efficiency or potency of the same drug and at the same time reduces its systemic toxic effects. Thus, niosomes entrapped through *insitu* hydrogel system has been developed to increase precorneal residence time, to minimize interference with blinking, enhance ocular bioavailability, and reduce frequency of the administration of a drug (Bharath, 2009; Sabyasachi, 2010).

Brimonidine tartrate is  $\alpha_2$ adrenergic agonist indicated in open angle glaucoma. Glaucoma is the leading cause of irreversible blindness in the world. It is a disease characterized mainly by an increase in intraocular tension, if sufficiently high and persistent, leads to irreversible blindness. (Prabhuet.al., 2010). The global burden of glaucoma possess a challenge to the researchers, ophthalmologists and general practitioners to detect, prevent and effectively treat this visual disability and make safer drugs available to making at an affordable price.

## MATERIALS AND METHODS

Brimonidine tartrate was a gift sample from Centaur pharmaceuticals Pvt Ltd, Mumbai and FDC Pvt Ltd, Goa. Span 20, Span 40, Span 60 and Span 80 were obtained from S.D fine chem. Ltd and Lobachemie Pvt. Ltd. Carbopol-940 and HPMC K15M were obtained from Dr. Milton Laboratories, Chennai. All other solvents and reagents used for study were of analytical grade. Rotary flask evaporator (Superfit rotary vacuum, Mumbai, India), Ultra sonicator (Vibronics ultrasonic

processor P2), Electronic balance (A and D company, Japan), Magnetic stirrer (Hotspin), UV-visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan), pH meter (Dalal, Chennai, India), Scanning electron microscopy (Hitachi S-450, Japan), Refrigerator (Kelvinator, India), Brookfield Viscometer model (LV DV2+Pro Brookfield), Tonometer (Shiotz, India) were used in this study.

### 1. PREPARATION OF NIOSOMES AND HYDROGELS

Non-ionic surfactant, Span series (Span 20, 40, 60 and 80) was used to prepare Brimonidine tartrate niosomes by thin film hydration method in a rotary flask evaporator. (Samar Mansour et.al, 2005) Various formulations were prepared as shown in the Table no.1. Surfactant and cholesterol were accurately weighed and dissolved in 15ml of Chloroform: Methanol (2:1 v/v) solvent mixture. Then it was vortexed in a round bottomed flask at temperature 60° to remove the solvent under reduced pressure in the rotary flask evaporator at 150rpm for 30-40 min. A thin layer or film formed inside the flask was then hydrated with aqueous phase containing the drug in 10ml of distilled water for 1h at temperature 60° to obtain yellowish white dispersion of niosomes (Mullaicharam A R and Murthy R S R, 2004). The resultant dispersion was then cooled in an ice bath, sonicated for 3min at 150v. Then the resultant niosomes which were stored at 4° in a refrigerator (Naseem Charoo A et.al, 2003) for further studies. For each formulation plain niosomes were also prepared by the same procedure.

**Table no.1**  
**Composition of niosomes**

Formulation	Surfactant	Ratio of	
		Surfactant	Cholesterol
F1	Span 20	1	1
F2	Span 20	2	1
F3	Span 20	3	1
F4	Span 20	1	2
F5	Span 40	1	1
F6	Span 40	2	1

F7	Span 40	3	1
F8	Span 40	1	2
F9	Span 60	1	1
F10	Span 60	2	1
F11	Span 60	3	1
F12	Span 60	1	2
F13	Span 80	1	1
F14	Span 80	2	1
F15	Span 80	3	1
F16	Span 80	1	2

The *insitu* gelling systems of Brimonidine tartrate niosomes were prepared by utilizing the phase transition properties of hydroxy propyl methyl cellulose (K15M) and carbopol940 indifferent ratios. These were prepared by adding appropriate amounts of polymer in acetate buffer pH5.0 (Doijad et.al, 2004;Gokul Gandhi M R et.al, 2007; Khandare J N, 2001).The niosomal dispersion equivalent was taken, mixed thoroughly with polymer to obtain a uniform dispersion in the aseptic chamber. The solution was made isotonic with sodium chloride (0.9%). Then Benzalkonium chloride was added as a preservative. The prepared gels were filled in amber colored glass vials refrigerated at 4 to 8°C.

## 2. PREPARATION OF NIOSOMAL DROPS

From the entrapment efficiency results and release studies, theniosomal dispersion which showed maximum entrapment efficiency and sustained release was selected for preparation of niosomal drops.The niosomaldispersion equivalent to 0.15%v/v of the drug was taken, mixed to phosphate buffer salinepH 7.4 containing sodium chloride and Benzalkonium chloride filled in an amber colored glass vial in an aseptic chamber.

## 3. EVALUATION OF NIOSOMES (Khandare J N, 2001)

### 3.1 Entrapment Efficiency

Entrapment efficiency was determined by dialysis method by allowing the drug to diffuse through dialyzing membrane (Spectra/Por dialysis membrane 12,000–14,000 Mwtcutoff).Niosomalpreparation taken in the dialysis tube was suspended suitably in a beaker containing 100ml of phosphate buffer saline which constantly stirred at 100 rpm on a magnetic

stirrer at 37°±1°C during the release studies. Samples werewithdrawn at various time intervals and assayed spectrophotometrically at 256nm using UV-Spectrophotometer (Shimadzu UV, Pharmaspec 1700, Japan). The time required to release untrapped drug was noted.

The entrapment efficiency was determined by the following formula: Entrapment efficiency (%) = (Amount of drug entrapped/Total amount of drug) X 100

### 3.2 Vesicle Shape and Size

Vesicle formation (shape) and size of niosomes were characterized by scanning electron microscopy.

### 3.3 InVitro Release Studies (Jain C P et.al, 2006; Khandare J N, 2001; Samar Mansour et.al,2005)

These studies were carried out by dialysis method as used for the entrapment efficiency determination. Total niosomal formulation was taken for the release studiesand the diffusion medium has been changed immediately at the time when untrapped drug was completely dialyzed. And then the release study was carried out for the entrapped drug from the vesicle. The collected samples were analyzedspectrophotometrically at 256nm using phosphate buffer saline as blank in a UV-visible spectrophotometer.

### 3.4 Stability Studies(ICH Q1A, 1993)

The best formulations were stored at different temperature 30°±2°C/60% RH ±5%RH and 4°±2°C for 10 weeks.At definite period intervals these formulations were evaluated for their drug content and mean vesicular diameter.

#### 4. EVALUATION OF HYDROGELS

The prepared gels were evaluated for their pH, drug content, *invitro* gelation studies, rheological studies and *in vitro* release studies. (Gokul Gandhi M R et.al, 2007)

##### 4.1 Visual Appearance, clarity and pH

Visual appearance and clarity were observed for the presence of any particular matter. The pH of *insitu* gels was measured using digital pH meter.

##### 4.2 Drug Content Analysis

It was carried out using UV-Spectrometric method and sufficient amount of 50% n-propanol was added to lyse the vesicles. Then 0.1ml of formulation was diluted to 100ml of simulated tear fluid pH 7.4 and the absorbance was measured at 256nm using simulated tear fluid pH 7.4 as blank.

##### 4.3 Rheological Studies (Dojad et.al, 2004; Hong Ru Lin and Sung K C, 2000; Jain C P et.al, 2006)

These studies were carried out in the Brookfield Viscometer LV DV2+ Pro with spindle SC 18 at 30° in a small sample adaptor.

##### 4.4 In Vitro Gelation Studies (Pandit J K et.al, 2007)

Gelling strength of formulations were evaluated by placing a drop of formulations in a test tube containing 2ml of freshly prepared simulated tear fluid pH 7.4. The time taken to form gelation and to dissolve was observed visually.

##### 4.5 In Vitro Drug Release Studies

The studies were done by placing the formulation in a circular plastic cup. This was then placed in an inverted USP basket kept inside a beaker containing 200ml of simulated tear fluid pH 7.4, stirred at 37° ± 1° in a magnetic stirrer. Then the known volume of the fluid removed at time period intervals to find the amount of drug release by measuring the absorbance in the UV-visible spectrophotometer (Shimadzu UV-1700, Pharmaspec, Japan) at 256nm. The volume removed was replaced by the same volume of fresh simulated tear fluid. (Jagadish, 2003; Pandit J K et.al, 2007)

#### 4.6 In Vivo Intra Ocular Pressure Lowering

##### Activity:

*In vivo* intraocular pressure lowering activity of selected niosomal preparation of Brimonidine tartrate was studied in normotensive male albino rabbits weighing 1.5-2kg. This study experimental protocol was approved by Institutional Animal Ethical Committee. The animals were housed under well controlled conditions of temperature (20-25°), humidity and given access to food and water. (Deepika Aggarwal and Indu Kaur P, 2005; Samar Mansour et.al, 2005)

Four groups of three rabbits received test formulation topically. The intra ocular pressure was measured with tonometer as a function of time. Ocular pressure (IOP) changes were recorded before drug administration and then after 30min and every hour for a period of 8h till the pressure difference between the control eye and treated eye is zero. Formulations were instilled on the corneal surface of one eye and contra lateral eye was remaining as control. The ocular hypotensive activity was expressed as the averaged difference IOP between '0' time to 't' time to minimize the diurnal, seasonal, individual variation commonly observed in rabbits.

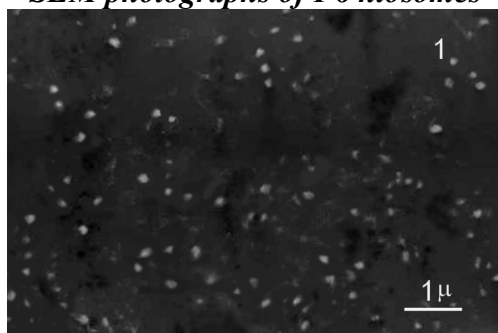
Change in IOP ( $\Delta$ IOP) = IOP<sub>'0' time</sub> – IOP<sub>'t' time</sub>

## RESULTS AND DISCUSSION

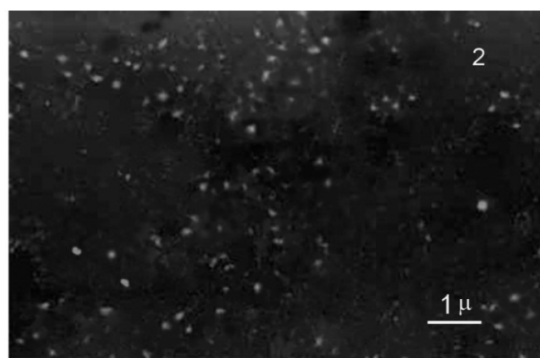
Brimonidine tartrate niosomes were prepared by thin film hydration method using non-ionic surfactants (Span 60, 40, 20 and 80) and cholesterol in different ratios of (S:C) (1:1, 1:2, 1:3 and 1:4) as shown in Table no.1. The selected formulation of the niosomes based on entrapment efficiency was observed and measured by Scanning Electron Microscopy. The small unilamellar vesicle 50 – 100 nm range was observed as shown in Fig.1 & 2. Most of the vesicles found to be spherical in shape. It has been observed that the formulations with increased cholesterol content (F<sub>4</sub>, F<sub>8</sub>, F<sub>12</sub>, and F<sub>16</sub>) showed decrease in entrapment efficiency as shown in Fig.3. This may be due to the cholesterol has the ability to cement the leaking space in the bilayer membranes. When the cholesterol content increases beyond a certain level, it starts disrupting the regular

bilayer structure that leads to decrease in the drug entrapment efficiency (Samar Mansour et.al, 2005)

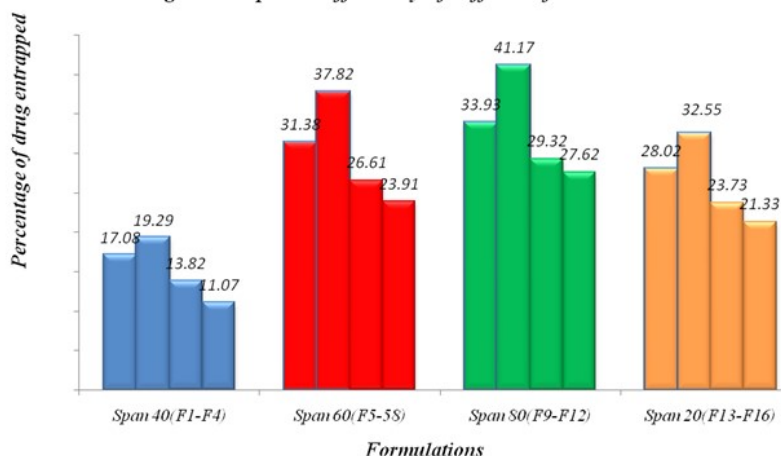
**Fig.1**  
**SEM photographs of F6 niosomes**



**Fig.2**  
**SEM photographs of F10 niosomes**



**Fig 3. Entrapment efficiency of different formulations**



Increase or decrease in surfactant concentration showed a nonlinear relationship with entrapment efficiency (Khandare J N, 2001). The entrapment efficiency differs depending upon the HLB value of

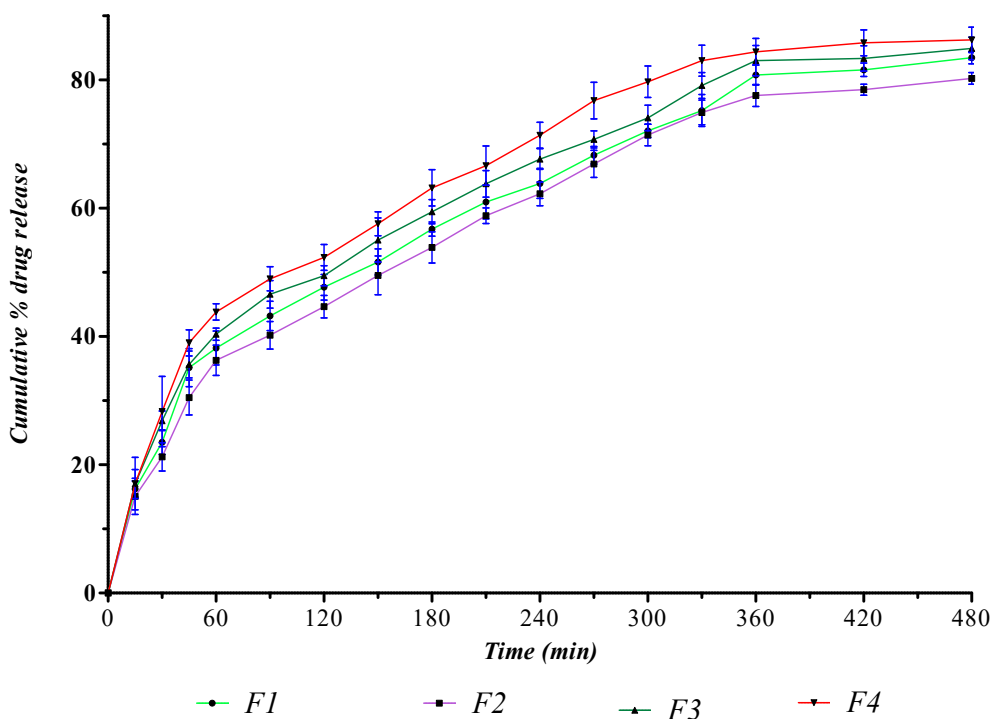
surfactants. It changes in the following order of span 60 > span 40 > span 80 > span 20 as shown in Fig.1. The decreased entrapment efficiency in Span 80 is an exception because of the presence of unsaturated

alkyl chain. Span 60 and span 40 showed higher entrapment efficiency than the other surfactants due to higher phase transition temperature (Alexander Florence T and Toshimistu-Yoshioka, 1994).

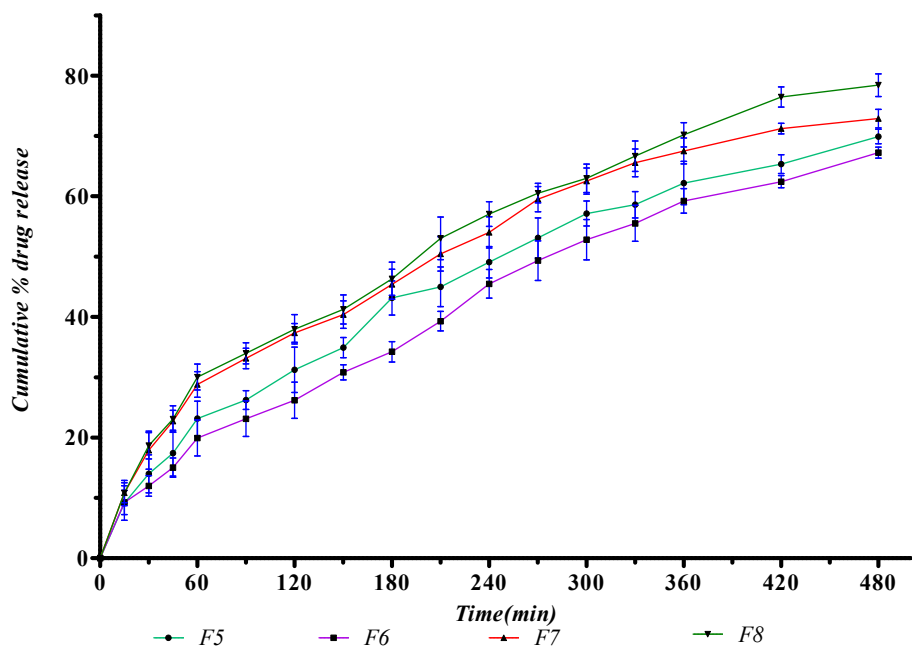
*In vitro* release studies of niosomal formulations showed that rate of drug release depends on the percentage of drug entrapment efficiency (Samar Mansour et al, 2005). Of all the formulations of different ratios (S/C 1:1, 2:1, 3:1, 1:2) the maximum drug release was observed in the formulations F4 (86.21%), F8 (78.42%), F12 (72.45%) and F16 (82.34) for span 20, 40, 60 and 80 (S/C 1:2) respectively in 8 hours due to lower entrapment efficiency as in Fig. 4, 5, 6, 7. The release studies also revealed that F2 (

S/C 2:1, 80.21%), F6 (S/C 2:1, 67.23%), F10 (S/C 2:1, 59.81%), F14 (S/C 2:1, 76.73%) showed slower and prolonged drug release than the other formulations due to higher entrapment efficiency. Further, F10 (span 60 S/C; 2:1) and F6 (span 40 S/C; 2:1) showed more prolonged release due to the ordered gel state and of span 40 and span 60 that decrease membrane permeability (Varghese V et al, 2004). The presence of higher alkyl chain length of span 40 and span 60 further prolongs the drug release (Samar Mansour et al, 2005). The release of formulations F2, F6, F10, and F14 was then compared with that of the pure drug showed the maximum drug release 99.26% in 4.5 h as shown in Fig. 8.

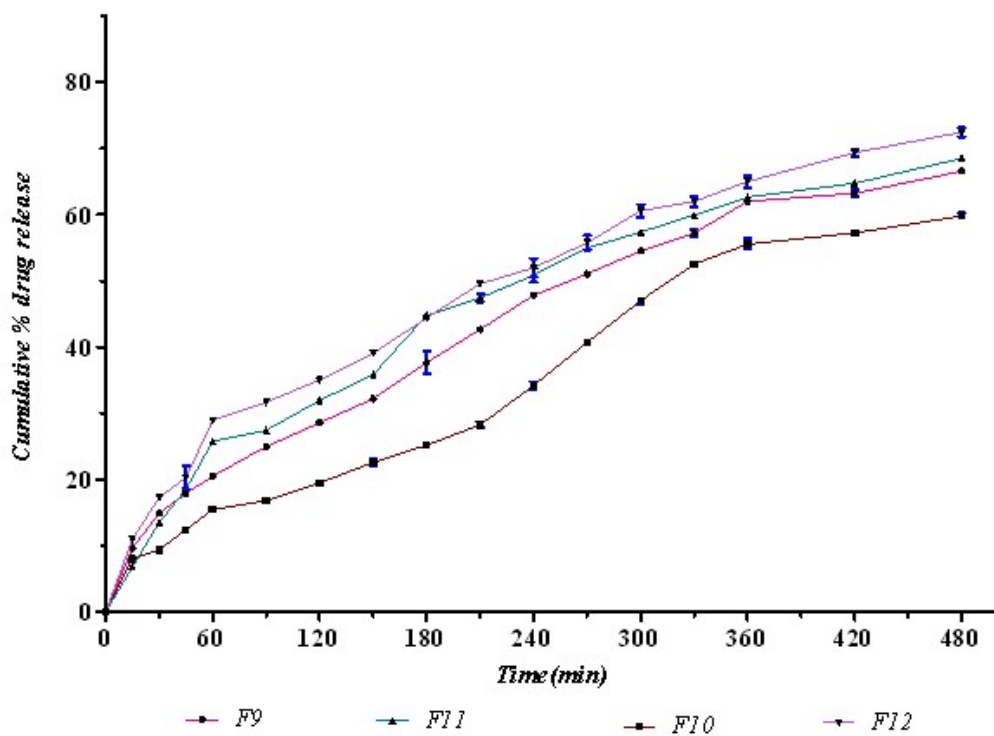
**Fig.4 Comparison of *in vitro* release of span 20 of different ratios of niosomes of brimonidine tartrate**

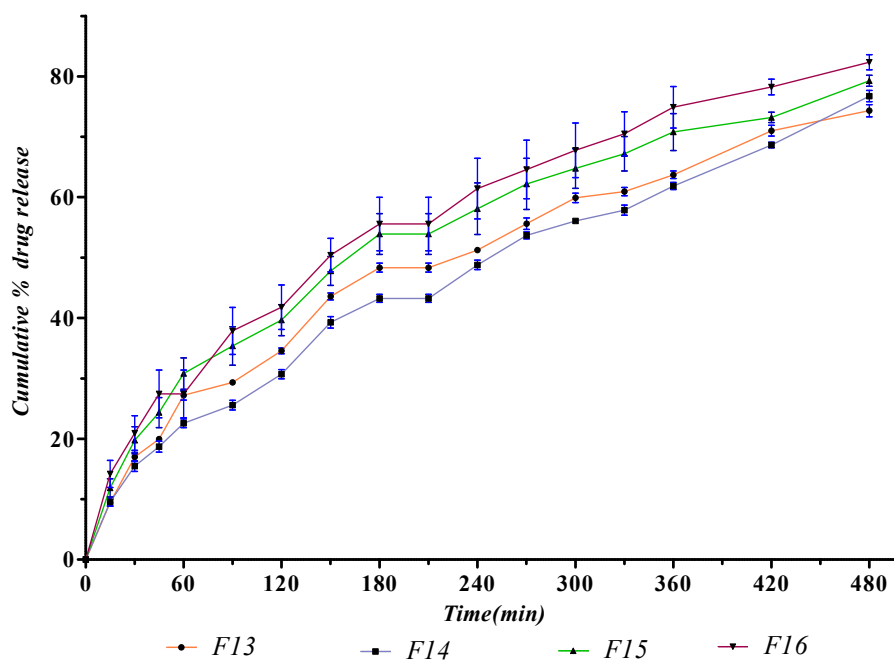
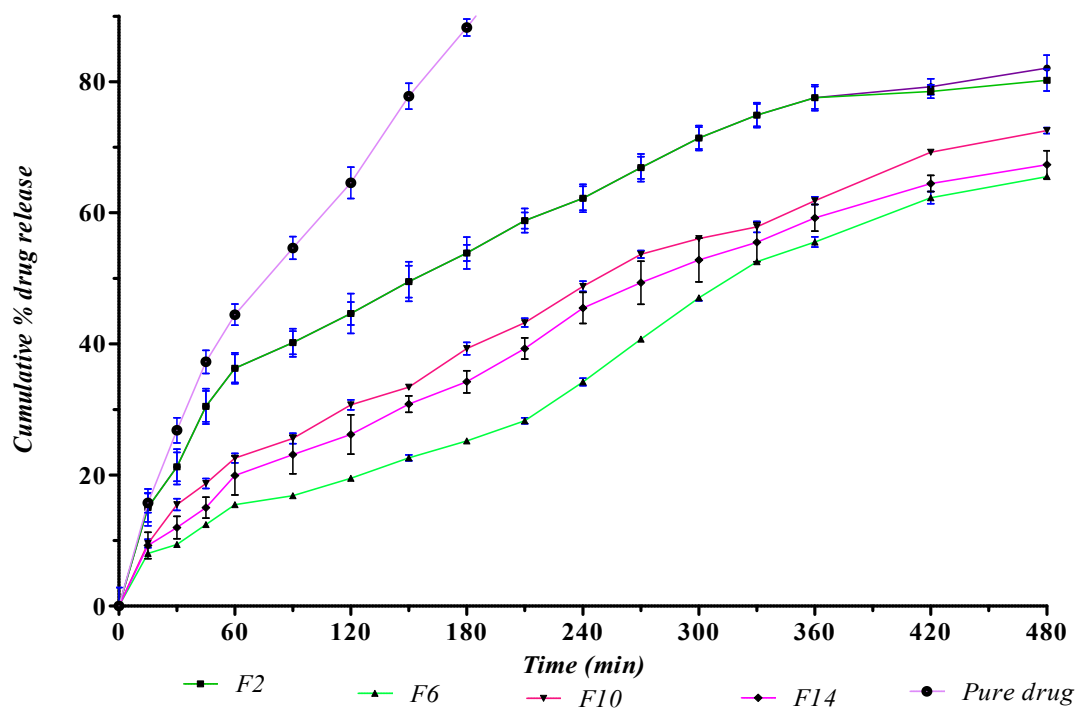


*Fig.5 Comparison of in vitro release of span 40 niosomes of different ratios*



*Fig.6 Comparison of in vitro release of span 60 niosomes of different ratios of Brimonidine tartrate*



*Fig.7 Comparison of in vitro release of span 80 niosomes of different ratios**Fig.8 Comparison of in vitro release of 2:1 niosomes of different surfactants with pure drug*



The niosomal formulation in each group of surfactant (Prolonged release and High entrapment efficiency) F2, F6, F10 and F14 showed that the drug retention capacity was more with niosomal preparation stored at  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  but increase in temperature and storage period decreased the drug retention capacity which was shown in Table.no.2a and 2b.

**Table no.2a**  
**Percentage of drug retained at temperature  $4^{\circ} \pm 2^{\circ}\text{C}$  (weeks)**

<b>Formulation</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>
F2	94.57	93.54	91.82	89.92	88.89	87.83	85.64	83.28	78.19	75.46
F6	95.83	95.12	94.59	93.90	91.80	90.39	87.19	84.48	80.13	78.69
F10	100.14	99.96	99.45	98.45	96.29	93.96	90.54	87.97	86.31	82.70
F14	96.30	95.36	93.18	92.36	90.78	87.43	85.48	85.18	83.29	80.16

**Table no.2b**  
**Percentage of drug retained at temperature  $30^{\circ} \pm 2^{\circ}/60\% \pm 5\%RH$**

<b>Formulation</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>
F2	93.48	92.86	89.79	86.43	81.28	77.03	71.73	68.12	56.19	47.94
F6	94.29	93.64	91.86	91.06	89.02	88.13	81.62	74.26	65.96	57.39
F10	100.99	98.0	97.10	94.0	92.26	87.43	79.90	74.01	69.20	58.69
F14	94.05	89.68	86.74	85.69	79.26	78.14	72.36	66.54	61.87	53.25

The best formulation F10 ( high entrapment and sustained release) was developed into an *in-situ* gelling system by utilizing the phase transition properties of HPMC K15M and carbopol 940 in different ratios as shown in Table no.3. The drug content of all the gel formulations revealed that drug was uniformly dispersed in the gel preparations which shown in Table no.4.

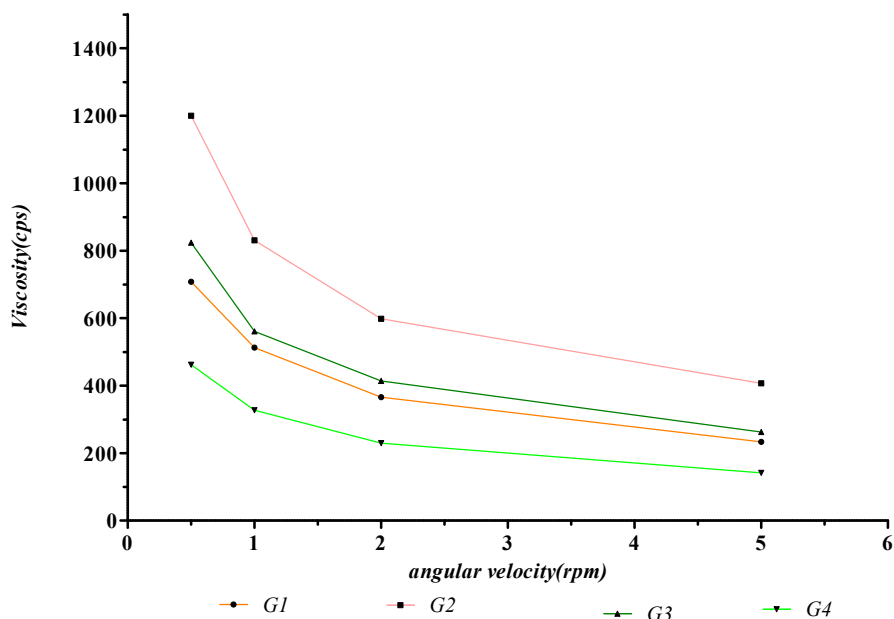
**Table no. 3**  
**Composition of in situ gelling system**

<b>Ingredients</b>	<b>Formulations</b>			
	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Niosomal dispersion eq. % v/v	0.15	0.15	0.15	0.15
Carbopol 940 %w/v	0.5	0.5	0.4	0.4
HPMC K15M % w/v	0.5	0.4	0.4	0.5
Sodium chloride % w/v	0.9	0.9	0.9	0.9
Benzalkonium chloride % v/v	0.001	0.001	0.001	0.001
Acetate buffer PH 5.0 %v/v	100	100	100	100

The pH of the gel formulation was in the acidic range of 4 – 5 and transformed into gel when it was instilled into the eye. The viscosity of the all gel formulations ranged from 141- 1200cps and it was shown in Fig.9. The rheological study of the formulations exhibited decrease in viscosity on increase in shear rate because of the pseudoplastic

behavior of the formulations. So, the gel formulations are preferred for ocular delivery since the ocular shear rate is very high ranging from  $0.03 \text{ s}^{-1}$  during interblinking periods to  $(4250 - 28,500) \text{ s}^{-1}$  during blinking (Aqil and Mohd, 2005; Khandare J N et.al, 2001).

Fig 9. Rheological studies of gel formulations



*In vitro* gelation studies revealed that the formulations G1, G2 and G4 showed immediate stiff gelation which remains for extended period of time while G3 showed immediate gelation which remains for 2 – 3 hours as shown in Table no.4.

Table no. 4  
*In vitro gelation studies and drug content of gel formulations*

Formulation	Gelation capacity	Drug content
G1	+++	97.61
G2	+++	98.14
G3	++	95.83
G4	+++	95.08

+++ - immediate stiff gelation

++ - immediate gelation

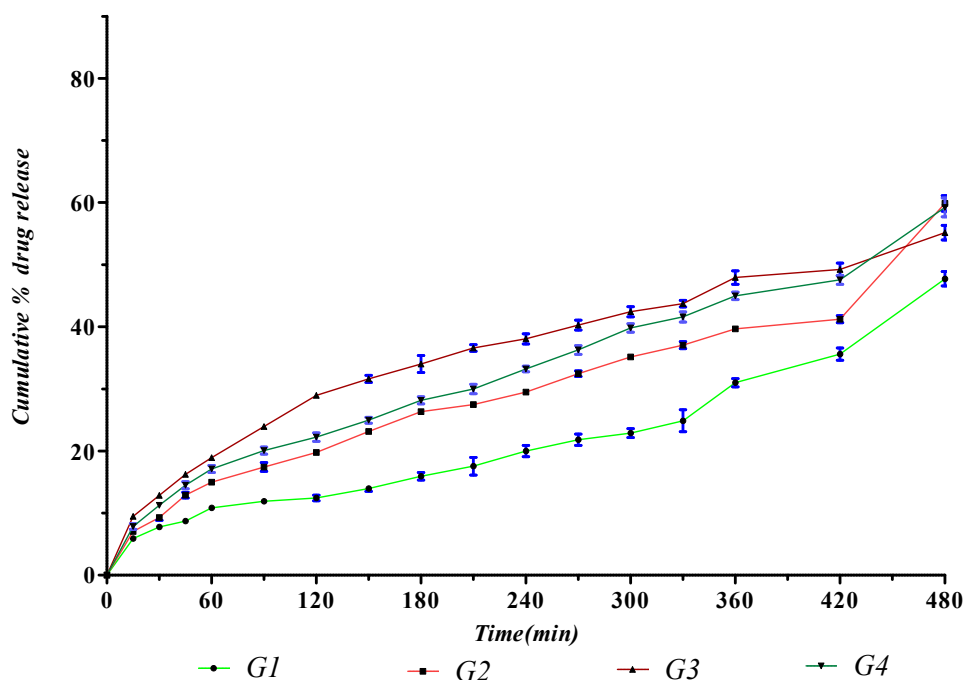
The prepared gel formulations released 47.73% (G1), 59.86% (G2), 55.16 % (G3), and 59.2% (G4) of drug after 8 hours as shown in Fig .10. Among all

formulations, G1 showed slower drug release due to high gelling capacity. The low gelling capacity of other formulations showed faster release than G1.

The in vitro release of G1 was then compared with niosomal drops and marketed drops. In marketed drops, the maximum drug (99.23%) was released at 5 hour when compared to niosomal drops and G1. In niosomal drops, the drug release was (81.27% at 5 hour) in a sustained manner compared to marketed drops due to entrapment of the drug in the

vehicle. Similarly, the release studies of G1 (47.73% at 8 hour) showed sustained release when compared to niosomal drops and marketed drops as shown in Fig.10. These indicate that the presence of polymer in niosomal gel showed prolonged release than niosomal drops due to gelling capacity and mucoadhesive properties of the gel.

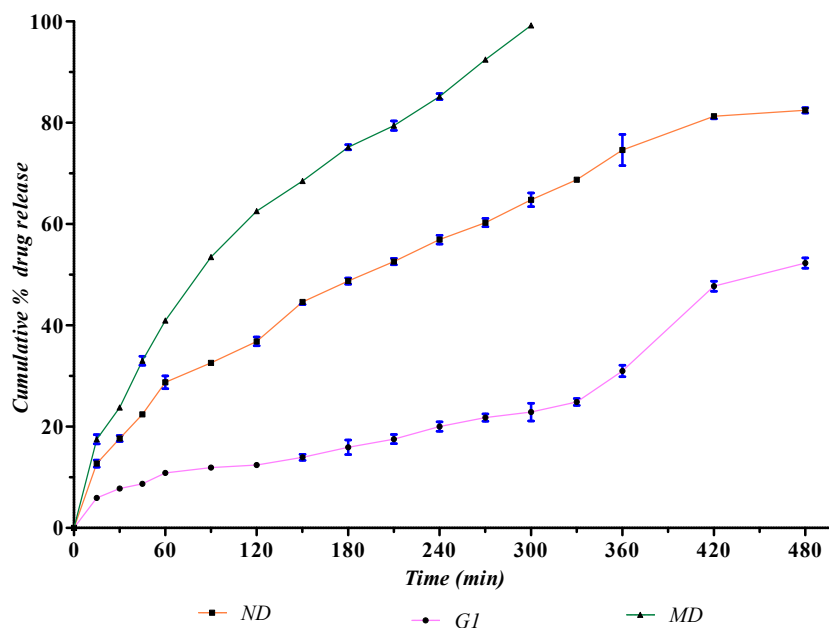
*Fig.10 Comparison of in vitro release of different ratios of gel formulations*



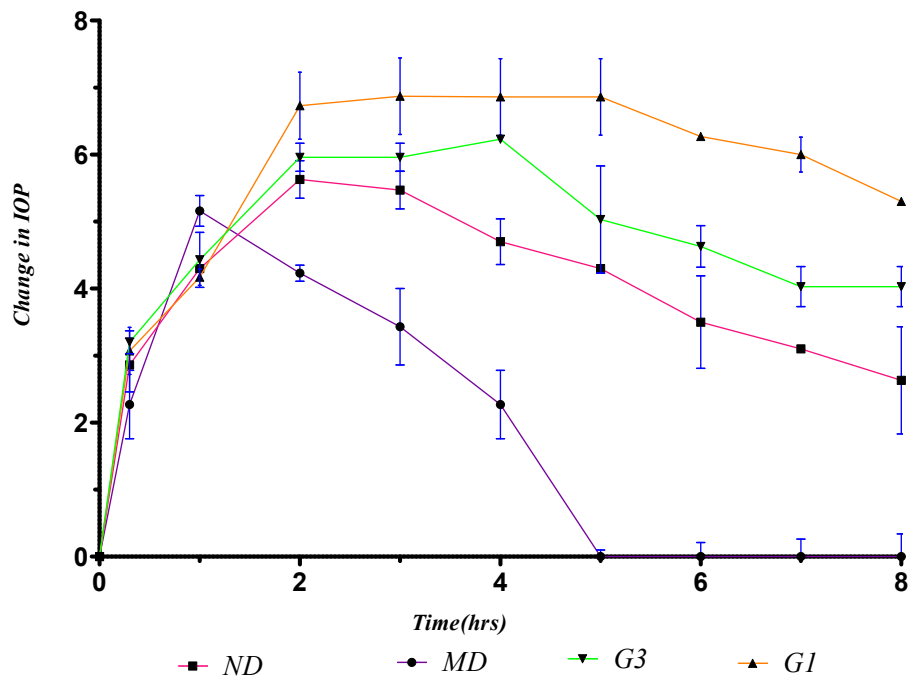
Two gel formulations (G3 faster, G1 slower drug release), niosomal drops and marketed drops were selected to determine anti-glaucoma activity for 8 hour. All three formulations ND, G3, and G1 showed significant anti-glaucoma activity as shown in Fig.11. The onset of action was started within 1 hour in all the formulations. The peak effect was observed at 1 hour and declined gradually, showed no effect after 5 hour of administration in marketed drops. The peak effect was observed at 2 hour and sustained up to 8 hour in niosomal drops, gel G1 and G3. Further

it was observed that the anti-glaucoma activity was in the following order  $G1 > G3 > ND > MD$ . Comparatively, the gel formulations showed more significant effect than the niosomal drops due to gelling capacity, mucoadhesive property of the polymer in the gel. Among the niosomal gels G1 showed better anti-glaucoma activity may be due to high entrapment of drug in niosomes than the gel G3. During the study, the formulations gelled in the form of transparent film over the corneal surface without any redness or inflammation.

**Fig.11 Comparison of in vitro release of G1 with niosomal drops and marketed drops**



**Fig.12 Anti-glaucoma activity for 8 hrs**



From the study, it was concluded that the niosomal gelling system is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence

time and ability to sustain drug release. In case of administration, decreased frequency of administration and resulting in better patient acceptance.

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