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FORMULATION AND EVALUATION OF TIMOLOL MALEATE OPTHALAMIC GEL FORMING SOLUTION WITH CARRAGENAN AND DIFFERENT PRESERVATIVE SYSTEM

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Abstract

The aim of this study was to develop a gel forming solution with carrageenan polymer which can give prolonged effect and evaluate it for different parameters such as pH, viscosity, osmolality, gelling capacity, assay of preservative and Timolol maleate, presence of related substances, preservative efficacy, in-vitro release and drug release kinetic studies. The results obtained during the evaluation of Timolol maleate ophthalmic gel forming solution with carrageenan and different preservative systems are summarised below: The pH of all the formulations was determined and it was found within the specified limits and this cleared that the formulation will not cause any irritation in the eye. Viscosity of all the formulations was found suitable and in-vitro gelling capacity test was performed. Formulation with parabens (GF6) and formulation with SOC (GF3) showed good gelling capacity. Osmolality of all the formulations was determined by Osmometer instrument and it was found that all formulations possessed osmolality within the specified limits indicating that the formulations will not cause any discomfort upon instillation. From the available preservatives, parabens (combination of methyl paraben sodium and propyl paraben sodium), SOC and sodium perborate tetrahydrate were used because of their compatibility with the other formulation ingredients. Benzalkonium chloride and benzododecinum bromide were failed because they caused thread formation in the formulation.

Keywords: - : osmolality, gelling, ophthalmic gel, parabens etc.

Introduction

Like propranolol and nadolol, timolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle and beta (2)-receptors in the bronchial and vascular smooth muscle. Beta (1)-receptor blockade results in a decrease in resting and exercise heart rate and cardiac output, a decrease in both systolic and diastolic blood pressure, and, possibly, a reduction in reflex orthostatic hypotension. Beta (2)-blockade results in an increase in peripheral vascular resistance. The exact mechanism whereby timolol reduces ocular pressure is still not known. The most likely action is by decreasing the secretion of aqueous humor. It is under the categories of antihypertensive agents, adrenergic beta antagonists and anti arrhythmia agents. The molecular weight of the drug is 316.42.

The chemical formula is $C_{13}H_{24}N_4O_3S$. the melting point of the drug is 201.5-202.5 °C. In its oral form it is used to treat high blood pressure and prevent heart attacks, and occasionally to prevent migraine headaches. In its ophthalmic form it is used to treat open-angle and occasionally secondary glaucoma. The IUPAC name of the drug is (S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1, 2, 5-thiadiazol-3-yl) oxy] propan-2-ol. The structure of the drug is given below:

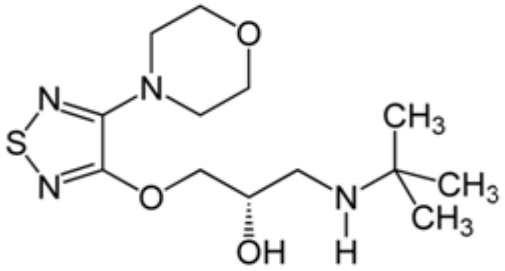


Figure 1: structure of timolol maleate

Table 1: Pharmacokinetic parameters of Timolol Maleate

Parameters	Values
Bioavailability	60 %
Metabolism	Primarily hepatic 80 %
Protein binding	10 %
Half life	2.5-5 hours

Materials and Methods

Timolol maleate was received from Ven petrochem. Tris buffer, sodium chloride, sodium bicarbonate, sodium hydroxide and hydrochloric acid were purchased from Merck. Sodium perborate tetrahydrate, stabilized oxychloro complex and calcium chloride dehydrate were purchased from Sigma Aldrich.Diethylene triamine penta methylene phosphonic acid hepta sodium salt (25% w/v aq. solution) was purchased from Sigma life science. Mannitol was purchased from Roquette france. Carrageenan gum was purchased from CP Kelco. Sodium carboxymethylcellulose was purchased from Signet chemical corporation pvt. ltd. Methyl paraben sodium and propyl paraben sodium were purchased from Gujrat organics ltd. Milli-Q water and 20 micron polypropylenen filter were purchased from Millipore.

Experimental Methods:

Formulation design of Timolol maleate *in situ* gel forming solution

Table 2: Formulation design of Timolol maleate *in situ* gel forming solution

S. No	Formulation Variation (% w/w)	Formulation code								
		GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	GF9
1.	Timolol maleate	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
2.	Iota Carrageenan	0.3	0.4	0.5	0.3	0.4	0.5	0.3	0.4	0.5
3.	Mannitol	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
4.	Tris buffer	0.25	0.25	0.25	0.18	0.18	0.18	0.25	0.25	0.25
5.	SOC	0.09	0.09	0.09	-	-	-	-	-	-
6.	Sodium CMC	0.10	0.10	0.10	-	-	-	-	-	-
7.	MPS	-	-	-	0.18	0.18	0.18	-	-	-
8.	PPS	-	-	-	0.02	002	0.02	-	-	-
9.	SPT	-	-	-	-	-	-	0.056	0.056	0.056
10.	Phosphonic acid	-	-	-	-	-	-	0.112	0.112	0.112
11.	Milli-Q water (q.s.)	100	100	100	100	100	100	100	100	100

Evaluation Parameters:

Pre compression parameters

Characterization of drug

Table 3: Characterisation of Timolol maleate

S. No.	Parameter	Observation
1.	Appearance	Crystalline powder
2.	Colour	white
3.	Odor	Odorless
4.	Melting point	198°C

Drug-excipient compatibility study

Drug was mixed with each excipient in ratio of 1:1 and then filled in the vials. These vials were observed for any physical change for 14 days. There was no physical interaction observed.

Table 4: List of mixtures kept for compatibility studies

S. No.	Mixture	Discoloration	Liquefaction	Clump formation
1.	Drug +carrageenan	-	-	-
2.	Drug+mannitol	-	-	-
3.	Drug+tris buffer	-	-	-
4.	Drug+SOC	-	-	-
5.	Drug+SPT	-	-	-
6.	Drug+MPS+PPS	-	-	-

+ Incompatibility; - Compatibility

Post compression evaluation parameters

Table 5: Strategy for the study to be done and formulation used

S. No.	Study to be done	Formulation code
1.	Appearance	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8,GF9
2.	pH	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8,GF9
3.	Osmolality	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8,GF9
4.	Viscosity	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8 ,GF9
5.	Gelling capacity	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8 ,GF9
6.	Content of Timolol maleate	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8,GF9
7.	Relative substance study	GF1,GF2,GF3,GF4,GF5,GF6,GF7,GF8,GF9
8.	Content of SOC	GF1,GF2,GF3
9.	Content of MPS and PPS	GF4,GF5,GF6
10.	Content of SPT	GF7,GF8,GF9
11.	Preservative efficacy test	GF10,GF11,GF12,GF3,GF6,GF9
12.	*in-vitro* drug release study	GF3,GF6,GF9, Marketed formulation
13.	Drug release kinetics	GF6
14.	Stability study	GF6

Test for Appearance

All formulations were checked against black and white background.

Table 6: Appearance and clarity parameters of formulations

Formulation code	Parameters	
	Appearance	Clarity
GF1	Transparent solution	Clear
GF2	Transparent solution	Clear
GF3	Transparent solution	Clear
GF4	Transparent solution	Clear
GF5	Transparent solution	Clear
GF6	Transparent solution	Clear
GF7	Transparent solution	Clear
GF8	Transparent solution	Clear
GF9	Transparent solution	Clear

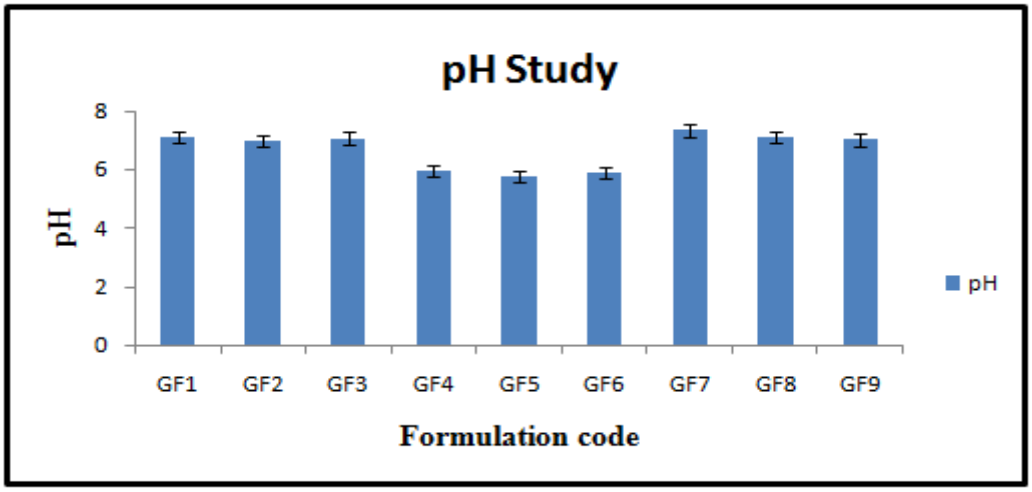
Determination of pH

The pH of all the formulations was checked by digital pH meter. The pH meter was calibrated before each use with buffer solutions of pH 4, pH 7 and pH 10. The pH of all the formulations was found within the range of 6-7.5 indicating that formulations will not cause irritation in eye.

Table 7: The pH of formulations GF1 to GF9

Formulation code	pH(mean ± SD)
GF1	7.12±0.06
GF2	6.98±0.06
GF3	7.07±0.05
GF4	6.35±0.05
GF5	6.37±0.03
GF6	6.51±0.07
GF7	7.15±0.06
GF8	7.10±0.07
GF9	7.02±0.08

Fig 2: The pH of formulations GF1 to GF9



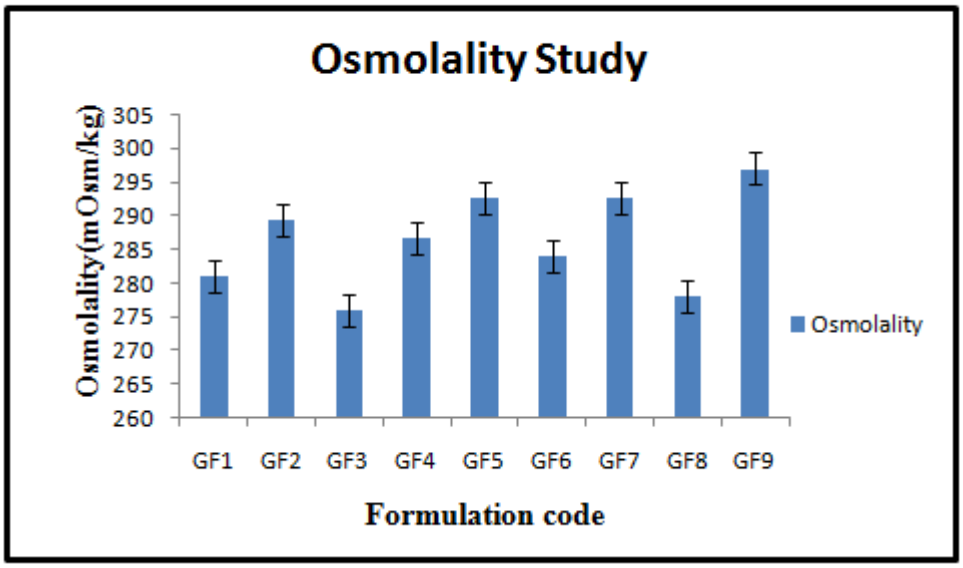
Determination of Osmolality

Osmolality of all the formulations was checked by osmometer. For this dilution of all the formulations was done to make the concentration one fifth and took 200 microlitre sample of each formulation to determine osmolality.

Table 8: Osmolality of all the formulations

Formulation code	Osmolality(mean±SD)
GF1	281.00±2.00
GF2	262.33±2.51
GF3	242.33±2.51
GF4	261.66±0.57
GF5	245.00±1.15
GF6	222.00±1.00
GF7	274.33±2.08
GF8	281.66±1.52
GF9	278.66±0.57

Fig 3: Osmolality of different formulations



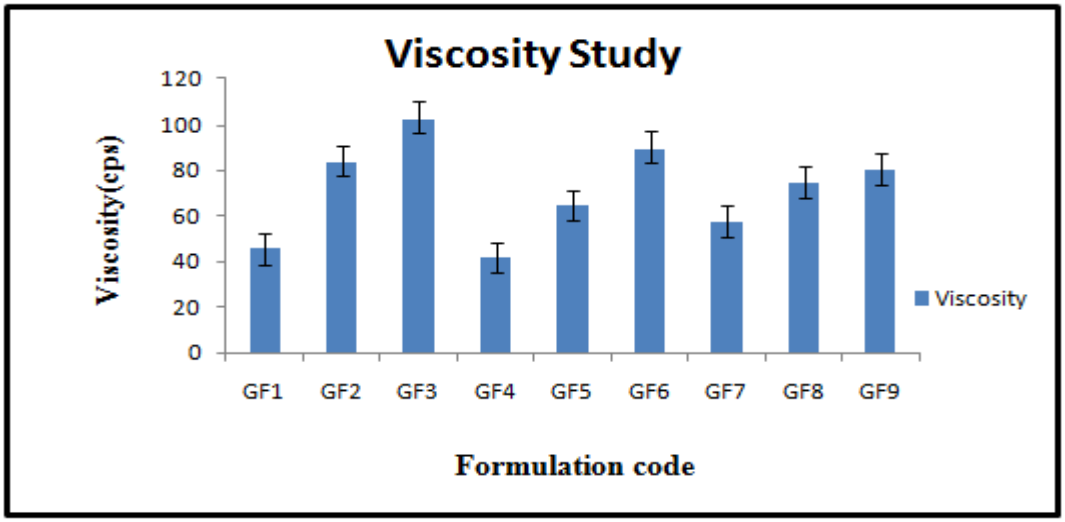
Viscosity Determination

The formulation should have an optimum viscosity that will allow for easy instillation into the eye, which would undergo a rapid sol to gel transition. So viscosity measurement was done. Viscosity of all the formulations was determined by Brookfield viscometer and it was found within the range of 41.73 to 103.03cps.

Table 9: Viscosity of all formulations at 90 rpm

Formulation code	Torque	Viscosity (cps) (mean±SD)
GF1	44.8%	45.66±1.89
GF2	62.9%	84.06±4.87
GF3	80.2%	103.03±3.51
GF4	23.3%	41.73±2.59
GF5	60.6%	64.53±4.85
GF6	29.6%	89.93±2.87
GF7	21.9%	57.33±3.30
GF8	55.9%	74.63±3.36
GF9	54.3	80.56±3.17

Fig. 4: Viscosity of formulations GF1to GF9



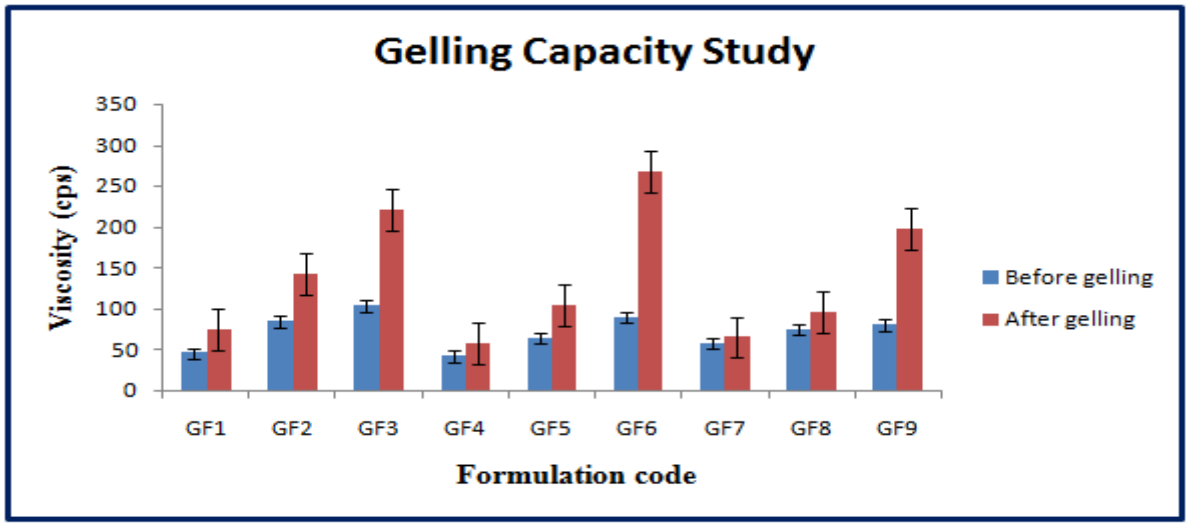
Determination of Gelling Capacity

Before gelling, viscosity was determined at 25±1°C and after gelling, viscosity was determined at 35 ±1°C with spindle no. S31.

Table 10: Viscosity of all formulations before gelling and after gelling

Formulation code	Viscosity in cps (before gelling) (mean±SD)	Viscosity in cps (after gelling) (mean±SD)
GF1	45.66±1.89	75.03±2.85
GF2	84.06±4.87	142.5±4.17
GF3	103.03±3.51	221.67±4.04
GF4	41.73±2.59	57.13±2.57
GF5	64.53±4.85	104.2±3.00
GF6	89.93±2.87	267.73±2.41
GF7	57.33±3.30	65.23±2.85
GF8	74.63±3.36	95.03±2.05
GF9	80.56±3.17	197.43±3.10

Fig. 5: Gelling capacity of all formulations



The flow behaviour of sample (formulation with tear fluid) was determined by various signs obtained by vis inspection. Flow behaviour with the "+" sign indicates the vehicle is in the liquid form and is very easy to f which show mild gelation after a few minutes and the gel dissolves rapidly. The "++" indicates that the vehicl in the liquid–gel like form and flows less rapidly and the gel remains for ≤1 hr. The flow behaviour with "+++" indicates that the sample is in the gel form and is difficult to flow which shows immediate gelation and gel remains for few hours.

Table 11: Gelling capacity of formulations

Formulation code	Gelling capacity
GF1, GF4, GF7	+
GF2,GF5,GF8,GF9	++
GF3,GF6	+++

Notice : + : Mild gelation after a few minutes and gel dissolves rapidly

++ : Gelation immediate and remains for ≤1 hr

+++ : Gelation immediate and remains for extended period (≥ 1 hr)

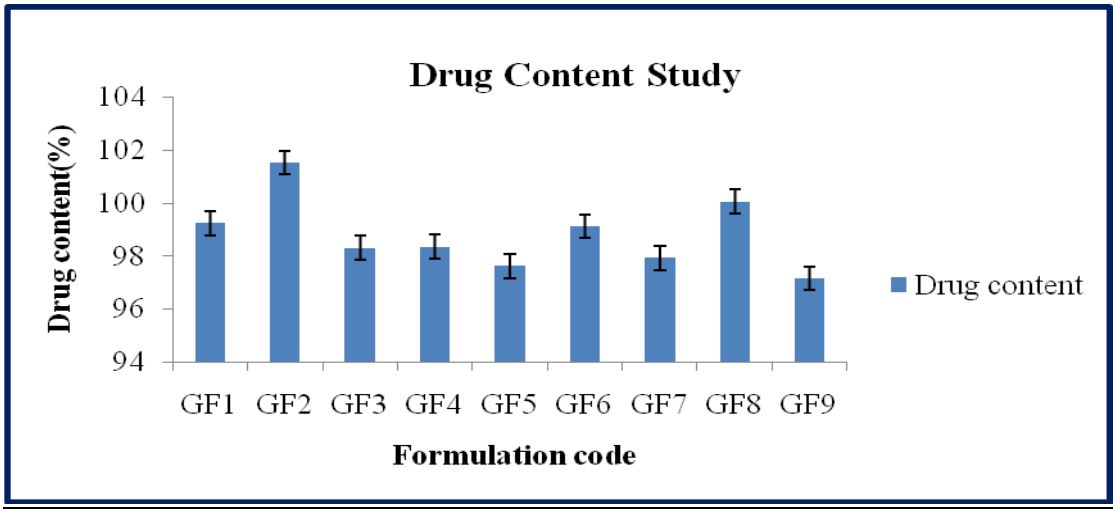
Drug Content Determination

The drug content of all formulations was determined by UV spectrophotometer by taking absorbance at 295 nm. Percent drug content of all the formulations are in the range of 97-102%.

Table 12: Percentage content of Timolol maleate

Formulation code	Percentage content(mean±SD)
GF1	99.23±0.43
GF2	101.52±2.49
GF3	98.30±1.24
GF4	98.34±1.30
GF5	97.61±1.08
GF6	99.12±0.62
GF7	97.92±1.34
GF8	100.04±1.40
GF9	97.15±1.67

Fig.6: Drug content of all the formulations GF1 to GF9



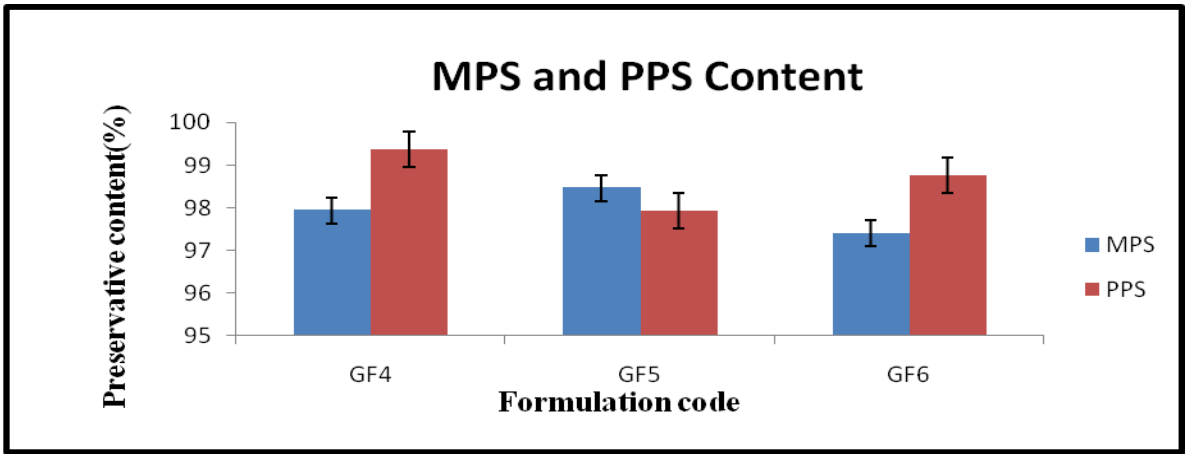
Determination of MPS and PPS Content

The content of MPS and PPS was determined by HPLC. HPLC results are given below. Percent of preservative content in all the three formulations are in the range of 97.4 to 98.46% in case of MPS and 97.92 to 99.37% in case of PPS.

Table 13: MPS and PPS content of formulations GF4, GF5 and GF6

S.No.	Formulation code	MPS content(%)(mean±SD)	PPS content(%)(mean±SD)
1	GF4	97.94±1.08	99.37±0.83
2	GF5	98.46±1.22	97.92±1.73
3	GF6	97.40±1.60	98.76±0.11

Fig. 7: Comparison of MPS and PPS content in formulations GF4, GF5 and GF6



From the above figure it is concluded that all the three formulations GF4, GF5 and GF6 have MPS and l content within the range 97-99.5%.

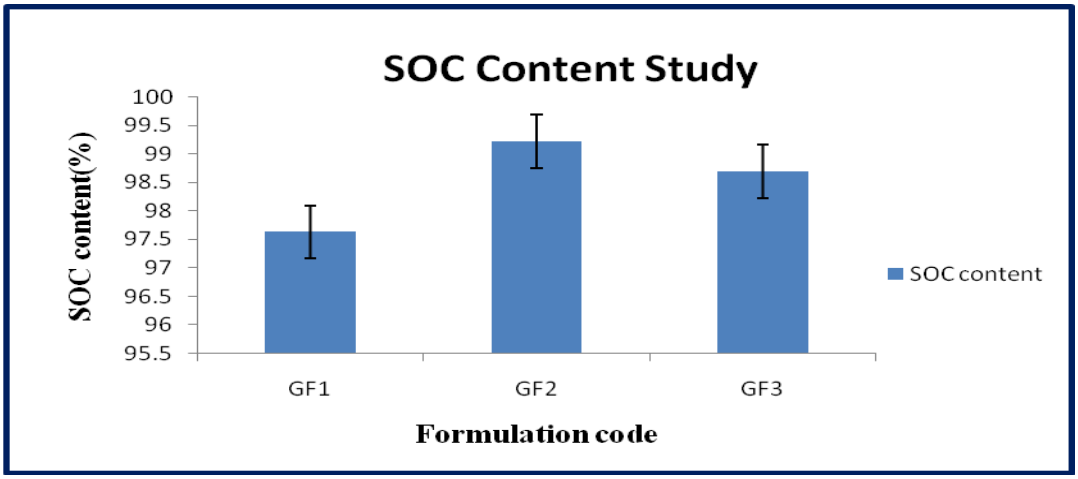
Determination of SOC Content

The content of SOC in three formulations GF1, GF2 and GF3 was determined by titrimetry. The results of SOC content are given in table below. Percent content of SOC in all the three formulations is in the range of 97.63 t 99.22%.

Table 14: Content of SOC in formulations GF1, GF2 and GF3

S. No.	Formulation code	SOC content(%)(mean ± SD)
1	GF1	97.63±0.80
2	GF2	99.22±0.64
3	GF3	98.69±1.07

Fig. 8: Content of SOC in formulations GF1, GF2 and GF3



From the above figure it is concluded that the three formulations have SOC content within the limits.

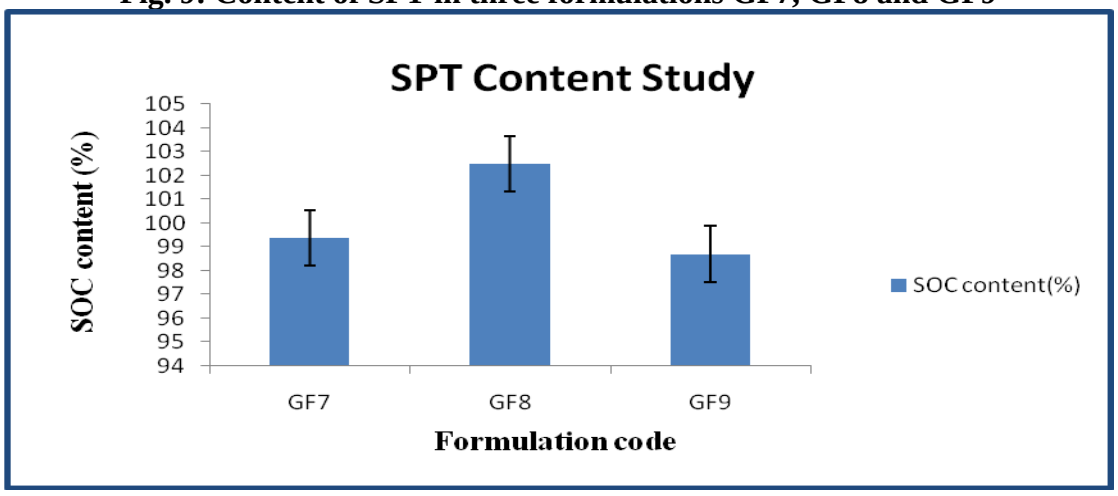
Determination of Sodium perborate tetrahydrate (SPT) Content

The SPT content in three formulations GF7, GF8 and GF9 was also determined by titrimetry. The results of SP content are given in the table below. Percentage of SPT in all the three formulations is in the range of 97.63 to 99.22%.

Table 15: Content of SPT in formulations GF7, GF8 and GF9

S.No.	Formulation code	SPT content(%)(mean±SD)
1	GF7	99.37±1.07
2	GF8	102.49±2.84
3	GF9	98.71±1.04

Fig. 9: Content of SPT in three formulations GF7, GF8 and GF9



From the above figure it is concluded that the three formulations have SPT content within the acceptable range.

Preservative Efficacy Test

In this study, formulations with different concentrations of SOC such as 50% SOC coded as GF10 and 100% SOC coded as GF3 were prepared. In the same way formulations with 50% parabens named as GF11 and 100% parabens coded as GF6 were prepared. Formulations with 50% Sodium perborate coded as GF12 and 100% Sodium perborate coded as GF9 were also prepared and all these six formulations were tested for their preservative effectiveness.

According to USP the ophthalmic products come under category 1.

Table 16: Testing parameters for formulations GF10, GF3, GF11, GF6, GF12 and GF9

Formulations code	Testing Parameters for formulations	
	Drug content (%) (mean ± SD)	Preservative content (%) (mean ± SD)
GF10	102.46±1.73	52.0±0.46
GF3	98.30±1.24	98.69±1.07
GF11	100.21±0.85	50.83±0.68(MPS) 51.03±0.56(PPS)
GF6	99.12±0.62	97.40±1.60(MPS) 98.76±0.11(PPS)
GF12	95.79±0.91	50.90±0.86
GF9	97.15±1.67	98.71±1.04

Table 17: Culture condition for inoculums preparation

Name of organism	Suitable Medium	Incubation Temperature	Inoculum's incubation time	Microbial recovery incubation time
E.coli ATCC8739	Soyabean-casein digestBroth; Soyabean-casein digest Agar	32.5± 2.5°C	18 to 24 hours	3 to 5 days
P.aeruginosa ATCC9027	Soyabean-casein digestBroth; Soyabean-casein digest Agar	32.5± 2.5°C	18 to 24 hours	3 to 5 days
S.aureus ATCC6538	Soyabean-casein digestBroth; Soyabean-casein digest Agar	32.5± 2.5°C	18 to 24 hours	3 to 5 days
C.albicans ATCC 10231	Soyabean-casein digestBroth; Soyabean-casein digest Agar	22.5± 2.5°C	44 to 52 hours	3 to 5 days
A.niger ATCC16404	Soyabean-casein digestBroth; Soyabean-casein digest Agar	22.5± 2.5°C	06 to 10 hours	3 to 7 days

Table 18: Microbial count observation of formulation GF10 containing 50% SOC

Name of organism	Counts (CFU/ml)					Specification
	Inoculum Count (CFU/ml)	Initial calculated	At "7th day"	At "14th day"	At"28th day"	
E.coli ATCC8739	$07x\ 10^8$	$07 x\ 10^6$	$08x\ 10^5$	$07x\ 10^4$	$04x\ 10^2$	Not specified
P.aeruginosa ATCC9027	$07 x\ 10^8$	$05 x\ 10^6$	$04 x\ 10^4$	$06x\ 10^3$	$04x\ 10^1$	Not specified
S.aureus ATCC6538	$06x\ 10^8$	$04x\ 10^6$	$06 x\ 10^5$	$04x\ 10^3$	$06x\ 10^2$	Not specified
C.albicans ATCC 10231	$08 x\ 10^8$	$03x\ 10^6$	$02 x\ 10^5$	$05x\ 10^3$	$03x\ 10^1$	Not specified
A.niger ATCC16404	$07 x\ 10^8$	$04x\ 10^6$	$04 x\ 10^4$	$04x\ 10^2$	$08x\ 10^1$	Not specified

Table 19: Microbial count observation of the formulation GF3 containing 100% SOC

Name of organism	Counts (CFU/ml)					Specification
	Inoculum Count (CFU/ml)	Initial calculated	At "7th day"	At "14th day"	At"28th day"	
E.coli ATCC8739	$07x\ 10^8$	$04x\ 10^6$	$05 x\ 10^5$	06×10^4	03×10^3	Not specified
P.aeruginosa ATCC9027	$07 x\ 10^8$	$02x\ 10^6$	$04 x\ 10^4$	06×10^3	22	Not specified
S.aureus ATCC6538	$06 x\ 10^8$	$08 x\ 10^6$	$06 x\ 10^5$	08×10^4	05×10^1	Not specified
C.albicans ATCC 10231	$08 x\ 10^8$	$05x\ 10^6$	$03 x\ 10^4$	05×10^2	12	Not specified
A.niger ATCC16404	$07 x\ 10^8$	$02 x\ 10^6$	$05 x\ 10^4$	04×10^2	08	Not specified

Table 20: Microbial count observation of formulation GF11 containing 50% Parabens

Name of organism	Counts (CFU/ml)					Specification
	Inoculum Count (CFU/ml)	Initial calculated	At "7th day"	At "14thday"	At"28th day"	
E.coli ATCC8739	$07x\ 10^8$	$06x\ 10^6$	$05 x\ 10^4$	$04 x\ 10^2$	$08\ x10^1$	Not specified
P.aeruginosa ATCC9027	$07 x\ 10^8$	$03 x\ 10^6$	$09x\ 10^3$	$05 x\ 10^2$	14	Not specified
S.aureus ATCC6538	$06 x\ 10^8$	$04x\ 10^6$	$09 x\ 10^4$	$03x\ 10^3$	$04\ x10^1$	Not specified
C.albicans ATCC 10231	$08x\ 10^8$	$05 x\ 10^6$	$04x\ 10^3$	$07x\ 10^1$	12	Not specified
A.niger ATCC16404	$07 x\ 10^8$	$02 x\ 10^6$	$08 x\ 10^3$	$04x\ 10^1$	09	Not specified

Table 21: Microbial count observation of formulation GF6 containing 100% Parabens

Name of organism	Counts (CFU/ml)					Specification
	Inoculum Count (CFU/ml)	Initial calculated	At "7th day"	At "14th day"	At"28th day"	
E.coli ATCC8739	$07x\ 10^8$	$04x\ 10^6$	$06 x\ 10^3$	$04 x\ 10^2$	08	Not specified
P.aeruginosa ATCC9027	$07 x\ 10^8$	$05 x\ 10^6$	$04 x\ 10^2$	$05 x\ 10^1$	Nil	Not specified
S.aureus ATCC6538	$06 x\ 10^8$	$08x\ 10^6$	$08 x\ 10^3$	$07x\ 10^1$	02	Not specified
C.albicans ATCC 10231	$08x\ 10^8$	$04 x\ 10^6$	$05x\ 10^3$	$04x\ 10^1$	Nil	Not specified
A.niger ATCC16404	$07 x\ 10^8$	$03 x\ 10^6$	$04 x\ 10^2$	$06x\ 10^1$	Nil	Not specified

Table 22: Microbial count observation of formulation GF12 with 50% SPT

Name of organism	Counts (CFU/ml)					Specification
	Inoculum Count (CFU/ml)	Initial calculated	At "7th day"	At "14th day"	At"28th day"	
E.coli ATCC8739	$08x\ 10^8$	$05 x\ 10^6$	$02 x\ 10^5$	$04 x\ 10^2$	14	Not specified
P.aeruginosa ATCC9027	$07 x\ 10^8$	$04 x\ 10^6$	$06 x\ 10^4$	$03 x\ 10^3$	$01 x\ 10^2$	Not specified
S.aureus ATCC6538	$05 x\ 10^8$	$05 x\ 10^6$	$08 x\ 10^4$	$04x\ 10^2$	$02 x\ 10^2$	Not specified
C.albicans ATCC 10231	$05x\ 10^8$	$04 x\ 10^6$	$08 x\ 10^3$	$05x\ 10^2$	$03 x10^2$	Not specified
A.niger ATCC16404	$07 x\ 10^8$	$05 x\ 10^6$	$04 x\ 10^3$	$04x\ 10^1$	$01 x\ 10^1$	Not specified

Table 23: Microbial count observation of formulation GF9 with 100% SPT

Name of organism	Counts (CFU/ml)					Specification
	Inoculum Count (CFU/ml)	Initial calculated	At "7th day"	At "14th day"	At"28th day"	
E.coli ATCC8739	$07x\ 10^8$	$03 x\ 10^6$	$04 x\ 10^3$	$02 x\ 10^1$	05	Not specified
P.aeruginosa ATCC9027	$07 x\ 10^8$	$04 x\ 10^6$	$08 x\ 10^3$	$04 x\ 10^2$	$01 x\ 10^2$	Not specified
S.aureus ATCC6538	$06 x\ 10^8$	$08 x\ 10^6$	$05 x\ 10^4$	$06x\ 10^1$	18	Not specified
C.albicans ATCC 10231	$08x\ 10^8$	$05 x\ 10^6$	$07 x\ 10^2$	Nil	Nil	Not specified
A.niger ATCC16404	$07 x\ 10^8$	$03 x\ 10^6$	$04 x\ 10^2$	Nil	Nil	Not specified

Acceptance Criteria: (As per USP)

Bacteria: not less than 1.0 log reduction from the initial calculated count at 7 days, not less than3.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days.

Yeast and Molds: No increase from the initial calculated count at 7, 14 and 28 days.

RESULT: Out of six formulations as mentioned above, results of GF10 and GF3 formulation did not meet criteria of U.S.P. compendia. Rest of four formulations meet U.S.P. compendia criteria but the best results were shown by formulation having 100% Parabens concentration i.e. GF6.

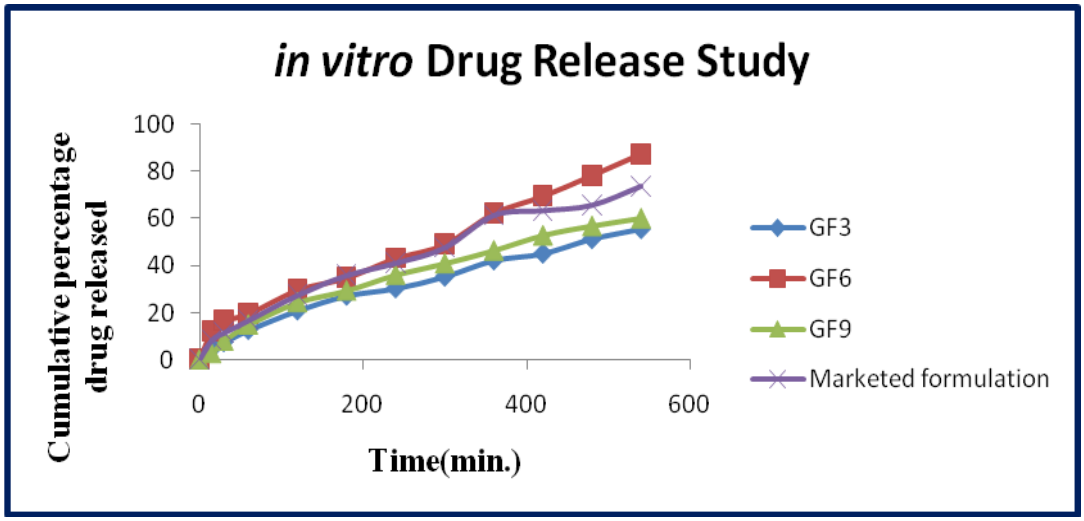
***In vitro* drug release study**

Drug release studies were performed and the results obtained are given below.

Table 5.24: Percentage drug release of formulations GF3, GF6, GF9 and Marketed formulation

Time (min.)	GF3 (mean±SD)	GF6 (mean±SD)	GF9 (mean±SD)	Marketed formulation (mean±SD)
0	0	0	0	0
15	4.42±0.10	12.13±0.05	2.71±0.08	8.45±0.07
30	7.44±0.09	16.73±0.03	8.01±0.08	11.55±0.07
60	12.59±0.07	19.58±0.03	14.89±0.10	16.78±0.03
120	20.89±0.06	29.64±0.06	24.34±0.07	27.48±0.05
180	27.16±0.12	34.9±0.06	29.33±0.08	35.83±0.06
240	30.25±0.10	43.09±0.09	36.0±0.06	41.04±0.08
300	35.22±0.06	48.97±0.09	40.84±0.07	47.56±0.11
360	42.11±0.05	62.15±0.05	46.37±0.18	61.27±0.07
420	44.98±0.11	69.45±0.09	52.73±0.07	63.28±0.03
480	51.18±0.08	78.23±0.05	56.76±0.10	65.55±0.07
540	55.41±0.12	87.34±0.06	60.00±0.14	73.58±0.04

Fig. 5.10: Comparision of *in vitro* drug release of formulations GF3, GF6, GF9 and marketed formulation



The *in vitro* drug release studies revealed that Formulation GF3 shows lowest drug release and formulation GF9 shows intermediate drug release. Formulation GF6 shows highest drug release out of the three formulations which is comparable to marketed formulation.

Drug Release Kinetic Study

The data obtained from *in vitro* release studies was fitted into different equations and kinetics models to calculate release kinetics of Timolol maleate from gelling system.

Table 25: Drug release kinetic data of formulation GF3

S. No.	Time (min.)	Square root of time	Log time	Cumulative percent drug released ± SD	Log cumulative percent drug release	Cumulative percent drug remaining	Log cumulative percent drug remaining
1	0	0	-	0	-	100	2
2	15	3.872	1.176	12.13±0.05	1.083	87.77	1.943
3	30	5.477	1.477	16.73±0.03	1.223	83.27	1.920
4	60	7.745	1.778	19.58±0.03	1.291	80.42	1.905
5	120	10.954	2.079	29.64±0.06	1.471	70.36	1.847
6	180	13.416	2.255	34.9±0.06	1.542	65.10	1.813
7	240	15.491	2.380	43.09±0.09	1.634	56.91	1.755
8	300	17.320	2.477	48.97±0.09	1.689	51.03	1.707
9	360	18.973	2.556	62.15±0.05	1.793	37.85	1.578
10	420	20.493	2.623	69.45±0.09	1.841	30.55	1.485
11	480	21.908	2.681	78.23±0.05	1.893	21.77	1.337
12	540	23.237	2.732	87.34±0.06	1.941	12.66	1.102

Fig.11: Cumulative percentage drug released vs time plot (Zero order)

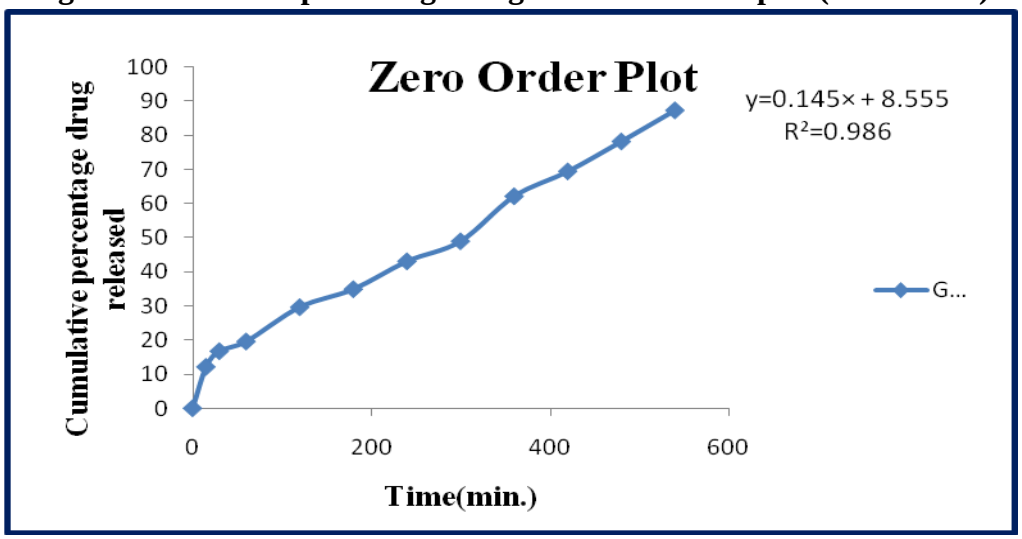


Fig. 12: Log cumulative percentage drug remaining vs time plot (First Order)

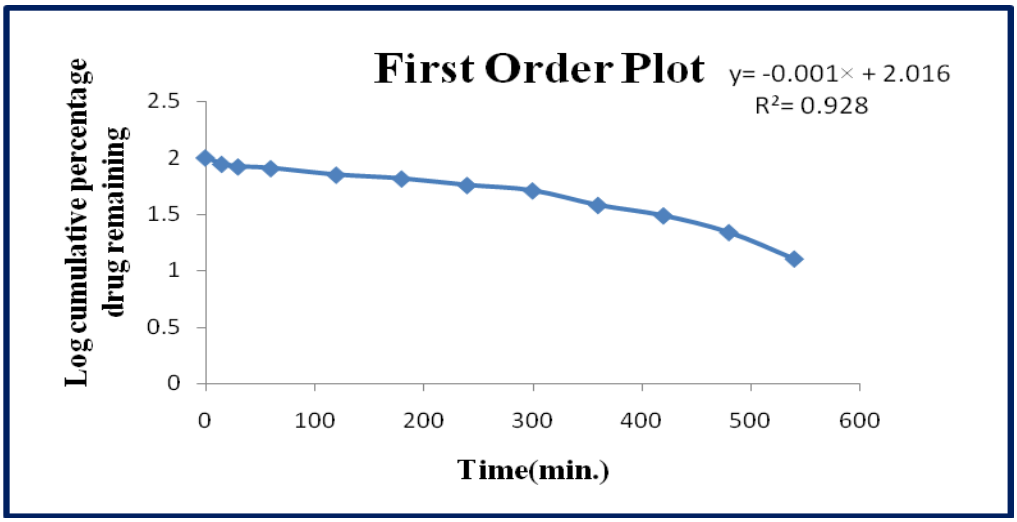


Fig.5.13: Cumulative percentage drug released vs square root of time (Higuchi's plot)

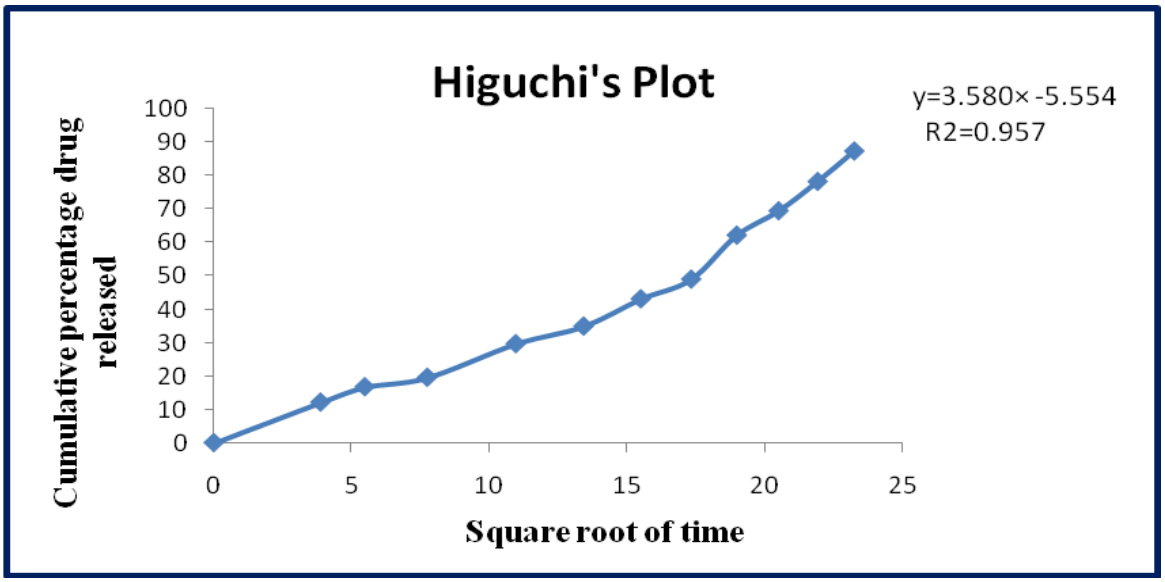
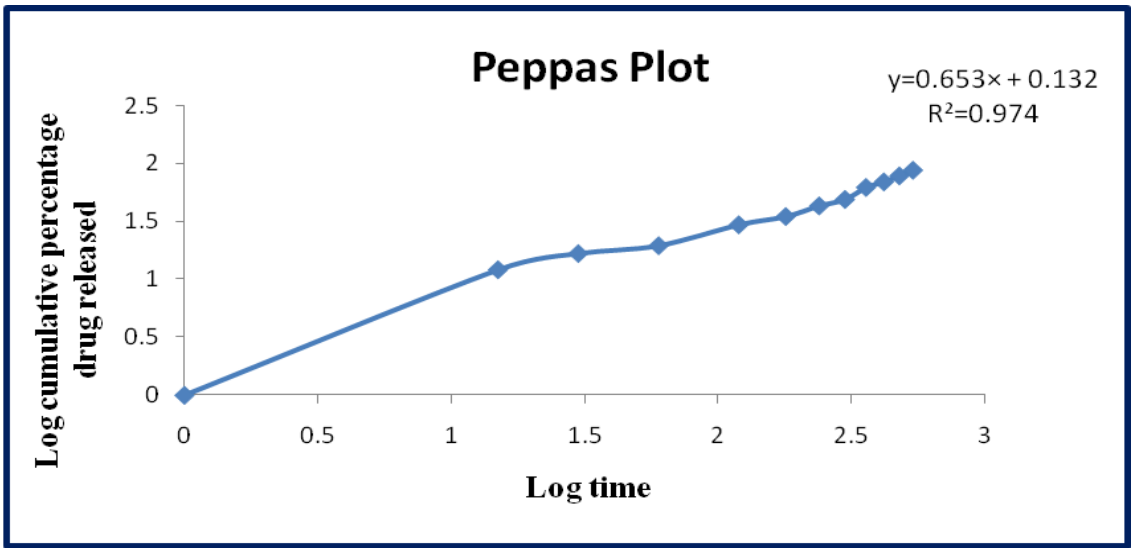


Fig.5.14: Log cumulative percentage drug released vs log time (Peppas plot)



Linear regression analysis and model fitting shows that formulation GF6 follows Zero-order kinetics, which has higher value of correlation coefficient (r^2).

Table 26: Regression coefficient (r^2) values obtained from various kinetic models

Formulation code	Zero order (r^2)	First order (r^2)	Higuchi model (r^2)	Korsemeyer-Peppas model (r^2)
GF3	0.986	0.928	0.957	0.974

Accelerated Stability Study

The optimized formulation was stored at 40±2ºC/75±5%RH for three months. Sample of formulation was taken out at the interval of one month and analyzed for drug and preservative content. The value of assay of drug was found within the specified limits.

Table 27: Three months stability data of the drug content for formulation GF6

Formulation Code	Initial (mean±SD)	01Month (mean±SD)	02 Month mean±SD)	03 Month mean±SD)
GF6	99.12±0.62	98.57±1.03	98.19±0.62	97.50±1.07

Fig. 15: Comparision of drug content w.r.t. time in 3 months Accelerated stability study

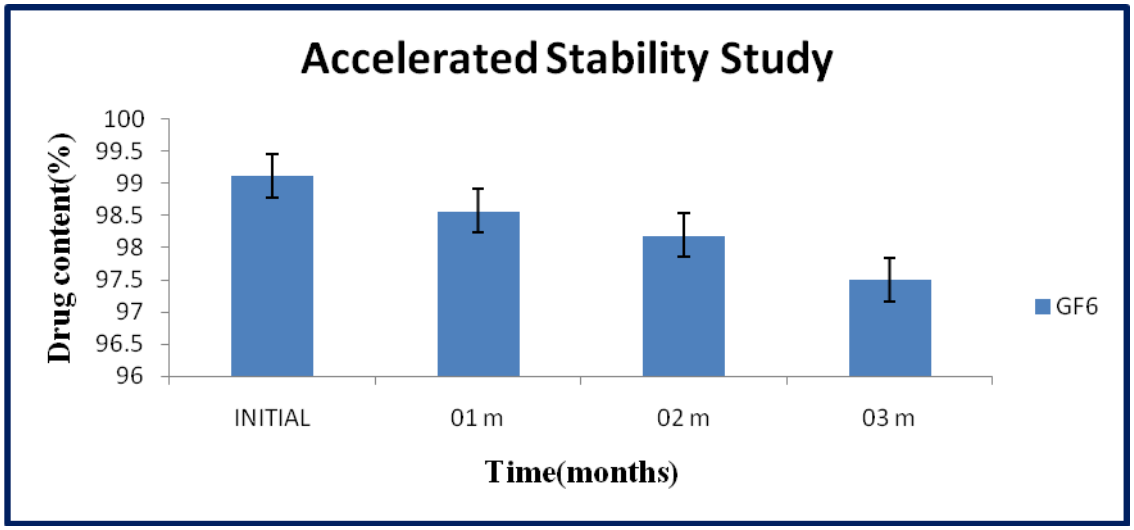
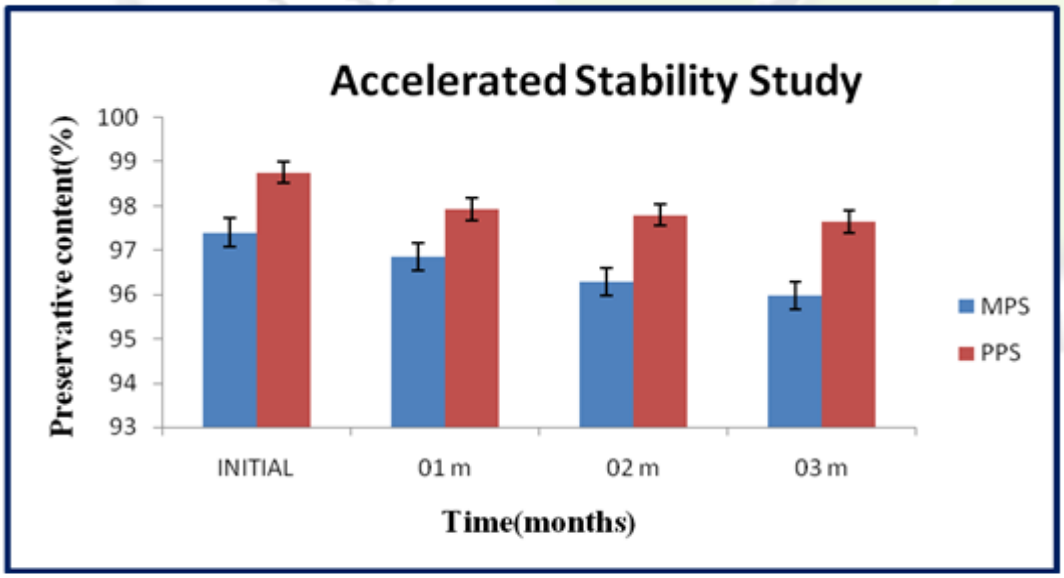


Table 28: Three months stability data of MPS and PPS content for formulation GF6

Formulation code	Preservative	Initial (mean ±SD)	01Month (mean ±SD)	02Month (mean ±SD)	03Month (mean ±SD)
GF6	**MPS**	97.40±1.60	96.86±0.11	96.29±0.14	95.97±0.34
	PPS	98.76±0.11	97.93±0.81	97.80±0.60	97.65±0.95

Fig. 16: Comparison of MPS and PPS content during Accelerated stability study.



From the figure it is concluded that there is more decrease in MPS content as compared to PPS content during accelerated stability study..

Stress Stability Study

The formulation GF6 was subjected to stress stability study. Samples were taken at the interval of 15 days. Drug and preservative content were determined and the values were found within the limits.

Table 29: Stress stability study data of drug content for the formulation GF6

Formulation code	Initial (mean±SD)	15 days (mean±SD)	30 days (mean±SD)
GF6	99.12±0.62	96.23±0.37	95.15±1.0

Fig.17: Comparision of drug content w.r.t. time in Stress stability study

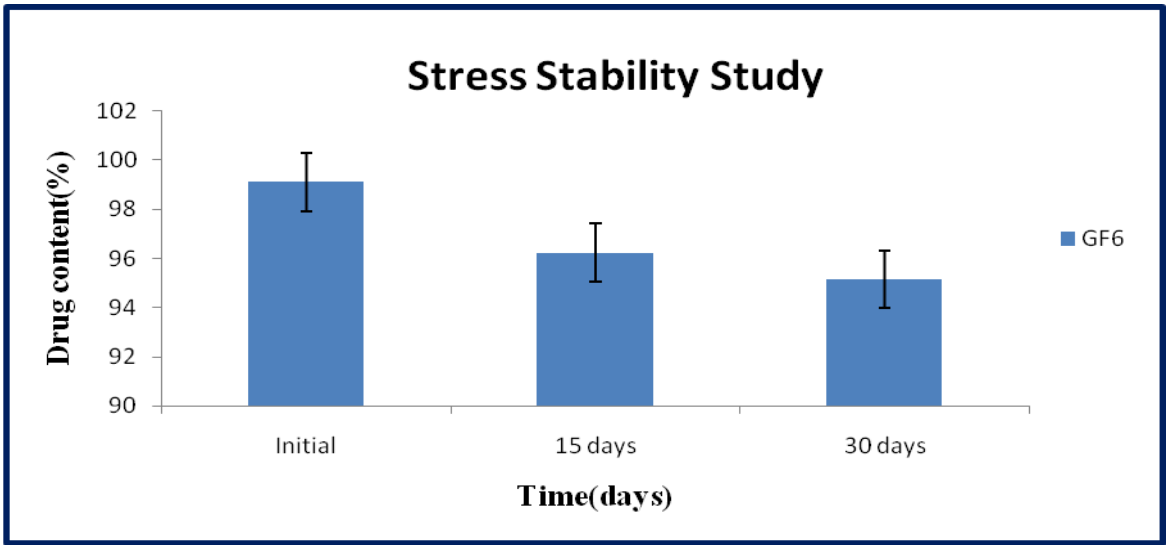
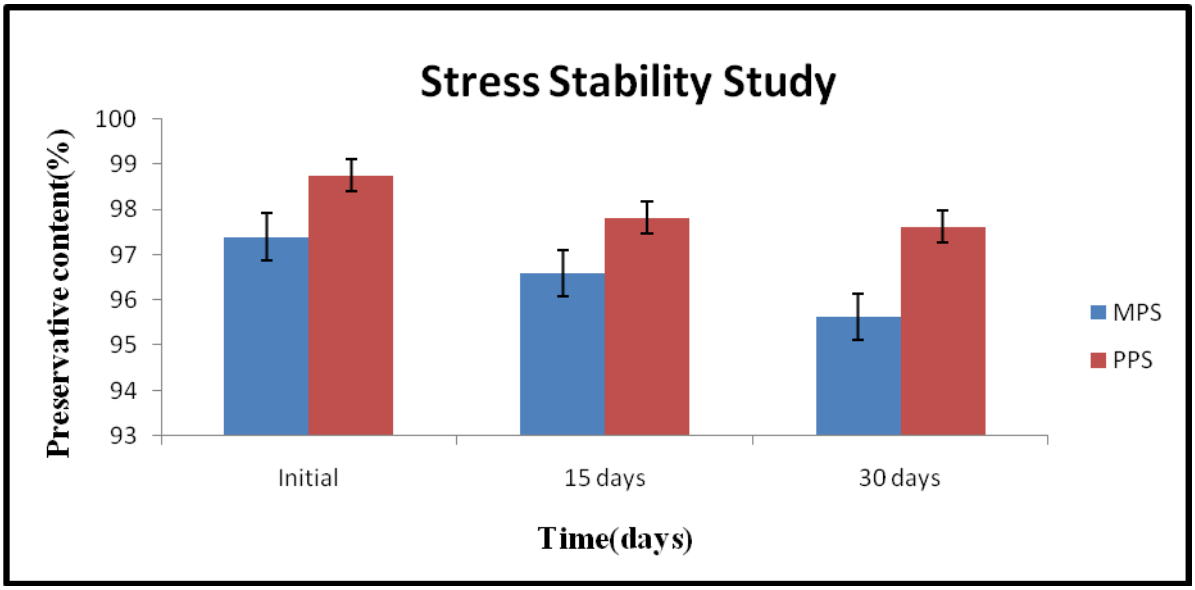


Table 30: Stress stability study data of preservative content for the formulation GF6

Formulation code	Preservative	Initial (mean±SD)	15 days (mean±SD)	30 days (mean±SD)
GF6	MPS	97.40 ±1.60	96.59±0.47	95.63±0.33
	PPS	98.76 ±0.11	97.83±0.37	97.62±0.52

Fig. 18: Comparision of MPS and PPS content w.r.t. time in Stress stability study



Summary

The formulation (GF6) was found to be stable upto three months on performing the accerlated stability studies. It was found that the vials did not absorb much drug through this period and the values of drug and preservative content were found within the specified limits. Stress stability study observation is the same as in accelerated stability study that is the formulation does not show significant absorption of preservative and drug. Preservative efficacy test was done to check the efficacy of preservative. This test was passed by four formulations out of six but the best result was found with 100% parabens formulation followed by 100% sodium perborate formulation. Stabilised oxychloro complex did not pass the test. All the formulations were sterilized by autoclaving and then filtered to make it sterilised and finally filled into Eto sterilized vials. In vitro drug release studies were performed and it was found that formulation GF6 with preservatives MPS and PPS gave 87.34% drug release which is better than marketed formulation results. Drug release data ofbest selected formulation (GF6) was subjected to zero order, first order, Higuchi's and Korsemeyer–peppas equation. Based on r^2 values it is concluded that drug follows zero order release pattern and Korsemeyer-Peppas model is the best fitted model.

Conclusion

All the formulations have been evaluated for different parameters such as pH, viscosity, osmolality, related substances, drug and preservative content. The pH and osmolality of all the formulations are within the limits concluding that they will not cause any discomfort in the eye. The formulation (GF6) shows good viscosity and gelling capacity as compared to others. The presence of any substance related to drug is determined through related substances test. There is no related substance found in all the formulations. The values of preservative and drug content are within the limits in all nine formulations. The efficacy of preservative is determined through preservative efficacy test. Formulation having a preservative combination of methyl paraben sodium and propyl paraben sodium is preferred because it shows maximum preservative effect. Preservative effect of sodium perborate tetrahydrate is better than stabilized oxychloro complex. Formulation with parabens as preservative system shows *in-vitro* release profile better than marketed formulation. Formulation with stabilised oxychloro complex shows minimum *in-vitro* release but it failed in preservative efficacy test. Formulation with sodium perborate tetrahydrate as a preservative shows good *in-vitro* release and it also passes preservative efficacy test. Drug kinetic studies show that drug release follows zero order release pattern. So it is concluded that out of three preservatives, parabens are the best followed by sodium perborate and the formulation (GF6) is suitable for the treatment of glaucoma.

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