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FORMULATION AND EVALUATION OF OPHTHALMIC IN-SITU GEL OF NATAMYCIN

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ABSTRACT

The aim of the present work was to formulate and evaluate in-situ gelling system of Natamycin. Natamycin is an antifungal agent which exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional ophthalmic suspensions. To overcome this, an attempt has been made to formulate pH-triggered in situ gelling system of Natamycin to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in pH. The Natamycin in- situ gelling system formulated by using poly acrylic acid (Carbopol 940) in combination with HPMC which acted as viscosity enhancing agent. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, In vitro diffusion study and antifungal activity. The developed formulation was stable and provided sustained release up to a time period of 8 hrs and it is a viable alternative to conventional eye drops.

Keywords: Natamycin; In-situ gel; Carbopol 940

INTRODUCTION

Eye is most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy **[1, 2].** A successful design of a drug delivery system, therefore, requires an integrated

knowledge of the drug molecule and the constraints offered by the ocular route of administration **[3, 4].** Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery; one of the ways to do so is by addition of polymers of various grades, development of in situ gel or colloidal suspension or using erodible or non-erodible insert to prolong the precorneal drug retention **[5].** Drugs administered by instillation must penetrate the eye and do so primarily through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera and appear to be particularly important for drugs that are poorly absorbed across the cornea **[6].**

MATERIAL AND METHODS [7, 8, 9, 10, 11, 12]

Material

Equipment: Magnetic stirrer, UV visible spectrophotometer, FTIR, viscometer, pH meter, incubator, dialysis membrane, hot air oven, sonicator, autoclave and stability chamber.

Chemicals and Reagents: Natamycin, Carbopol 940, HPMC K4M, HPMC E50LV, Sodium chloride, BKC, Sodium hydroxide.

Methods

PREPARATION OF IN-SITU GEL:

- ✓ Sodium chloride was dissolved in required quantity of distilled water, HPMC E50LV or HPMCK4M was added slowly to it and stirred with magnetic stirrer, care was taken that no lumps of HPMC was formed during stirring. Carbopol940 was sprinkled over this solution and allowed to hydrate overnight. Benzalkonium chloride was then added to it. The solution was again stirred with magnetic stirrer after 24hrs. From this solution 15 ml was withdrawn and used for further preparation.
- ✓ Natamycin was added in distilled water and sonicated for 15 min.
- ✓ Now, the drug solution was added to the carbopol-HPMC solution under constant stirring until a uniform solution was obtained. The pH of the formulation was then set to 5.5 using 0.1N NaOH. Distilled water was then added to make up the volume to 20ml [13]. Selection of Gelling agent (polymer) and its concentration is shown in **Table 1**.

Table 1: Selection of polymer and its concentration

Sr. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
1.	Natamycin	5	5	5	5	5	5	5	5	5	5	5	5	-
2.	Carbopol 940	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.7
3.	HPMC-K4M	0.5	1.0	1.5	-	-	-	0.5	1.0	1.5	-	-	-	-
4.	HPMC-E50LV	-	-	-	0.5	1.0	1.5	-	-	-	0.5	1.0	1.5	-
5.	Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
6.	Benzalkonium Chloride(ml)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
7.	Water (up to ml)	20	20	20	20	20	20	20	20	20	20	20	20	20

Analytical Methods:

Preparation of standard calibration curve of Natamycin: Accurately weighed 5 mg of Natamycin was dissolved in 100ml of 0.1% methanolic glacial acetic acid to get the stock solution of 50µg/ml. From this stock solution 20 ml was withdrawn and further diluted to 100 ml with 0.1% methanolic glacial acetic acid to obtain a concentration of 10 µg/ml. From this stock solution aliquots of 1, 2, 3, 4, 5, 6, 7, 8 & 9 ml were withdrawn and further diluted to 10 ml with 0.1% methanolic glacial acetic acid to obtain a concentrations range of 1 to 9 µg/ml. The absorbance of the solutions was measured at 304 nm by using UV-Visible spectrophotometer. The concentration of corresponding absorbance was given in below **Table 2**. Standard curve was plotted by taking concentration on X-axis and absorbance on Y-axis.

Table 2: Spectrophotometric data of Natamycin

Concentration (µg/ml)	Absorbance			Mean
	I	II	III	
0	0	0	0	0
3	0.256	0.258	0.260	0.258±0.002
4	0.336	0.337	0.344	0.339±0.004
5	0.424	0.425	0.420	0.423±0.002
6	0.519	0.518	0.526	0.521±0.004
7	0.623	0.621	0.622	0.622±0.001

Preformulation Studies:

Drug-Excipient Compatibility Study (FTIR): The study was designed to determine compatibility of drug with different co-processed excipients. The procedure consisted of dispersing a sample (drug alone, polymers alone and mixture of drug and polymers) in KBr to prepare 10% of mixture and ground generally in mortar-pestle with KBr before being compressed into pellets. This pellet was placed in light path and spectrum was recorded at a resolution of 2 cm^{-1} over a frequency range of 4000 to 400 cm^{-1}. The background spectrum of KBr was used as blank for determination.

Evaluation of in-situ gel:

Colour and Clarity: Colour and clarity of the formulations were determined visually **[13].**

pH: The pH of the formulations was adjusted by using pH meter.

Gelling capacity: The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid (pH 7.4) and equilibrated at 35°C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The composition of artificial tear fluid was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride $2H_2O$ 0.008 g and purified water q.s. 100 g **[14]**.

Drug content: The drug content of in situ gel was determined by taking sample (1ml) of in-situ gel in a 10ml volumetric flask and diluted with 0.1% methanolic glacial acetic acid. Then the absorbance was measured at max (304nm) using UV-spectrophotometer to calculate the percentage of drug content.

Viscosity: The viscosity of the in situ gel was measured by a brookfield viscometer using a spindle No.64.

***In vitro* Drug release:** The release of Natamycin from formulations was assessed using a dialysis bag under sink condition for 7 hr. Samples of formulation (1.0 mL) containing 50 mg Natamycin were enclosed in dialysis bags (mw cut-off 13000-14000), were incubated in 200 mL of artificial tear fluid, pH 7.4 at 370C under mild agitation . In order to increase the solubility and maintain sink condition, 1.0% (v/v) tween 80 was added into dissolution medium. Aliquots (5.0 mL) were collected at predetermined intervals and replaced with equal volume of artificial tear fluid to maintain sink conditions. The amount of the drug in the receiving solution was analysed by UV spectrophotometer at 304 n m **[15]**.

Kinetic modeling: In order to determine drug release mechanism that provide the best description to the pattern of drug release, the in vitro drug release data were fitted to zero order, first order, Hixon Crowell equation, Higuchi matrix model and korsmeyer peppas model. Regression coefficient was calculated for each model.

Sterilization: The formulation was filled in amber ampoules under aseptic conditions and sterilized in the autoclave (121 °C and 15 psi) for 20 minutes and further evaluation was carried out.

Sterility testing: IP method (2010) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating formulations for not less than 14 days at 30 to 350° C in the alternative thioglycollate medium to find the growth of bacteria and at 20 to 250° C in the soyabean-casein digest medium to find the growth of fungi in the formulations.

Antifungal activity: Antifungal efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against fungus. This was determined in the agar diffusion medium employing "Disc diffusion technique". Sterile suspension of marketed Natamycin eye drops was used as a standard. A layer of nutrient agar (20 ml) seeded with the test microorganism was allowed to solidify in the Petri plate. Discs containing different concentration of drug were placed on the solidified agar layer with the help of a sterile forceps. After keeping Petri plates at room temperature for 4 hr, the plates were incubated at 37 °C for 24 hours. The zone of inhibition (ZOI) was compared with that of the standard **[15]**.

Osmolality: The osmolality of the in situ gel was measured by a osmometer **[16]**.

Accelerated stability studies: Short term accelerated stability study was carried out for the period of 45 days for the formulations. The samples were stored at different storage conditions of room temperature, elevated temperature such as 40°C at 75% relative humidity and refrigerator (2 to 8°C). Samples was withdrawn after month and analyzed for visual appearance, clarity, pH, gelling capacity and drug content.

immediate and remains for an extended period)

RESULTS AND DISCUSSION

Standard calibration curve of Natamycin: Standard calibration curve obeyed Beer`s law in the concentration range of 1 - 9 µg/ml and the value of regression coefficient was found to be 0.998 which showed a linear relationship between concentration and absorbance in Phosphate Buffer pH 5.8 was shown in **Figure 1**.

FTIR Compatibility Studies: FTIR spectra of Natamycin alone, polymers mixture (Carbopol940, HPMC K4M) and physical mixture of drug and polymer were obtained. There was no interaction between Natamycin and polymers were shown in **Figure 2, Figure 3 and Figure 4.**

Evaluation of In-situ gels: The finished product of all the batches (F1 - F12) were evaluated for color and clarity, pH, Gelling capacity, drug content, osmolality and viscosity and data showed in the **Table 3.** The pH of all the formulations was adjusted to 5. 5, the drug content of all the formulations was within the range of 90.10% to 94.50%. The osmolality of the F-11 was found to be 295 mOsm kg^{-1}.

In-vitro Drug Release Studies: Results indicated that, the drug release was significantly prolonged by using the in situ gelling system due to the addition of the polymers carbopol 940 and HPMC (E50LV). From the results it is concluded that the high viscosity plays important role in controlling the release of drug from the

formulations. When the polymer concentration increases drug release decreases, and when polymer concentration decreases drug release from the formulation increases. Drug release study of formulation F1 to F6, F7 to F12 and Drug release study of batch F11 and marketed formulation are given in below **Figure 5, Figure 6 and Figure 7.**

Kinetic Modeling: The release kinetics of all the formulations (F1-F12) were shown in **Table 4**. The release kinetics of the optimized formulation was well fitted to Korsmeyer-Peppas model based on the concepts of the highest regression coefficient (R2) value. The 'n' value for the optimized formulation was found to be 0.579. The 'n' value obtained from Peppas equation was greater than 0.5, which indicated that the formulation showed drug release by non- fickian diffusion mechanism.

Sterilization: Autoclaving is a suitable method for sterilization of product. There was no significant difference in the drug content of natamycin which was performed before and after autoclaving. Drug content of natamycin before and after autoclaving was found to be 92.30% and 92.36% respectively.

Sterility test : The formulation F-11 passed the test for sterility as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 14 days at 30-35°C in case of alternative thioglycolate medium and at 20-250 C in the case of soyabean casein digest medium.

Antifungal activity: The results of the antifungal efficacy tests were shown in **Table 5**. The study indicated that the natamycin retained its antifungal efficacy even after formulated as an in situ gelling system. Results are given in **Table 5**.

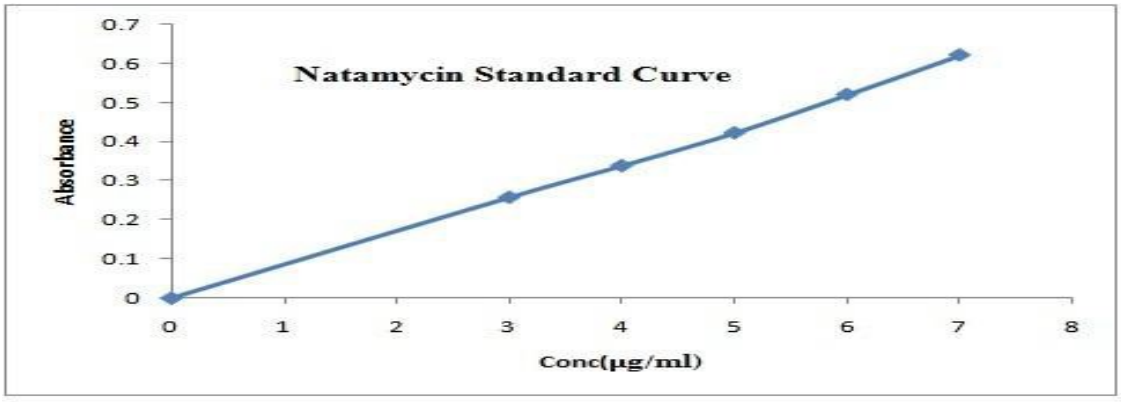

Figure 1: Calibration curve of Natamycin

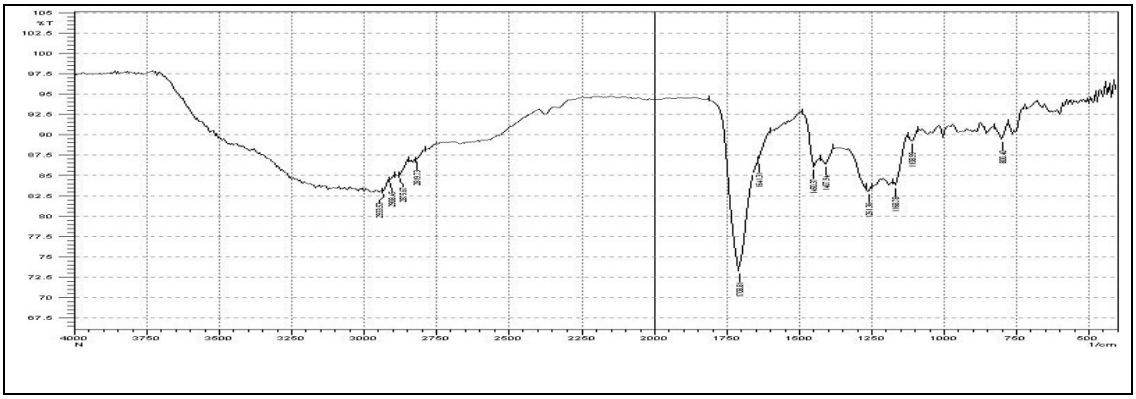

Figure 2: FTIR spectra of Natamycin

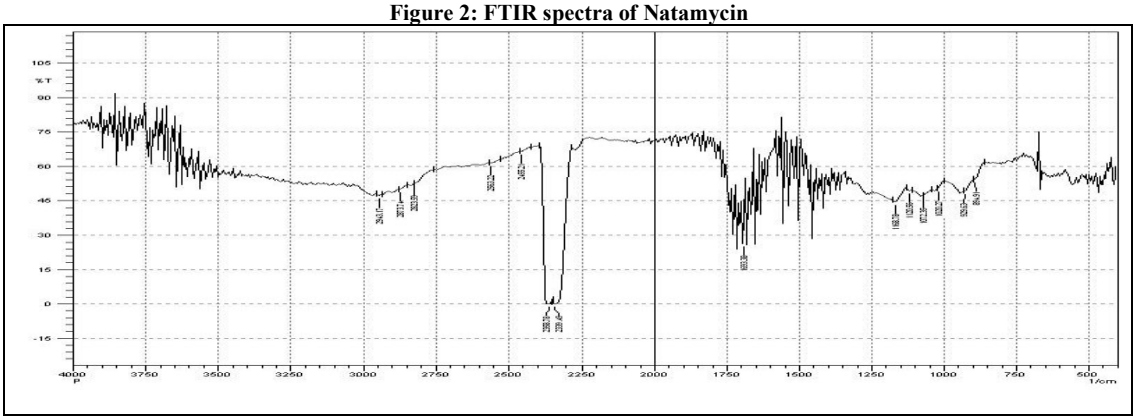

Figure 3: FTIR spectra of polymer mixture

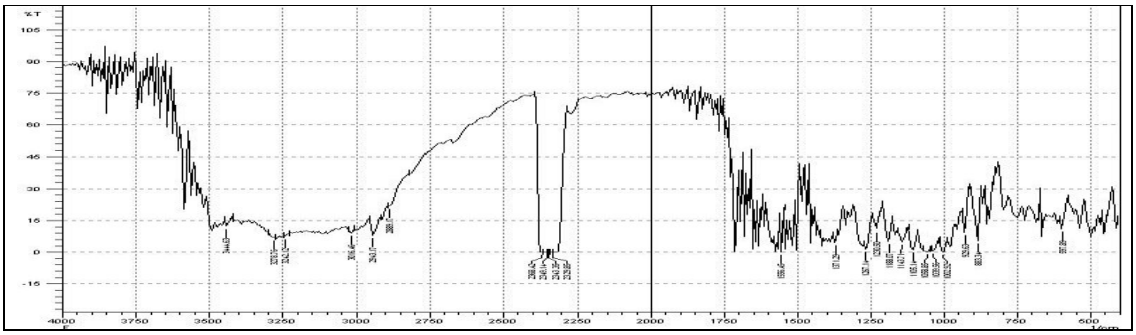

Figure 4: FTIR spectra of physical mixture

Table 3: Evaluation of In-situ gels

Batch No.	Color	Clarity	pH	Gelling time (Seconds)	Gelling capacity	Drug Content±S.D(%)	Viscosity	
							30 RPM	60 RPM
F1	Light Yellow	Cloudy	5.5	2	+++	90.10±0.77	700	500
F2			5.5	2	+++	90.54±0.55	800	650
F3			5.5	2	+++	90.32±0.67	900	750
F4			5.5	2	+	92.30±0.80	500	400
F5			5.5	2	+++	91.86±0.59	600	500
F6			5.5	2	+++	93.40±0.63	700	650
F7			5.5	2	+	93.84±0.48	350	300
F8			5.5	2	+++	90.98±0.87	400	350
F9			5.5	2	+++	94.50±0.41	600	500
F10			5.5	2	+	93.62±0.65	250	250
F11			5.5	2	+++	92.30±0.32	400	350
F12			5.5	2	+++	94.06±0.13	500	400

(+: Gel dissolves fastely, ++: Gelation immediate and remains for a 1-2 hours, +++: Gelation

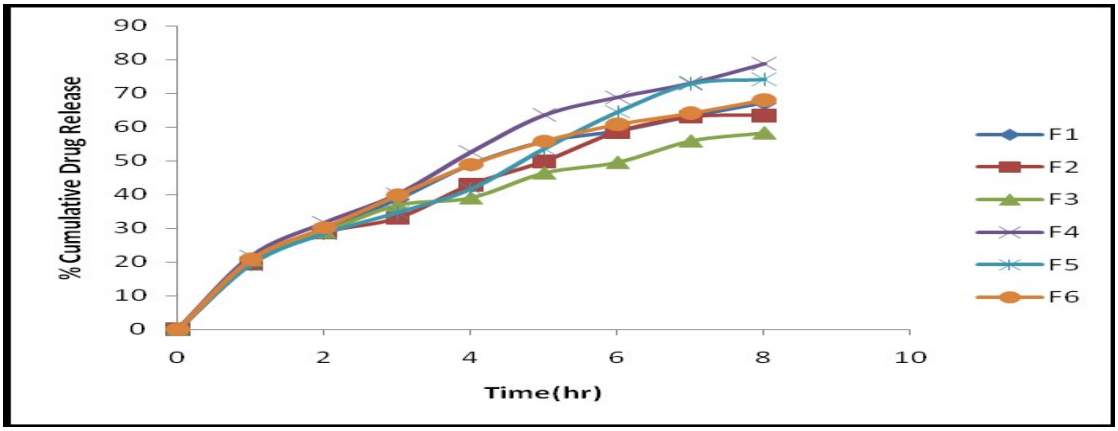

Figure 5: Drug release study of batch F1 to F6

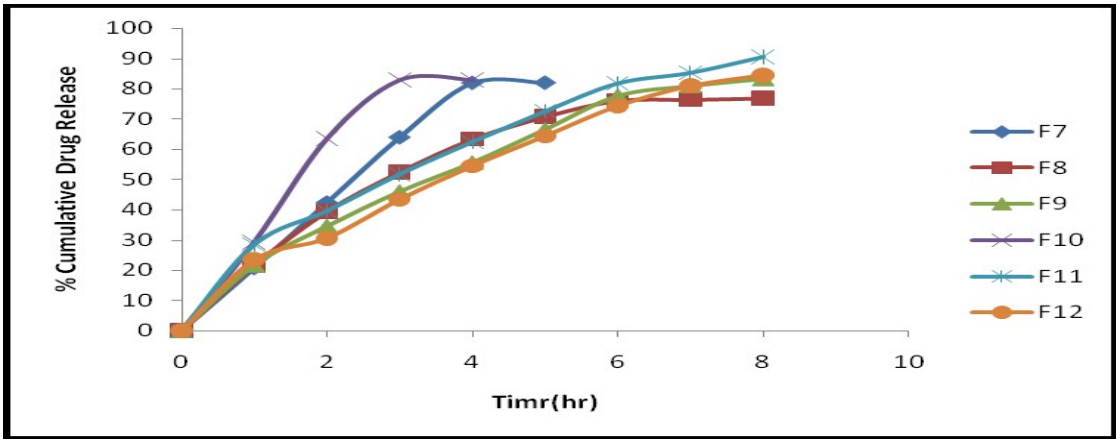

Figure 6: Drug release study of batch F7 to F12

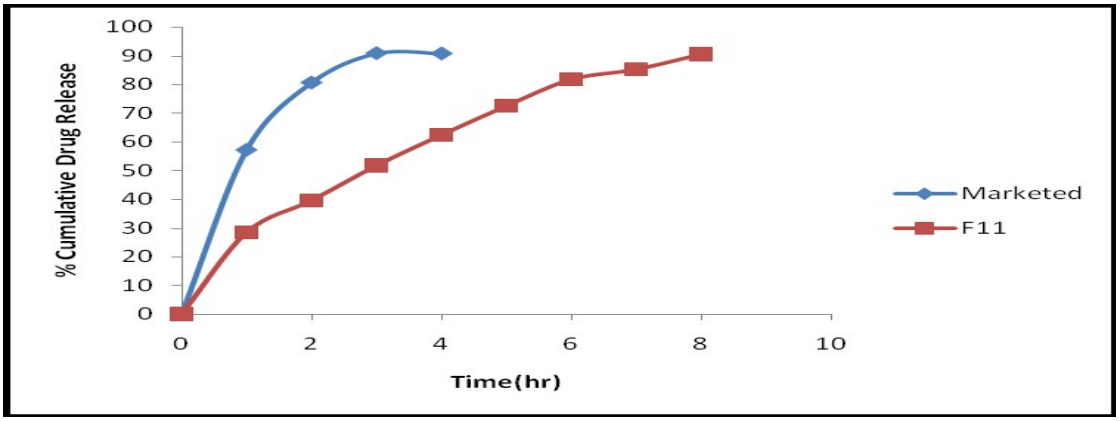

Figure 7: Drug release study of batch F11 and marketed formulation

Table 4: Release kinetic of formulations

Batch	F1	F2	F3	F4	F5	F6
ZERO ORDER						
Slope	6.678	6.472	5.276	8.339	8.082	6.871
Intercept	16.992	15.446	17.111	15.676	11.674	16.844
R^2	0.9836	0.9856	0.9962	0.9911	0.9932	0.9838
FIRST ORDER						
Slope	0.071	0.072	0.061	0.079	0.083	0.072
Intercept	1.316	1.287	1.306	1.326	1.259	1.316
R^2	0.9521	0.9602	0.9737	0.9622	0.978	0.9526
HIGUCHI MODEL						
Slope	26.064	25.152	20.356	32.334	30.947	26.816
Intercept	-5.483	-6.127	-0.185	-11.974	-14.367	-6.277
R^2	0.9970	0.9948	0.9981	0.9980	0.9876	0.9972
HIXSON CROWELL MODEL						
Slope	-2.226	-2.157	-1.758	-2.779	-2.694	-2.290
Intercept	27.669	28.184	27.629	28.107	29.442	27.718
R^2	0.9836	0.9857	0.9962	0.9911	0.9932	0.9838
KORSE MEYER PEPPAS MODEL						
Slope	0.581	0.584	0.492	0.639	0.658	0.590
Intercept	-0.685	-0.711	-0.689	-0.672	-0.732	-0.684
R^2	0.9972	0.9975	0.9985	0.9882	0.9928	0.9977
Batch	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
ZERO ORDER						
Slope	8.738	7.949	9.144	6.527	9.169	9.196
Intercept	21.472	22.044	16.183	40.949	22.083	14.845
R^2	0.9433	0.9386	0.9819	0.800	0.9879	0.9913
FIRST ORDER						
Slope	0.078	0.073	0.082	0.051	0.071	0.081
Intercept	1.387	1.390	1.341	1.588	1.442	1.333
R^2	0.8887	0.8928	0.9506	0.7586	0.9630	0.9702
HIGUCHI MODEL						
Slope	34.842	31.781	35.625	27.32	35.614	35.476
Intercept	-9.366	-6.172	-14.468	-15.406	-8.440	-15.299
R^2	0.9768	0.9746	0.9935	0.8696	0.9966	0.9932
HIXSON CROWELL MODEL						
Slope	-2.912	-2.65	-3.048	-2.179	-3.056	-3.065
Intercept	26.175	25.98	27.939	19.683	25.97	28.384
R^2	0.9433	0.9387	0.982	0.80	0.988	0.9913
KORSE MEYER PEPPAS MODEL						
Slope	0.670	0.631	0.671	0.479	0.579	0.6537
Intercept	-0.631	-0.626	-0.660	-0.442	-0.558	-0.659
R^2	0.977	0.9806	0.9970	0.9002	0.9973	0.9912

Table 5: Results of antifungal activity

Concentration (µg/ml)	Standard Zone of Inhibition(mm)	Test(F11)	
		Zone of Inhibition(mm)	Percentage efficacy
Candida albicans			
5	13	12	92
50	22	20	91
500	25	23	92
Aspergillus fumigates			
5	7	7	100
50	16	10	63
500	30	24	80

Table 6: Stability study data

Storage Condition	Time Period (Week)	Evaluation Parameters(F6)			
		Appearance	Ph	Gelling Capacity	Drug Content (%)
2^0 To 8^0c	0	Light Yellow	5.5	+++	93.40
	2		5.6	+++	92.78
	4		5.6	+++	92.20
	6		5.5	+++	92.28
Room Temperature	0		5.5	+++	93.40
	2		5.4	+++	92.96
	4		5.3	+++	92.69
	6		5.4	+++	92.18
At 40^0c (75% Relative Humidity)	0		5.5	+++	93.40
	2		5.1	+++	93.48
	4		5.2	+++	91.00
	6		5.3	+++	92.20

CONCLUSION

The novel ophthalmic pH-triggered in situ gelling drug delivery was successfully formulated by using carbopol 940 and HPMC (K4M and E50LV). The formulated in-situ gelling systems were characterized for appearance, clarity, pH, gelling capacity, rheological character, in vitro release in simulated tear fluid. The formulation was liquid at the formulated pH (5.5) and underwent rapid gelation upon raising the pH to 7.4. The pH-triggered in situ gelling system showed sustained drug release upto 8 hr. So, this formulation has potential for using as an alternate to the conventional natamycin ophthalmic suspension to improve the bioavailability through its longer precorneal residence time and ability to sustain drug release. The patient compliance may be improved due to the decrease in frequency of drug administration.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article

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REFERENCES

[1] Sasaki H, Yamamura K, Nishida K, Nakamurat J and Ichikawa M, "Delivery of drugs to the eye by topical application." Progress in Retinal and Eye Research, 1996; 15(2): 553-620.

[2] Lee V, "Review: Topical ocular drug delivery: recent developments and future challenges.", J. Ocular Pharmaco., 1986; 2: 67-108.

[3] Raghava S, Hammond M and Kompella U, "Periocular routes for retinal drug delivery." Expert Opinion on Drug Delivery, 2004; 72: 99-114.

[4] Greaves J and Wilson C, "Treatment of diseases of the eye with mucoadhesive delivery systems." Advance Drug Delivery Review, 1993; 21:349-383.

[5] Robinson J, "Ocular anatomy and physiology relevant to ocular drug delivery." Ophthalmic Drug Delivery Systems, 1993; 41: 29-57.

[6] Ahmed I and Patton T, "Disposition of Timolol and inulin in the rabbit eye following corneal versus non-corneal absorption." International Journal of Pharmaceutis, 1987;2: 9-21.

[7] Drug Today, April-June 2012, Volume: II, 994.

[8] USP 31, NF 26, Asian edition, 2008, volume.III, 2767-2768.

[9] The Merck Index, 14th edition, 6430.

[10] Martin Dale by Sean C. Sweetman, 34th edition, 406.{20}

[11] Analytical profiles of drug substances by Klaus Florey, volume: 10,513-561.

[12] Raymond C, Paul J, and Marian E, "Handbook of Pharmaceutical Excipients", 5th edition, 61-63,111-115,346-349,671-674.

[13] Mohanambal E and Arun K, "Formulation and Evaluation of pH triggered in situ gelling System of levofloxacin." Indian Journal of Pharmaceutical Education and Research, 2011;45(1):58-64.

[14] Zhidong L and Jiawei L, "Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin." International Journal of Pharmaceutics, 2006; 315(1-2): 12–17.

[15] Bhatta R and Chandasana H, "Mucoadhesive nanoparticles for prolonged ocular delivery of natamycin." International Journal of Pharmaceutics, 2012; 432(1–2):105–112.

[16] Gratieri T, Gelfuso G.M, Rocha E.M, Sarmento V.H, O.D Freitas and R.F.V Lopez, "A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery." European Journal of Pharmaceutics and Biopharmaceutics, 2010; 75:186-193.