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Original Article

FORMULATION AND EVALUATION OF CETIRIZINE HYDROCHLORIDE pH TRIGGED *IN-SITU* OCULAR GEL

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ABSTRACT

Objective: In the present research work, the aim was to prepare pH trigged *in-situ* ocular gel of Cetirizine Hydrochloride (CTZ) to improve its local bioavailability at the eye surface.

Methods: CTZ in-situ ocular gel was prepared by the pH-trigged method. *In-situ* CTZ ocular gel was prepared by a pH-sensitive gelling agent (Carbomer) with a one-viscosity builder polymer (HPMC E4M). All formulation was evaluated for appearance, pH, viscosity at different pH, gelling capacity, % drug content, and drug release. Nine formulations were prepared and optimized successfully using 3^2 factorial designs. Optimization was done by DoE software version Version 13.0.10.064.

Results: All nine formulations of in-situ ocular gel were subjected to evaluation. Out of 9 formulations, F3 had a good gelling capacity with the minimum amount of polymer. The appearance of the optimized formulation was translucent and homogenous. The pH of the F3 formulation is 5.55±0.07, which is good for maintaining formulation in the solution stage. Viscosity at 20 RPM of F3 formulation at pH 5.5 is 837.30±1.00 cps; this range of viscosity has good flow properties. Viscosity at 20 RPM of F3 formulation at pH 7.4 is 6800.74±1.58cps; this range of viscosity has a good gelling capacity which helps to drug retain at the eye surface. Drug content is 100.16±0.53%. Drug release at 300 min is 69.22±2.12, it can say that the drug may be retained for more than 300 min at the eye surface, which is good for reducing dosing frequency.

Conclusion: CTZ was successfully formulated in pH triggered *in-situ* gelling system using Carbomer 974P in combination with HPMC E4M. The prepared in-situ gel is easily converted from solution stage to gel stage at the pH of the eye so we can say that the drug in the in-situ ocular gel is more bioavailable than conventional ophthalmic solution *In vitro* results indicated that the *in-situ* gel system is a viable alternative to conventional ocular drops by virtue of its ability to sustain drug release.

Keywords: Carbomer, HPMC, pH trigged *in situ* ocular gel, Bioavailability

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INTRODUCTION

CTZ, a potent second-generation antihistamine, is widely used orally for the treatment of allergy, hay fever, angioedema, and urticaria. Recently in May 2017, its first topical formulation (ophthalmic solution) was approved by FDA for the treatment of ocular itching associated with allergic conjunctivitis. CTZ is available as an ophthalmic solution in the international market. The ophthalmic solution has a problem of short retention time at the eye surface and can say CYZ ophthalmic solution has low local bioavailability so it requires enhancement in retention time to increase its local bioavailability [1].

pH trigged *in situ* ocular gel is a potential tool for improving the local bioavailability of drugs at the eye surface. pH trigged *in situ* ocular gel is a solution of a drug, polymer, buffering agent, and purified water. *In situ* ocular gel spontaneously covert from solution to gel at physiological pH of the eye [2].

In the present research work, CTZ shall be formulated as pH trigged *in situ* ocular to overcome the short retention time (Low local bioavailability) of conventional ophthalmic solution at the eye surface the.

MATERIALS AND METHODS

CTZ was procured from Glochem Industries Pvt Ltd., Hyderabad (Telangana) as a gift sample. Carbomer (Acrypol 974P) (Corel Pharma Chem), HPMC E4M (DuPont), and Deionised water (In-house), other chemicals, and solvents were of analytical grade/IP/BP/USP equivalent grade available in the laboratory.

Formulation development of CTZ pH trigged *in situ* ocular gel

Preparation of CTZ *in situ* ocular gel

HPMC E4M was dissolved in purified water and then Carbomer (Acrypol 974P) was slowly added to the solution with continuous stirring. The dispersion was then kept at room temperature for 5 h to hydrate.

Aqueous solutions of CTZ and buffer salts were prepared in a small quantity of purified water in a separate beaker and then were mixed into the polymer solution with continuous magnetic stirring. Benzalkonium chloride (50% solution) was added before the volume makes up and then the volume was made up using purified water [3, 4].

In the present research work, a full 32-factorial study will be used to study the effect of two critical variables: Carbomer (Acrypol 974P) (X1) and HPMC E4M (X2), at three levels of their concentrations. The variables and their levels are presented in table 1. A total of 9 formulations (32; 3X3=9) will be prepared. The composition of the formulations (full factorial design) for CTZ pH trigged *in situ* ocular gels is presented in table 2.

Table 1: Variables and their levels for the preparation of CTZ pH trigged *in situ* ocular gels

Levels Factors	Low (-1)	Medium (0)	High (+1)
Carbomer (Acrypol 974P) (X1)	0.4	0.6	0.8
HPMC E4M (X2)	0.4	0.6	0.8

Table 2: Full 3^2 factorial design for the preparation of CTZ pH trigged *in situ* ocular gels

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
CTZ (g)	0.285	0.285	0.285	0.285	0.285	0.285	0.285	0.285	0.285
Carbomer 974P (g)	0.4	0.6	0.8	0.4	0.6	0.8	0.4	0.6	0.8
HPMC E4M (g)	0.4	0.4	0.4	0.6	0.6	0.6	0.8	0.8	0.8
Citric acid (gm)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Disodium hydrogen phosphate (g)	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
Benzalkonium chloride sol (50 %) (g))	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Purified water q. s. (ml)	100	100	100	100	100	100	100	100	100
Sodium Hydroxide	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH
Hydrochloric Acid	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH	q. s. to pH

Evaluation of the prepared CTZ *in situ* oular gels

Physical examination

The prepared CTZ pH trigged *in situ* ocular gels were visually evaluated for their color and homogeneity [4].

Measurement of pH

The pH of the prepared *in situ* ocular gel was determined using a digital pH meter [5].

Rheological studies using brookfield viscometer

The viscosity of the prepared CTZ pH trigged *in situ* ocular gel was evaluated using Brookfield viscometer, spindle 62. For evaluation, the formulations were allowed to stand at room temperature (25 ºC) for 30 min. Viscosity was measured at rpm 5, 10, 20, and 50. An average of three readings at different positions were taken. The viscosity of the formulations was measured as such pH 5.5 and then at pH 7.4. pH of the formulations was changed from 5.5 to 7.4 using a 0.1M Sodium hydroxide solution [6-8].

Gelling capacity

The gelling capacity of the prepared *in situ* gels was determined by using simulated tear fluid (STF). 2 ml of STF was taken in a test tube and warmed to 35 ºC. To it, 3-4 drops of the preparation were added and the gel formation was observed visually [9, 10].

Drug content determination

5 ml (by weight) of the formulation was taken and was diluted to 500 ml with purified water. An aliquot of 5 ml was withdrawn and further diluted to 500 ml with purified water. CTZ concentration was determined at 230 nm [11].

***In vitro* drug release studies**

The *in vitro* release studies were performed on 6 units using Dissolution Apparatus (12 Units, DS14000 Smart with Auto Sampler). The temperature was adjusted to 36±0.2ºC to simulate the ocular surface temperature. A plastic tube with both sides open was selected and one side of it was sealed with a dialysis membrane (soaked in phosphate buffer saline pH 7.4 for 24 h before use). 2 g of each formulation was placed in the plastic tube from the other side. The tubes were attached to the paddle of the dissolution apparatus such that the tube was just immersed in the reservoir. The paddle was rotated at a speed of 50 rpm in 250 ml phosphate buffer saline pH 7.4. 5 ml samples were withdrawn at predetermined time intervals of 15, 30, 45, 60, 120, 180, 240, and 300 min. A fresh medium was replaced to ensure constant volume. The samples were analyzed spectrophotometrically to determine percentage release [12-14].

Kinetic treatment of release data

The profile of dissolution data of drug passage in solution was fit into different release models like zero-order (cumulative percentage of drug released vs. time), first-order (log cumulative percentage of drug release vs. time), Higuchi's (cumulative percentage of drug released vs. square root of time), Hixson Crowell's (cube root of a percentage of drug unreleased vs. time) and Korsmeyer's Peppas (fraction of drug release vs. time) equations. Kinetic constant (k) and diffusional release exponent (n) were also computed based on the relationship proposed by Korsmeyer and Peppas [15].

Data analysis

Multiple regression analysis

Multiple regression analysis is used for establishing approximate mathematical models in which the variables are screened by a stepwise selection method and the final model would be used to predict the relationship between different variables and their levels. MRA is important to understand the complexity of pharmaceutical formulations [16].

The dependent response is measured and then either a simple linear equation (Equation 1), or interactive equation (Equation 2), or a quadratic model (Equation 3) is fitted by carrying out MRA to identify statistically significant terms:

$$Y = b_0+b_1X_1+b_2X_2+b_3X_3 \ldots\ldots\ldots \quad (1)$$

$$Y = b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{123}X_1X_2X_3 \ldots\ldots\ldots \quad (2)$$

$$Y = b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_1^2X_{11}+b_2^2X_{22}+b_3^2X_{33}+b_{123}X_1X_2X_3 \ldots\ldots\ldots \quad (3)$$

Where,

Y–estimated response;

b_0–constant;

b_1, b_2, b_3–linear coefficients;

b_{12}, b_{23}, b_{13}–interaction coefficients; and

b_1^2, b_2^2, b_3^3–quadratic coefficients

Response surface methodology (RSM)

Response surface methodology is a collection of mathematical data and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable like Viscosity and Drug release) which is influenced by several independent variables (concentration of gelling agents and viscosity builder). The response can be represented graphically, either in the three-dimensional space or as contour plots that help visualize the shape of the response surface. Contours are curves of constant response drawn in the xi, xj plane keeping all other variables fixed [17-21].

The polynomial regression results were demonstrated using 3-D response surface plots and 2-D contour plots with the help of Design Expert software (Version 13.0.10.064).

RESULTS AND DISCUSSION

A picture of the prepared formulation is presented in fig. 1

The prepared *in situ* gels were evaluated for their physical appearance and it was found that the prepared formulations were translucent and homogenous [4]. The results are presented in table 3.

Fig. 1: Prepared CTZ pH trigged *in situ* ocular gel

Table 3: Physical examination of the prepared cetirizine hydrochloride *in situ* ocular gels

Formulation	Colour	Homogeneity
F1	Translucent	Homogenous
F2	Translucent	Homogenous
F3	Translucent	Homogenous
F4	Translucent	Homogenous
F5	Translucent	Homogenous
F6	Translucent	Homogenous
F7	Translucent	Homogenous
F8	Translucent	Homogenous
F9	Translucent	Homogenous

The pH of the prepared *in situ* gels was found to be between 5 to 6, which is good to maintain the formulations in the solution state and suitable for application in the eye [5]. The results are presented in table 4.

Table 4: pH of the prepared cetirizine hydrochloride *in situ* ocular gels

Formulation	pH
F1	5.52±0.05
F2	5.61±0.06
F3	5.55±0.07
F4	5.57±0.05
F5	5.52±0.01
F6	5.49±0.06
F7	5.65±0.04
F8	5.61±0.01
F9	5.65±0.02

(Results: mean±SD, n=3)

Table 5: Viscosity of the prepared *in situ* gels at pH 5.5

RPM	5	10	20	50
F1	200.40±1.20	195.60±1.00	172.50±1.00	133.10±0.50
F2	271.40±1.50	210.30±1.10	184.90±0.58	158.30±0.90
F3	1372.00±2.00	1153.00±1.20	837.30±1.00	542.30±1.23
F4	324.60±0.75	261.20±0.39	206.70±1.04	170.40±0.59
F5	460.80±1.08	409.30±1.50	371.70±0.56	261.31±1.10
F6	1451.00±1.10	1220±1.00	940.30±0.50	681.30±1.10
F7	581.80±1.00	443.70±1.08	380.70±2.51	337.80±0.55
F8	681.90±1.52	539.70±1.08	481.90±2.49	435.90±1.53
F9	2071.00±1.00	1664.25±1.51	1350.16±1.65	874.00±1.16

*Viscosity unit-cps (Results: mean±SD, n=6)

Table 6: Viscosity of the prepared *in situ* gels at pH 7.4

RPM	5	10	20	50
F1	1616.21±1.10	1556.52±1.00	1411.41±1.00	1100.52±1.53
F2	2220.42±1.00	1705.75±1.00	1509.42±1.48	1287.52±1.58
F3	11111.15±1.00	9332.51±1.00	6800.74±1.58	4431.52±1.58
F4	2585.85±0.58	2030.73±1.00	1768.95±1.10	1284.53±1.00
F5	3636.31±1.10	3211.52±1.00	3013.19±1.30	2055.86±1.00
F6	11457.53±1.10	9876.12±0.58	7503.32±1.68	5412.86±1.58
F7	4532.42±1.65	3448.62±1.00	3001.85±1.23	2614.62±1.42
F8	5325.76±2.08	4151.85±1.53	3295.19±1.26	3317.93±1.52
F9	16584.32±1.58	11018.11±1.13	7680.85±1.06	4886.76±1.00

*Viscosity unit-cps (Results: mean±SD, n=6)

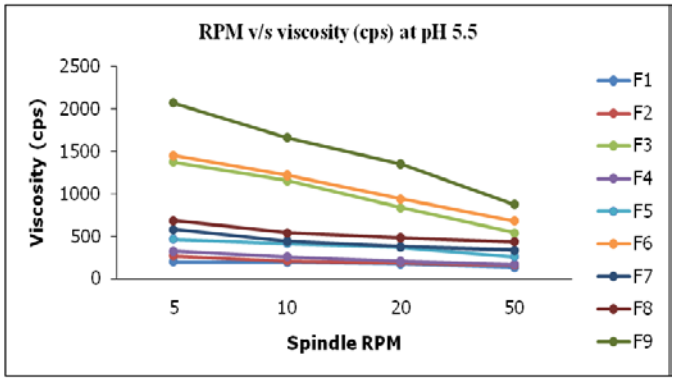


Fig. 2: Effect of rpm on viscosity of prepared CTZ *in situ* ocular gel at pH 5.5, *Viscosity unit-cps (Results: mean, n=6)

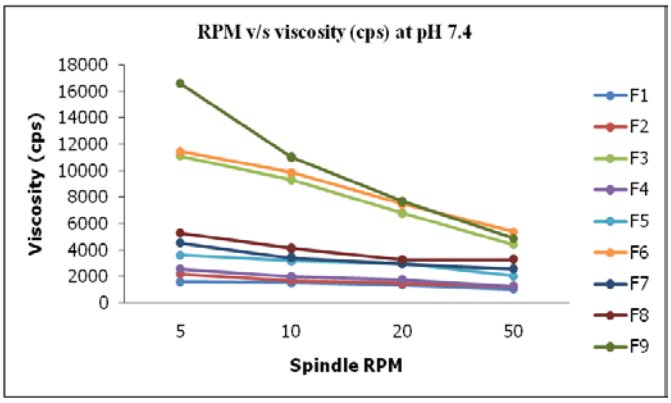


Fig. 3: Effect of rpm on viscosity of prepared CTZ *in situ* ocular gel at pH 7.4, *Viscosity unit-cps (Results: mean, n=6)

The viscosity of the prepared CTZ *in situ* ocular gels was evaluated at different rpm. The results are presented in Tables 5 and 6 and fig. 2 and 3. It was found that with an increase in the concentration of viscosity enhancer, HPMC E4M, the viscosity of the formulations increased linearly. With an increase in the concentration of the gelling agent, Carbomer (Acrypol 974P), the viscosity of the formulations increased non-linearly [7, 8].

It was also found that viscosity of the formulations increased considerably with an increase in the pH of the formulations [10].

A shear thinning effect, that is, a decrease in viscosity on increasing shear rate from 5 to 50 rpm was observed. The prepared *in situ* gel formulations exhibited a shear thinning behavior since the viscosity decreased with increasing the shear rate. As the shear stress is increased, the disarranged molecules get arranged in a pattern, thereby reducing resistance to flow, i.e., viscosity. This is desirable as the ocular surface is subjected to high shear rates during blinking and low shear rates between blinks [11, 12].

Viscosity data were analyzed by ANOVA and it was found that the increase in concentration of HPMC E4M from 0.4% to 0.8% resulted in an insignificant (P = 0.64, *df* = 2, F = 0.73 and F_{crit} = 5.143) increase in viscosity at pH 5.5. At pH 7.4, the increase in concentration of HPMC E4M from 0.4% to 0.8% also resulted in an insignificant (P = 0.83, *df* = 2, F= 0.180 and F_{crit} = 5.143) increase in viscosity.

ANOVA results also showed that the increase in the concentration of Carbomer (Acrypol 974P) from 0.4% to 0.8% resulted in a significant (P = 0.004, *df* = 2, F = 15.25 and F_{crit} = 5.143) increase in viscosity at pH 5.5. At pH 7.4, the increase in concentration from 0.4% to 0.8% also resulted in a significant (P = 0.0003, *df* = 2, F = 41.16 and F_{crit} = 5.143) increase in viscosity.

The average effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) on the viscosity of the prepared CTZ *in situ* ocular gels at pH 5.5 at 20 rpm is shown in table 7 and fig. 4.

The main effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) on the viscosity of the prepared CTZ *in situ* ocular gels at pH 5.5 at 20 rpm is shown in fig. 5.

Table 7: Average effect of varied concentrations of hydroxy propyl methyl cellulose E4M and Carbomer (Acrypol 974P) at 20 rpm at pH 5.5

Name of polymer	Concentration of polymer	Formula for calculation of average effect	Calculation of average effect	Average effect (cps)
Carbomer (Acrypol 974P)	0.4	(F1+F4+F7)/3	(172.50+206.70+380.70)/3	253.30
	0.6	(F2+F5+F8)/3	(184.90+371.70+481.90)/3	346.16
	0.8	(F3+F6+F9)/3	(837.3+940.3+1350.16)/3	1042.58
Hydroxy Propyl Methyl Cellulose E4M	0.4	(F1+F2+F3)/3	(172.50+184.90+837.30)/3	398.23
	0.6	(F4+F5+F6)/3	(206.70+371.70+940.30)/3	506.23
	0.8	(F7+F8+F9)/3	(380.70+481.90+1350.16)/3	737.58

*Viscosity unit-cps (Results: mean±SD, n=6)

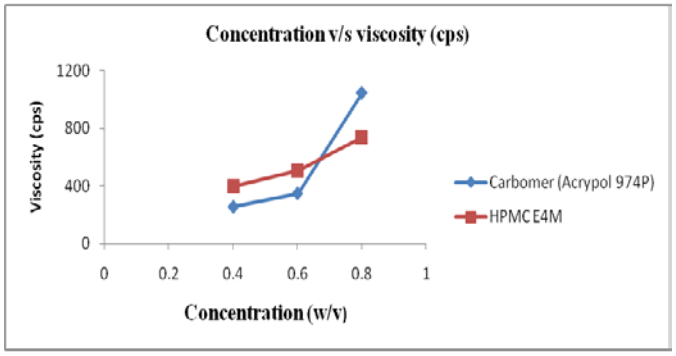


Fig. 4: Average effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) at 20 rpm at pH 5.5, *Viscosity unit-cps (Results: mean, n=6)

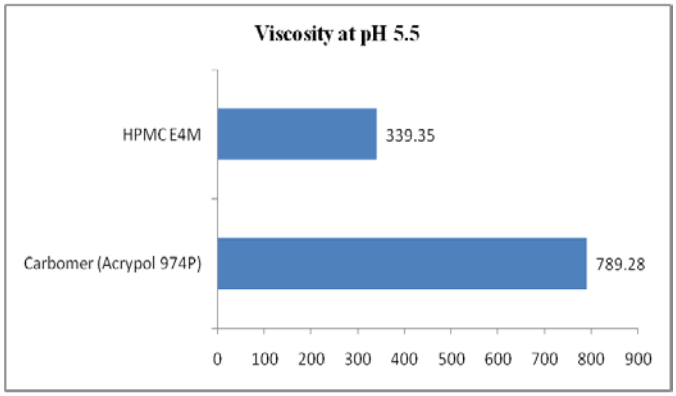


Fig. 5: Main effect of concentration of HPMC E4M and Carbomer (Acrypol 974P) on Viscosity at 20 RPM at pH 5.5

The average effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) on the viscosity of the prepared CTZ *in situ* ocular gels at pH 7.4 at 20 rpm is shown in table 8 and fig. 6.

The main effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) on the viscosity of the prepared CTZ *in situ* ocular gels at pH 7.4 at 20 rpm is shown in fig. 7.

Table 8: Average effect of varied concentrations of Hydroxy propyl methyl cellulose E4M and Carbomer (Acrypol 974P) at 20 rpm at pH 7.4

Name of polymer	Concentration of polymer	Formula for calculation of average effect	Calculation of average effect	Average effect (cps)
Carbomer (Acrypol 974P)	0.4	(F1+F4+F7)/3	(1411.41+1768.95+3001.85)/3	2060.73
	0.6	(F2+F5+F8)/3	(1509.42+3013.19+3295.19)/3	2605.93
	0.8	(F3+F6+F9)/3	(6800.74+7503.32+7680.85)/3	7328.30
HPMC E4M	0.4	(F1+F2+F3)/3	(1411.41+1509.42+6800.74)/3	3240.52
	0.6	(F4+F5+F6)/3	(1768.95+3013.19+7503.32)/3	4095.15
	0.8	(F7+F8+F9)/3	(3001.85+3295.19+7680.85)/3	4659.29

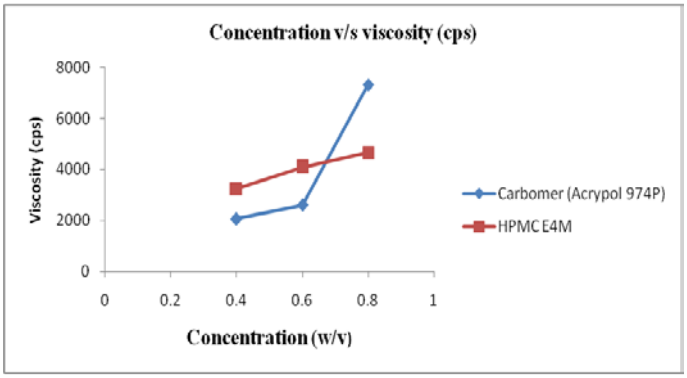


Fig. 6: Average effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) at 20 rpm at pH 7.4, *Viscosity unit-cps (Results: mean, n=6)

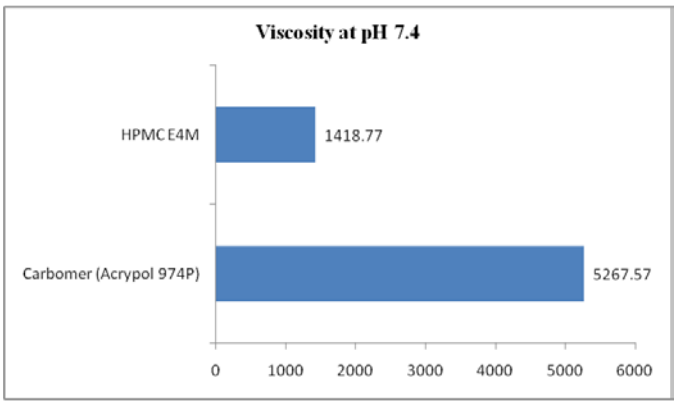


Fig. 7: Main effect of concentration of HPMC E4M and carbomer (Acrypol 974P) on viscosity at 20 RPM at pH 7.4

Fig. 8 shows the interaction effect between Carbomer (Acrypol 974P) and HPMC E4M on viscosity at 20 rpm at pH 5.5.

Fig. 9: shows the interaction effect between Carbomer (Acrypol 974P) and HPMC E4M on viscosity at 20 rpm at pH 7.4.

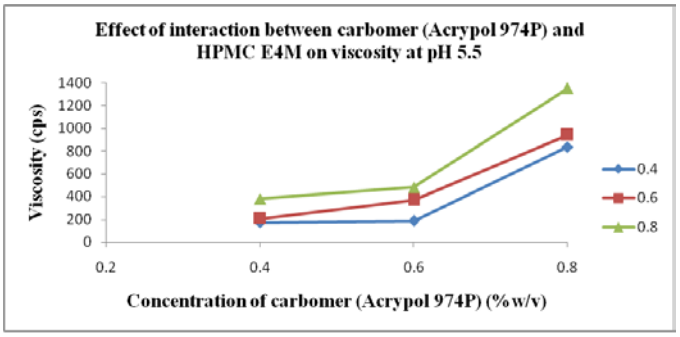


Fig. 8: Effect of interaction between Carbomer (Acrypol 974P) and HPMC E4M on viscosity of prepared CTZ *in situ* ocular gel at 20 rpm at pH 5.5

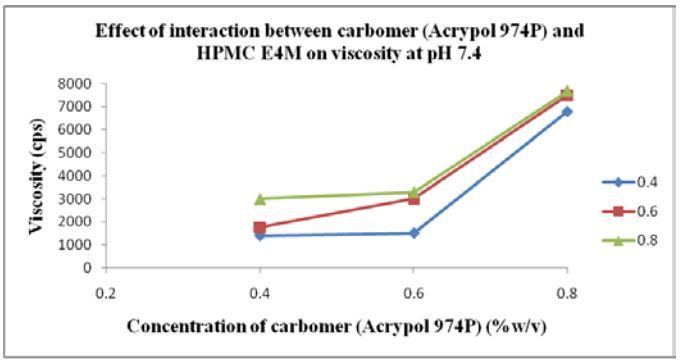


Fig. 9: Effect of interaction between carbomer (Acrypol 974P) and HPMC E4M on the viscosity of prepared CTZ *in situ* ocular gel at 20 rpm at pH 7.4; the observations of gelling capacity are presented in table 7

Table 9: Gelling capacity of the prepared *in situ* gels

Formulations	Gelling capacity	Result
F1	+	Gels form, but dissolve rapidly
F2	++	Gels form, and remain up to 4 h
F3	+++	Gels form, and remain up to 7 h
F4	++	Gels form, and remain up to 4 h
F5	++	Gels form, and remain up to 4 h
F6	+++	Gels form, and remain up to 7 h
F7	++	Gels form, and remain up to 4 h
F8	+++	Gels form, and remain up to 7 h
F9	+++	Gels form, and remain up to 7 h

Note: +Gels form, but dissolve rapidly; ++Gels form, and remain up to 4 h; +++Gels form, and remain upto 7 h.

Table 10: Drug content (Assay) of the prepared *in situ* gel

Formulation	Drug content (Practical/Theoretical content)*100
F1	101.01±1.2
F2	102.31±1.01
F3	100.16±0.53
F4	101.60±0.44
F5	101.5±0.75
F6	101.29±0.83
F7	99.90±0.92
F8	99.28±0.52
F9	100.35±1.05

(Results: mean±SD, n=3)

Drug content (Assay) of the prepared CTZ *in situ* ocular gels was determined and it was found in the range of 99.00 % to 102.00 %. It can say that % drug content is good for all formulations. No degradation was observed during manufacturing [11, 12]. The results of drug contents are presented in table 10.

In vitro release studies were performed and results are presented in table 11 and fig. 10. After 300 min, the release from the formulations F1, F2, F3, F4, F5, F6, F7, F8 and F9 were found to be 84.21, 81.23, 69.22, 78.33, 73.21, 72.17, 64.23, 61.34 and 58.13 respectively. The release from the formulations can be arranged in the following decreasing order: F1>F2>F4>F5>F6>F3>F7>F8>F9.

Release data were analyzed by ANOVA and it was found that the increase in concentration of Carbomer (Acrypol 974P) from 0.4% to 0.8% resulted in insignificant (P = 0.52, *df* = 2, F = 0.721 and F_{crit} = 5.143) decrease in release at 300 min, while the increase in the concentration of HPMC E 4M from0.4% to 0.8% resulted in significant (P = 0.017, *df* = 2, F = 8.65 and F_{crit} = 5.143) decrease in release at 300 min.

The average effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) on the drug release of the prepared CTZ *in situ* ocular gels at 300 min is shown in table 12 and fig. 11

The main effect of varied concentrations of HPMC E4M and Carbomer (Acrypol 974P) on the drug release of the prepared CTZ *in situ* ocular gels at 300 min is shown in fig. 12.

Table 11: *In vitro* drug release from the prepared *in situ* gels

Time (min)	Cumulative % release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	09.12±1.23	5.10±1.25	4.89±3.50	6.23±2.25	4.83±3.51	4.50±4.12	4.31±2.16	4.22±1.56	4.98±2.65
30	17.16±2.15	8.81±2.00	7.45±3.12	9.79±1.56	7.65±2.62	7.60±3.14	8.70±2.70	9.55±1.89	4.86±2.02
45	20.78±3.12	17.61±2.15	10.63±2.65	13.15±3.65	12.29±4.62	11.48±3.16	12.82±1.50	11.43±2.23	8.08±1.59
60	33.53±1.25	21.23±3.12	15.81±2.60	24.01±2.41	16.76±2.63	15.43±1.45	14.54±1.78	13.67±2.89	11.12±3.12
90	47.67±1.56	31.92±2.96	19.34±1.78	28.63±2.41	26.15±2.63	26.63±3.12	21.72±3.10	20.63±1.52	15.67±2.75
120	59.53±1.85	45.73±1.86	46.35±2.63	48.72±1.89	40.43±3.61	41.62±1.12	42.34±3.12	28.78±1.31	20.53±2.15
180	67.93±1.95	57.48±2.10	51.23±1.26	57.43±2.45	51.92±2.41	49.31±1.56	43.84±1.85	37.76±1.63	34.76±1.62
240	73.13±1.56	70.21±2.63	59.43±3.41	70.55±1.25	64.23±3.12	59.73±1.25	60.10±2.12	43.36±3.34	46.49±2.12
300	84.21±1.26	81.23±2.41	69.22±2.12	78.33±1.45	73.21±2.63	72.17±2.10	64.23±2.11	61.34±3.10	58.13±1.86

(Results: mean±SD, n=6)

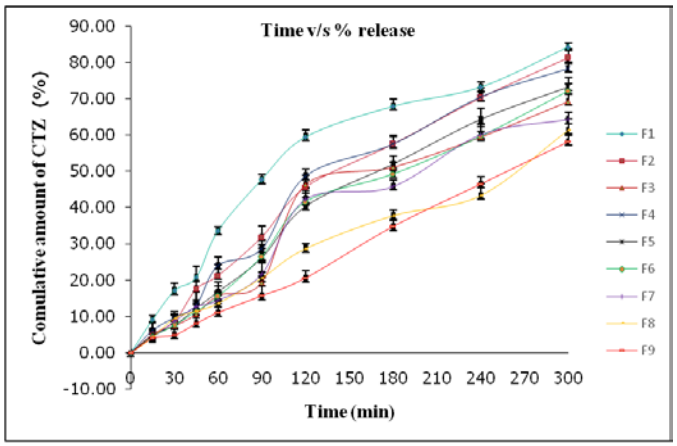


Fig. 10: *In vitro* release of CTZ from the prepared *in situ* gels, (Results: mean±SD, n=6)

Table 12: Average effect of varied concentrations of hydroxy propyl methyl cellulose E4M and carbomer (Acrypol 974P) on drug release

Name of polymer	Concentration of polymer	Formula for calculation of average effect	Calculation of average effect	Average effect (%)
Carbomer (Acrypol 974P)	0.4	(F1+F4+F7)/3	(84.21+78.33+64.23)/3	75.59
	0.6	(F2+F5+F8)/3	(81.21+73.21+61.34)/3	71.92
	0.8	(F3+F6+F9)/3	(69.22+72.17+58.13)/3	65.50
Hydroxy Propyl Methyl Cellulose E4M	0.4	(F1+F2+F3)/3	(84.21+81.23+69.22)/3	78.22
	0.6	(F4+F5+F6)/3	(78.33+73.21+72.17)/3	74.57
	0.8	(F7+F8+F9)/3	(64.23+61.34+58.13)/3	61.23

(Results: mean±SD, n=6)

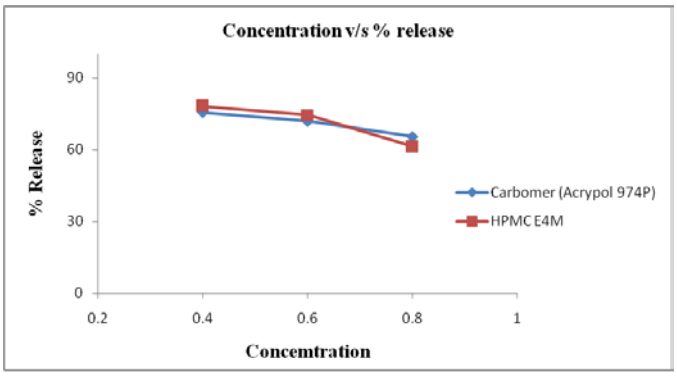


Fig. 11: Average effect of varied concentrations of carbomer (Acrypol 974P) and HPMC E4Mon release at 300 min

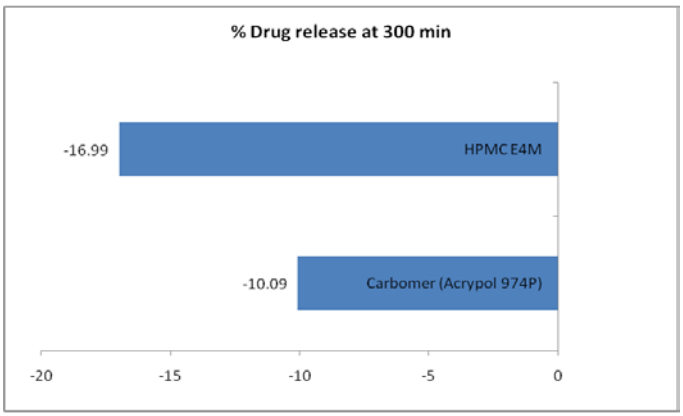


Fig. 12: Main effect of concentration of HPMC E4M and Carbomer (Acrypol 974P) on the release of CTZ at 300 min

From the above data, it was found that HPMC E4M showed a greater effect on the release of a drug. The increase in the concentration of HPMC E4M causes the formation of strong hydrophilic matrices, which results in retardation of drug release from the formulations. HPMC E4M is a polymer with a viscosity of 4000 cps, which in turn is a function of its molecular weight and chain length [12-14].

HPMC hydrophilic matrix is a swellable matrix from which the drug gets diffused out slowly depending on its solubility in water. The polymer matrix itself erodes slowly simultaneously. At the end of the drug release, the matrix is completely dissolved, suggesting that the overall release time is controlled by polymer erosion [14].

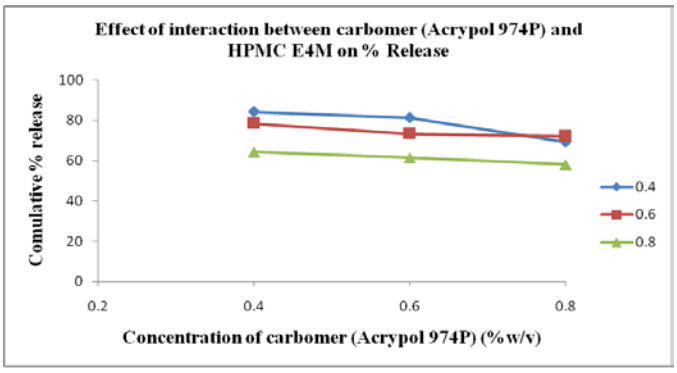


Fig. 13: Effect of interaction between Carbomer (Acrypol 974P) and HPMC E4M on percent release of prepared CTZ *in situ* ocular gel at 300 min

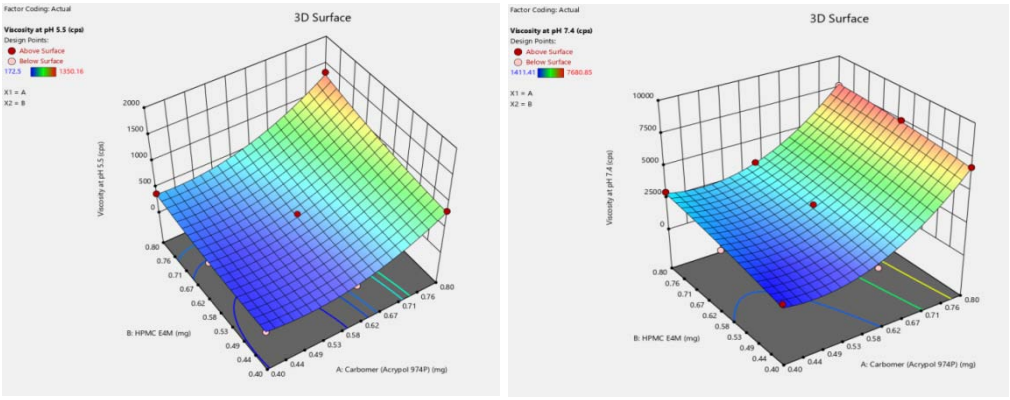
Formulations F1, F2, F3, F4, F5, F6, and F7 were best fitted to Higuchi's model, while formulations F8 and F9 were best fitted to the zero-order model.

The formulations best fitted to the zero-order model suggest that release was concentration independent. The formulation best fitted Higuchi's model, suggesting that release was diffusion controlled, and to Hixon-Crowell model, which suggests that release was dissolution rate limited. The 'n' value obtained from the Korsmeyer-Peppas equation was less than 0.43 (no formulation), which indicated that release from formulation was based on the Fickian diffusion mechanism and between 0.43-0.85 (for F1), which indicated that release was following anomalous (non Fickian) transport or between 0.-0.85-1 (for F2-F9) which indicated that release belonged to Case II transport [15]. The results of Release kinetic model fitting are presented in table 13.

Table 13: The release kinetics of various formulations

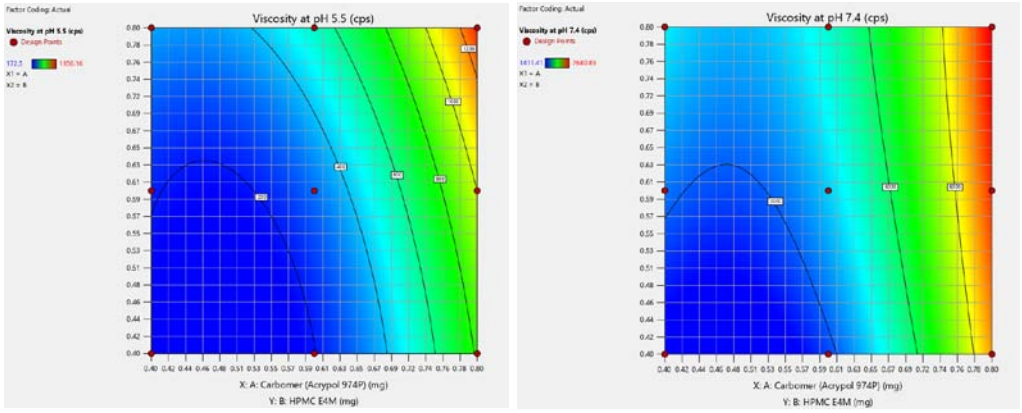
Release kinetics-model fitting							
Formulation code	**Co-relation coefficient for the model**				**Korsemeyer-Peppas (M_t/M_∞ vs T)**		
	0-order R% vs T	**1-order log R% vs T**	**Highuchi R% vs $T^{1/2}$**	**Hixon-crowell ($100^{1/3}$-$R\%^{1/3}$) vs T**	**r**	**n**	**k**
F1	0.950	0.857	0.985	0.950	0.966	0.754	0.003
F2	0.988	0.887	0.997	0.988	0.985	0.948	0.004
F3	0.967	0.909	0.974	0.967	0.965	0.963	0.003
F4	0.979	0.900	0.990	0.979	0.977	0.906	0.005
F5	0.989	0.911	0.993	0.989	0.989	0.967	0.003
F6	0.986	0.905	0.991	0.986	0.989	0.967	0.003
F7	0.974	0.901	0.983	0.974	0.980	0.935	0.003
F8	0.993	0.921	0.983	0.993	0.990	0.85	0.004
F9	0.998	0.955	0.976	0.998	0.990	0.955	0.002

The following equations were derived to describe the relationship between viscosity and drug release (Y) and concentration of Carbomer (Acrypol 974P) (X_1) and HPMC E4M (X_2)



(a) **(b)**

Fig. 14: Three-dimensional response surface plots showing the effect of concentration of Carbomer (Acrypol 974P) and HPMC E4M on the viscosity of CTZ *in situ* ocular gel at (a) pH 5.5 and (b) at pH 7.4



(a) **(b)**

Fig. 15: Contour plot showing the effect of concentration of Carbomer (Acrypol 974P) and HPMC E4M on the viscosity of CTZ *in situ* ocular gel at (a) pH 5.5 and (b) pH 7.4

Model for viscosity

At pH 5.5

$Y = 2568.65 - 8222.56X_1 - 2144.39X_2 + 1904.13X_1X_2 + 7544.42\ X_1^2 + 1541.92\ X_2^2$

($R^2 = 0.989$)

At pH 7

$Y = 8565.10 - 46824.95X_1 + 10567.97X_2 - 4439.56X_1X_2 + 52214.67\ X_1^2 - 3631.08\ X_2^2$

($R^2 = 0.991$)

Model for percent drug release

$Y = 82.77 - 29.70X_1 + 69.50X_2 + 55.56X_1X_2 - 21.96\ X_1^2 - 121.08\ X_2^2$

($R^2 = 0.967$)

Based on the high values of R^2, it can be concluded that a good fit was found for both responses. These polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the sign (+or-) it carries. Amount of Carbomer (Acrypol 974P) (X_1) and HPMC E4M (X_2) are significant model terms which significantly affect viscosity and drug release.

The response for viscosity at pH 5.5 and 7.4 was used to construct the 3-dimensional response surface as shown in fig. 14 and the form of 2 D contour plot in fig. 15.

The response for percent drug release at 300 min was used to construct the 3-dimensional response surface as shown in fig. 16 and the form of 2 D contour plot in fig. 17

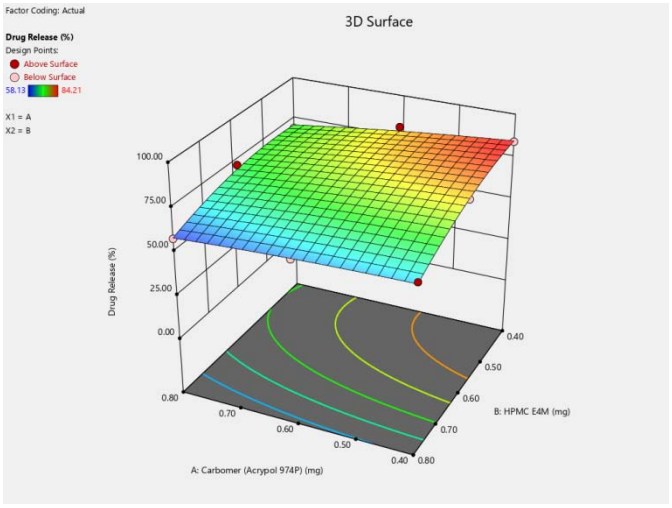


Fig. 16: Three-dimensional response surface plots showing the effect of concentration of carbomer (Acrypol 974P) and HPMC E4M on percent drug release of CTZ *in situ* ocular gel at 300 min

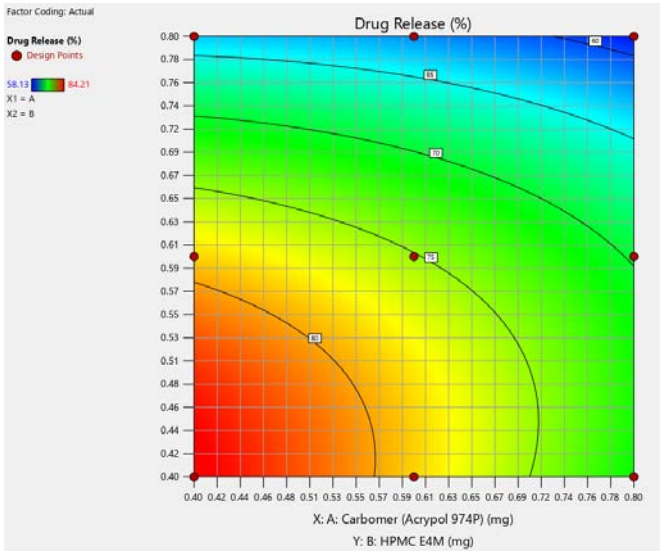


Fig. 17: Contour plot showing the effect of concentration of carbomer (Acrypol 974P) and HPMC E4M on percent drug release of CTZ from *in situ* ocular gel at 300 min

CONCLUSION

CTZ was successfully formulated in pH triggered *in situ* gelling system using Carbomer 974P in combination with HPMC E4M. It was seen that HPMC E4M is important for *in situ* gel behaviour along with Carbomer 974P on the basis of main effect of concentration of HPMC E4M and Carbomer 974P. *In vitro* results indicated that the *in situ* gel system is a viable alternative to conventional ocular drops by virtue of its ability to sustain drug release. The major pertinent findings of the present study is the increase in concentration of HPMC E4M from 0.4% to 0.8% resulted in insignificant (P = 0.64, *df* = 2, F = 0.73 and F_{crit} = 5.143) increase in viscosity at pH 5.5. At pH 7.4, the increase in concentration of HPMC E4M from 0.4% to 0.8% also resulted in insignificant (P = 0.83, *df* = 2, F= 0.180 and F_{crit} = 5.143) increase in viscosity and Increase in concentration of Carbomer (Acrypol 974P) from 0.4% to 0.8% resulted in significant (P = 0.004, *df* = 2, F = 15.25 and F_{crit} = 5.143) increase in viscosity at pH 5.5. At pH 7.4, the increase in concentration from 0.4% to 0.8% also resulted in a significant (P = 0.0003, *df* = 2, F = 41.16 and F_{crit} = 5.143) increase in viscosity.

it was found that the increase in the concentration of Carbomer (Acrypol 974P) from 0.4% to 0.8% resulted in an insignificant (P = 0.52, *df* = 2, F = 0.721 and F_{crit} = 5.143) decrease in release at 300 min, while the increase in the concentration of HPMC E 4M from0.4% to 0.8% resulted in significant (P = 0.017, *df* = 2, F = 8.65 and F_{crit} = 5.143) decrease in release at 300 min.

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Nil

AUTHORS CONTRIBUTIONS

All authors were contributed equally. All authors approved the final version of the manuscript.

CONFLICT OF INTERESTS

Declared none

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