Kanoujia et al., International Current Pharmaceutical Journal 2012, 1(3): 43-49
http://www.icpjonline.com/documents/Vol1Issue3/01.pdf

International Current Pharmaceutical Journal

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Formulation and characterization of a novel pH-triggered *in-situ* gelling ocular system containing Gatifloxacin

*Jovita Kanoujia, Kanchan Sonker, Manisha Pandey, Koshy M. Kymonil, Shubhini A. Saraf

Department of Pharmaceutics, Babu Banarasi Das National Institute of Technology & Management Sector I, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, U.P. India-227105

ABSTRACT

The present research work deals with the formulation and evaluation of *in-situ* gelling system based on sol-to-gel transition for ophthalmic delivery of an antibacterial agent gatifloxacin, to overcome the problems of poor bioavailability and therapeutic response exhibited by conventional formulations based a sol-to-gel transition in the cul-de-sac upon instillation. Carbopol 940 was used as the gelling agent in combination with HPMC and HPMC K15M which acted as a viscosity enhancing agent. The prepared formulations were evaluated for pH, clarity, drug content, gelling capacity, bioadhesive strength and *in-vitro* drug release. *In-vitro* drug release data of optimized formulation (F12) was treated according to Zero, First, Korsmeyer Peppas and Higuchi kinetics to access the mechanism of drug release. The clarity, pH, viscosity and drug content of the developed formulations were found in range 6.0-6.8, 10-570cps, 82-98% respectively. The gel provided sustained drug release over an 8 hour period. The developed formulation can be used as an *in-situ* gelling vehicle to enhance ocular bioavailability and the reduction in the frequency of instillation thereby resulting in better patient compliance.

***Key Words*:** *In-situ* gelation; Gatifloxacin; Carbopol 940; HPMC K15M.

INTRODUCTION

Ophthalmic drug delivery is one of the most attractive and challenging field facing the pharmaceutical scientist. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage (Mitra, 2003). Most of the ocular treatments call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity (Gorle and Gattani, 2009). The most conventional ocular dosage forms for the delivery of drugs are eye drops (solution, suspension) and ophthalmic ointments. Short residence time, pulsed dosing of drug, frequent instillation, and large drainage factor are the limitation associated with conventional ocular dosage form. Newer ocular drug delivery systems are being explored to develop extended duration and controlled release strategy (Rathore and Nema, 2009). Formulation of *in-situ* ocular gel of gatifloxacin is a fourth generation fluoroquinolone derivative used to treat external infections of the eye, using biodegradable polymers is the approach to overcome the drawbacks of conventional eye preparations (Zhidong *et al.*, 2006; Mishra *et al.*, 2008; Pundir *et al.*, 2009; Kalam *et al.*, 2009). Carbopols are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity increasing agents. Formulations include creams, gels and ointments for use in ophthalmic, rectal and topical preparations. HPMC is widely used in oral and topical pharmaceutical preparations as coating agent, film formers, rate controlling polymers for sustained release, stabilizing agents, viscositifier etc. (Raymond *et al.*, 2004; Edsman *et al.*, 1996).

The objective of this study was to develop an optimized *in-situ* ophthalmic gel - a viscous liquid that shift to a gel phase upon exposure to physiological condition (Rathore and Nema, 2009; Doijad *et al.*, 2006). To achieve the objective, independent formulation variables such as, polymer-to-polymer ratio, and different viscosity grades of HPMC (K4M and

***Corresponding Author**:
Jovita Kanoujia, Department of Pharmaceutics
Babu Banarasi Das National Institute of Technology and Management, Sector I, Dr. Akhilesh Das Nagar, Faizabad Road
Lucknow, U.P., India-227105
E-mail: *jovita_kanoujia@rediffmail.com*
Contact No.: +91 9336 829 995

Table 1: Level of Investigated Variables.

Factors	Independent Variable	
	Ratio of Polymer (HPMC:HPMC K15M)	Amount of Carbopol 940
Levels	1:1	1%
	1:2	2%
	1:3	3%
	1:4	4%

K15M) were examined. The dependent variables included gelling capacity, percentage of gatifloxacin release at 8 hours, viscosity and bioadhesive strength was performed to identify the best formulation using 4^2 full factorial designs.

MATERIALS AND METHODS

Materials

Gatifloxacin was obtained as a gift sample from Syntho Pharmaceuticals Pvt. Ltd., Lucknow (India). Hydroxypropylmethyl cellulose (HPMC) and HPMC K15M were obtained from SD Fine Chemicals Limited, Mumbai, India and Carbopol 940 was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals/reagents used were of analytical grade, available commercially and used as such without further processing. A UV/Vis spectrophotometer (Systronics, Double beam UV-VIS Spectrophotometer: 2201) was used for drug analysis.

Experimental Design

A 4^2 full factorial design was adopted to optimize the variables and 16 experiments were conducted in total. In this design, two factors were evaluated each at 4 levels (Madan *et al.*, 2009). The polymer-to-polymer (HPMC, HPMC K15M) (X1) and the amount of bioadhsive polymer (Carbopol 940) (X2) were chosen as independent variables and Y as dependent variables (viscosity, drug content, bioadhesive strength and *in-vitro* drug release). The levels of independent variables are shown in Table 1 (Narendra *et al.*, 2006)

Preparation of Formulations

Accurately weighed 0.1g of HPMC was dispersed in 50ml of purified water, HPMC K15M was added, carbopol 940 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer and buffer salts were dissolved in the solution. Gatifloxacin was dissolved in small quantity of acidic medium (HCl in water), benzylkonium chloride (BKC) was added to this solution; the drug solution was added to the polymer solution. Purified water was then added to make up the volume to 100ml and the prepared formulations were sterilized in an autoclave at 121°C for 20 min (Mohan *et al.*, 2009). Formulation ingredients of formulation F1 to F16 are represented in Table 2.

Evaluation Studies

Physical appearance

Physical appearance of the formulations was visually observed which included the color, homogeneity, consistency and phase separation. The prepared ophthalmic gel formulations (Figure 1) were inspected visually for physical properties (Mohan *et al.*, 2009; Mohamed, 2004).

pH determination

0.3g gel was dissolved in 100ml distilled water and the pH was measured in triplicate (pH Meter, E I Instruments, Model 111E) (Mohamed, 2004; Quinones and Ghaly, 2008).

Determination of viscosity

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. Viscosity of the samples was determined using a Brookfield digital viscometer (Model-RVT) with spindle number 3 and angular velocity run from 10-100 r/min (Abraham *et al.*, 2009; Patel *et al.*, 2010).

Table 2: Composition of *in-situ* gel as per 4^2 Factorial Design.

Composition	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
Gatifloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
HPMC	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HPMC K15M	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4
Carbopol 940	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4

Each formulation contains 0.407g of citric acid; 1.125g of disodium hydrogen phosphate; 0.02g of benzalkonium chloride, 100ml purified water; all values are expressed in gram.

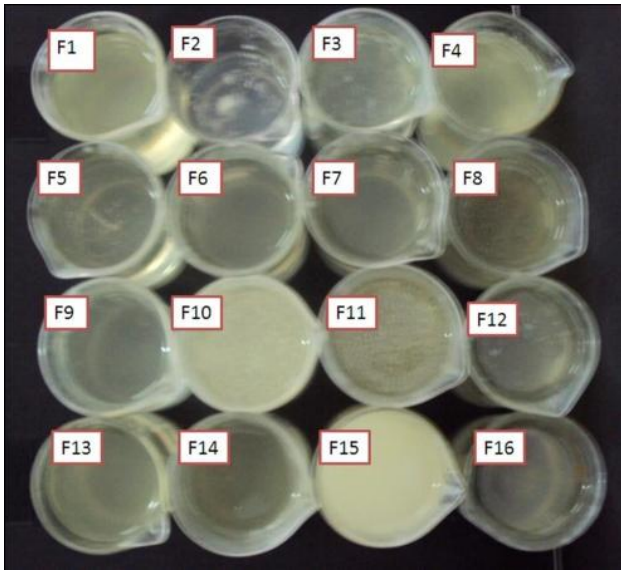

Figure 1: Representative photograph of macroscopic appearance of ophthalmic gel.

Gelling capacity

Determination of *in-vitro* gelling capacity was done by visual method. Colored solutions (1% Congo Red solution in water) of *in-situ* gel forming drug delivery system were prepared. The *in-vitro* gelling capacity of prepared formulations was measured by placing 5ml of the gelation solution (pH 7.2 buffer) in glass test tube and maintained at 37±1°C temperature. Oneml of colored formulation solution was added with the help of pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which the formed gel remains as such (Figure 2). Color was added to give visualized appearance to formed gel. The *in-vitro* gelling capacity was graded in three categories on the basis of gelation time and time period for which the formed gel remains as such.

Drug content

The drug content was determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. Aliquot of 5ml was withdrawn and further diluted to 25ml with distilled water. Gatifloxacin concentration was determined at 293nm by using UV-Visible spectrophotometer (Abraham *et al.,* 2009).

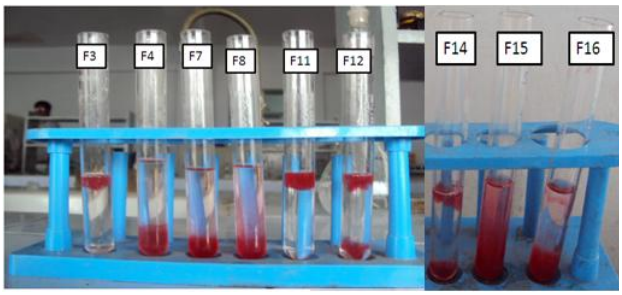

Figure 2: Visual observation of gel formation in formulations. (+) Gel forms after few minutes, disperses rapidly
(++) Immediate gelation, remains for few hours
(+++) Immediate gelation, remains for an extended period.

Bioadhesive strength

The bioadhesive strength was measured using a modified two arm balance as shown in figure 3. The biological membrane was fixed to the outer surface of the bottom of the 50ml beaker with cynoacrylate adhesive and then placed in a 100ml beaker. Phosphate buffer pH 7.4 was added into the beaker up to the upper surface of the gastric mucosa such that the media remains just above the mucosa. Accurately measured 1ml gel was put between the bottom of modified stainless steel pan and beaker. A preload of 50g was placed to the pan for 5 min (preload time) to establish adhesion bonding between gel and biological membrane (Figure 3). The preload

Table 3: Physicochemical parameters of *in-situ* ocular gel.

Formulation	pH	Clarity	Viscosity in cps at 100 rpm	Gelation Capacity
F1	6.8	Clear	10	+
F2	6.4	Clear	30	+
F3	6.2	Clear	75	++
F4	6.1	Clear	220	++
F5	6.5	Clear	30	+
F6	6.3	Clear	60	+
F7	6.2	Clear	110	++
F8	6.2	Clear	270	++
F9	6.2	Clear	50	+
F10	6.3	Clear	100	+
F11	6.1	Clear	240	++
F12	6.0	Clear	470	+++
F13	6.3	Clear	35	+
F14	6.5	Clear	140	++
F15	6.4	Clear	270	++
F16	6.8	Clear	510	+++

+ gel forms after few minute, disperses rapidly;
++ immediate gelation, remains for few hours;
+++ immediate gelation, remains for extended period of time.

and preload time were kept constant for all the formulations. After completion of preload time, preload was removed from the pan and another beaker placed to the pan. The addition of water was stopped when the other pan detached from the membrane (Figure 4). The mass, in grams (weight of empty beaker and weight of beaker with water), required to detach the pan from membrane gave the measure of bioadhesive strength (Patel *et al.*, 2010).

In-vitro release of gatifloxacin from gel

The *in-vitro* release of gatifloxacin from the prepared formulations was studied through cellophane membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium used was pH 7.4 buffer. Cellophane membrane previously soaked overnight in the dissolution medium was tied to one end of a specifically designed glass cylinder (open at both ends of 5 cm diameter). A 2ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic drive shaft and suspended in 100ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The shafts was rotated at 50 r/min. Aliquots each of 1ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with receptor medium and absorbance was measured at 293nm using UV-Visible spectrophotometer (Mohan *et al.*, 2009).

Statistical analysis

Statistical analysis of data was performed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to find out the effect of independent variable (concentration of carbopol 940 and HPMC K15M) on the dependent variables (*in-vitro* drug release, drug content, bioadhesive strength and viscosity), assuming confidence level of 95% ($p<0.05$) for statistical significance.

Kinetics of drug release

Three kinetic models, the zero order release equation (Eq. (1)), Higuchi equation (Eq. (2)), and first order equation (Eq. (3)), were applied to process the *in-vitro* data of formulation F-12 to find the equation with the best fit and to investigate the mechanism of gatifloxacin release from *in-situ* gel.

$Q = k_1 t$ (1)

$Q = k_2(t)^{0.5}$ (2)

$Q = 100(1 - e^{-k3t})$ (3)

where Q is the percentage release at time t. k_1, k_2 and k_3 are the rate constants of zero order, Higuchi, and first order model, respectively. Further, to confirm the mechanism of drug release, first 60% of drug release was fitted in Korsmeyer-Peppas model

$$\frac{M_t}{M_\alpha} = K_p t^n \quad (4)$$

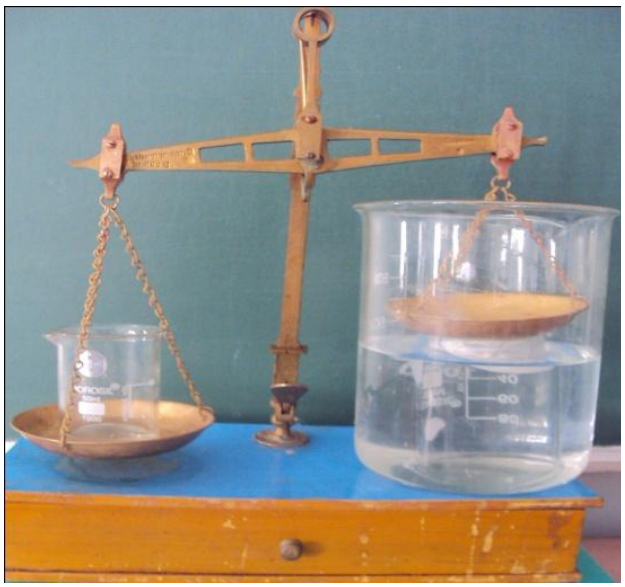

Figure 3: Modified two arm balance used for bioadhesive test (preload was put on modified arm for attachment of gel to the biological membrane).

Figure 4: Modified two arm balance used for bioadhesive test (detachment of pan from the membrane on the addition of water to the other pan).

Table 4: Table for Analysis of Variance & Dunnett's Multiple Comparison Test.

Concentration of Carbopol 940 (Independent variable)

Dunnett's Multiple Comparison Test	Carbopol vs *in-vitro* release	Carbopol vs drug content	Carbopol vs bioadhesive strength	Carbopol vs viscosity	
Mean Diff.	-92.87	-88.97	-23.78	-252.8	
Significant? P < 0.05?	Yes	Yes	Yes	Yes	
ANOVA Table	**SS[a]**	**Df[b]**	**MS[c]**	**Calculated F Value**	**Tabulated F Value**
Treatment (between columns)	351898	4	87975	19.50	2.45
Residual (within columns)	180414	40	4510		
Total	532312	44			

Concentration of HPMC K15M (Independent variable)

Dunnett's Multiple Comparison Test	HPMC K15M vs *in-vitro* release	HPMC K15M vs drug content	HPMC K15M vs bioadhesive strength	HPMC K15M vs viscositiy	
Mean Diff.	-93.54	-89.63	-24.44	-253.4	
Significant? P < 0.05?	Yes	Yes	Yes	Yes	
ANOVA Table	**SS[a]**	**Df[b]**	**MS[c]**	**Calculated F Value**	**Tabulated F Value**
Treatment (between columns)	350795	4	87699	19.44	2.45
Residual (within columns)	180406	40	4510		
Total	531201	44			

a: sum of square, b: degree of freedom, c: mean squares, calculated F value is greater or equal to tabulated F value indicate model terms are significant

where Mt/Mα is the fraction of the drug release at time t, K_P is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released (Mt / Mα) vs log of time (t) (Behera *et al.,* 2008).

RESULTS AND DISCUSSIONS

Physical appearance and pH

The formulations were light yellowish in color and clear. The pH value of all the prepared formulations ranged from 6.0 to 6.8, which is considered acceptable to avoid the risk of irritation upon application to the eye. Physicochemical data presented in table 3 shows pH, clarity, viscosity and gelation capacity of the prepared gels.

Viscosity & gelling capacity

The two main fundamentals of gelling system are viscosity and gelling capacity. The viscosity of the different formulations was compared as shown in Table 4. The viscosity was directly dependent on the polymeric content of the formulations. The data indicated that the viscosity increased with increase in concentration of HPMC K15M and carbopol 940 (1 to 4%). F16 showed the maximum viscosity of 510cps at 100rpm (HPMC:HPMC K15M:Carbopol 940 was 1:4:4) whereas the minimum viscosity at 100 rpm was shown by F1(HPMC:HPMC K15M: Carbopol 940 was 1:1:1). Except for the formulations F1, F2, F5, F6, F9, F10 and F13, all the formulations gelled instantaneously on addition to the simulated tear fluid and extended for few hours. The *in-situ* formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally.

Drug content, bioadhesive strength and in-vitro release studies

On the basis of physicochemical properties (viscosity and gelation capacity) nine formulations (F3, F4, F7, F8, F11, F12, F14, F15 and F16) were selected and evaluated for drug content, bioadhesive strength and *in-vitro* dissolution. The drug content of all the formulations was in range (82-98%). The bioadhe-

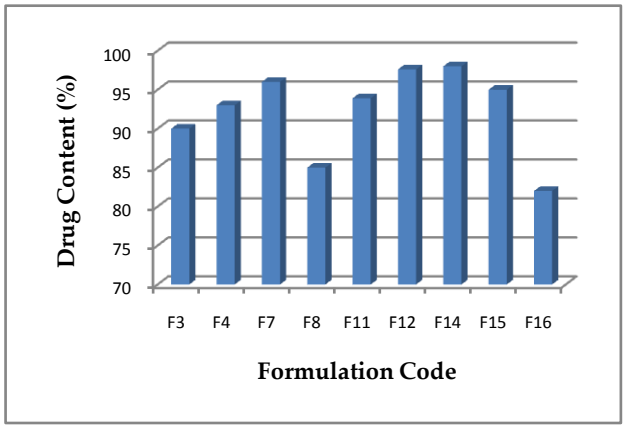

Figure 5: Comparison of drug content of formulations.

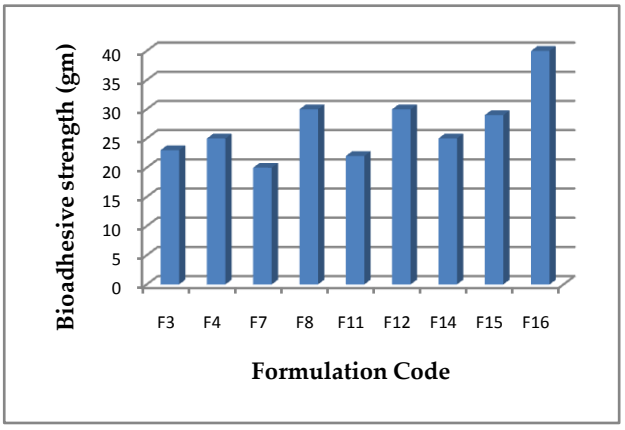

Figure 6: Comparison of bioadhesive strength of formulations.

sive strength was found to be satisfactory, maximum bioadhesive strength was 40gm for formulation F16. The evaluation results are shown in figure 5 and 6.

Figure 7 shows the cumulative amount of gatifloxacin released versus time profiles for different drug-containing solutions. In the case of formulation F12, approximately 74% of gatifloxacin was released from the solution (1% HPMC, 3% HPMC K15M, 4% Carbopol 940 1:3:4) after 90 min. This indicates that formulation F12 has a better ability to retain drugs than the individual polymer solution. These results also suggest that the HPMC. HPMC K15M, Carbopol 940 aqueous system can be used as an *in-situ* gel-forming system for ophthalmic drug delivery

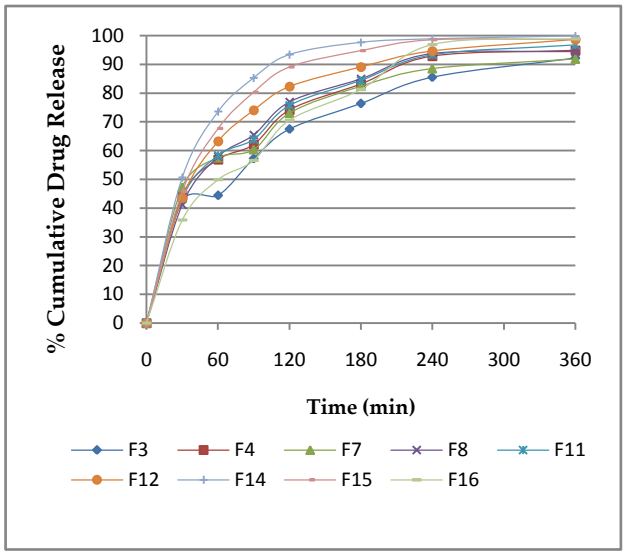

Figure 7: *In-vitro* release profile of *in-situ* gel formulations.

systems. The release of drug from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower rate followed by a second phase of moderate release.

Statistical analysis

The results obtained from the experiment were statistically analyzed for response variables by using Graph Pad Prism Demo 5 (version 5.03, Graph Pad Software Inc.). Statistical analysis (One way ANOVA) however revealed that batches were significantly different (calculated F value is greater than tabulated F value). Dunnett's multiple comparison tests predicted that there was a significant effect of independent variable (concentration of carbopol 940 & HPMC K15M) on the dependent variables (*in-vitro* drug release, drug content, bioadhesive strength and viscosity) as shown in table 4.

Kinetics of release

The *in-vitro* release profiles were fitted to various kinetic models in order to find out the mechanism of drug release. The rate constants were calculated from the slope of the respective plots. High correlation (R^2=0.9031) was observed in the Higuchi plot rather than first-order (R^2=0.3273) and zero-order (R^2=0.6485) models. The drug release was proportional to square root of time, indicating that the drug release from *in-situ* gel was diffusion controlled. The data obtained was also fit in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value (0.8029) obtained from Korsmeyer-Peppas was more than 0.5, which indicated that the mechanism of the

drug release was Anomalous and Non Fickian diffusion controlled.

CONCLUSION

HPMC, HPMC K15M, Carbopol 940 ocular *in-situ* gel of Gatifloxacin showed appreciable gel forming properties on application in eye. The gels were found to be uniform, clear, viscous and bioadhesive. On the basis of *in-vitro* drug release, drug content and gelation capacity studies, it could be concluded that Gatifloxacin could be successfully administered through gel forming controlled release ocular formulation for treatment of bacterial keratitis and conjunctivitis and also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance. The statistical analysis revealed that the factor, concentration of carbopol 940 and HPMC K15M did significantly affect the studied dependent variables.

REFERENCES

Abraham, S., Furtado, S. Bharath, S. Basavaraj, B.V. Deveswaran, R., Madhavan, V. (2009). Sustained Ophthalmic Delivery of Ofloxacin from An Ion-Activated In Situ Gelling System. Pakistan Journal Pharmaceutical Sciences, 22: 175-179. PMid:19339228

Behera, B.C., Sahoo, S.K. Dhala, S. Barika, B.B., Gupta, B.K. (2008). Characterization Of Glipizide-Loaded Polymethacrylate Microspheres Prepared By An Emulsion Solvent Evaporation Method, Tropical Journal of Pharmaceutical Research, 7(1): 879-885. [DOI]

Doijad, R.C., Manvi, F.V. Malleswara Rao, V.S.N. Alase, P. (2006). Sustained Ophthalmic Delivery of Gatifloxacin from insitu Gelling System. Indian Journal Pharmaceutical Sciences, 68: 814-818. [DOI]

Edsman, K., Carlfors, J. Harju, K. (1996). Rheological evaluation and ocular contact time of some carbomer gels for ophthalmic use. International Journal of Pharmaceutics, 137: 233-241. [DOI]

Gorle, A.P., Gattani, S.G. (2009). Design & Evaluation of polymeric ocular drug delivery system. Chemical & Pharmaceutical Bulletin, 57: 914-919. [DOI]

Kalam, M.A., Sultana, Y., Ali, A. Aqil, M. Mishra, A.K., Chuttani, K. (2009). Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system, Journal Drug Targeting, 18: 191-204. PMid:19839712 [DOI]

Madan, J., Sharma, A.K., Singh, R. (2009). Fast Dissolving Tablets of Aloe Vera Gel, Tropical Journal of Pharmaceutical Research, 8(1): 63-70. [DOI]

Mishra, D.N., Gilhotra, R.M. (2008). Design and characterization of bioadhesive in-situ gelling ocular inserts of gatifloxacin sesquihydrate. DARU Journal of Pharmaceutical Sciences, 16(1): 1-8.

Mitra, A.K. (2003). Ophthalmic Drug Delivery Systems. Marcel Dekker, (pp. 1-3). Inc, New York, Basel.

Mohamed, M.I. (2004). Optimization of Chlorphenesin Emulgel Formulation. The AAPS Journal, 6: 1-7. PMid:15760111 PMCid:2751251 [DOI]

Mohan, E.C., Kandukuri, J.M. Allenki, V. (2009). Preparation and Evaluation of In-Situ-Gels for Ocular Drug Delivery, Journal of Pharmacy Research. 2(6): 1089-1094.

Narendra, C., Srinath, M.S., Ganesh, B. (2006). Optimization of Bilayer Floating Tablet Containing Metoprolol Tartrate as a Model Drug for Gastric Retention. AAPS PharmSciTech, 7(2): 1-7. PMid:16796352 PMCid:2750284 [DOI]

Patel, P.R., Dadhani, B. Ladani, R. Baria, A.H., Patel, J. (2010). Formulation, evaluation and optimization of stomach specific in-situ gel of clarithromycin and metronidazole benzoate. International Journal Drug Delivery, 2: 141-153. http://dx.doi.org/10.5138/ijdd.2010.0975.0215.02023

Pundir, R., Pandey, M., Verma, R.K., Pandian, C.S., Saraf, S.A. (2009). Biodegradable Orthopedic Implant: Fabrication and Evaluation. International Journal of Current Pharmaceutical Research, 1(1): 8-14.

Quinones, D., Ghaly, E.S. (2008). Formulation and characterization of nystatin gel. The Puerto Rico Health Sciences Journal, 27(1): 61-67.

Rathore, K.S., Nema, R.K. (2009). An Insight into Ophthalmic Drug Delivery System. International Journal of Pharmaceutical Sciences and Drug Research, 1(1): 1-5.

Rathore, K.S., Nema, R.K. (2009). Review on Ocular Inserts. International Journal of PharmTech Research, 1(2): 164-169.

Raymond, R.C., Sheskey, P.J., Weller, P. J. (2004). Handbook of Pharmaceutical Excipient, 4th ed., pp. 89-91.

Zhidong, L., Jiawei, L. Shufang, N. Hui, L. Pingtian, D. Weisan, P. (2006). Study of an alginate/HPMC-based in-situ gelling ophthalmic delivery system for gatifloxacin. International Journal of Pharmaceutics, 315: 12–17. PMid:16616442 [DOI]