THERAPEUTIC EFFECT OF MUCOADHESIVE NANOGEL OF GATIFLOXACIN IN THE TREATMENT OF BACTERIAL KERATITIS

Neha Pal^{*1}, Rajiv Malviya¹, Subhendu S. Mishra²

¹Radharaman College of Pharmacy, Ratibad, Bhopal, M.P.

²Gayatri College of Pharmacy, Jamadarpali, Sambalpur, Odisha.

*Corresponding Author's E mail: nehapal849@gmail.com

Received 10 Aug. 2019; Revised 19 Aug. 2019; Accepted 29 Aug. 2019, Available online 15 Oct. 2019

ABSTRACT

Keratitis is a condition in which the eye's cornea becomes inflamed. The causes of disease, include infection, dry eyes, physical or chemical injury, and other diseases. The objective of the present study to formulate nanosized dispersion of gatifloxacin ionotropic gelation method and then incorporate gelling agent consist of carbopol to produce nanogel. The formulations characterized by size and surface diameter using SEM and Zetasizer. FTIR and DSC studies also determine the compatibility of drug and excipient. The formulations are also characterized by *invitro* diffusion and skin permeability study. he nanogel formulation showed the promising treatment for bacterial characterized.

Keywords: Keratitis, Nanogel, Gatifloxacin, *invitro* diffusion.

INTRODUCTION

Keratitis is a disease in which eye's cornea, the front part of eye become infected. In this disease has many causes, including infection, dry eyes, physical and chemical injury, and underlying medical disease. Keratitis may be mild, moderate or severe and may be associated with inflammation of other parts of the eyes ¹⁻².

Keratitis is a condition in which the eye's cornea becomes inflamed. The causes of disease, include infection, dry eyes, physical or chemical injury, and other diseases. Keratitis may be mild, moderate or severe and may be associated with inflammation of other parts of the eyes. Infection is the most frequent causes of keratitis in which some causative microorganism bacteria, viruses, fungi, and parasites may infect the cornea ^{3,4}.

Gatifloxacin is a fourth-generation antibiotic which has a fluoroquinolone family. Fluoroquinolones are synthetic broad-spectrum antibiotics. They inhibit DNA gyrase (topoisomerase II) and topoisomerase IV enzyme, which are key enzymes involved in DNA replication and transcription. Topoisomerase IV is the main target for most Gram-positive bacteria. DNA gyrase, on the other hand, is the main target for Gram-negative bacteria ⁵⁻⁷.

Parmar *et al.*,⁸ compare the bacteriologic and clinical efficacy of gatifloxacin and ciprofloxacin for the treatment of bacterial keratitis. It was observed that A total of 104 eyes of 104 patients with bacterial keratitis significantly higher proportion of ulcers in the gatifloxacin group inhibited completely compared to ciprofloxacin. So that I choose gatifloxacin nanogel for long term treatment for keratitis.

MATERIALS AND METHODS:

Gatifloxacin sesquihydrate was a gift sample from zydu Cadila. Carbapol, Triethyl citrate, Acrylic resin purchased from S.D. Fine chemical, New Delhi, Propylene glycol, Lecithin, Ethanol was purchased from Hi-Media Laboratory, Mumbai and other chemical purchase from local market.

Method of Preparation:

Gatifloxacin nanoparticles were prepared using chitosan and acetic acid. The nanoparticles prepared by ionotropic gelation method. In this preparation, chitosan (0.4% w/v) will be dissolved in aqueous acetic acid solutions (1% v/v) (pH 6.1), while TPP (0.1% w/v) will be dissolved in deionized water.⁹ gatifloxacin solution will be premixed with chitosan solution before the addition of the TPP solution dropwise into the chitosan solution under magnetic stirring (600 rpm) at ambient temperature for 2-4 hr. The obtained nanoparticles formulation will be lyophilized and stored in 4-8° C until it will further use. The gel is been prepared by dispersion method by dispersing carbopol 934 in water for 2 hours for swelling. Once the carbopol is been swelled it is been kept on magnetic stirrer for stirring and the prescribed amount of nanoparticles dispersion or the separated nanoparticles is been added in the carbopol mixture, along with it propylene glycol which acts as the penetration enhancer and Triethanolamine which acts as the humectant and viscosity modifier is ben added into the mixture.

Table 1: Formulation of Nanogel using different concentrations of carbopol 934

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 934	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3
Propylene Glycol	1	1	1	1	1	1	1	1	1
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water (ml)	100	100	100	100	100	100	100	100	100

Characterization of nanogel:

Physical Characteristic:

The Physical Characteristic was checked for hair gel formulations (color, clogging, homogeneity and texture) and observations were shown in Table 2.

Determination of pH:

The pH of the hair gels was determined by a digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped into gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.¹⁰

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25⁰C. The sufficient quantity of gel was filled in an appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow dipping the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 ±1⁰C) before the measurements.¹¹

Spreadability¹²

Spreadability is determined by apparatus suggested by Mutimer. It consists of wooden block, which is provided by a pulley at one end. By this method, spreadability is measured on the basis of “Slip” and “Drag”. A ground glass slide is fixed on this block. A sample of 0.1 g of nanogel under study is placed on this ground slide. The gel is fixed on the beach formula was pressed between two slides and a 1 kg weight is placed on the top of two slides and left for about 5 min to expel air and to provide a uniform film of the nanogel between two slides. Excess of the gel is scrapped from edges. The top plate is then subjected to pull the weight. With help of string attaches to the hook and the time required by top slide to cover the distance is noted. A shorter interval indicate better spreadability, spreadability was calculated by using the formula,

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l = length of glass slide (6cms).

t = time taken in seconds.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminum collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.^{13,14}

In vitro drug release:

The in-vitro drug release studies were carried out using Franz diffusion cell. The formulation was applied on the dialysis membrane which was placed between the donor and receptor compartment of the Franz diffusion cell. The temperature of the cell was maintained at 37°C by circulation jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. A similar blank set was run simultaneously as a control. Sample 5 ml was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution media. Samples were analyzed chromatographically at 374 nm and % drug release and control were used as the actual reading in each case. % drug release of each is shown in table no 8.¹⁵

Drug release kinetics^{16,17}

Several drug release data on mathematical models can be obtained, hence drug release profile can be correlated with drug release kinetic models. Zero-order release kinetics According to the principles of pharmacokinetics, drug release from the dosage form can be represented by the below equation.

$$C_0 - C_t = K_0 t \dots\dots\dots \text{Eq.1}$$

$$C_t = C_0 + K_0 t \dots\dots\dots \text{Eq.2}$$

C_t is the % amount of drug released at time t , C_0 is the % CR drug at time $t=0$ K_0 is the zero-order rate constant Graph is plotted against % cumulative drug release vs. time.

First-order release kinetics

The release of drug which follows first-order kinetics is given below, it is the first-order process whose rate is directly proportional to the concentration of drug undergoing reaction

$$\log C = \log C_0 - K_1 t / 2.303 \dots\dots\dots \text{Eq.3}$$

K_1 is the first-order rate equation expressed in time-1 or per hour. C_0 is the initial concentration of drug C is the percent of drug remaining at time t Graph is obtained by $\log$ % of CR vs. Time.

Higuchi model

Today it is considered as one of the widely used and most well-known controlled release equation, which is represented below

$$Q = KH \times t^{1/2} \dots \dots \dots \text{Eq.4}$$

KH is the Higuchi constant Graph is plotted as %CR vs. square root of time.

Korsmeyer Peppas

Once it has been ascertained that the prime mechanism of drug release is diffusion-controlled from Higuchi plot then it comes the release of drug follows which type of diffusion, to understand the dissolution mechanisms from the matrix the release data were fitted using the empirical equation.

$$F = (M_t / M) = K_m t^n \dots \dots \dots \text{Eq.5}$$

F = Fraction of drug released at time, t^n

M_t = Amount of drug released at time, t^n

M = Total amount of drug in dosage form

K_m = Kinetic constant

n = Diffusion or release exponent

t = Time in hours Graph is plotted log % CDR vs log time in hours

RESULTS AND DISCUSSION:

Psychorheological Characteristic:

The Psychorheological Characteristic was checked for hair gel formulations (color, clogging, homogeneity and texture) and observations were shown in Table. 2.

Table No. 2: Psychorheological Characteristic

Formulation	observation
F1	white viscous cream
F2	white softy cream
F3	white viscous cream
F4	white softy cream
F5	white viscous cream
F6	white softy cream
F7	white viscous cream
F8	white softy cream

Determination of pH

The pH of the nano gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped into gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Determination of Viscosity:

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25⁰C. The sufficient quantity of gel was filled in an appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow dipping the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±1⁰C) before the measurements.

Table No. 3: Determination of Viscosity and pH

Formulation	Viscosity(cps)	pH
F1	4251	6.12
F2	4256	6.23
F3	4358	6.14
F4	4345	6.22
F5	4350	6.18
F6	4360	6.21
F7	4948	6.45
F8	4115	6.32

Fig 1: Viscosity of Different formulation of Gatifloxacin Nanogel

Spreadability coefficient:

The spreadability of various concentrations of carbopol 934 is been, out of which F7 showed better spreadability than the other formulations.

Polydispersity Index:

The values of polydispersity indices were found in the range from 0.476 – 1. 263. The variation in the values of polydispersity indices may be due to the number of stabilizers used in the formulation. They act at the interface of nanosized particles and prevent them from aggregation.

Table 4: Evaluation of Gatifloxacin Nanogel batches

Formulation code	Polydispersibility index	Zeta potential (Mv)± S.D	Spreadability (g.cm/s)
F1	0.411±2.16	-27.77±1.55	0.61±0.06
F2	0.389±2.54	-24.84±0.79	0.82±0.04
F3	0.420±2.36	-20.29±1.03	0.68±0.12
F4	0.385±2.19	-25.44±0.92	0.93±0.06
F5	0.325±2.25	-21.07±1.75	0.73±0.08
F6	0.370±2.46	-24.57±0.16	0.81±0.02
F7	0.386±2.38	-23.84±0.86	0.76±0.06
F8	0.342±2.47	-22.57±1.32	0.84±0.04

IN VITRO RELEASE STUDIES

Dialysis membrane diffusion technique was used to study in-vitro diffusion of the drug from the prepared nanogel formulations. The receptor medium used was freshly prepared phosphate buffer pH 7.2. *In vitro* drug release from the semisolid preparation of gatifloxacin nanogel optimized formulation F7 shows significantly improved in drug release rate as compared to other formulations. It was concluded that developed formulations deliver the drug for the treatment of bacterial disease. Hence it could be concluded that the gatifloxacin based semisolid preparation would provide local onset of action without need of any device for their application on eye. The preparation of nanogel has potential advantages over-marketed preparation as they improved patient compliance rapid local onset of action for longer period

with cost-effectiveness. The pediatric and geriatric populations are the primary ones whose problems are easily targeted.

Table 5: *In Vitro* Release from Nanogel Formulation

Time (Hrs.)	F1	F2	F3	F4	F5	F6	F7	F8
1	18.36	17.27	16.87	18.56	19.73	18.36	19.05	18.53
2	32.28	28.46	30.75	31.37	31.93	33.58	29.38	33.58
3	41.28	38.86	42.49	41.33	38.19	39.29	39.61	42.65
4	56.73	54.28	56.71	53.73	54.58	52.37	51.73	48.58
5	71.33	68.39	69.74	71.56	67.71	69.21	65.46	62.14
6	86.27	85.19	86.33	91.28	87.89	88.53	89.37	87.47
8	97.46	96.89	94.54	96.31	95.58	98.43	98.85	97.89

Figure 2: *In Vitro* Release of drug from Nanogel Formulations

Release Kinetic Study:

The release data of all formulations were fitted to different models mentioned above. F7 has improved the release rate as compared to other formulation. The best fit with the highest determination

R^2 coefficients was shown by both zero-order models and Higuchi model followed by Korsmeyer Peppas which indicate the drug release via diffusion mechanism. Zero-order rate equations, which describe the system where release rate is independent of the concentration of the dissolved species. The Korsmeyer Peppas equation is used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

From the results, it was confirmed that all the formulations are following the Higuchi model which indicate the drug release *via* diffusion mechanism.

Fig 3: Zero order release kinetic of Nanogel formulation

Figure 4: First order release kinetics Nanogel formulation

Figure 5: Higuchi release kinetics Nanogel formulation

Figure 6: Pappas release kinetics Nanogel formulation

Antibacterial activity of Nanogel:

The present investigation in this research work, the Antimicrobial activity of nanogel formulation were evaluated against microbial pathogens used under the present study. Nanogel formulation to suitably dilute up to the concentrations of 30, 20 and 10 μg per ml and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in Table 6, which clearly shows the Antimicrobial activity of formulation.

Table 6: Antimicrobial activity of standard and nanogel on selected microbes

S. No.	Name of microbes	Zone of inhibition		
		10µg/ml	20 µg/ml	30µg/ml
Standard (Gatifloxacin)				
1.	<i>Escherichia coli</i>	7±0.57	8±0.28	10±0.47
2.	<i>Staphylococcus aureus</i>	10±0.76	12±0.57	16±0.47
Nanogel				
1.	<i>Escherichia coli</i>	8±0.86	9±0.76	11±0.57
2.	<i>Staphylococcus aureus</i>	11±0.57	14±0.47	17±0.28

Photo plates of Antimicrobial activity of standard and nanogel on selected microbes

Figure7: Photoplates of Antimicrobial activity of standard and nanogel on selected microbes
 AJPER Oct – Dec. 2019, Vol 8, Issue 4 (21-33)

Conclusion

In this research work, in-situ gel will be prepared which will consist of gatifloxacin. Gatifloxacin is an antibiotic of the fourth generation fluoroquinolone inhibits the bacterial enzymes. The formulation will remain drop form and become gel in ocular cavity when administered. The in-situ gel will help to retain drug in ocular cavity form longer period which will manage the Keratitis and also reduce the dose and dosing frequency.

Reference

1. Onlen Y, Tamer C, Oksuz H, Duran N, Altug ME and Yakan S. Comparative trial of different anti-bacterial combinations with propolis and ciprofloxacin on *Pseudomonas* keratitis in rabbits. *Microbiol Res.* 2007; 162, 62–68.
2. Sensoy D, Cevher E, Sarıcı A, Yılmaz M, Ozdamar A and Bergisadi N. Bioadhesive sulfacetamide sodium microspheres: Evaluation of their effectiveness in the treatment of bacterial keratitis caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a rabbit model. *Eur. J. Pharm. Biopharm.* 2009; 72: 487–495.
3. Schaftenaar E, Peters RPH, Baarsma GS, Meenken C, Khosa NS, Getu S et al. Clinical and corneal microbial profile of infectious keratitis in a high HIV prevalence setting in rural South Africa. *Eur J Clin Microbiol Infect Dis.* 2016;35:1403-9.
4. Zhou L, Zhao SZ, Koh SK, Chen L, Vaz C, Tanavde V et al. In-depth analysis of the human tear proteome. *J Proteomics.* 2012; 75:3877-85.
5. Forman D, Webb P and Parsonnet J. *Helicobacter pylori* and gastric cancer *Lancet.* 1994; 343: 243-244.
6. Megraud F and Lamouliatte H. *Helicobacter pylori* and duodenal ulcers. *Dig Dis Sci,* 1992;37:769-772.
7. Nethaji R, Natesan G, Surya Prakash TNK and Subramanian N. Formulation and evaluation of gatifloxacin niosomes using sorbitan monoesters. *Acta Pharmaceutica Scientia.* 2011; 53(4):619-634.
8. Parmar P, Salman A, Kalavathy CM, Kalamurthy J, Prasanth DA, Thomas PA and Jesudasan CA. Comparison of topical gatifloxacin 0.3% and ciprofloxacin 0.3% for the treatment of bacterial keratitis. *Am J Ophthalmol.* 2006;141(2):282-286.
9. Desai KG. Chitosan Nanoparticles Prepared by Ionotropic Gelation: An Overview of Recent Advances. *Crit Rev Ther Drug Carrier Syst.* 2016;33(2):107-58.

10. Chaudhary H, Rohilla A, Rathee P and Kumar V. Optimization and formulation design of carbopol loaded Piroxicam gel using novel penetration enhancers. *Int J Biol Macromol.* 2013;55: 246-253.
11. Jain S, Padsalg BD, Patel AK and Moale V. Formulation development and evaluation of fluconazole gel in various polymer bases. *Asian J Pharm.* 2007;1(8):63-68.
12. Phatak A, Jorwekar P and Chaudhari P. Nanosuspension a promising nanocarrier as a drug delivery system. *Research J Pharm Dosage Forms and Tech.* 2011; 3:176.
13. Adhikari B, Cherukuri S, Reddy SC, Chinthaginjala H, Bhatta H and Naidu R. Recent Advances in Nanogels in Drug Delivery System. *World J Pharm Pharm Sci.* 2016; 5(9):505-530.
14. Talele S, Nikam P, Ghosh B, Deore C, Jaybhav A and Jadhav A. Nanogel as Topical Promising Drug Delivery for Diclofenac Sodium. *Indian Journal of Pharmaceutical Education and Research.* 2017;51(4S):5680-5685.
15. D'Souza S. A Review of In Vitro Drug Release Test Methods for Nano-Sized Dosage Forms. Hindawi Publishing Corporation. 2014. Article ID 304757.
16. Gouda R, Baishya H, Qing Z. Application of Mathematical Models in drug Release Kinetics of Carbidopa and Levodopa ER Tablets. *J Develop Drugs.* 2017;6(2):1-8.
17. <https://www.slideshare.net/sagarsavale1/drug-releasekinetics> accessed on dated February 12, 2018.