Acta Pharmaceutica Sciencia
50:111-117 (2008)

Scintigraphic Assessment of the *In situ* Gelling Formulation of Gatifloxacin for Monitoring Ocular Residence : Enhancement of Ocular Residence Time

M. Abul Kalam[1], Y. Sultana[1*], A. Ali[1], M. Aqil[1], M. Sharma[2], A. K. Mishra[3], and K. Chuttani[3]

[1]Department of Pharmaceutics and [2]Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi110062, India
[3]Department of Radiopharmaceuticals, Institute of Nuclear Medicine and Allied Sciences (INMAS), Brig. S.K. Majumdar Road, Delhi-110054, India.

Abstract

This study was carried out to determine ocular residence time of *in situ* gelling formulation of gatifloxacin. *In situ* gelling formulations of gatifloxacin were prepared and optimized on the basis of *in vitro* release studies and their rheological behaviors. Scintigraphic assessment of the optimized formulation was done to monitor ocular residence time in rabbit eye. The optimized formulation containing 0.5 % w/v Gelrite® demonstrated favorable *in vitro* release and rheological properties. It did not cause any irritation and showed residence time of 10 minutes in rabbit eye. The developed formulation has ability to enhance pre-corneal residence time and improve ocular bioavailability.

Keywords: Scintigraphy, i*n situ* gelling, Gatifloxacin, ocular irritation, ocular residence time

Introduction

Conjunctivitis known, as "Pink eye" is an inflammation of the membrane (conjunctiva) that covers the inner surface of the eyelid. Hyperactive acute bacterial conjunctivitis is most often caused by *N. gonorrhoeae* and *N. meningitides*. Acute bacterial conjunctivitis is caused by *Staphylococcus* species, *Streptococcus* species, *Pseudomonas, Haemophilus influenza, E.coli.* The symptoms include hyperemia, increased secretion, itching, redness, sensitivity to light, swelling of lids, gritty sensation and matting of eyelids. The discharge may be purulent or mucopurulent. Gatifloxacin, a fourth generation fluoroquinolone (FQs), is a pyridone carboxylic acid derivative, which exerts a broad-spectrum antibacterial effect. It inhibits the enzyme bacterial DNA Gyrase and has *in vitro* activity against a broad spectrum of Gram positive and Gram-negative bacteria (Blondeau, 2004). *In vitro* study indicated that fourth generation FQs appear to cover bacterial resistance to the second and third generation FQs, were more potent than the second and third generation FQs for gram-positive bacteria, and are equally potent for gram-negative bacteria (Mather *et al.*, 2002).

The inherent drawbacks of conventional ophthalmic drug delivery systems are precorneal drug loss due to the lacrimal flow, rapid drainage, palpebral blinking and high tear turnover. Due to which frequent administration of the dosage form is required which can cause toxicity due to

* Corresponding author: yas2312003@yahoo.com

nasolacrimal drainage, problems related to the poor patient compliance and sometimes incorrect management of the therapy. The ophthalmic availability can be improved by enhancing the precorneal residence of the instilled dose of the formulation (Sandri *et al.*, 2006). One way to achieve this is the use of *in situ* gel forming systems, which upon instillation as drops into the eye undergo a sol-gel transition in the cul-de-sac. This may result in better ocular availability of the drug.

Gelrite® is a gellan gum that is a high molecular mass, linear anionic heteropolysaccharide produced aerobically from the bacterium *Auromonas (pseudomonas) elodea*, renamed *Sphingomonas paucimobilis*. The polymer backbone is comprised of a tetrasaccharide repeat unit of glucose, glucuronic acid and rhamnose (Jansson *et al.*, 1983). Deacetylation of the polysaccharide enables extensive intermolecular association to take place and the formation of strong brittle gels with cations to occur (Morris *et al.*, 1996). Human tears contain bicarbonate, chloride, potassium, and calcium ions. Gellan gum forms clear gel in the presence of mono or divalent cations. Because of its ability to form strong clear gels at physiological ion concentration, gellan gum has been widely investigated for use as an *in situ* gelling agent in ocular formulations. It has a characteristic property of temperature dependent and cation induced gelation. This gelation involves the formulation of an ordered state of gellan chains. X ray diffraction studies have confirmed that a double helix of gellan chains is formed by complexation with cations and hydrogen bonding with water (Sanzgiri *et al.*, 1993).

A number of studies have been done utilizing *in situ* gelling polymers like Gelrite® (Balasubramaniam *et al.*, 2003, Balasubramaniam and Pandit, 2003, Sultana *et al.*, 2004 and Sultana *et al.*, 2006a), carbopol-methyl cellulose (Sultana *et al.*, 2006b), carbopol-pluronic (Lin and Sung, 2000), pluronic F127 (El-Kamel, 2002), alginate-pluronic (Lin *et al.*, 2004) and carbopol-HPMC (Kumar and Himmelstein, 1995).

The objective of the study was to monitor ocular residence time of *in situ* gelling formulation of gatifloxacin in rabbit eye using scintigraphy.

Materials and Methods

Materials

Gatifloxacin was obtained as a gift sample from Lupin, Pune, India and Gelrite® was a gift from Kelco division of Merck, USA. Other formulation excipients were of pharmaceutical grade and obtained from standard commercial suppliers.

Preparation of formulation

Different concentration of Gelrite® (0.1-0.7% w/v) were prepared in 0.01M Boric acid buffer (pH 4.7) by continuous stirring at room temperature. Gatifloxacin (0.27% w/v) was dissolved in buffer and added to the polymer solutions and stirred until dissolved. Finally, preservatives were added and formulations were filled in amber colored glass vials, capped with rubber bungs and sealed with aluminum caps. The formulations, in their final pack were subjected to terminal sterilization by autoclaving at 121°C and 15 psig for 20 minutes. The antimicrobial effectiveness of formulation was tested before and after sterilization by this method. Simulated tear fluid (STF) was prepared according to the electrolyte composition of tear fluid (Rozier *et al.*, 1989).

The formulations were optimized based on the results of *in vitro* release and rheological studies. The drug release studies were done using flow through apparatus. The dissolution medium used was simulated tear fluid at pH 7.4. Samples were then analyzed for the drug content of gatifloxacin at 286 nm. using apparatus double beam UV spectrophotometer, Hitachi-110 Japan. Viscosity measurement was carried out using Cone and Plate Viscometer (Physica Rheolab, Austria) using MK-22 spindle.

In Vivo Studies

Rabbits weighing 2-3 kg were used. Animals were housed 1 per cage and maintained at 20-30% humidity in natural light and dark cycle, with free access to food and water. Permission for the use of animals was obtained from Animal Ethics Committee, of Institute of Nuclear Medicine and Allied Sciences (INMAS) Delhi, India.

a) Ocular Irritation Studies

Six rabbits were used in the study. The albino rabbit has historically been the animal of choice for testing potential eye irritants, because its large eyes make it easy to observe any damage and it has large conjunctival sac that easily accepts test material and holds it against the eye. Discomfort of the rabbit eyes were graded, so that slight irritation was characterized by half closed eyelids and severe irritation by firm closure of the eyelid. The eyelid closure was expressed as the sum of full closure and half closure times of the eyes. Mucoidal discharge was scored from 0 to 2, where 0 is normal, any clear discharge different from normal is 1, and milky discharge moistening the lids scores 2 (Suhonen *et al.*, 1996). Observations are given in Table1.

Table 1. Observations showing Ocular Irritation Studies (Mucoidal discharge and Eyelid closure of marketed formulation and Gelrite® based formulation of Gatifloxacin).

S.No. of Rabbits	Mucoidal discharge (Marketed Formulation)	Mucoidal discharge (Gelrite® Formulation)	Eyelid closure (Marketed Formulation)	Eyelid closure (Gelrite® Formulation)
1	0	1	0	1
2	0	0	1	1
3	1	0	0	0
4	0	1	1	1
5	0	1	0	1
6	1	0	1	1

b) Scintigraphic Studies

In vivo precorneal drainage of radionuclide was studied using single photon emission computing tomography (SPECT, starcam 3200I X/R General Electric, USA) auto tuned to detect the 140 KeV radiation of ^{99m}Tc .The optimized formulation containing Gelrite® 0.5% (w/v) was assessed in three rabbits with a minimum washout period of 3 days. Radiolabelled formulation was prepared by adding aqueous stannous chloride into the formulation as a reducing agent; pH was set at neutral and after that activity was added. The stability of binding was determined by instant thin layer chromatography by calculating percentage binding from a gamma counter (Electronic Corporation, India). The rabbit was positioned 5 cm in front of the probe and exactly 50 µl of radiolabelled test formulation (^{99m}Tc, specific activity of approximately 200 MB/ml) (Alany *et al.*, 2006) which were stored at 20°C for about half an hour before use was instilled in the lower conjunctival sac of left eye of each rabbit and blinked the eye manually to distribute the formulation throughout the corneal surface. The right eye of the rabbit served as a control to monitor the irritation. A static sequence of images was recorded after instillation of the radiolabelled formulation according to the following schedule: 6 frames at 5 sec, 2, 4, 6, 8 and 10 minutes using 128 x 128 pixel matrix. Images were analyzed qualitatively using a dual head gamma camera (Gencoglu *et al.*, 2005) as shown in Fig. 1. For qualitative analysis all the graphs were divided

into three regions of interest (ROIs) as shown in Fig. 2, and the movement of the gamma emitting material accurately followed within zone.

Results and Discussion

Different formulae of *in situ* gel forming systems were prepared and sterilized by autoclaving at 121°C and 15 psig. for 20 minutes. The results of antimicrobial test conducted before and after sterilization led us to conclude that sterilization method does not cause any change in effectiveness of the formulation. It was found that formulation containing 0.5% (w/v) Gelrite® demonstrated favorable *in vitro* drug release and rheological properties and hence this formulation was selected as an optimized formulation.

a) Ocular Irritation Studies

The results of ocular irritation studies indicated that formulation was non irritant. Excellent ocular tolerance was noted. Abnormal clinical signs to cornea, iris, or conjunctiva were not seen, which were supported by paired 1t- test, the two tailed P value is 0.6952, considered not significant, t = 0.4152 with 5 degree of freedom for mucoidal discharge and two tailed P value is 0.1747, considered not significant t = 1.581 with 5 degree of freedom for eye lid closure. Results of ocular irritation studies (Mucoidal discharge and Eyelid closure) are given in Table 2.

Table 2. Summary of Data of Paired t-test for Mucoidal discharge and Eyelid closure.

Parameters	Mucoidal discharge (Marketed formulation)	Mucoidal discharge (Gelrite® based formulation)	Eyelid closure (Marketed formulation)	Eyelid closure (Gelrite® based formulation)
Mean	0.3333	0.5000	0.5000	0.8333
No. of Points	6	6	6	6
Std. Deviation	0.5164	0.5477	0.5477	0.4082
Lower 95 % CI[a]	- 0.2087	- 0.07489	-0.07489	0.4048
Upper 95 % CI[a]	0.8753	1.075	1.075	1.262

[a] = CI is Confidence Interval

b) Scintigraphic Studies

Gamma Scintigraphy is a well-established technique for monitoring the ocular residence time of drug in the formulations. Gelrite® 0.5% (w/v) showed at all times a significantly higher retention of the radioactive tracer in the regions of interest as shown in Figure 1.

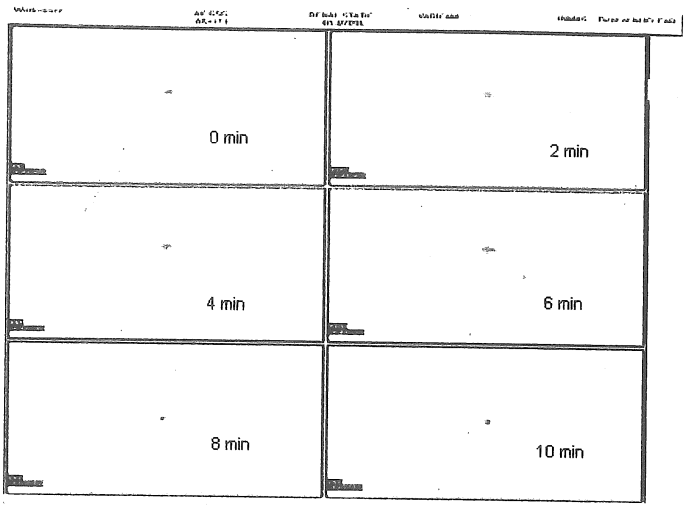

Figure 1. Gamma camera image of rabbit eye for 10 minutes after instillation of 99mTc labeled Gelrite® based formulation.

Presence of polymer in the optimized formulation prolonged the residence of the radioactive tracer in the precorneal region; Fig. 2 shows the passage of radioactive tracer in the three regions of interest which are corneal surface, inner canthus and nasolacrimal duct.

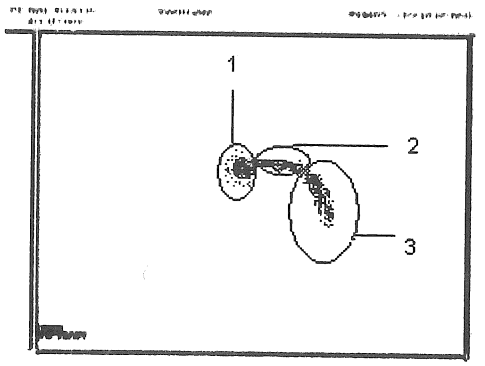

Figure 2. Scintigraphic image divided into three region of interest (ROIs); 1.Corneal surface, 2.Inner canthus, 3. Nasolacrimal duct. Showing passage of radioactive tracer in these regions of rabbit's eye.

The presence of radiolabelled formulation could be marked in these regions of interest up to 10 minutes after the instillation of the formulation. This is due to anionic nature of the polymer Gelrite® which would interact with cations present in the tear fluid to demonstrate increased ocular retention. Visual examination revealed good retention of the test formulation over the entire area of the rabbit eye. The transit of the formulation between the identifying non-ocular ROIs was monitored. The non ocular ROI represents the inner canthus and lacrimal sac, showed drainage down the nasolacrimal duct and hence it is not considered relevant to pre-corneal residence. It was difficult to differentiate the retention of the radiolabelled formulation

between the inner canthus and lacrimal sac due to poor resolution, however the transit of the radioactivity from the site of instillation to the nasolacrimal duct was obvious.

Conclusion

In situ ocular sol to gel system of gatifloxacin was successfully formulated using Gelrite® as ion-activated polymer. Gelrite® 0.5% (w/v) based formulation was non-irritating, possess excellent ocular tolerance as determined by ocular irritation studies. The images obtained in the study indicated that the formulation remained associated with the eye for a significant period of time (10 minutes) as assessed by scintigraphic measurements. Hence, this can be viewed as a viable alternative to conventional eye drops by virtue of its ability to enhance pre-corneal residence time and thereby increasing ocular bioavailability.

Acknowledgements

The authors are grateful to Lupin Research Park (India) for the generous gift of Gatifloxacin and Kelco Division of Merck (USA) for the Gelrite®. The first author acknowledges the Fellowship grant by the University Grants Commission (UGC), New Delhi, India.

References

Alany, R.G., Rades, T., Nicoll, J., Tucker, I.G. and Davies, N.M. (2006). W/O microemulsions for ocular delivery: Evaluation of ocular irritation and precorneal retention. *J. Cont. Rel.*111:145-152.

Balasubramaniam, J., Kanth, S. and Pandit, J.K. (2003). In-vitro and *in vivo* evaluation of the Gelrite® gellan gum-based ocular delivery system for indomethacin. *Acta. Pharm.* 53:251-261.

Balasubramaniam, J. and Pandit, J.K. (2003). Ion activated *in situ* gelling system for sustained ophthalmic delivery of ciprofloxacin hydrochloride. *Drug. Deliv.* 10:185-191.

Blondeau, J.M. (2004). Fluoroquinolones: mechanism of action, classification, and development of resistance. *Surv. Ophthalmol.* 49 (Suppl 2):S73-S78.

El-Kamel, A.H. (2002). *In vitro* and *in vivo* evaluation of Pluronic F127-based ocular delivery system for timolol maleate. *Int. J. Pharm.* 241 (1):47-55.

Gencoglu, E.A., Dursun, D., Akova, Y.A., Cengiz, F., Yalcin, H. and Koyuncu, A. (2005). Tear clearance measurement in patients with dry eye syndrome using quantitative lacrimal scintigraphy. *Ann. Nucl. Med.* 19 (7):581–587.

Jansson, P.E., Lindberg, B. and Sandfor, P.A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea. Carbohydr. Res.* 124:135-139.

Kumar, S. and Himmelstein, K.J. (1995). Modification of in situ gelling behaviour of carbopol solution by HPMC. *J. Pharm. Sci.* 84:334-337.

Lin, H.R. and Sung, K.C. (2000). Carbopol/pluronic phase change solutions for ophthalmic drug delivery. *J. Cont. Rel.* 69 (3):379-388.

Lin, H.R., Sung, K.C. and Vong, W.J. (2004). *In situ* gelling of alginate/pluronic solutions for ophthalmic delivery of pilocarpine. *Biomacromolecules* 5(6):2358-2365.

Mather, R., Karenchak, L.M., Romanowski, E.G. and Kowalski, R.P. (2002). Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. *Am. J. Ophthalmol.* 133:463-466.

Morris, E.R., Gothard, M.G.E., Hember, M.W.N., Manning, C.E. and Robinson, G. (1996) Conformational and rheological transitions of Welan, Rhamsan and acetylated gellan. *Carbohydr. Polym* 30:165-175.

Rozier, A., Mazuel, C., Grove, J. and Plazonnet, B. (1989). Gelrite®, A novel ion activated, *in situ* gelling polymer for ophthalmic vehicles. Effects of bioavailability of timolol. *Int. J. Pharm.*57:163-168.

Sandri, G., Bonferoni, M.C., Chetoni, P., Rossi, S., Ferrari, F., Ronchi, C. and Caramella, C. (2006). Ophthalmic delivery systems based on drug-polymer-polymer ionic ternary interaction: *In vitro* and *in vivo* characterization. *Eur. J. Pharm. Biopharm.* 62:59-69.

Sanzgiri, Y.D., Maschi, S., Crescenzi, V., Callegaro, L., Toppa, E.M. and Stella, V.J. (1993). Gellan based systems for ophthalmic sustained delivery of methylprednisolone. *J. Cont. Rel.* 26:195-201.

Suhonen, P., Jarvinen, T., Lehmussaari, K., Reunamaki, T. and Urtti, A. (1996). Rate control of ocular pilocarpine delivery with bispilocarpic acid diesters. *Int. J. Pharm.*127: 85-94.

Sultana, Y., Jha, M.C., Ali, A. and Aqil, M. (2004). Three-way comparative study on the efficacy of twin sol to gel systems and marketed eye drops of pefloxacin mesylate. *J. Ocul. Pharmacol. Ther.* 20:363-371.

Sultana, Y., Ali, A. and Aqil, M. (2006a). Ion-activated, Gelrite® based *in situ* ophthalmic gels of pefloxacin mesylate: comparison with conventional eye drops. *Drug. Deli.* 13:1-5.

Sultana, Y., Ali, A., Aqil, M. and Zafar, S. (2006b). Evaluation of Carbopol-Methyl Cellulose Based Sustained-Release Ocular Delivery System for Pefloxacin Mesylate using Rabbit eye Model. *Pharm. Dev. Technol.* 11:313-319.

Received: 28.02.2008
Accepted: 10.04.2008