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Formulation and characterization of ofloxacin opthalmic GEL

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Abstract

Ofloxacin is a fluoroquinolone broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria for the treatment of infections. Ofloxacin is being used as eye drops in the treatment of eye infections. Rapid elimination and poor bioavailability is the disadvantage associated with eye drops. An attempt was made to prolong the retention of ofloxacin in the eye by formulating into gel using HPMC K100, Carbopol 940 and Sodium Alginate individually and in combination and the prepared formulations were evaluated.

Keywords: Oflioxacin gel, Opthalmic gels, Occular drug delivery.

Introduction

Ophthalmic route of drug administration is the most interesting and challenging as the anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. The eye is a unique organ containing several widely varied structures with different physiological functions. The conventional drug delivery such as suspension, ointment, solution show some drawbacks like increased pre-corneal drainage, blurred vision, low bioavailability low residence time. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye and by other concomitant factors like, drainage of the instilled solutions, lachrymation and tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, limited corneal area and poor corneal permeability, binding by the lachrymal proteins. A major goal in ocular therapeutics is to circumvent structural obstacles and protective mechanisms of the eye to elicit desired pharmacological response.

Most conventional ophthalmic dosage forms are simplistic. It is usual that water-soluble drugs are delivered through topical administration in an aqueous solution and water-insoluble drugs are administered topically as an ointment or aqueous suspension.

Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage and the high efficiency of the blood–ocular barrier. It is standard for only 1% or less of a topically applied dose to be absorbed across the cornea and thus reach the anterior segment of the eye. Pulse entry is a common and yet highly undesirable, pharmacokinetic characteristic associated with eye drops. The initial high drug concentration found in tears, followed by a rapid decline, poses a potential risk of toxicity and suggests a requirement for frequent dosing.

Therefore, it seems promising to develop a dosage form that can aim at increasing the bioavailability in the ocular region by increasing the retention time in the eye.

Materials and Method

Materials were obtained from commercial suppliers and were used. IR spectra were recorded in KBr discs on a Bruker analyzer.

Standard Graph of Ofloxacin: Standard graph of Ofloxacin was plotted in phosphate buffer pH 6.8 by making serial dilutions from the stock solution of 1mg/ml. Prepared solutions were analyzed at 292 nm using UV-visible spectrophotometer.

Drug-Excipient Compatibility Study: Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, to promote the consistent release and bioavailability of the drug and protect it from degradation. API and excipients were thoroughly mixed in predetermined ratio. FTIR studies were carried out to determine the compatibility of excipients with the drug.

Preparation of Gels: The weighed quantities of polymers were kept for swelling overnight in distilled water and dissolved (heated, if necessary) using a magnetic stirrer. Aqueous solution of Ofloxacin was added in to the polymeric solution with continuous stirring. Benzalkonium chloride was added to the resulting solution. The pH of the formulation was adjusted to 7.4 using 0.1 N NaoH/0.1 N HCl. The gel formulations are depicted in Table 1.

Table 1: Formulation of gels

Formulation	F1	F2	F3	F4	F5	F6
Ofloxacin	0.3g	0.3g	0.3g	0.3g	0.3g	0.3g
HPMC K100	-	1g	-	0.5g	-	0.5g
Carbopol	1g	-	-	0.5g	0.5g	-
Sod. Alginate	-	-	1g	-	0.5g	0.5g
Benzalkonium Chloride	0.02	0.02	0.02	0.02	0.02	0.02
Water q.s	100 gm	100 gm	100 gm	100 gm	100 gm	100 gm

Sterilization of Prepared Formulations: Prepared solutions were sterilized by autoclaving at 121° C for 15 min and stored in aseptic conditions until use.

Evaluation

Physical parameters:

Clarity Test: The formulations were visually checked for the clarity by visual inspection under a good light against a black and white background, with the contents set in motion with a swirling action.

pH Determination: pH of each formulation was determined immediately after preparation by using digital pH meter which was previously calibrated by pH 4 and pH 7 standard buffers.

Determination of Percent Drug Content: Accurately weighed quantities of gels sample equivalent to 30 mg of ofloxacin were separately added to 100 ml of distilled water in stoppered conical flasks. The solution was filtered, diluted suitably and was assayed by a UV-Vis spectrophotometer for drug content at 292 nm.

$$\text{Amount of drug} = \frac{\text{Concentration X dilution factor X volume of dissolution}}{1000}$$

$$\text{Percent drug content} = \frac{\text{Actual amount present}}{\text{Amount expected}} \times 100$$

In-vitro drug diffusion study: In-vitro drug release studies of the formulations were conducted using Franz diffusion cell apparatus. Egg membrane isolated from egg by placing in dil. HCl was used to study the in-vitro diffusion of the formulations. The receptor chamber was filled with freshly prepared buffer of pH 6.8. The donor compartment was loaded with 1gm of formulation. Aliquots of receptor medium were withdrawn and replenished with fresh medium at specific intervals of time and analysed spectrophotometrically for the drug content.

Sterility Test: The sterility test was carried out using three sets of agar medium where the first set was a negative control containing sterile media, second set was a positive control for this sterilized media inoculated with Staphylococcus aureus was used and third set was a test.

1gm of sterile optimized formulation was taken and this formulation was diluted with 100ml sterile water for injection, from this 10ml test solution was added to the medium and incubated for a period of 7 days at 20-25° C to find out growth of bacteria.

Antibacterial Activity: Agar diffusion method was used for the determination of antibacterial activity of formulations. Standard Petri dishes containing medium to a depth of 0.5 cm were used. The inoculum (0.5ml) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. The bores of 0.5 cm diameter were prepared and 100mg of formulations were added in the bores. After incubation at 35°C for 24 hrs, the zone of inhibition around the bores was measured.

Results and Discussion

Construction of Calibration Curve of Ofloxacin: The data standard graph of Ofloxacin has shown good linearity over a concentration range of 0-10µg/ml with R^2 value of 0.9977. The equation was y=0.0782x. This was utilized in the estimation of Ofloxacin samples.

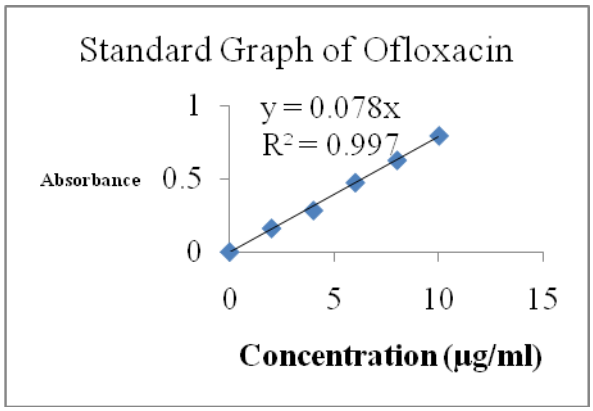

Fig. 1: Standard graph of Ofloxacin

Drug-Excipient Compatibility Study: Infrared spectroscopy studies of Ofloxacin alone and their physical mixture with HPMC, Carbopol 934 and Sodium alginate revealed that, Ofloxacin is compatible with all the polymers used.

The functional groups present in the drug were identified. The wavenumbers of drug were compared with final formulated product IR spectrum. The resulted peaks revealed that there was no significant disturbance in the principle peaks of pure drug Ofloxacin. From the interpretation it was understood that there was no major shifting in the frequencies of Ofloxacin which indicated that there is no chemical interaction in the formulations. This further confirms the integrity of pure drug and compatibility of it with excipients.

Table 2: Principle IR Peaks of Ofloxacin

Ofloxacin	Ofloxacin + excipients	Functional group
2926	2932	OH group
1004	1005	C-F
2785	2786	CH3
1709	1705	C=O
3037	3038	C-H(AROMATIC)

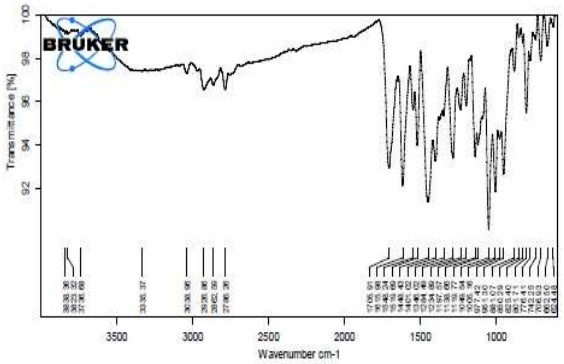

Fig. 2: FTIR of Ofloxacin

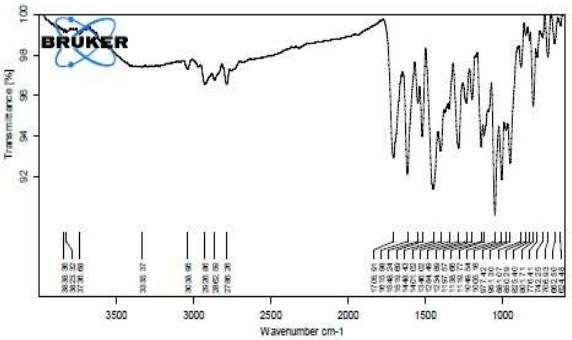

Fig. 3: FTIR of ofloxacin with polymers

Evaluation of Ophthalmic Gel Formulation

Physical parameters

Clarity: On careful visual inspection against dark and white background, all the prepared ophthalmic gel formulations were found to be free from any suspended particulate matter.

pH Determination: The pH of the prepared gels was found to be in the range of 7.40-7.53 with a standard deviation of 0.025 to 0.197. All the formulations have shown a pH with not much deviation.

Drug Content of Ofloxacin: The drug content of the prepared gels was found to be in the range 96-99 indicating the application of the present method for the preparation of gels with high content uniformity.

Table 3: Physical parameters and drug content of the formulations

S. No	Formulation code	Appearance	Observed pH (±S.D.)	Assay
1	F1	Clear	7.40±0.025	97.61 ± 0.92
2	F2	Clear	7.52±0.134	96.01 ± 0.23
3	F3	Clear	7.51±0.178	98.33 ± 1.48
4	F4	Clear	7.40±0.197	95.16 ± 0.36
5	F5	Clear	7.53±0.115	99.32±0.25
6	F6	Clear	7.43±0.145	98.31±0.38

In-vitro Drug Diffusion Study: The percentage drug release observed from the in vitro diffusion studies are shown in Table 4. Out of the formulations containing 1% of polymer, by the end of six hours, formulation F1 with carbopol as polymer has shown good release characteristics than those with HPMC K 100 and Sodium alginate.

When the combination of polymers were used, the F5 containing Carbopol and Sodium alginate each at 0.5% and F6 formulation containing HPMC K 100 and Sodium alginate each at 0.5% have shown a decrease in drug release may be due to hindrance of release by interaction of two polymers used in combination.

The formulation F4 containing HPMC K 100 and Carbopol each at 0.5% as polymer has shown a release of 98.89% by the end of 6th hour. Thus it is considered as optimised formulation as it has shown maximum drug release by the end of six hours.

Table 4: Cumulative percentage of drug release

Time (hrs)	Cumulative % drug released					
	F1	F2	F3	F4	F5	F6
0.5	21.60±0.84	17.21±0.62	19.14±1.43	24.14±1.30	12.60±1.08	14.06±0.99
1	34.71±0.61	26.01±0.30	31.30±0.58	46.96±3.28	21.46±0.79	19.31±1.39
2	45.52±1.07	36.17±0.64	40.98±1.35	62.37±1.57	29.41±0.53	30.7±0.52
3	51.84±0.96	44.53±0.94	47.32±2.27	72.01±2.43	37.21±0.73	39.96±0.63
4	69.51±0.96	54.58±0.88	61.18±1.28	88.71±1.28	45.90±0.57	46.71±1.13
5	82.88±1.45	59.66±0.93	69.84±0.43	92.52±1.12	47.35±0.40	52.66±0.97

6	90.48±0.84	64.46±0.91	76.76±1.17	98.89±1.13	50.12±1.79	61.37±1.17

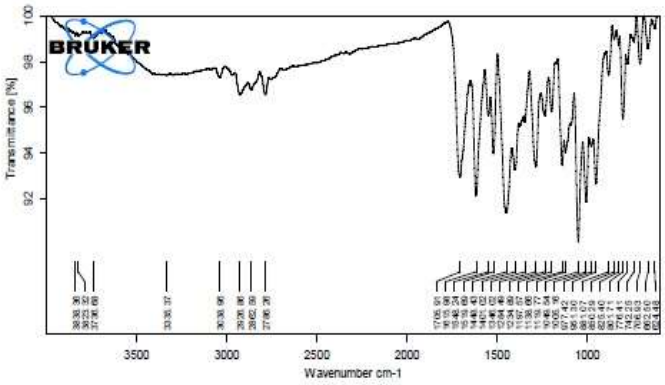

Figure 4: Cumulative drug release of F1-F6 formulations

Sterility test: The formulations showed no evidence of microbial growth when incubated for more than 7 days and cleared the sterility test.

Table 5: Sterility test data of prepared gels

Formulation	Days of incubation					
	1	2	3	4	5	6
F1	-	-	-	-	-	-
F2	-	-	-	-	-	-
F3	-	-	-	-	-	-
F4	-	-	-	-	-	-
F5	-	-	-	-	-	-
F6	-	-	-	-	-	-

*-ve sign indicates no growth.

Antibacterial Activity: Antimicrobial efficacy study was performed on formulations using Gram +ve S. aureus and Gram -ve E. coli organism. Clear zones showing inhibited zone of growth were observed. The zones of inhibition of the formulations were shown in the Table 6. The study indicated Ofloxacin retained its antimicrobial activity when formulated as gel against both selected S. aureus and E. coli.

Table 6: Zone of Inhibition of prepared In situ gels

Microorganism	Zone of Inhibition(mm)						
	Standard (Pure Drug)	F1	F2	F3	F4	F5	F6
S. aureus	29	28	28	27.5	25.5	28.5	26
E. coli.	30	27.5	28.5	29.5	27.5	28	27.5

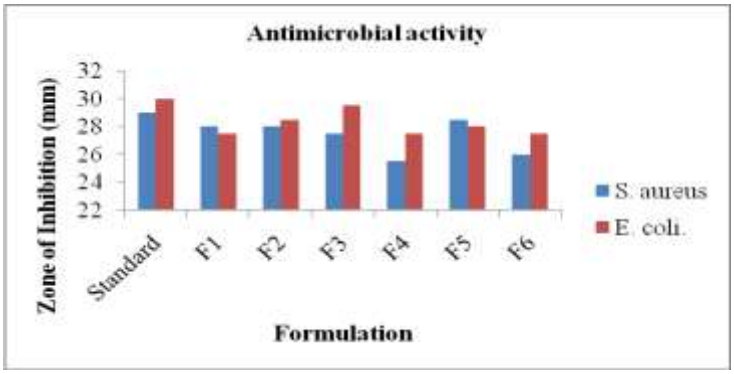

Fig. 5: Zone of Inhibition of prepared In situ gels

Conclusion

Opthalmic gels of ofloxacin were prepared by using the polymers HPMC K100, Carbopol and Sodium Alginate alone and in combination. Comparative drug release was estimated. Of all the formulations, the formulation having HPMC K100 and Carbopol at 0.5% each has shown a sustained release of drug for the estimated six hours of time.

Therefore the combination of HPMC K100 and Carbopol was found to be optimized for ophthalmic gel of ofloxacin for a six hour release.

References

1. Ahuja M., Singh G. and Dipak K., Effect of formulation parameters on corneal permeability of Ofloxacin, *Science Pharma,* (2008); 76: 505-514.
2. Ashim K., Ophthalmic drug delivery system. Vol. 58. New York: Marcel Dekker Inc; (1993). pp. 105–10.
3. Chelladurai S., Mishra M. and Mishra B., Design and evaluation of bioadhesive *in-situ* nasal Gel of ketorolac tromethamine, *Chemical and Pharmaceutical Bullettin*. (2008); 56(11):1596–1599.
4. Chu K., Chen L. and Xu W., Preparation of A paeonol-containing temperature-sensitive *in- situ* gel and its preliminary efficacy on allergic rhinitis, *International Journal of Molecular Sciences*, (2013);14(3):6499–6515.
5. Deshmukh K. and Ravindra A., Study of a novel environmentally responsive ophthalmic drug delivery syste*m, International Journal of Pharma and Bio Sciences*, (2010); 1(2): 1-13.
6. Dholakia M., Thakkar V., Patel N. and Gandhi T., Development and characterisation of thermo reversible mucoadhesive moxifloxacin hydrochloride *in-situ* ophthalmic gel, *Journal of Pharmacy and Bioallied Sciences*, (2012); 4:S42–S45.
7. Geethalakshmi A., Karki R., Jha S., Venkatesh D. and Nikunj B., Sustained ocular delivery of brimonidine tartrate using ion activated *in-situ* gelling system, *Current Drug Delivery*, (2012);9(2):197–204.
8. Gokulgandhi M., Parikh J., Megha Barot M. and Modi D., A pH triggered *in-situ* gel forming ophthalmic drug delivery system for tropicamide, *Drug Delivery Technology*, (2007);5:44–49.
9. Gupta S. and Vyas S., Carbopol/chitosan based pH triggered *in-situ* gelling system for ocular delivery of timolol maleate, *Scientia Pharmaceutica*, (2010); 78(4):959–976.
10. Hemant K., Gupta M. and Gupta R., pH sensitive *in-situ* ocular gel: A review, *International Journal of Scientific Research and Reviews*, (2014); 3(2): 260-273.
11. Ikeagwu I., Amadi E.and Iroha I., Antibiotic sensitivity pattern of Staphylococcus aureus in Abakaliki, Nigeria, *Pakistan Journal of Medical Sciences.,(*2008); 24(2): 231-235.
12. Indu P., Manjit S. and Meenakshi K., Formulation and evaluation of ophthalmic preparations of acetazolamide, *International Journal of Pharmaceutics*, (2000);199:119–127.
13. Johan C., Katarina E., Roger P. and Katarina J., Rheological evaluation of gelrite *in situ* gel for opthalmic use, *European Journal of Pharmaceutical Sciences*, (1998);6:113–116.
14. Katarina E., Johan C. and Roger P., Rheological evaluation of poloxamer as an *in-situ* gel for ophthalmic use, *European Journal of Pharmaceutical Sciences*, (1998);6:105–112.
15. Kaur I., Garg A., Singla A. and Aggarwal D., Vesicular systems in ocular drug delivery an overview, *International Journal of Pharmaceutics*,(2004);269:1–14.
16. Mahesh A. and Nair B., Simultaneous determination of Ofloxacin and Cefixime by first and ratio first derivative UV spectrophotometry, *Chronicles of Young Scientists*, (2014); 2(3): 144-148.
17. Mohanambal E., Arun K. and Sathali A., Formulation and evaluation of pH-triggered *in- situ* gelling system of levofloxacin, *Indian Journal of Pharmaceutical Education and Research*, (2011);45(1):58–64.
18. Nagesh C., Patil M., Chandrashekhara S.and Sutar R., A novel *in-situ* gel for sustained ophthalmic delivery of ciprofloxacin hydrochloride and dexamethasone design and characterization, *Pharm. Letter*, (2012);4(3):821–827.
19. Pandit D., Bharathi A., Srinatha R. and Singh S., Long acting ophthalmic formulation of indomethacin, Evaluation of alginate gel systems, *Indian Journal of Pharmaceutical Sciences,(*2007);69:37–40.
20. Pawar P., Rawat, Mahajan, Galgatte and Maheshwari, Formulation, development and evaluation of aqueous injection of poorly soluble drug made by novel application of mixed solvency concept, *International Journal of Drug Delivery*, (2013);5(2):152-166.
21. Piyush B., Divyesh H., Pragna K., Shukla A. and Gaurang B. Design and characterization of ofloxacin mucoadhesive *in- situ* hydrogel, *African Journal of Pharmacy and Pharmacology*, (2012); 6(23): 1644-1652.
22. Sharma Jitendra P., Banik A. and Dixit S., A new trend: ocular drug delivery system, *Pharma Science Monitor: An International Journal of Pharmaceutical Sciences*, (2011); 2(3): 1-25.
23. Sireesha D., Prabha K. and Prasanna P., Advanced approaches and evaluation of ocular drug delivery system, *American Journal of Pharmatech Research*, (2011); 1(4): 72-92.
24. Srividya B., Cardoza R. and Amin P., Sustained ophthalmic delivery of ofloxacin from a pH triggered *in-situ* gelling system, *Journal of Control Release*, (2001);69:379–388.
25. The Indian Pharmacopoeia, The Indian Pharmacopoeia Commission: Ghaziabad, India, Published by Ministry of Health and Public Welfare, Government of India, New Delhi vol. 2, (2010), 1032.
26. Thimmasetty M., Mandal S., Prabhushankar G.L. and Geetha M., Formulation and evaluation of an *in-situ* gel of amoxicillin trihydrate, *International Journal of Pharmaceutical Investigation*, (2012);2(2):78–82.
27. Thorsteinn L. and Tomi J., Cyclodextrins in ocular drug delivery, Advanced *Drug Delivery Reviews*, (1999);36:59–78.
28. Verma L., Sakir M., Singh N., Mehra R. and Mehan S., Development of phase change solutions for ophthalmic drug delivery based on ion activated and pH induced polymers. *International Journal of Pharma Professional's Research.,* (2010);1(2):127-134.
29. Vijaya C., Goud K., Ion-activated *in-situ* gelling ophthalmic delivery systems of azithromycin, *Indian Journal of Pharmaceutical Sciences*,(2011);73(6):615–620.
30. Zhidong L., Jiawei L., Shufang N., Hui L., Pingtian D. and Weisan P., Study of an alginate/HPMC based *in-situ* gelling ophthalmic delivery system for gatifloxacin, *International Journal of Pharmaceutics*, (2006);315:12–17.