International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

Formulation and Evaluation of ion sensitive in situ ophthalmic gel of Moxifloxacin Hydrochloride

Abhishek*, Nitan Bharti Gupta
Department of Pharmaceutics, Sri Sai College of Pharmacy,BadhaniPathankot-145001, India

Date of Submission: 25-10-2021 Date of Acceptance: 03-10-2021

ABSTRACT: The aim of this study was to develop in situ gel of Moxifloxacin Hcl. The poor bioavailability and therapeutic response of conventional ophthalmic solutions due to short precorneal residence time and rapid elimination may be overcome by use of in situ gel forming system. It is instilled as eyedrop and later on they undergo sol to gel transition in cul-de-sac. Hence in situ gel by ion sensitive method was prepared using gelling agent sodium alginate and the viscosity increasing agents like HPMC K100M,CMC sodium and Xanthum gum to provide a sustained release . Suitable preservative like benzalkonium chloride was added. The in situ gels were further evaluated for clarity, pH, drug content, viscosity, gellation studies, sterility studies, Microbiological studies, In vitro diffusion studies, Ocular irritancy test. The results were highly agreeable showing in situ gels as an alternative in ophthalmic drug delivery showing improved bioavailability, increased precorneal residence time and providing sustained release upto 7 hours.

In situ gels should be easy to instill as eye drops and later on it gel in cul-de-sac forming in situ gel hence increasing contact time. The various approaches that are used in preparation of in situ gelling mechanism are as follows[4,5,6]

Ion activated gels are formed due to ions change. Various polymers like sodium alginate act as such ion activated gel. Gellan gum is another such polymer. They can be used alone or in combination with certain other viscosity increasing agents (HPMCK100M, CMC sodium, polyvinyl pyrrolidone, xanthum gum). Eyes have presence of Calcium ions and Magnesium ions which help in gelling of Sodium alginate[7].

The objective of the present study was to develop an ion activated in situ gelling for Moxifloxacin hydrochloride, used to treat external infections of the eye such as acute and sub acute bacterial conjunctivitis, conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers which can prevent frequent drug administration and enhance

Keywords: Moxifloxacin hcl, sodium alginate, gelling solution,in situ gel, ion sensitive, sustained release,sustained release.

I. INTRODUCTION

The field of Ocular drug delivery is one of most challenging endeavors facing pharmaceutical world. The physiological constraints offered by barriers of eye results in low absorption of drug.Various eye drops and eye suspensions are not that efficacious in terms of bioavailability The major loss is due to nasolachrhymal drainage. It is drained as soon as it is instilled into eyes. Thus one main concern is that there is systemic absorbtion instead of ocular absorption. Systemic absorption takes place from conjuctival sac via local blood capillaries. Ocular drug delivery can be significantly improved if residence time is increased. This problem can be overcome by use of insitu gels which can be instilled as eye drops and then they undergo sol to gel transition in cul-de-sac thereby increasing the contact time[1,2,3] patient compliance. Sodium alginate was used as the gelling agent in combination with(HPMCK100M, CMC sodium, Xanthum Gum) as the viscosity enhancer for the formulation of Moxifloxacin Hydrochloride eye drops which undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug.

II. MATERIALS AND METHODS

Moxifloxacin Hydrochloride was supplied as gift sample from Eden Drugs Pvt. Ltd, Amritsar. (Sodium alginate, Xanthum gum,CMC sodium), was purchased from S.D. Fine chemicals. HPMC K100M was purchased from Sigma Aldrich. All the chemicals were of analytical grade.

Preparation of insituophthalmiic gel:

1.Sodium alginate, HPMC K100M and xanthum gum was dissolved in 40ml distilled water.

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

2.Moxifloxacin Hcl was dissolved in 0.1N HCL. Further it was added to above solution under constant stirring. Required amount of Benzalkonium chloride was added.
3.Now distilled water was added to make the volume upto 100ml. The solution was then filtered using 0.45um filter paper. The formulations was filled in vials and sterilized in autoclave(121℃ at 15lb) for 20 mins.
Moxifloxacin hcl gel was formulated using excipients Table 1

Evaluation:
Clarity:Gels formed are checked for clarity against light under white and black backgrounds. For ophthalmic, Clarity is an issue as it should be clear[8].
Gelling Capacity: The gelling capacity is determined by placing 2ml of formulation in simulated tear fluid Table 2 and then noting time for gelation. Generally Simulated Tear Fluid consists of Calcium Chloride, Sodium Bicarbonate and Sodium chloride[9].
Drug Content: The drug content is determined by taking 1ml of sample and then diluting it with 50ml of STF maintained at buffer 7.4. Then further 5ml was taken and then again diluted with 50ml. Then samples were analysed at (295nm) using UV visible spectrophotometer[10].
Rheological Studies: Rheological studies of the formulation was carried out by using brookefield viscometer using spindle 61 at liquid formulations at (pH 5.5). Alternately T-bar spindle was used at (pH 7.4) in the gel form.The viscosity of the formulation was measured at different speeds (5, 6, 10, 12, 20, 30, 50 rpm)[11].
Sterility Studies: Ophthalmic preparations should be sterile. Direct inoculation method was used. Samples was taken out with sterile pipette or with sterile syringe and then aseptically transferred into the Fluid thioglycolate medium and soyabean casein digest medium and then incubated at (30-35℃) for 7 days for fluid thioglycolate medium and at (20-25℃) for soyabean casein digest medium[12].

Antimicrobial Studies: Antimicrobial efficiency studies were carried out to ascertain thebiological activity of sol-to-gel systems againstmicroorganisms. This was determined in the agar diffusion medium employing Cup platetechnique. Sterile solution of marketed Moxifloxacin Hcl eye dropswas used as a standard. The standard solution and the developedformulations (test solution) were taken into separate cups bored into sterile SCDM Agar previously seeded with organisms(Staphylococcus aureus and Pseudomonas aeruginosa). After allowing diffusion of solutions for two hours, the plates were incubated for 24hrs at 37℃. The zone of inhibition (ZOI) was compared with that ofthe standard (17, 19, 22). Each sample was tested in triplicate[13].
In vitro drug Release Studies: The in vitro drug release studies of moxifloxacin hydrochloride was studied through a cellophane membrane using modified (USP XIII) dissolution testing apparatus. The dissolution medium employed was artificial tear fluid prepared(7.4). Cellophane membrane used was soaked overnight in dissolution medium was tied to one end of glass cylinder (which was open at both ends and of 5cm diameter). An 1ml of the formulation was accurately pipette into the cylinder. The cylinder was then attached to metallic driveshaft and then suspended in 50 ml of the dissolution medium maintained at 37℃ and was stirred by magnetic stirrer at 50 rpm employing a magnetic stirrer. Aliquots each of 1ml volume was withdrawn after hourly intervals and then it was analysed at (290 nm) using Uv spectrophotometer[14].
Stability Studies: Then the formulation was subjected to stability studies. Sterlized formulations was filled in glass vials stoppered with grey butyl rubber closures sealed with aluminium caps. Short term accelerated stability studies was carried out for a period of 45 days. The samples were stored at room temperature and at elevated temperature such as 40℃ at 75% RH and refrigerator at(2-8)℃. Samples were withdrawn on weekly interval and analysed for drug content, visual appearance, clarity,pH[15].

III. RESULTS AND DISCUSSION

The appearances of all formulations were light yellow in color and were clear. Terminal sterilization by autoclaving had no effect on the formulations. The haziness observed during autoclaving due to precipitation of HPMCK100M at elevated temperature was found to disappear and the clarity was regained after overnight standing. The pH of all the formulations was found to be within the range of 6.0 to 6.5, which is desirable for the ophthalmic formulations.Table 3

The viscosity and gelling capacity plays important role for in situ gelling system. The formulation should have an optimum viscosity for easy instillation into the eye as a liquid which

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

undergo sol-to-gel transition. Prepared in situ gelling systems were evaluated for the in vitro gelation capacity. All the formulations gave satisfactory results.Table 4

The drug content of all the formulations was within the range of 97.45%(w/v) to100.43%(w/v).Table 5

The rheological study of the formulations exhibited decrease in viscosity on increase in shear rate because of the pseudoplastic behavior of the formulations. Moreover, the pseudoplastic property of these formulations may be in favor of sustaining the release of drug in the conjunctival sac of the eye.Table 6,7

All the prepared in situgelling systems were evaluated for the sterility. After 7 days of Incubation the results showed no microbial growth in all formulations.Table 8

The optimized in situ gelling formulations showed antimicrobial activity when tested microbiologically by the Cup-Plate technique. Clear zones of inhibition were obtained in all the formulations.Table 9

The in vitro release studies indicated that amongst all the formulations,(F-4,F-5, F-6) showed sustained drug release for 7hours(Figure 1), this may be due to higher concentration of sodium alginate alongwith polymers. The standard marketed moxifloxacin eye drops showed complete release within 3 hours whereas only 42% drug was released of in situ gel formulations.Table 10

The stability studies indicated that all the formulations was physically and chemically stable with no significant change in any of the parameters evaluated when stored at the ambient humidity conditions between (2-8℃), ambient temperature and 40℃ except for a slight decrease in the pH with time at 40℃. From stability studies it was observed that the in situgelling system of moxifloxacin was stable.

ACKNOWLEDGEMENTS

The authors thank Eden Drugs, Amritsar, for providing Moxifloxacin hydrochloride as gift sample for this work. They also thank Prof Preet Mohinder Singh Bedi, Pawan Sachdeva for providing facilities to carry out resaech work.

REFERENCES

[1]. Worakul N, Robinson J. Ocular pharmacokinetics and pharmacodynamics. Eur. J. Pharm. Biopharm1997; 44: 71-83

[2]. Jarvinen k, Jarvinen T, Urtti A. Ocular absorption following topical delivery. Adv. Drug Del. Rev 1995; 6: 3-19

[3]. Schoenwald T. Ocular drug delivery pharmacokinetic considerations.Clinical Pharmacokinetics 1990; 18: 255-269.

[4]. Middleton D, Leung S,Robinson R. Bioadhesive drug delivery Systems. CRC Press Boca Raton 1990; 21: 179-202.

[5]. Katrina E, Johan C, Roger P. Rheological evaluation of polaxaomer as an in situ gel for ophthalmic use . Eur J Pharm Sci 1998; 42:1056-12

[6]. Vidya B, Cardoza R,Amin P. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J control Release 2001;69:379-68

[7]. Patil A, Tagalpallewar A, Rasve G, Bendre A, Khapekar G. A novel ophthalmic Drug delivery system in situ gel.International Journal of Pharmaceutical Sciences and Research2012; 3(9): 2938-2946.

[8]. Padma J, Karthika K, Rekha N , Elshafie K. Formulation and evaluation of in situ ophthalmic gel of diclofenac sodium. J Chem Pharm Res 2010; 2(3):528-535.

[9]. Vodithala S, Khatry S, Shastri N, Sadanandam M. Development and evaluation of thermo reversible ocular gels of ketorolac tromethamine. Int J of Biopharm 2010; 1(1): 39-45.

[10]. Basavaraj K, Manjappa A, Murthy R,Yuvaraj D. A novel pH-triggered in situgel for sustained ophthalmic delivery of ketorolac tromethamine.Asian Journal of Pharmaceutical Sciences 2009; 4(3):189-199

[11]. Pandya T, Modasiya M,Patel V.Sustained ophthalmic delivery of Ciprofloxacin Hydrochloride from an ion-activated in situgelling system.Der Pharmacia Lettre 2011; 3(3): 404-410

[12]. Mondol S, Thimmashetty K,Prabhushankar L. Formulation and evaluation of in situ gelling ophthalmic formulation of moxifloxacin hcl. International Journal of Pharmaceutical Investigation 2012; 2:77-82

[13]. Khan M, Noorulla S, Muqtader M, Roshan S, Sadath A. Development of pH based phase change solutions for ophthalmic drug delivery of gatifloxacin. Int J Pharm 2013; 3(1): 66-72

[14]. Abraham S, Furtado S, Bharath S, Basavaraj B, Deveswaran R, Madhavan V. Sustained ophthalmic delivery of ofloxacinfrom an

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

ion-activated in situgelling system. Pak. J. Pharm. Science 2009;22(2):175-179

[15]. Nagargoje S,Phatak A, Bhingare C, Chaudhari S.Formulationand evaluation of ophthalmic delivery of fluconazole from ion activated in situgelling system.DerPharmacia Lettre 2012; 4(4):1228-1235

[16]. Rozier A, Manuel C, Groove J. A novel ion-activated in situgelling polymer for ophthalmic vehicles effect on bioavailability of timolol. Int. J. Pharm1989; 57: 163-168

[17]. Gao J, Ding P, Zhang L. Study of a pingyangmycin delivery system zein-saib in situgels. Int. J.Pharm.2007; 328: 57-64.

[18]. Pandit D, Bharathi A, Srinatha R. Long acting ophthalmic formulation of indomethacin evaluation of alginate gel systems. Indian J Pharma Sci2007; 69:37-40

TABLE 1: FORMULATION OF MOXIFLOXACIN HCLIN SITU OPHTHALMIC GEL

Ingredients%w/v	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Moxifloxacin Hcl	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Sodium alginate	1.0%	1.1%	1.0%	1.5%	1.5%	1.5%
CMC Sodium	0.5%	-	1.34gm	0.5%	-	-
Xanthum gum	-	0.5%	.4gm	-	0.5%	
HPMC K100M	-	-	.800gm	-	-	1.3%
Benzalkonium Chloride	0.02%	0.02	0.02%	0.02%	0.02%	0.02%
Distilled Water q.s. to	100ml	100ml	100m	100ml	100ml	100ml

Ingredients	**Quantity**
Sodium Chloride	1.34gm
Sodium Bicarbonate	.4gm
Calcium Chloride	.800gm
Distilled Water	Upto 200ml

TABLE 2: COMPOSITION OF SIMULATED TEAR FLUID

TABLE 3: CLARITY OF FORMULATIONS

Formulation Code	**Visual Appearance**	**Clarity**	**pH**
F1	Light Yellow	Clear	6.5
F2	Light Yellow	Clear	6.6
F3	Light Yellow	Clear	6.5
F4	Light Yellow	Clear	6.5
F5	Light Yellow	Clear	6.6
F6	Light yellow	Clear	6.8

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

TABLE 4: GELLING CAPACITY

Formulation	Gelling Capacity
F1	++
F2	+
F3	++
F4	+++
F5	+++
F6	+++

TABLE 5: DRUG CONTENT

Formulation	Drug Content
F1	97.4%
F2	98%
F3	99%
F4	98%
F5	99.67%
F6	98.40%

TABLE 6: VISCOSITY(IN CPS) BEFORE GELLING SPINDLE NO. 61

RPM	F1	F2	F3	F4	F5	F6
1	240	255	275	1100	1155	1230
1.5	232	223	252	1055	1095	1210
2	237	220	239	994	1015	1138
2.5	230	211	227	947	960	1088
3	230	210	223	860	890	1050
4	237	194	215	850	860	990

TABLE 7: VISCOSITY(IN CPS) AFTER GELLING SPINDLE NO. 61

RPM	F1	F2	F3	F4	F5	F6
1	390	404	450	1760	1850	1901
1.5	370	385	437	1590	1678	1722
2	356	371	422	1480	1550	1639
2.5	336	354	416	1390	1475	1555
3	316	339	411	1385	1390	1510
4	210	300	399	1370	1370	1460

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

TABLE 8: STERILITY TESTING

Formulation Code	Incubation Days						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-

TABLE 9: ANTIMICROBIAL STUDIES

Microorganism	Concenteration mcg/ml	Standard Pure Drug	F1	F2	F3	F4	F5	F6
Staphlyococcus aureus Gram+ve	2µg/ml	30	29	28	28	28	29	28
E.coli Gram-ve	2µg/ml	31	29	27	29	30	30	29

TABLE 10: IN VITRO DIFFUSION STUDIES

Time(Hr.)	F1	F2	F3	F4	F5	F6
1	25%	24%	23%	21%	19%	19%
2	75%	44%	43%	33%	31%	33%
3	96%	66%	66%	45%	49%	47%
4		84%	80%	60%	64%	61%
5		95%	96%	75%	77%	78%
6				90%	88%	85%
7				96%	98%	97%

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1214-1220www.ijprajournal.com ISSN: 2249-7781

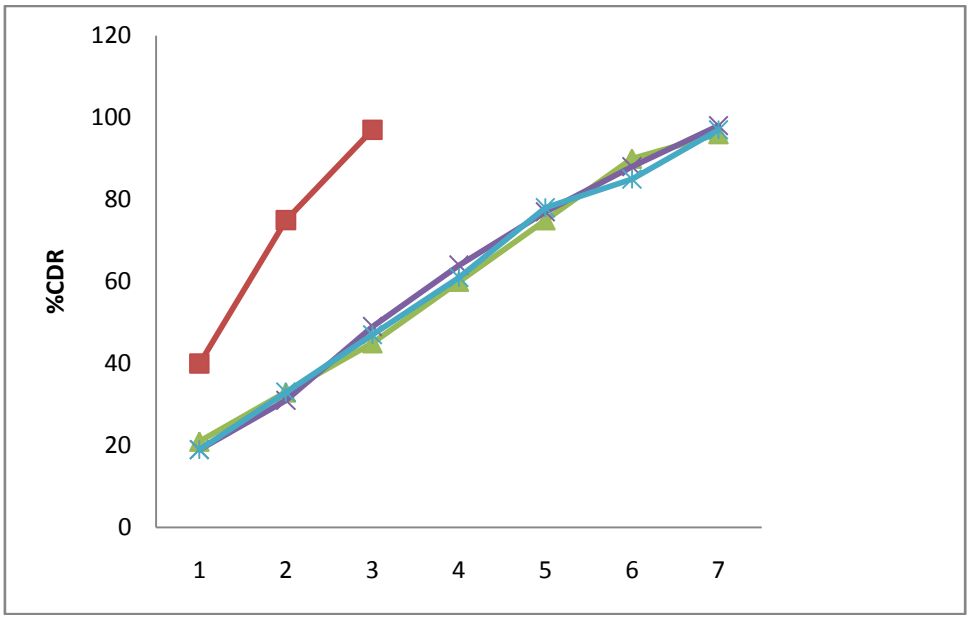

FIGURE 1: FORMULATION MARKETTED FORMULATION ( ),FORMULATION F-4(Δ), FORMULATION F-5 (-+-), FORMULATION F-6 (-*-)

TABLE 1: FORMULATION OF MOXIFLOXACIN HCL IN SITU OPHTHALMIC GEL
TABLE 2: COMPOSITION OF SIMULATED TEAR FLUID
TABLE 3: CLARITY OF FORMULATIONS
TABLE 4: GELLING CAPACITY
TABLE 5: DRUG CONTENT
TABLE 6: VISCOSITY(IN CPS) BEFORE GELLING SPINDLE NO. 61
TABLE 7: VISCOSITY(IN CPS) AFTER GELLING SPINDLE NO. 61
TABLE 8: STERILITY TESTING
TABLE 9: ANTIMICROBIAL STUDIES
TABLE 10: IN VITRO DIFFUSION STUDIES
FIGURE 1: FORMULATION MARKETTED FORMULATION ( ),FORMULATION F-4(Δ), FORMULATION F-5 (-+-), FORMULATION F-6 (-*-)