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Research Article

Therapeutic Efficacy of Moxifloxacin Mucoadhesive Hydrogel for Bacterial Keratitis

Nipuna Kumar Bhoi^{1*}, A. K. Singhai¹, Gaurav Jain¹, Subhendu S. Mishra²¹Lakshmi Narain College of Pharmacy, Bhopal, M.P, India²Kalinga Biomedical Research Institute & Training Establishment, Sambalpur, Odisha, India

ABSTRACT

Bacterial keratitis is a hypothetically devastating corneal infection due to the opportunity of fast development; corneal devastation either to be completed in 24–48 hours with even more contagious bacterial aetiological agents. Moxifloxacin mucoadhesive Hydrogel was prepared by using polymer Hydroxy Propyl Methyl Cellulose E50 LV by hydration method. Moxifloxacin was dissolved in small quantity of water and Benzalkonium Chloride was added to the Polymer solution. The formulations were evaluated for clarity, pH measurement, spread-ability test, drug content estimation, viscosity study, in vitro diffusion study and antibacterial activity. The developed formulation exhibits the sustained release over a period of 10 hour. The optimized formulation was further evaluated with antimicrobial activity. The results of the in-vitro antimicrobial activity of hydrogel were satisfactory.

Keywords: Corneal Infection, Hydrogel, Moxifloxacin, invitro release.

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*Address for Correspondence:

Mr. Nipuna Bhoi, M. Pharm Scholar, Lakshmi Narain College of Pharmacy, Bhopal, M.P, India

INTRODUCTION:

Keratitis, a spectrum of ocular diseases, affecting cornea and pathogenically resulting from bacterial, fungal, and protozoal etiologic organisms can potentially cause ocular morbidity and disability¹. Mostly corneal infection is reliant on three factors: the degree of pathogenicity of the etiologic organism, the underlying condition of the cornea, and the immunological state of the individual².

It is a condition where the cornea the clear, round dome layer. The eye's iris and the pupil become swollen or reddened and painful. It is also defined as a corneal ulcer. Contact lens wearers essential to evoke that infectious keratitis³⁻⁶. The numeral of multi-resistant CoNS (Coagulase-negative staphylococci) recognized in patients with lively ocular blemish has also sustained to upsurge in topical years⁷. The clear diagnosis after bacterial keratitis rest on the size, locality, and depth of the ulcer, as well as on the prospects and the bacteria remoteds⁸. Bacterial keratitis, due to its high rate and hypothetical hitches, is one of the extreme visual aggressive ocular infective pathologies. The avascular corneal stroma is especially vulnerable to bacterial infection. Corneal damages, which have been

particularly, invasive pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*⁹.

Mucoadhesion is thought to be caused by two mechanisms: the contact stage and the consolidation stage. Mucoadhesive that attaches to a mucus membrane may result in swelling of the formation. This condition leads to the spread of the formation along the surface of the mucosal layer. This may facilitate penetration of hydrogel inside mucosal layers¹⁰.

Moxifloxacin Mucoadhesive hydrogel drug delivery system can enhance the bioavailability of therapeutic agents by avoiding drug degradation. Hydrophobic and hydrophilic molecules can be integrated inside a core-shell assembly while the surface can be further functionalized by ligands and other molecules to increase its cellular attachment. This system is suitable for multidrug encapsulation. The physicochemical properties of mucoadhesive systems (e.g., swelling, expanding, and shrinking), are advantageous to reaction with mucosal layers, allowing hydrogel to be internalized in tissues¹¹⁻¹³.

The standard initial treatment consists of frequent instillation of eye drops containing a broad-spectrum antibiotic. However, poor retention of a drug (tear-out from

the ocular cavity) and poor ocular bioavailability of drugs in form of a drop has remained one of the greatest challenges to treating these clinical conditions. It has the greatest importance in providing sustained ocular drug delivery. By exhibiting elastic properties, they resist ocular drainage of the drug leading to longer contact times.

The objective of the present investigation to formulate in-situ gelling thermosensitive mucoadhesive hydrogel by an antibacterial agent, Moxifloxacin using a combination of Carbopol-934 with different mucoadhesive polymers such as HPMC and Sodium alginate to increase gel strength and adhesion force and thereby increased precorneal contact time and bioavailability of the drug.

MATERIALS AND METHODS:

Materials:

Moxifloxacin is a gift sample from Alkem pharmaceutical company, Sikkim (India). Carbopol – 934 was purchased from Alpha chemika Pvt. Ltd, Mumbai. HPMC E50LV was

purchased from Avra Synthesis Pvt. Ltd. Hyderabad. Sodium Alginate, Benzalkonium Chloride, Buffer capsule were procured from Research-lab fine chem industries Mumbai. Sodium hydroxide was purchased from HiMedia Laboratories Pvt. Ltd. of Mumbai.

Methods:

The formulation procedure for Moxifloxacin mucoadhesive Hydrogel drug delivery system was charted below. Formulation ingredients with their extents were as specified in table-1. The buffer salts were dissolved in 75 mL of purified water, Hydroxy Propyl Methyl Cellulose E50 LV was added & allowed for hydration, Carbopol – 934 were sprinkled over the solution and allowed for hydration overnight. The solution was stirred with an overhead stirrer with 1000 rpm. Moxifloxacin was dissolved in a small quantity of water and Benzalkonium Chloride was added to the Polymer solution. Then make up the volume up to 100 mL, then the solution was filtered through Whatman filter paper¹⁴.

Table:1-Composition of Mucoadhesive Hydrogel

Formulations	Moxifloxacin (g)	Carbopol-934 (g)	HPMC E50LV	Sodium Alginate	Benzalkonium Chloride	Sodium Hydroxide	Distilled water (ml)
F1	0.4	0.2	0.1	-	1	0.01	100
F2	0.4	0.3	0.2	-	1	0.01	100
F3	0.4	0.4	0.3	-	1	0.01	100
F4	0.4	0.2	0.4	-	1	0.01	100
F5	0.4	0.3	-	0.1	1	0.01	100
F6	0.4	0.4	-	0.2	1	0.01	100
F7	0.4	0.2	-	0.3	1	0.01	100
F8	0.4	0.3	-	0.4	1	0.01	100

Evaluation of Hydrogel

a) Clarity and Visual appearances:

Clarity and visual appearance can be determined by observing transparent or white particles against the black background & dark or black particles against the white background.

b) pH determination:

The pH of the gel formulations was determined by using a pH meter. For pH determination, 1% of hydrogel formulation in deionized water was prepared and pH was determined¹⁵.

c) Spread-ability Test:

Spreadability can be determined by applying the gel over an even surface and observed for the gritty nature of the hydrogel if present¹⁶.

d) Viscosity Study:

The viscosity of gels was determined by using a Brook Field viscometer DV-II model. T-Bar spindles in combination with a Heli path stand were used to measure the viscosity.

e) Drug content:

From the developed moxifloxacin hydrogel formulation, 1ml of suspension is a mixture in the 10 ml of 1.2 pH buffer. The amount of insulin was determined using a UV spectrophotometer at 272nm¹⁷.

f) Measurement of the gel strength:

The gel sample of 25 mL was put in a 50 mL graduated cylinder. A weight of 14.33 g was placed on the gel surface.

The gel strength, which is an expression for the ophthalmic gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel. All measurements were performed in triplicate (n=3).

g) In-vitro diffusion study:

An in-vitro drug release study was performed utilizing altered Franz diffusion cells. The dialysis layer (Hi-Media, Molecular weight 5000 Daltons) was put among receptor and donor compartments. In-situ gel proportional to 100 mg of moxifloxacin hydrogel was set in the donor compartment and the receptor compartment was loaded up with phosphate buffer, pH 7.4. The dispersion cells were kept up at 37±0.5°C with blending at 50 rpm all through the investigation. At various time interim, 5 ml of aliquots were pulled back from the recipient compartment through the side cylinder and examined for medication content by UV Visible spectrophotometer¹⁸.

Sterility study:

The well diffusion method was used to determine the antibacterial activity of the mucoadhesive hydrogel using the standard procedure. The drug used in standard preparation was Moxifloxacin of IP grade. The antibacterial activity was performed by using 24hr culture of *Staphylococcus aureus*. There was 3 concentration used which are 1%, 2% and 3% for antibiogram studies. The plates were incubated at 37°C for 24 hours and then examined for clear zones of inhibition around the wells impregnated with a particular concentration of the drug¹⁹.

RESULTS AND DISCUSSION:

The characterizations of the hydrogel, Physical appearance of the formulations were light white and clear. Table-2 shows the gelling capacity of all formulations, which is depicted as + (gel forms in 40 to 50 seconds and dissolves rapidly) and ++ (gel forms within 60 seconds and remains stable for 3 hours). The gelling capacity increases with increasing concentration of gelling agents both at a higher and lower concentration. The pH value of all the prepared formulations ranged from 6.9 ± 0.03 to 7.3 ± 0.06 , which is considered acceptable to avoid the risk of irritation upon application to the eye. The viscosity of the different formulations was compared as shown in Table-2. It was shown that the viscosity was an increase when the concentration of Carbopol increased. Eight formulations were evaluated for drug content and in vitro dissolution study. The drug content was in between 95.42 ± 0.04 to 98.46 ± 0.04 in different formulation. All the data was reported in table-2.

The invitro cumulative % drug release of the mucoadhesive hydrogel of moxifloxacin was 82.89 ± 0.049 % to 91.89 ± 0.021 % at the end of 10 h, respectively as shown in Table-3 and Fig-4. The kinetic release profile of hydrogel shows that formulation MHG6 was the best formulation and it follows the Higuchi release profile. In vitro release pattern was fitted to the Higuchi model Which recommends that Ocular in situ hydrogel released drug in a controlled release manner in 10 hours.

The antibacterial activity of the mucoadhesive hydrogel was assayed to determine the zone of inhibition against different bacteria. that antimicrobial activity of at three different concentrations 1%, 2%, and 3% by disc diffusion method. It indicated that the formulation displayed a variable degree of antimicrobial activity. The inhibitory effect increases when the increase of the formulation concentration.[Table – 5]

Table 2: Evaluation of Moxifloxacin in situ hydrogel Gel Formulations

Code	Clarity	pH	Spreadability (gm.cm/sec)	Viscosity (Cps)	Drug Content (%)	Gel strength
MHG1	Clear	7.3 ± 0.06	10.83 ± 0.75	6514 ± 1.25	96.61 ± 0.02	+
MHG2	Clear	6.9 ± 0.06	11.53 ± 0.42	5428 ± 0.83	95.42 ± 0.04	+
MHG3	Clear	7.1 ± 0.06	11.25 ± 0.56	5175 ± 1.82	98.15 ± 0.01	++
MHG4	Clear	7.2 ± 0.03	11.29 ± 0.81	7245 ± 2.34	96.51 ± 0.08	+
MHG5	Clear	7.1 ± 0.05	10.91 ± 0.36	5487 ± 0.71	98.14 ± 0.04	+
MHG6	Clear	6.9 ± 0.03	11.38 ± 0.62	6547 ± 1.15	97.45 ± 0.07	++
MHG7	Clear	7.3 ± 0.05	11.73 ± 0.56	5048 ± 2.48	98.46 ± 0.04	++
MHG8	Clear	7.3 ± 0.04	11.23 ± 0.82	7542 ± 1.15	96.76 ± 0.04	+

Table 3: In-vitro drug release study of moxifloxacin

Cumulative % of drug release								
Time (hour)	MHG1	MHG2	MHG3	MHG4	MHG5	MHG6	MHG7	MHG8
0	0	0	0	0	0	0	0	0
1	8.27 ± 0.028	7.46 ± 2.25	10.24 ± 0.037	9.82 ± 0.037	12.21 ± 0.077	10.05 ± 0.049	9.53 ± 0.045	13.54 ± 0.054
2	18.56 ± 0.015	20.56 ± 1.13	19.87 ± 0.046	18.56 ± 0.083	26.58 ± 0.014	25.45 ± 0.061	24.58 ± 0.052	23.56 ± 0.046
3	23.58 ± 0.011	32.25 ± 1.75	30.25 ± 0.075	28.87 ± 0.075	35.45 ± 0.073	34.47 ± 0.035	33.65 ± 0.068	32.45 ± 0.091
4	38.58 ± 0.015	42.56 ± 1.08	40.78 ± 0.019	38.78 ± 0.093	48.78 ± 0.093	47.98 ± 0.044	46.58 ± 0.042	45.58 ± 0.083
6	56.89 ± 0.018	55.48 ± 1.59	53.47 ± 0.078	51.15 ± 0.057	63.45 ± 0.051	62.14 ± 0.062	60.14 ± 0.073	60.01 ± 0.093
8	78.45 ± 0.022	72.45 ± 2.57	68.78 ± 0.045	65.56 ± 0.077	75.74 ± 0.046	74.56 ± 0.071	73.47 ± 0.072	72.56 ± 0.069
10	91.89 ± 0.021	89.45 ± 1.58	85.45 ± 0.067	83.98 ± 0.037	87.56 ± 0.043	85.85 ± 0.049	82.89 ± 0.049	83.89 ± 0.046

Table 4: Regression analysis data

Code	Zero Order	First Order	Higuchi's Model	Korsmeyer Peppas Equation
	R ²	R ²	R ²	R ²
MHG1	0.959	0.993	0.953	0.699
MHG2	0.968	0.983	0.968	0.706
MHG3	0.978	0.994	0.965	0.681
MHG4	0.968	0.981	0.951	0.653
MHG5	0.969	0.965	0.963	0.693
MHG6	0.973	0.979	0.978	0.705
MHG7	0.970	0.986	0.953	0.680
MHG8	0.977	0.991	0.962	0.702

Table 5: Zone of Inhibition of Formulated Hydrogel & Marketed in situ Hydrogel

S. No.	Product Details	Zone of inhibition by using <i>S. aureus</i>			
		0	1%	2%	3%
1.	Moxifloxacin in situ Hydrogel	0	9±0.57	10±0.28	12±0.47
2.	Marketed in situ Hydrogel	0	8±0.76	9±0.17	9±0.87

Fig-1 Viscosity of all formulation **Fig-2 Spreadability and pH** **Fig-3 drug content of all formulation** **Fig-4 In-vitro release study** **Fig-5 Zero-order release kinetic study** **Fig-6 First order Kinetic Study** **Fig-7 Higuchi Equation** **Fig-8 Korsmeyer-Peppas Equation**

[Moxifloxacin in situ Hydrogel]

[Marketed in situ Hydrogel]

Fig-9 Zone of Inhibition of Plate**CONCLUSION:**

Moxifloxacin Hydrochloride is a broad-spectrum fluoroquinolone antibiotic. Moxifloxacin binds besides inhibiting the bacterial enzymes' DNA gyrase (topoisomerase II) and topoisomerase IV resulting in inhibition of DNA replication, repair, and cell death in sensitive bacterial species. Hydrogel produced by mucoadhesive polymers has the advantage of being immobilized at the mucosal surface by an adhesion mechanism. The mucoadhesive action depends on the physical reaction of hydrogel moieties with the structure of mucosal content and results in the swelling and expansion of the hydrogel. The ophthalmic in situ hydrogel was formulated using Carbopol-934 (g), HPMC E50LV, and Sodium Alginate. The clarity of the prepared formulation was satisfactory. The pH was ranging between 6.9 ± 0.03 to 7.3 ± 0.06 . the drug content of the prepared formulation was satisfactory. The formulations of MHG1 to MHG8 showed sustained drug release for 10 hours. Formulation MHG6 showed the maximum sustained drug release. Based on drug content, pH, Spreadability, and In-vitro release data were found to be satisfactory. The results of the in-vitro antimicrobial activity of hydrogel were also satisfactory.

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Conflicts of Interest:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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