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R E S E A E C H A R T I C L E

Design and evaluation of Moxifloxacin microbeads by covalent cross - linking method

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A B S T R A C T

The aim of this study was to prepare novel ocular mucoadhesive microbeads of Moxifloxacin HCl to increase its residence time on the ocular surface and to enhance its therapeutic efficacy in ocular bacterial keratitis. Microbeads were fabricated with Microcrystalline cellulose (MCC) as polymer. Microbeads were evaluated for their particle size, surface morphology, encapsulation efficiency, FTIR, DSC and in vitro drug release studies. The average particle size of Microbeads was found to be less than 12.1 µm. MCC Microbeads were found to have a smoother surface. Entrapment efficiency was enhanced with an increased polymer concentration and viscosity. In vitro release of Moxifloxacin HCl from Microbeads was retarded with increased viscosity and concentration of polymers, and was controlled by diffusion as well as polymer relaxation. By comparing profiles of all the formulations, the formulation F6 showed the smallest particle size of 12.1 µm and also showed the controlled drug release of 8hrs. These optimized microbeads showing controlled drug release can be further incorporated into bioadhesive polymer to prepare ophthalmic gel. Controlled release with this formulation may reduce dose frequency and side effects as well as improve the patient compliance. As a result aesthetic appeal of the final formulation was improved.

Keywords: Microbeads, Moxifloxacin HCl, MCC.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Different strategies have been developed to increase the drug bioavailability by prolonging the contact time of the formulation with the corneal / conjunctival epithelium. One of the strategies is to develop ocular mucoadhesive systems to increase the residence time of the drug in the cul de sac. Different natural and synthetic mucoadhesive polymers interact with the precorneal mucin layer and show good potential to increase the bioavailability by increasing the precorneal residence time of the drug [1]. This will help to reduce the frequency of administration of the drug dose as well as improve patient compliance.

Topical application of drugs to the eye is the most popular and well-accepted route of administration for the treatment of various eye disorders. The bioavailability of ophthalmic drugs is very poor due to efficient protective mechanisms of the eye. Blinking, baseline as well as reflex lachrymation and nasolacrimal drainage, removes drug rapidly from the surface of the eye [2]. Whenever a drug is applied topically to the anterior segment of the eye, only a small amount (about 5%) actually penetrates the cornea and reaches the internal anterior tissue of the eye. Therefore, frequent instillation of eye drops is necessary to maintain a therapeutic drug level in the tear film or at the site of action. But the frequent use of highly concentrated solutions may induce toxic side effects and cellular damage at the ocular surface. It is one of the reasons of non-compliance and failure of therapy. To enhance the amount of active substance reaching the target tissue to exert a local effect in the cul de sac, the residence time of the drug in the tear film should be lengthened [3].

Moxifloxacin HCl is a fourth-generation fluoroquinolone, having broad-spectrum antibiotic activity, with efficacy against various Gram-positive and Gram-negative microorganisms including Staphylococci, S. pneumoniae, members of the family Enterobacteriaceae, P. aeruginosa, H. influenza, and Moraxellaspecies, through the inhibition of DNA gyrase and topoisomerase IV. It is commonly used to treat ocular infections and is a pre- and post-operative prophylactic agent in intraocular surgery to prevent endophthalmitis. It has superior corneal and aqueous penetration ability leading to higher therapeutic levels, more effective antimicrobial activity, and better clinical outcomes [8]. Studies revealed that it has improved activity against Gram-positive, atypical, and Gram-negative organisms compared to second- and third-generation fluoroquinolones [4-7] (i.e. ofloxacin, ciprofloxacin, levofloxacin). It is available as drops (0.5% w/v) for ophthalmic use, and its FDA-approved dosing regimen for the treatment of acute bacterial conjunctivitis is one drop twice a day for seven days. In these formulations, the dosing frequency is quite high. To reduce the dosing frequency and to increase its precorneal residence time, an ocular mucoadhesive system is required. Literature was reviewed for Moxifloxacin HCl ocular dosage forms; only in situ hydrogel systems [9, 10] were prepared previously. In this study, a successful attempt was made to formulate and evaluate more efficacious ocular mucoadhesive microspheres of Moxifloxacin HCl.

2. Materials and Methods

Materials:

Moxifloxacin Hydrochloride was gifted by Orex Pharma, Dombivali India. The polymer Microcrystalline cellulose was procured from the S D Fine chemicals. All the other reagents used were of analytical grade.

Preparation of microbeads:

The solution of microcrystalline cellulose (total polymer concentration 3 % w/v) was prepared homogeneously using a magnetic stirrer [11]. An accurately weighed quantity of Moxifloxacin was added to the above solution and mixed uniformly. 20 ml of the solution was extruded in the form of droplets into aqueous solution of $CaCl_2$ solution using 25 ml hypodermic syringe through a needle (number 23) under constant stirring. After incubating for additional 15 minutes in $CaCl_2$ solution, the beads were removed and dried at 40^{o}C for 10 h. Further, the beads were placed in a solution containing different concentrations of glutaraldehyde and 1N HCl for 30 min at 50 ^{o}C. Then the beads were removed and washed with distilled water. The beads were dried at 40^{o}C for 10hrs and stored in a closed container. The formulation details are given in Table 1.

A) Measurement of microbeads size by optical microscopy:

The particle size of the microbeads was determined by an optical microscope fitted with an ocular micrometer. The ocular micrometer was calibrated previously with the stage micrometer. The microbeads were mounted in liquid paraffin and a diameter of 50 microbeads was measured randomly by the optical microscope [12].

B) Estimation of drug entrapment efficiency (DEE):

Known amount of microbeads were added to 100 ml USP phosphate buffer of pH 6.8 for complete swelling at 37 ^{o}C. The beads were crushed in a glass mortar with pestle, the solution was then heated gently for 2 h to extract the drug completely and centrifuged to remove polymeric debris [13]. The clear supernatant solution was analyzed for drug content using UV-visible spectrophotometer at 289 nm.

C) Swelling study:

The dynamic swelling behavior of the microbeads was studied by the 50 mg of beads were incubated with 25 ml phosphate buffer solution pH 6.8 at 37^{o}C. The beads were taken out at different time intervals and blotted carefully without pressing hard to remove the excess surface liquid. The swollen beads were weighed using the electronic microbalance. The percent water uptake (Q) at different time intervals was calculated [14].

D) In-vitro drug release study:

In-vitro drug release study was carried out using a rotating dissolution tester USP XIV-II. The dissolution was measured at 37.0 ± 0.5^{o}C and 50 rpm speed. The dissolution medium consists of simulated intestinal fluid (pH 7.4

phosphate buffer) for 8 hours. At predetermined time 5ml aliquots containing the micropellets in the apparatus were removed every 30mins and an equivalent amount of fresh dissolution equilibrated at the same temperature was replaced. The amount of drug released was analysed at 289nm spectrophotometrically [15].

E) Data analysis (curve fitting analysis):

To analyze the mechanism of the drug release kinetics of the dosage form, the data obtained were fitted to various kinetic equations of zero order, first order, Higuchi model and Korsmeyer - peppas model and plotted as [16]:

1. Cumulative percent drug released Vs time (Zero order plots)
2. Log cumulative percent drug remaining Vs time(First order plots)
3. cumulative percent drug release Vs square root of time(Higuchi plots)
4. log cumulative percent drug release *Vs* log time(Korsmeyer-Peppas Plots)

Zero order kinetics:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q_0 - Q_t = K_0 t$$

Where, Q_t is the amount of drug dissolved in time *t,*

Q_0 is the initial amount of drug in the solution

(most times, $Q_0 = 0$)

K_0 is the zero order release constant expressed

in unitsof concentration/time.

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with slope equal to K_0.

First order kinetics:

The release of the drug which followed first order kinetics can be expressed by the equation:

$$\log C = \log C_0 - K_t / 2.303$$

Where, C_0 is the initial concentration of drug,

k is the first order rate constant

t is the time.

The data obtained are plotted as log cumulative percentage of drug remaining *vs*. time which would yield a straight line with a slope of -K/2.303.

Higuchi model:

The release of the drug which follows higuchi kinetics can be expressed by the equation:

$$Q = K_H * t_{1/2}$$

Where, K_H is the Higuchi dissolution constant

Q is the amount of drug released in time t

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Korsmeyer-Peppas model:

To find out the mechanism of drug release, drug release data were fitted in Korsmeyer-Peppas equation which is expressed as:

$$Q/Q_0 = k t^n$$

Where, K_0 to K_2 were release rate constants

Q/Q_0 was fraction of drug released at time t,

K was constant and n was diffusion constant that indicates general operating release mechanism. For Fickian (diffusion controlled) n ≤0.5; for non Fickian (anomalous/zero order) release 'n' value is in between 0.5 to 1.0; for zero order release n=1.0; for super case transport II, n >1.0.

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release *versus* log time

F) Scanning electron microscopic studies (SEM):

The microbeads were mounted onto stubs using double sided adhesive tape and sputter coated with platinum using a sputter coater (Edward S 150, UK). The coated beads were observed under SEM (JEOL, JSM-6360, Kyoto, Japanatthe required magnification at room temperature. The acceleration voltage used was 10 kV with the secondary electron image as a detector [17].

G) Fourier transmitted infrared spectroscopy (FTIR):

The samples were crushed with KBr to make pellets under hydraulic pressure of 600 kg, and then the FTIR spectra were recorded between 400 and 4000 cm^{-1}.

H) Differential scanning calorimetric analysis (DSC):

The sample were heated from 0-3000C at heating rate of 100C/min underARGON atmosphere using a micrometer (DSC Q20 V24.4 Build 116, TAInstruments, USA) and then thermo grams were obtained [18].

3. Results and Discussions

The size of the beads was determined using digital micrometer and recorded in Table 2. The average bead size was found to be in the range of 171 to 250µm. It was noticed that as the concentration of glutaraldehyde was increased, the bead size decreases. Whereas, by increasing the microcrystalline cellulose concentrations in the beads, an increase in size of the beads was observed, also, by increasing the amount of drug, an increase in size of the beads was observed.Percentage yield was found to be in range of 45% to 65%. Maximum percentage yield was found to be 65% for F6 formulation. Percentage yield increases with increase in concentration of the polymer added to formulation.

The drug entrapment efficiency (DEE) of the prepared microbeads was studied, and the results are summarized in Table 2.The drug entrapment efficiency was found to be in the range of 63- 90 %. The DEE was increased as the concentration of $CaCl_2$ was increased in the beads. The swelling study of the prepared microbeads was carried out in phosphate buffer pH 6.8 and the results are shown in Table: 3 &Figure: 1. The swelling behavior of beads was expressed as the ratio of initial weight of beads to the final weight of swollen beads as a function of time. The swelling of microbeads depends upon the concentrations of microcrystalline cellulose and glutaraldehyde in the beads. The swelling of the beads increased with an increasing amount of microcrystalline cellulose in the beads and swelling decreased with an increasing amount of glutaraldehyde.

The in-vitro drug release study was performed using dissolution rate test apparatus in phosphate buffer pH 7.4). The dissolution profiles of Moxifloxacin are given in Figure 2.The in-vitro drug release studies of different formulations

cumulative percentage drug release was observed in the range of 82% to 65%. The formulations F1, F2, F3, F4, F5, F6 containing microcrystalline cellulose respectively showed a release of 82%, 80%, 75%, 73%, 68% and 65% after 8 hours. This shows that more sustained release was observed with the increase in percentage of microcrystalline cellulose. The best formulation was observed as F6, by the observation of all results of the six formulations Moxifloxacin microbeads.

Data analysis (curve fitting analysis):

In order to know the mechanism of drug release from ocular mucoadhesive microspheres of Moxifloxacin HCl, data of *in vitro* release studies were extrapolated by the zero order, first order, Higuchi's and Korsmeyer-Peppas. The applicability of all of these equations was tested and summarized in Table. 4. The rate constants were also calculated from the slope of the plot of the respective models. From the dissolution data of all of the formulations when fitted in accordance with Higuchi's square root equation, a linear relationship was obtained with an 'r' (correlation coefficient) value close to unity (0.9253 to 0.9789) and higher than 'r' obtained from the zero order equation (0.9276 to 0.9902) and the first order equation (0.6728 to 0.8769). To find out the exact mechanism, dissolution data of all formulations were fitted in the Korsmeyer-Peppas equation. All formulations showed good linearity (0.8754 to 0.9509), with slope (n) values ranging from 0.445 to 0.725. The zero order rate equation describes the system, where release rate is independent of the concentration of the dissolved species. The first order equation describes the release from the systems where dissolution rate is dependent on the concentration of the dissolving species. The rate laws predicted by the different mechanisms of dissolution both alone and in combination, have been discussed by Higuchi. 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. All formulations were best fitted in the Higuchi equation, indicating diffusion to be the predominant mechanism of drug release.

Scanning Electron Microscopy of Moxifloxacin Microbeads:

The obtained beads were spherical in shape and freely flowing. The surface morphology was examined by scanning electron microscopy studies (SEM). The SEM microphotographs of beads showed that the prepared beads are spherical, having smooth surface along with surface foldings (Figure: 3). The figure tells that range of particle falls between 5.63µm to 16.3µm. hence all the particles are in micron range.

FT-IR Studies:

FTIR spectra of moxifloxacin hydrochloride (**Figure: 6& 7**) showed aromatic C=C stretching at 1621, 1515 and 1454 cm^{-1} and C–H bending for substituted benzene at 873 cm^{-1}. Besides, spectra also showed carboxylic acid C=O stretching at 1705 cm^{-1}, C–N stretching at 1350 cm^{-1}, stretching of monofluorobenzene at 1183 cm^{-1}. Spectra of a physical mixture of the drug and MCC showed peaks at 3520 cm^{-1}due to stretching of the hydroxyl group, 2920 cm^{-1} due to C–H stretching and 1023 cm^{-1} due to C–O–C stretching (which appears to be contributed by MCC), along with drug peaks. All the coated inserts showed aromatic C=C stretching at usual positions, indicating incorporation of moxifloxacin and peaks for ester at 1730 cm^{-1}, since acrylate polymers are esters. Major characteristic peaks of Moxifloxacin were found in the entire coated ocular insert, confirming the presence of the drug in the polymer without interaction.

DSC analysis:

The DSC analysis of plain moxifloxacin, drug- polymer microcrystalline cellulose physical mixture and drug-loaded beads was carried out and the results are shown in Figure: 8. The plain moxifloxacin has shown a sharp endothermic peak at 240^{o}C, due to melting of the drug, this peak is seen in the drug-loaded beads. The polymer microcrystalline cellulose has shown an endothermic peak at 265^{o}C indicating melting temperature of the polymer, whereas drug-loaded beads showed an endothermic peak at 165^{o}C and 255^{o}C. This indicates that the drug was uniformly dispersed in an amorphous state in the polymer matrix.

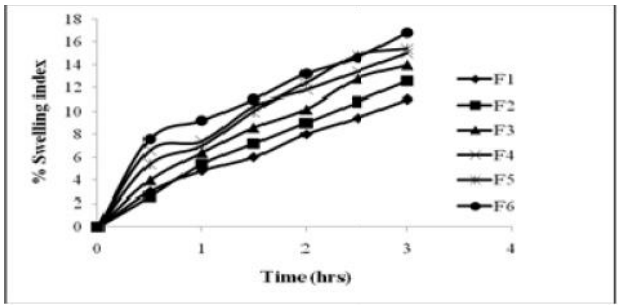

Fig 1: Swelling Behaviour of Moxifloxacin Microbeads in Phosphate Buffer pH 6.8

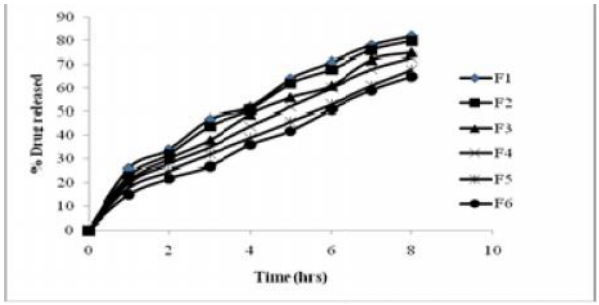

Fig 2: In-vitro release profile of moxifloxacin from prepared microbeads in phosphate buffer pH 7.4

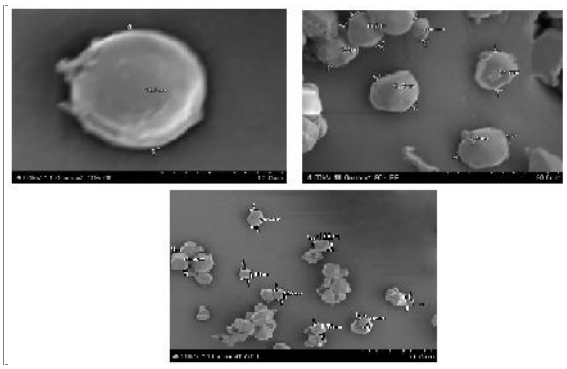

Fig 3: SEM Photographs of Optimised Formulation

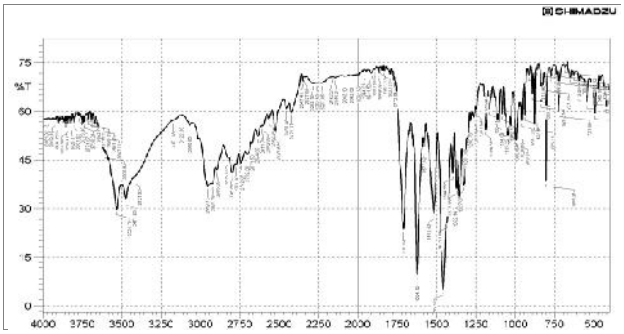

Fig 4:FT-IR Spectrum of Pure Drug Moxifloxacin

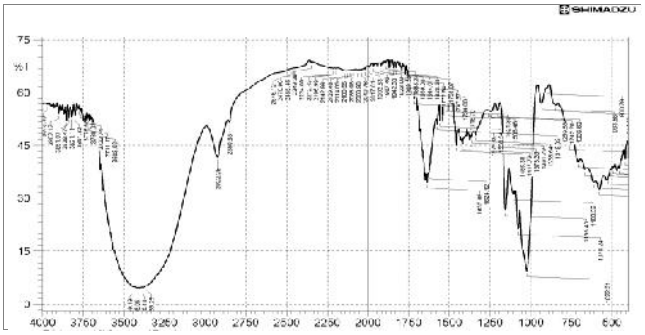

Fig 5: FT-IR spectrum of Moxifloxacin microbeads

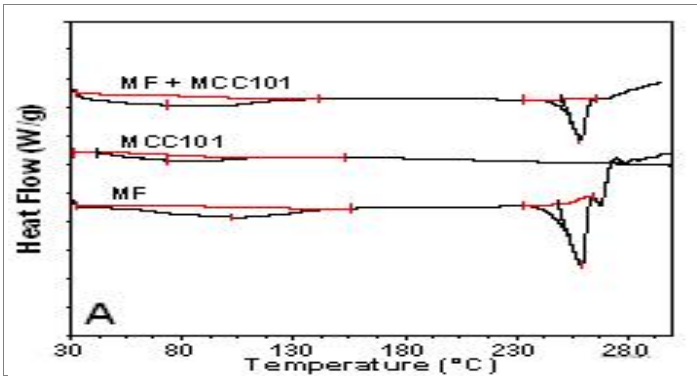

Fig 6:DSC spectrum of drug, polymer and formulation

Table 1: Formulation Document of Moxifloxacin Microbeads

Ingredients (gm)	F1	F2	F3	F4	F5	F6
Moxifloxacin HCl	0.5	0.5	0.5	0.5	0.5	0.5
MCC	0.5	1.0	1.5	2.0	2.5	3.0
$CaCl_2$	3	3	3	4	4	5
Glutaraldehyde	5	4	4	3	3	3

Table 2: Particle size, percentage yield and entrapment efficiency (DEE) of moxifloxacin microbeads

Formulation code	Particle Size (µm)	Percentage yield (%)	Drug Entrapment Efficiency (%)
F1	182	45	63
F2	171	47.5	67
F3	190.72	50.1	73
F4	245.91	54.8	83
F5	250	63	85
F6	234	65	90

Table 3: Swelling data of microbeads in Phosphate Buffer pH 6.8

Time (hrs)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	3	2.6	4	6.6	5.4	7.6
1.0	4.8	5.4	6.4	7.4	7	9.2
1.5	6	7.2	8.6	10.4	10	11
2.0	8	9	10.2	11.8	12.4	13.2
2.5	9.4	10.8	12.8	13.4	14.8	14.6
3.0	11	12.6	14	15	15.4	16.8

Table 4: Model fitting data of release profile for formulations F1 to F6

Formulation code	Zero order	First order	Higuchi model	Korsemeyer peppas model	
				r^2	n
F1	0.9672	0.8653	0.9253	0.8754	0.445
F2	0.9356	0.7842	0.9532	0.8867	0.526
F3	0.9276	0.6728	0.9432	0.8965	0.654
F4	0.9789	0.8245	0.9576	0.9233	0.725
F5	0.9846	0.7463	0.9657	0.9463	0.716
F6	0.9902	0.8769	0.9789	0.9509	0.503

4. Conclusion

The Moxifloxacin loaded MCC microbeads were prepared successfully. The microbeads were pale yellow in color, spherical; smooth surfaced, free flowing with rigid morphology. Microbeads showed high percentage yield in the range of 45-65%. The drug loaded microbeads showed 63- 90% of entrapment and in-vitro drug release up to 8 hrs. The differential scanning calorimetry thermographs showed stable character of drug in the drug-loaded microbeads and revealed the absence of drug-polymer interactions. The best-fit release kinetics was achieved with Higuchi plot and followed Higuchi model of matrix diffusion controlled drug release. By analyzing all the evaluation parameters of F1-F6 batches it was observed that batch F6 was better in terms of particle size 12.1µm , drug content of 65%, entrapment efficiency of 90% and in-vitro drug release up to 8 hrs. As microspheres are intended for ocular drug delivery and eye being a very sensitive organ the large particles could cause irritation. Thus the particle size of microbeads is a critical parameter. F6 batch showed the smallest particle size of 12.1 µm and also showed the controlled drug release of 8hrs. These optimized microbeads showing controlled drug release can be further incorporated into bioadhesive polymer to prepare ophthalmic gel. Controlled release with this formulation may reduce dose frequency and side effects as well as improve the patient compliance. As a result aesthetic appeal of the final formulation was improved.

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