

Original Article

DESIGN AND EVALUATION OF MODIFIED CHITOSAN BASED *IN SITU* GEL FOR OCULAR DRUG DELIVERY

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ABSTRACT

Objective: The object of the present study was to formulate and evaluate *in-situ* gel of modified chitosan by using temperature triggered method to improve bioavailability.

Methods: Modified chitosan-based moxifloxacin HCl was prepared by cold method. polaxomer 407 adding in distilled water and this solution kept in the refrigerator. Modified chitosan and moxifloxacin HCl was dissolved separately in distilled water and added to the polymeric solution with continuous stirring until thoroughly mixed. Prepared formulation was evaluated for drug content, gelling capacity, rheological study, *in vitro* drug release behavior, measurement of phase change temperature, antibacterial study, release kinetics, statistical analysis.

Results: The prepared formulations were evaluated for their, drug content, gelation temperature, *in vitro* drug release studies, rheological study and release kinetics. All batches of *in situ* formulations had satisfactory pH ranging from 6.2±0.2, drug content between 98.8±0.2 showing uniform distribution of drug. As the concentration of each polymeric component was increased, there was a decrease in phase change temperature. The *in vitro* drug release decreased with increase in polymeric concentrations. The antibacterial efficiency of the selected formulation against *staphylococcus aureus* confirmed that designed formulation has prolonged effect and retained its properties against bacterial infection.

Conclusion: The prepared *in situ* gelling formulation had the appropriate combination of polaxomer 407 and modified chitosan were suitable satisfactorily sustained the drug release from moxifloxacin HCl *in situ* gel. The prepared formulation of moxifloxacin HCl appears to be promising drug delivery for bacterial infectious disease.

Keywords: Ocular drug delivery, *In situ* gel, Modified chitosan, Polaxomer 407, Moxifloxacin HCl

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INTRODUCTION

The current situation of ophthalmic drug delivery is highly competitive and rapidly developing. The conventional ocular drug delivery systems like solutions, suspensions and ointments have drawbacks such as increased precorneal elimination, high changeability and blurred vision, low residence time. Poor bioavailability of drugs from ocular dosage forms is mainly due to tear production, transient residence time and impermeability of corneal epithelium [1]. Several *in situ* gelling system have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. These systems consist of polymers that exhibit sol to gel phase transitions due to change in specific physicochemical parameters (pH, temperature) in their environment, the cul-de-sac in this case [2]. Chitosan is a biodegradable polymer that has demonstrated excellent ocular compatibility. Chitosan solutions have been successfully used in prolonging contact time with the ocular surface. Therefore, a combination of this polymer with poloxamer would be very promising for ocular administration, as the *in situ* mechanical strength of the formulation would be higher than that of both polymers alone [3]. The aim of the present work was to obtain an ophthalmic delivery system with improved retention time for the treatment of ocular diseases.

MATERIALS AND METHODS

Materials

Moxifloxacin HCl was received as gift sample from Emcure Pharmaceutical Hinjavadi, Pune. Modified chitosan was purchased from Sea Foods Kochi, India. Polaxomer 407 was purchased from S D Lab Chem, Mumbai. All other chemicals used were of analytical grade. Freeze-dried strains *staphylococcus aureus* was obtained from Yashvantrao Chavan Institute of Science, Satara.

Methods

Preparation of *in Situ* gel formulation

In situ gel was prepared by the cold method. A weighed amount of polaxomer 407 was slowly added to 15 ml distilled water (at 4±2 °C) in a beaker with continuous stirring using a magnetic stirrer at a speed of 500 rpm for 2 h. The temperature of the water was maintained at 4±2 °C throughout the preparation. This solution was kept overnight in a refrigerator at 4 °C results in clear solution. Modified chitosan was initially dissolved in distilled water and it is then kept in the refrigerator and then it is added to the above solution. Moxifloxacin HCl was weighed separately and dissolved in distilled water and added to the polymeric solution with continuous stirring until thoroughly mixed. Benzalkonium chloride (0.01%) was added as a preservative. Finally, the volume was adjusted with distilled water up to 50 ml. The composition of the *in situ* gel formulations is shown in table 1 [4, 5].

Evaluation

Drug content

Drug content of moxifloxacin HCl was determined by taking 1 ml sample of *in situ* gel into 100 ml volumetric flask and diluting with 100 ml of simulated tear fluid then analyzed using UV-visible spectrometer at 287 nm [6].

Determination of pH

The pH of the gel was determined using calibrated pH meter. Determinations were carried out in triplicate and an average of these determinations was taken as the pH of the gel [7].

Measurement of phase change temperature

The gelation temperature was determined by placing the 2 ml cold sample solution in the test tube was dipped in a water bath whose

temperature was maintained at 37 ± 5 °C for 2 min. The temperature at which solution was converted to gel was noted down by placing the thermometer in the test tube. The maximum limit for gelation

was checked up to 60 °C. The gel was said to have formed when there was no flow of the formulation. Each sample was measured in triplicate [8].

Table 1: Composition of *in situ* gel formulations

Formulation code	Drug(g)	Polaxomer(g)	Modified chitosan(g)	Distilled water(ml)
F1	0.250	7	0.25	50
F2	0.250	7	0.5	50
F3	0.250	7	0.75	50
F4	0.250	8	0.25	50
F5	0.250	8	0.5	50
F6	0.250	8	0.75	50
F7	0.250	9	0.25	50
F8	0.250	9	0.5	50
F9	0.250	9	0.75	50

Number of experiments=9

Determination of gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling was noted [9].

Rheological studies

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed to gel at physiological temperature and then viscosity was measured at 37 °C using Brookfield viscometer model in spindle number 62 at 100 rpm. The average of three determinations was taken [10].

In vitro drug release study

The dialysis bag method was used for *in vitro* studies. The *In vitro* release rate of moxifloxacin HCl from sustained release *in situ* gel was performed in USP apparatus paddle method with STF (pH 7.4) as dissolution medium by using 2 cm diameter cylinder test tubes open at both ends for placing test sample under study. Dialysis membrane previously soaked for 8 h in STF (simulated tear fluid pH 7.4) was tied to one end of the glass cylinder and 2 ml volume of the formulation was accurately weighed and placed. The glass cylinder was attached to the shaft of USP apparatus, in the place of the paddle. The cylinder was suspended in 100 ml of dissolution medium and the stirring rate was maintained at 50 rpm. The samples were withdrawn from release media at intervals of 1, 2, 3, 4, 5, 6, 7, 8 and 9 replaced with an equal volume of fresh STF. The concentration of moxifloxacin HCl was determined by UV spectrophotometer at 287 nm. The percentage of cumulative release was calculated and a graph of percent cumulative release against time was plotted [11].

Antibacterial study

The sustained release *in situ* gel of moxifloxacin HCl was subjected to antibacterial studies by agar cup plate method. A sterile solution of marketed moxifloxacin HCl eye drops (0.2 mg/ml) was used as a standard. The strain of *staphylococcus aureus* was obtained from the department of microbiology Yashvantrao Chavan Institute of Science, Satara. This study was performed using *staphylococcus aureus* for antibacterial studies. Three samples tested for minimum inhibitory concentration, and they are standard, modified chitosan and F5 formulation. The nutrient agar media (25 ml) was prepared and sterilized by autoclaving at 121 °C, 15 lbs pressure for 15 min. immediately 25 ml of the agar suspension was poured into petriplate. After solidification of the media, the seeded test organism spread on the solid media using a spreader. Three cups of 5 mm diameter were made using sterile borer. The aliquot test samples were poured to cup aseptically and labeled accordingly. After allowing diffusion of the solution for 2 h, the plate was incubated at 37 °C for 24 h. The diameters for *Staphylococcus aureus* were measured after 24 h respectively [12].

Drug release kinetics

Drug release kinetics was studied by curve fitting method to different kinetic models of zero order, first order, Higuchi and Hixson Crowell models to find out the mechanism of drug release, 60 % drug of release data was first fitted in the *Korsmeyer-Peppas model* and the value of 'n' the diffusion exponent was determined. According to this model if 'n' is b/w 0.45 to 0.5 the Fickian mechanism, 0.5 to 0.8 the Non-Fickian and if 0.8 to 1.0 Case-II transport i.e. a zero-order mechanism is governing the drug release mechanism from the gels [13].

Statistical analysis

All values presented in the study were an average of triplicate experiments for the same time points. Differences in viscosity *in vitro* drug release profile of moxifloxacin HCl *in situ* gel under different conditions were tested statistically using one-way analysis of variance (ANOVA) followed by Dunnett's test at the different level of significance. (†Statistically significant difference at $P < 0.05$; ††statistically significant difference at $P < 0.01$; †††statistically significant difference at $P < 0.001$ from control) [14, 15].

RESULTS AND DISCUSSION

Drug content

The drug content of the sample was the range from 90% to 98% (table 2). The result indicated that the process employed to prepare gels in this study was capable of producing gels with uniform drug content.

pH

All the formulation should have satisfactory pH ranging from 5.8 to 6.2 which is acceptable for ocular drug delivery (table 2).

Measurement of phase change temperature

The two main requirements of an *in situ* gelling system are viscosity and gelling capacity. The optimum thermosensitive *in situ* gel should have a gelation temperature higher than room temperature and a shift to gel at the conjunctival sac temperature (35.0 °C) after mixed with artificial tears. Based on the suitable range of gelation temperature (25-35 °C), polaxomer chosen as the gelling agent. Polaxomer 407 is reported to be the less toxic of the commercially available polymer. It was found that increase in the concentration of polaxomer decreases the phase transition temperature. Modified chitosan in the range of 0.25, 0.5 and 0.75% did not affect the gelation temperature of formulation but it forms viscous formulation. The gelation temperature for the formulations was found between of 25.63 ± 0.14 °C to 38.33 ± 0.71 °C. The minimum gelation temperature was observed for batch F9 that is 25.63 ± 0.14 °C (table 2). This might be an effect of higher concentration of polaxomer 407 (9%) and with combination modified chitosan (0.75%). In order to obtain an *in-situ* gel having increased muco-adhesiveness when modified chitosan was added into the formulation [8].

Gelling capacity

The formulation should have an optimum gelling capacity, so that after installation into the eye as a liquid (drops), it would undergo a rapid sol-to-gel transition and would preserve its integrity without dissolving or eroding for a prolonged period of time. Gelling capacity increases with increasing concentration of gelling agent both at higher and lower concentration of viscofying agent. Gelling capacity

observed by visual observation. The gelation capacity data of prepared formulation presented in table 2 represent that the formulation F1, F2, F3 and F4 had gel after few minutes and dissolves rapidly. F5 and F6 had immediate gelation and underwent rapid dissolution. On the other hand the formulations F7, F8 and F9 had immediate gelation but exist for an hour [19]. This indirect that by increasing the concentration of polymer, transition time was decreased and the erosion time of formed gel was increased.

Table 2: Shows evaluation of *in situ* gel formulation

Formulation code	pH	Drug content %	Gelation temperature (°C)	Gelling capacity	Viscosity (cps)
F1	5.8±0.01	94.2±0.6	38.33±0.71	+	264.3±5.9
F2	6.2±0.01	95.1±1.04	37.22±0.56	+	281.3±3.71
F3	6.1±0.4	92.1±0.33	37.5±0.22	+	354±7.51
F4	6.0±0.11	95.1±0.12	36.80±0.75	+	426.3±7.84
F5	6.2±0.2	98.8±0.2	32.0±0.51	++	444.6±10.3
F6	5.8±0.12	94.6±0.1	30.67±0.72	++	568±11.4
F7	5.9±0.1	92.4±0.14	29.41±0.73	+++	772±10.5
F8	6.0±0.01	90.7±0.33	27.5±0.41	+++	875.6±8.69
F9	6.2±0.14	96.2±0.01	25.63±0.14	+++	986.3±6.39

Number of experiments=9, Each observation means is the mean±SD of three observations, +:gel after few minutes dissolves rapidly, ++: immediate gelation remains for few min, +++: immediate gelation remains for nearly an h.

Rheological studies

For thermosetting gels, the viscosity at various conditions is an important rheological parameter involved in its utilization and its *in vitro* performance. For example, if viscosity is too high it will lead to difficult installation; on the contrary, if viscosity is too low it will give rise to increased drainage. Previous papers have shown that a polaxomer solution under non-physiological or physiological conditions demonstrated a Newtonian flow behaviour and that the shear increased linearly with an increase in shear rate. The viscosity values of all formulation were shown in table 2. The formulations exhibited pseudoplastic rheology as evidenced by shear thinning and an increase in shear stress with increased angular velocity. Since the ocular shear rate is about 0.03 s⁻¹during inter-blinking periods and 4250-28500 s⁻¹during blinking. So, the viscoelastic fluids having high viscosity under low shear rates and low viscosity under high shear rates called as pseudoplastic fluid are often preferred [16]. Among all the formulation, F9 formulation provides maximum viscosity and statistically significant (P<0.01) value that is, 986.3±6.39 cps at 100 rpm. The F1, F2 and F3 batch has a very low viscosity at 35 °C that it did not improve the contact time of drug with eye. Batch F6, F7,F8, F9 containing 8 % and 9% of polaxomer 407 and 0.25, 0.5, 0.75% modified chitosan showed very high viscosity that it forms a very stiff gel which may leads to the difficulty for installation and also retard the release of the drug.

Batch F4 and F5 showed good results but F5 showed better viscosity. Viscosity is an important factor to determine the residence time of drug in the eye [18]. The viscosity increased with increasing concentration of modified chitosan and polaxomer 407 was observed.

In vitro drug release study

The cumulative percent released versus time profiles of moxifloxacin HCl *in situ* gel containing polaxomer 407 and modified chitosan in different ratios are shown in fig. 2. The entire formulation containing 0.25% (w/v) moxifloxacin HCl. The *in vitro* drug release was ranging from 84% to 68 % in 8 h. The drug release from the batch F6, F7,F8,F9 having 8% and 9% polaxomer 407 and 0.25, 05 and 0.75% of modified chitosan showed a lesser cumulative percentage release in comparison to other formulations. The formulation F5 containing 8% w/v of polaxomer and 0.5% modified chitosan, showed a cumulative release of 92% at the end of 8 h which indicated that F5 provided sustained release. From *in vitro* drug release studies and gelation temperature for all formulations F5 showed sustained release up to 8 h and have gelation temperature closer to the ocular surface temperature that is 35 °C hence the formulation was selected for further study [11]. The results are shown in fig. 1 showing statistically significant values (P<0.01) which indicate that the *in vitro* release from *in situ* gel of moxifloxacin HCl was sustained for 8 h.

Table 3: Shows *in vitro* drug release

Time h	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	30.42	20.48	24.78	19.87	32.45	17.55	14.43	13.28	12.56
2	42.51	31.76	32.54	26.6	45.8	24.56	27.76	22.67	20.43
3	51.43	43.73	40.3	34.2	53.2	36.97	31.54	32.54	28.54
4	66.15	54.27	48.45	43.85	68.43	47.34	42.43	45.76	36.89
5	74.35	62.4	60.56	53.47	76.85	55.76	50.87	57.43	48.65
6	79.32	69.46	68.97	61.43	82.45	63.78	62.94	59.55	52.84
7	82.5	79.15	73.67	67.56	87.54	72.65	72.65	69.73	61.95
8	84.21	82.45	80.9	78.4	92.56	78.43	78.43	72.54	68.4

Number of experiments=9, mean *in vitro* drug release at 8 h.

Antibacterial study

The optimized *in situ* gelling formulation showed antibacterial activity tested microbiologically by cup-plate technique. The antibacterial efficiency of the selected sustained release moxifloxacin HCl formulation F5 was calculated against *staphylococcus aureus*. The zones of inhibition of standard and ophthalmic formulation were

found to be 24 mm and 21 mm. The zone of inhibition of standard and ophthalmic formulation was found to be almost similar. The modified chitosan also showed a zone of inhibition of 18 mm. The inhibition zones were evaluated after 24 h and reduction in the growth of microorganisms was clearly observed in fig. 2. The zone of inhibition increased significantly as the amount of moxifloxacin HCl diffused from the *in situ* gel was increased [12].

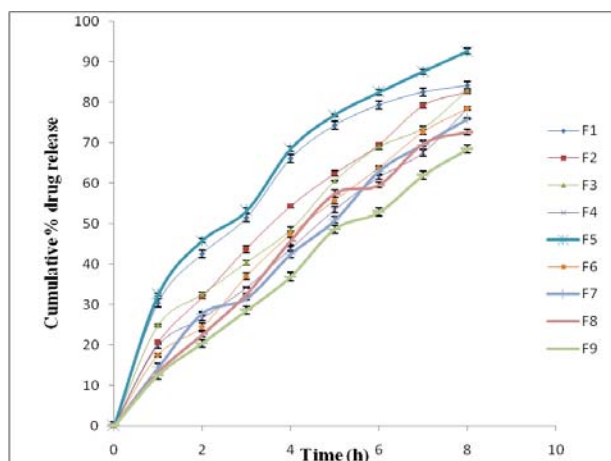


Fig. 1: *In vitro* dissolution profile of moxifloxacin HCl from *in situ* gelling system, number of experiments=9

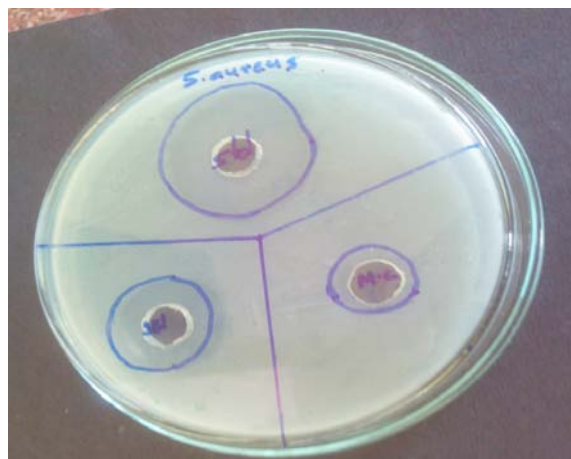


Fig. 2: Antimicrobial activity of standard, formulation and modified chitosan, number of experiments=1

Table 4: Antimicrobial activity of standard, formulation and modified chitosan

Microbial strain	ZOI (mm) Standard	ZOI (mm) F5	ZOI(mm) Modified chitosan
S. aureus	24.2±0.45	21.5±0.02	18.4±0.26

Values are expressed as mean±SD of three observations

Release kinetic

The *in vitro* release profile was fitted to various kinetic models in order to find out the mechanism of drug release. The goodness of fit was evaluated using regression coefficient (r^2) values. To explore the drug release mechanism, *in vitro* release results were further fitted to the K-P equation. The regression coefficient (r^2) for all the formulations using

different kinetics equation is listed in table 5. The values of r^2 for all formulations were found higher for Zero order model. It was found that all formulation mostly followed the zero order models as best fit model because of the higher value of r (correlation coefficient). The release profile of *in situ* gelling formulations were treated with Korsmeyer Peppas equation and slope value $n > 0.85$ were indicating Case-II transport drug release involving non Fickian diffusion [17].

Table 5: Kinetics of *in vitro* drug release

Formulation	Zero order	First order	Higuchi model	Hixon crowell	Korsmeyer-peppas		Mechanism of drug release
	r^2	r^2	r^2	r^2	r^2	N	
F1	0.994	0.911	0.974	0.959	0.991	0.94	Case-II transport
F2	0.995	0.88	0.975	0.944	0.993	0.873	Case-II transport
F3	0.968	0.981	0.983	0.986	0.993	0.905	Case-II transport
F4	0.994	0.93	0.984	0.974	0.989	0.869	Case-II transport
F5	0.994	0.855	0.978	0.941	0.992	1.054	Case-II transport
F6	0.99	0.902	0.967	0.953	0.986	0.941	Case-II transport
F7	0.94	0.939	0.97	0.973	0.997	0.892	Case-II transport
F8	0.993	0.939	0.97	0.971	0.987	0.863	Case-II transport
F9	0.994	0.911	0.966	0.959	0.987	0.841	Non Fickian

Number of experiments=9, * r-coefficient of correlation, n-kinetic constant

Statistical analysis

The results obtained from the experiment were statistically analyzed for response variables by using Graph Pad instat (version 3.1, Graph Pad Software Inc.). Statistical analysis (One way ANOVA) however revealed that batches were significantly different. Dunnett's multiple comparison tests predicted that there was a significant effect of concentration of poloxamer 407 and modified chitosan on the *in vitro* drug release and viscosity.

CONCLUSION

In this study, we suggest that the appropriate combination of poloxamer 407 and modified chitosan were suitable satisfactorily sustained the drug release from moxifloxacin HCl *in situ* gel, thereby potentially lowering corneal toxicity. We demonstrated that poloxamer/modified chitosan formulation in a concentration of 8/0.5% w/w showed an optimal gelation temperature (32 °C) and was able to withstand low shearing forces at 35 °C. The gels proved

to possess a mucoadhesive ability that is influenced by modified chitosan concentration. Therefore, the developed delivery system seems to be a promising tool for ophthalmic use, as it is easily administered and shows a prolonged ocular contact time.

AUTHOR CONTRIBUTION

Anuja Kadam developed a method, experimental part of work and writing of the manuscript. Pradnya Salunke is a technical executive in antibacterial activity research. Rahul Jadhav and Satwashila Kadam contributed in its manuscript correction.

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CONFLICT OF INTERESTS

Declared none

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