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Research Article

DEVELOPMENT AND EVALUATION OF *IN SITU* GELS OF MOXIFLOXACIN FOR THE TREATMENT OF PERIODONTITIS

Hareesh Babu Kunche[1], Mohammed Gulzar Ahmed[1*], Narayana Charyulu Rompicharla[2]

[1]Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G. Nagara, India.
[2]Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Mangalore , India.

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*Corresponding Author
Mohammed Gulzar Ahmed

Email : mohammedgulzar1 @gmail.com

ABSTRACT

Gel dosage forms are successfully used as drug delivery systems to control drug release and protect the medicaments from a hostile environment. The main objective of the present investigation is to formulate and evaluate *in situ* gels of moxifloxacin for the treatment of periodontitis using gellan gum and sodium alginate based on the concept of ion activated systems. The system utilizes polymers that exhibit sol-to-gel phase transition due to change in specific physico-chemical parameters. Sol-to-gel transformation occurred in the presence of monovalent /divalent cations. It was found that increase in the concentration of calcium ions produced stronger gels. Formulations were evaluated for gelling capacity, drug content, clarity, viscosity, gel strength, spread ability, microbiological studies and *in vitro* release. The results shown by the above characterization studies were found to be satisfactory. Experimental part showed that viscosity of sols and gel strength was increased with increase in the concentration of polymers and the sustained release of drug was observed. The formulations were therapeutically efficacious, sterile and provided sustained release of the drug over a period of time. These results demonstrated that the developed system is an alternative to conventional drug delivery systems, provides patient compliance and economical.

Key words: *in situ* gels, moxifloxacin, periodontitis, polymers, Ion activation.

INTRODUCTION

Dental diseases are recognized as the major public problem throughout the world and these are amongst the most widespread chronic disorders affecting the mankind (Swati *et al.,* 2010). Periodontal disease refers to the inflammatory processes that occur in the tissues surrounding the teeth in response to bacterial accumulations or dental plaque on the teeth (Jemin and Salomon 2006). It is characterized by loss of bone, collagen support of affected teeth. The progression of the disease is related to the colonization of microorganisms, including *Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Tannerella forsythia* and *Treponema denticola* (Mohammed *et al.,* 2008, 2009).

To eliminate bacterial infections, antibiotics are administered systemically but the repeated and long term use of systemic antibiotics is associated with toxic adverse effects. These consequences can be solved by delivering the drug at the desired target site (i.e. periodontal pocket) to show local therapeutic action. The main advantage of the local route of the drug delivery is the reduction of drug dose and possibility to increase the concentration of the drug in the periodontal pockets, at the same time keeping comparatively low systemic drug concentrations (Mohammed *et al.,* 2008; Pragati *et al.,* 2009).

At present various local drug delivery systems available for treating periodontitis like fibers, films, strips and gels etc,. Among this gel formulations have some advantages over conventional dosage forms as sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the *in situ* gel formulations can make them more acceptable and excellent drug

delivery systems (Nirmal *et al.,* 2010; Vyas *et al.,* 2011).

Moxifloxacin is a fourth generation fluoroquinolone with a broad antibacterial activity against Gram-positive and Gram-negative bacteria. Moxifloxacin shows bactericidal, concentration dependent, anti-infective. It interferes with bacterial survival by binding to DNA gyrase (topoisomerase II) and topoisomerase IV, essential bacterial enzymes involved in the replication, translation, repair and recombination of deoxyribonucleic acid (Ingo *et al.,* 2002; Harish *et al.,* 2009).

Hence, the aim of present work is to develop and evaluate the *in situ* gel containing moxifloxacin as a local drug delivery within the periodontal pockets for the treatment of periodontitis.

METHODOLOGY

Materials and reagents

Moxifloxacin was kindly obtained from Aurabindo pharmaceuticals, Hydrabad. Gellan gum was provided by Hi Media laboratories Ltd, Mumbai and sodium alginate was obtained from S.D. Fine-Chem limited, Mumbai. All the other materials used were of analytical grade.

Preparation of *in situ* gelling system

Gellan gum and sodium alginate solutions of various concentrations were prepared by adding the gum to deionised water containing 0.17% w/v sodium citrate and heated to 90°C while stirring. After cooling to below 40°C, moxifloxacin was dissolved in ethanol water and was added to the solution. The mixture was stirred by using a magnetic stirrer to ensure thorough mixing (Shastri *et al.,* 2010) (Table I).

Evaluation of gels

Compatibility studies carried out by FTIR showed, both drugs and polymers were compatible. The clarity of formulations determined by visual inspection under black and white background (Nirmal *et al.,* 2010) and the pH of the *in situ* sols was determined using a calibrated pH meter.

In vitro gelling capacity

The gelling capacity of the formed gel was determined by visual inspection and grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time and $CaCl_2$ required for each formulation for the sol-gel transition was noted (Shastri *et al.,* 2010).

Spreadability (Harish *et al.,* 2009)

For the determination of spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 min. Weight (50 g) was added to the pan and the time required for separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (S).

Spreadability (g.cm/s) (S)=M×L/T
Where M = weight tide to upper slide
L = length moved on the glass slide
T = time taken

In situ release studies

Drug release studies from the *in situ* gel were carried out by using a cellophane membrane. Apparatus was designed as it is a glass tube had a length of 10.5 cm and a diameter of 2.1 cm. The lower base was tied with cellophane membrane containing *in situ* gel and this was placed in a beaker containing 100 mL of phosphate buffer pH 6.8 (simulated salivary pH) as diffusion medium which is maintained at 37°C with 50 RPM. Samples (5 mL) were withdrawn at different time intervals from the reservoir till the gel was completely eroded (3 hours). The cumulative percent drug release was determined by measuring the absorbance at 289 nm (Himanshu *et al.,* 2009).

Measurement of viscosity of sols

Viscosity determinations for the prepared formulations were carried out on a Brookfield digital viscometer using spindle No 4. Viscosity of *in situ* gelling solutions was measured at different angular velocities at a temperature of 37°C. A typical run comprised changing of the angular velocity from 0.0 to 100 rpm. The averages of two readings were used to calculate the viscosity (Shastri *et al.,* 2010).

Determination of gel strength

The method by which the properties of polymeric system may be conveniently determined is texture profile analysis.

Table I. Formulation of *in situ* gel of Moxifloxacin with various polymers at different concentrations

Formulation code	G1	G2	G3	S1	S2	S3
Gellan gum (% w/v)	0.4	0.5	0.6	-	-	-
Sodium alginate (% w/v)	-	-	-	1.5	2	2.5
Sodium citrate (% w/v)	0.17	0.17	0.17	0.17	0.17	0.17
Moxifloxacin (µg)	50	50	50	50	50	50

Table II. Characteristics of various *in situ* gel formulations

Formulations code	pH	Spreadability g*cm/s	Drug content (% w/v)	Viscosity (Pa*s)	Gelling capacity	Gel strength (g)
G1	7.1	39.70	95.03	12.65	+	4.1
G2	6.9	29.34	95.10	15.00	+++	6.5
G3	7.2	23.27	97.16	16.50	+++	7.1
S1	7.2	45.00	95.31	12.02	+	4.0
S2	7.1	35.52	97.23	15.54	++	6.0
S3	7.0	25.96	96.17	16.20	+++	6.5

+ - Average; ++ - Good; +++ - Excellent

Table III. Release exponent values and rate constant values for different formulations

Formulation code	Kinetic models				
	Zero order	First order	Higuchi	Korsmeyer *et al.*	
	R^2	R^2	R^2	n	R^2
G1	0.969	0.951	0.719	0.359	0.956
G2	0.989	0.983	0.802	0.290	0.981
G3	0.912	0.910	0.915	1.155	0.906
S1	0.937	0.917	0.708	0.364	0.938
S2	0.969	0.963	0.778	0.472	0.974
S3	0.979	0.970	0.792	0.472	0.946

A TA-XT2 Texture analyzer is used. The experiment was done by placing the gels in standard Beaker below the probe. In this an analytical probe is then immersed into the sample. The Texture Analyzer was set to the 'gelling strength test' mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per seconds and a trigger force of 5 g were selected. An aluminum probe of 7.6 cm diameter was used for all the samples. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of g (Shastri *et al.,* 2010).

Microbiological studies

The microbiological studies were carried out on the plain drug solution and optimized formulation G3 of different concentrations as 100, 200, 300 and 400 µg/mL against different microorganisms. *E.coli, Salmonella, klebsiella* and *Pseudomonas* were used as the test microorganisms. A layer of nutrient agar (20 mL) seeded with the test microorganism (0.2 mL) was allowed to solidify in the petriplate. Cups were made on the solidified agar layer with the help of sterile borer at 6 mm diameter. Appropriate amount of drug solution was poured into the cups.

After keeping petriplates at room temperature for 4 hours, the plates were incubated at 37°C for 24 hours. The zone of inhibition was obtained. The diameter of zone of inhibition was measured by an antibiotic zone finder (Himanshu and Aarti, 2009).

RESULTS AND DISCUSSION

In the present investigation, an attempt was made to develop and evaluate *in situ* gel formulation of moxifloxacin having controlled release characteristics for direct placement into the periodontal pocket. The two main prerequisites of an *in situ* gelling system are viscosity and gelling capacity. To instill easily at the affected site the formulation must possess optimum viscosity, which then undergoes a rapid sol-gel transition due to ionic interaction. Sol to gel transformation of *in situ* gels occurs in the presence of either monovalent or divalent cations at the site of administration. In these formulations the calcium chloride was a source of divalent cation for internal ionotropic gelation for polymers.

Experimental part (Table II) have showed that the formulation G3 (gellan gum 0.6 % w/v) and formulation S3 (sodium alginate 2.5 % w/v) were satisfactory to cause gelation. Drug content of all formulations was found between 95.1 to 97.23 % w/w. Clarity test for the prepared formulations has done by visual inspection under black and white background, there was no evidence of contamination, the entire formulations passes clarity test. Aqueous gellan gum and sodium alginate sols exhibited pH values in the range of 6.9 to 7.2 at 25°C. Spreadability was decreasing by increasing the concentration of polymer. Formulation G3 shows least Spreadability (23.27 g cm/s) and formulation S1 shows more Spreadability (45 g cm/s).

The viscosity of *in situ* sols was determined by Brookfield viscometer (Table II). Among the all formulations, S1 (Sodium alginate 1.5%) shows least viscosity (12.02 Pa·s) and G3 was more (16.5 Pa·s). The formulations S2 and G2 were nearly same (15.54 and 15.0 Pa·s respectively). This says increase in polymer concentration causes increase in viscosity of the sols. The gel strength of *in situ* gels was determined by A TA-XT2 Texture analyzer. Among the all formulations, S1 shows least gel strength (4.0 g/s) and G3 was more (7.1 g/s). The formulations S2 and G2 were showed moderate gel strength (6.0 and 6.5 g/s respectively). This says increase in polymer concentration causes increase in gel strength (Table II).

The antibacterial activity of the best formulations G3 was compared with the reference standard (pure drug). It was found that the zone of inhibition (in diameters) of formulation G3 was equal to that of the reference standard. Results have showed that moxifloxacin is more active against microorganisms like *Salmonella Sp.* and *Pseudomonas Sp.* as compared to *Kelebsiella Sp.*, least active against *E.coli.*

The *in vitro* diffusion profile of moxifloxacin from the gels containing different concentration of gellan gum and sodium alginate based gels is shown in fig.1. The release of drug from these gels was characterized by an initial phase of high release (burst effect), the remaining drug was released at a slower rate (second phase). This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration. The formulations G1 and S1 released maximum amount of drug (87.76 and 82.22 % respectively) in 3 hours as compared to G2 and S2 (80.24 and 78.9 % respectively). The formulations G3 and S3 have shown delayed release than other formulations, among these two formulations G3 have shown least drug release in 3 hours, this says G3 was the best formulation than other. Finally, it was found that drug release was get sustaining by increasing the polymer concentration.

Release kinetics

The examination of the correlation coefficient 'r' indicated that the drug release followed diffusion controlled mechanism from the *in situ* gels (Table III), as the values of 'r' for first order (ranged from 0.910 to 0.983) found to be less in comparison to zero order (ranged from 0.912 to 0.989) and Higuchi's square root of time (ranged from 0.708 to 0.915). It was understood to be predominant zero order release pattern. Further, to understand the drug release mechanism, the data were fitted into Peppas exponential model $M^t/M^\infty=Kt^n$, where

Table IV. Release exponent values and rate constant values for different formulations

Formulation code	Kinetic models				
	Zero order	First order	Higuchi	Korsmeyer *et al.*	
	R^2	R^2	R^2	*n*	R^2
G1	0.969	0.951	0.719	0.359	0.956
G2	0.989	0.983	0.802	0.290	0.981
G3	0.912	0.910	0.915	1.155	0.906
S1	0.937	0.917	0.708	0.364	0.938
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S3	0.979	0.970	0.792	0.472	0.946

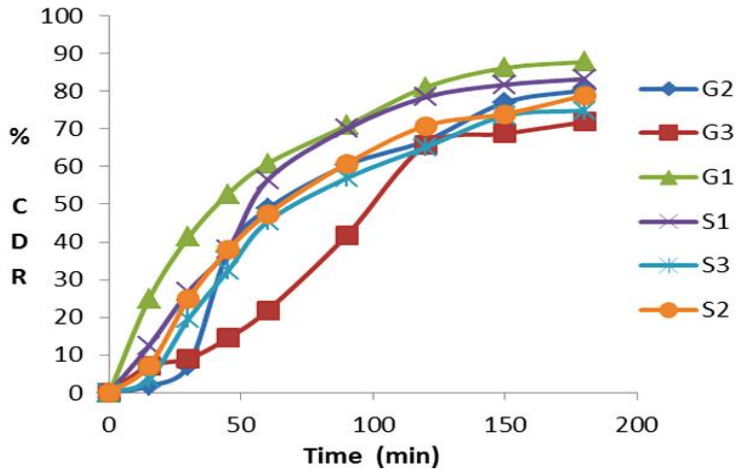

Figure 1. Comparative drug release profile of the formulations (G1, G2, G3, S1, S2 and S3)

M^t/M^∞ is the fraction of drug released after time 't' and 'K' is kinetic constant and 'n' is release exponent which characterizes the drug transport mechanism. The values 'n' were in the range of 0.290 to 1.155. The formulations G1, G2 and S1 were following fickian release mechanism ('n' values are less than 0.45), S2 and S3 are indicating non-fickian release mechanism ('n' values are between 0.45-0,89) and G3 is following super II release ('n' values are more than 0.89).

CONCLUSION

From the above studies it can be concluded that local drug delivery may be an advantageous in treatment, since it would probably eliminate side effects, which occur with systemic dosing. The local drug delivery system in present study is simple and easy to use. Its syringeability allows easy insertion of gel formulation in to periodontal pocket. *In situ* gel formulation of Moxifloxacin with mucoadhesive properties is useful to prolonging local residence time at periodontal pocket. This type of drug delivery system can serve as a novel approach for treating periodontal diseases with better patient compliance. The developed formulation can release the drug at controlled rate for prolonged duration.

An *in vivo* study of the formulation is necessary factor to evaluate the efficacy and safety of the novel drug delivery system.

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