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Sustained Ocular Delivery of Sparfloxacin from pH Triggered *In Situ* Gelling System

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Abstract

Poor bioavailability of ophthalmic solutions caused by dilution and drainage from the eye can be overcome by using *in situ* forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible liquid-gel phase transitions. This may result in better ocular availability of the drug. In present work *in situ* gels of sparfloxacin have been developed by using HPMC and carbopol based on the concept of pH triggered gelation systems. Sol-to-gel transformation occurred in the presence of stimulated tear fluid of pH 7.4. Sparfloxacin has *in vitro* activity against a wide range of gram-negative and gram-positive microorganisms. Formulations were evaluated for gelling capacity, drug content, clarity, viscosity and *in vitro* release. Experimental part showed that viscosity of sols was increased with increase in the concentration of polymers and the solutions shown pseudoplastic behaviour. The antimicrobial studies against *Staphylococcus aureus* and *in vivo* ocular gelation studies using suitable animal models were performed. All the results were found to be satisfactory. The formulations were therapeutically efficacious, sterile and provided sustained release of the drug over a period of time. These results demonstrate that the developed system is an alternative to conventional drug delivery system, patient compliance, industrially oriented and economical.

Keyword: *In situ*, Sparfloxacin, pH trigger, HPMC, Carbopol, *In vivo* studies.

INTRODUCTION

Eye diseases are commonly encountered in day to day life, which are cured or prevented through the conventionally used dosage forms like eye drops, ointments etc., Delivery to the internal parts of the eye still remains troublesome due to anatomical and protective structure of the eye.[1]

Poor ocular bioavailability of drug which is less than 1% from conventional eye drops is due to the physiological barriers of the eye.[2] Most of the topically applied drugs in the form of eye drops are washed off from the eye by various mechanisms include lacrimation, tear dilution and decrease in residence time[3]. The development of eye ointments had an added advantage over ophthalmic solution with their long residence contact time in eye. Ointments also has disadvantages such as sticking of eyelids, blurred vision, poor patient compliance, drug choice limited by partition coefficient[4].

A significant increase in the pre-corneal residence time of drug and consequently better bioavailability can be achieved by using delivery systems based on the concepts of *in-situ* gel formation.[5] Ocular *in-situ* drug delivery systems consists of polymer that exhibit sol-to-gel phase transitions due to change in specific physicochemical parameters (pH) and results in ease of application, reduction in frequency of administration, improved patient compliance and comfort.[6]

Sparfloxacin is a synthetic broad spectrum flouroquinolone group of antibacterial agent, acts by inhibition of DNA gyrase and topoisomerase IV, which is required by bacteria for DNA replication, transcription repair and recombination. The minimum inhibitory concentration for sparfloxacin is found to be less than 0.25-10µg/ml[7,8]. Sparfloxacin appears to possess excellent in vitro activity against *erythromycin-resistant S. pneumoniae* that were often highly resistant to beta-lactams.[9]

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Hence, the aim of present work is to develop a formulation for ophthalmic delivery with prolong precorneal drug residence time by using an antimicrobial drug sparfloxacin which have broad spectrum of activity.

MATERIALS AND METHODS

Sparfloxacin was procured from Yarrow chem. Products (Mumbai). HPMC K4M and Carbopol 934 were purchased from S.D Fine Chem. Ltd (Mumbai). Sodium chloride and benzalkonium chloride were purchased from S.D Fine Chem. Ltd (Mumbai).

Required quantity of sodium chloride was dissolved in 50 ml of distilled water. HPMC K4M was added to the above solution and stirred slowly with magnetic stirrer; Care was taken that no lumps of HPMC were formed during stirring. Carbopol 934 was sprinkled over this solution and allowed to hydrate overnight. The solution was again stirred with magnetic stirrer after 24 hrs. Sparfloxacin was dissolved in 5 ml distilled water, benzalkonium chloride (BKC) was then added and the solution was filtered through 0.2 µm cellulose acetate membrane filter. The drug solution was added to the Carbopol-HPMC solution under constant stirring until a uniform solution was obtained pH of the formulation was then set to 5.4 by adding 0.1N NaOH solution. Distilled water was then added to make up the volume to 100 ml. The developed formulations were filled in 5 ml capacity ambered glass vials, closed with gray butyl rubber closures and sealed with aluminium caps. The formulations in their final pack were subjected to terminal sterilization by autoclaving at 121 °C at 15 psi for 20 minutes[10].

Table 1. Formulation design of *in situ* gel

Name of ingredient	Quantity in 100ml (% w/v)		
	S1	S2	S3
Sparfloxacin %W/V	0.3	0.3	0.3
HPMC K4M %W/V	1.0	1.0	1.5
Carbopol 934 %W/V	0.4	0.5	0.4
sodium chloride %W/V	0.9	0.9	0.9
Benzalkonium chloride %W/V	0.01	0.01	0.01
Distilled water	q.s	q.s	q.s

EVALUTION OF IN SITU GEL

1. Clarity

Clarity is one of the prime characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.[11]

2. Determination of pH

pH is one of the most important parameter involved in the ophthalmic formulation. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH using digital pH meter (Chemi line).[12]

3. In Vitro Gelling Capacity

The prepared in situ gelling system was evaluated for gelling capacity in order to identify the composition suitable for use as in situ gelling system. The in situ gelling system was mixed with simulated tear fluid to find out the gelling capacity of the ophthalmic product. The gelation was then assessed visually by noting the time

for the gelation. The gelling capacity of the formed gel was determined by visual inspection and the different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time.[13]

4. Viscosity and Rheology of in situ sols Studies

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. The developed formulations were poured into the small sample adaptor of the Brookfield Synchrolectric viscometer and the angular velocity increased gradually from 0.5 to 50 rpm. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity.[14]

5. Drug Content

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1 ml of the formulation to 100 ml with stimulated tear fluid solution pH 7.4. Aliquot of 0.1 ml was withdrawn and further diluted to 50 ml with tear fluid solution. Sparfloxacin concentration was then determined at 291 nm by using UV-Vis spectrophotometer (Shimadzu UV"1700, Japan).[15]

6. In vitro drug release study

The in vitro release of Sparfloxacin from the formulation was studied using a modified USP XXIII dissolution testing apparatus. Freshly prepared Simulated Tear Fluid (STF; pH 7.4, ionic strength of 0.188) was used as the dissolution medium. Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). 1 ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1˚C so that the membrane just touched the receptor medium surface. The shaft was rotated at 50 rpm. At hourly time intervals, 5 ml of solution was withdrawn from the cell and replaced with an equal volume of fresh dissolution medium to provide sink condition. The samples were diluted with the receptor medium and analyzed by UV spectrophotometer at 291 nm. Each experiment was performed in triplicate.[15]

Further investigation for the drug release from the pH triggered in situ gels was done by studying the release data with zero order, first order kinetics and Higuchi equation. The release mechanism was understood by fitting the data to Korsemeyer Peppas model.16,17

7. Effect of Sterilization study

The selected formulations were filled in 50 ml capacity ambered glass bottles, closed with grey butyl rubber closures and sealed with aluminium caps. The vials were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min. The formulations were evaluated for drug content, viscosity, clarity and pH before and after the terminal sterilization.

8. Antimicrobial efficacy studies

This was determined by the agar diffusion test employing 'cup plate technique'. The microbiological studies were carried out on the plain drug solution (standard sols) and optimized formulations S1, S2, S3 (test sols) of different concentrations as 100, 200, 300 and 400 μg/ml against different microorganisms. *Klebsiella* and *Staphylococcus aureus* was 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 hrs, the plates were incubated at 37°C for 24 hrs. The zone of inhibition was obtained. The diameter of zone of inhibition was measured by an antibiotic zone reader. The zone of inhibition (ZOI) measured around cup was compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit.[18]

9. *In vivo gelation studies*

The in vitro release studies were completely devoid of complication by variability in precorneal factors such as blinking, lacrimation, tear turnover. It is therefore necessary to study the in vivo ocular gelation studies for the optimised formulations. The study was carried out on three male albino rats, weighing 150-200 grams, and with no signs of ocular inflammation or gross abnormalities. All animals were maintained according to CPCSEA guidelines. 3 rats (total 6 eyes) were divided into 3 groups; i.e. 2 eyes in each group. Each group received a topical administration of S1, S2 & S3 respectively.[19]

10. Stability Studies

Optimized formulations were tested for stability studies. Both the formulations were stored at storage conditions of elevated temperature such as 40°C ± 1.0°C / 75% RH. The samples were withdrawn at 7 days interval for 56 days and were evaluated for parameters like clarity, pH, gelling capacity, drug content and *in vitro* drug release.[20]

RESULTS AND DISCUSSION

1. Clarity test

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 passed clarity test.

2. Determination of pH

The pH of in situ gels was determined using a calibrated pH meter (Chemi line). The readings were taken for average of 3 samples. The pH values of formulations were found to be in the range of 5.4 to 6 at 25°C.

3. In vitro gelling capacity

It was found that the gel intensity was increased when the concentration of polymers were increased. Experimental part has showed that the formulations S1 and S2 were satisfactory to cause gelation which is tabulated in Table 2.

Table 2. *In vitro* gelling capacity of the in situ gels

Formulation code	Gelling capacity	Time (seconds)
S1	+++	54
S2	+++	41
S3	++	67

4. Viscosity and Rheology of the in situ sols

The viscosity of *in situ* sols was determined by Brookfield viscometer and the results were tabulated in Table 3. The formulation S1 shows least viscosity and S3 was more viscous. This says increase in polymer concentration causes increase in viscosity of the sols.

Table 3. Viscosity of the *in situ* sols

Shear rate (RPM)	Viscosity of the formulation (cps)		
	S1	S2	S3
10	1340	1832	1934
20	910	1043	1123
30	567	698	732
40	365	436	512
50	203	278	312

The rheological studies of the optimum formulations were studied by plotting a graph of shear rate vs. viscosity which was shown in Figure 1. This showed that the viscosity of the formulations decreased with increase in shear rate, which indicates the character of pseudoplastic fluids.

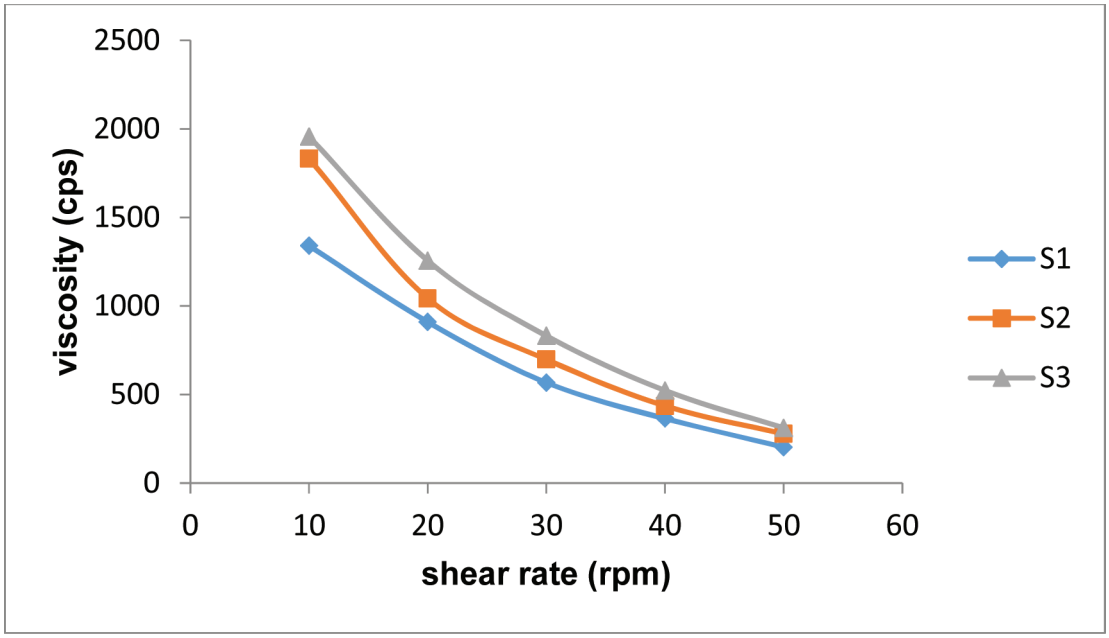

Figure 1. Rheological profile of the in situ gelling systems

5. Drug content:

The drug content estimation was done and the absorbances were measured by UV spectrophotometer (Shimadzu UV" 1700), drug content was calculated. Drug content of formulations was found to be in the range of 94.54 to 97.24 % w/w.

6. In vitro release studies

The *in vitro* diffusion profile of Sparfloxacin from the gels containing different concentration of HPMC and Carbopol is tabulated in Table 4 and shown in Figure 2. Formulation S2 have shown least drug release (69.10 %) in 8 hrs compared to formulation S1 that is 73.34% and formulation S3 have shown maximum drug release (74.88 %).

Table 4. *In vitro* release studies of in situ formulations

Time (min)	% Cumulative drug release		
	S1	S2	S3
0	0.000±0.00	0.000±0.00	0.000±0.00
1	10.00±0.011	3.330±0.012	6.000±0.011
2	16.50±0.024	12.17±0.022	10.30±0.023
3	27.30±0.013	18.77±0.012	18.53±0.015
4	33.26±0.015	27.00±0.013	31.68±0.011
5	43.40±0.016	35.60±0.015	41.58±0.018
6	51.23±0.013	44.57±0.014	51.64±0.016
7	61.48±0.022	57.23±0.021	63.03±0.022
8	72.34±0.019	69.10±0.023	74.88±0.024

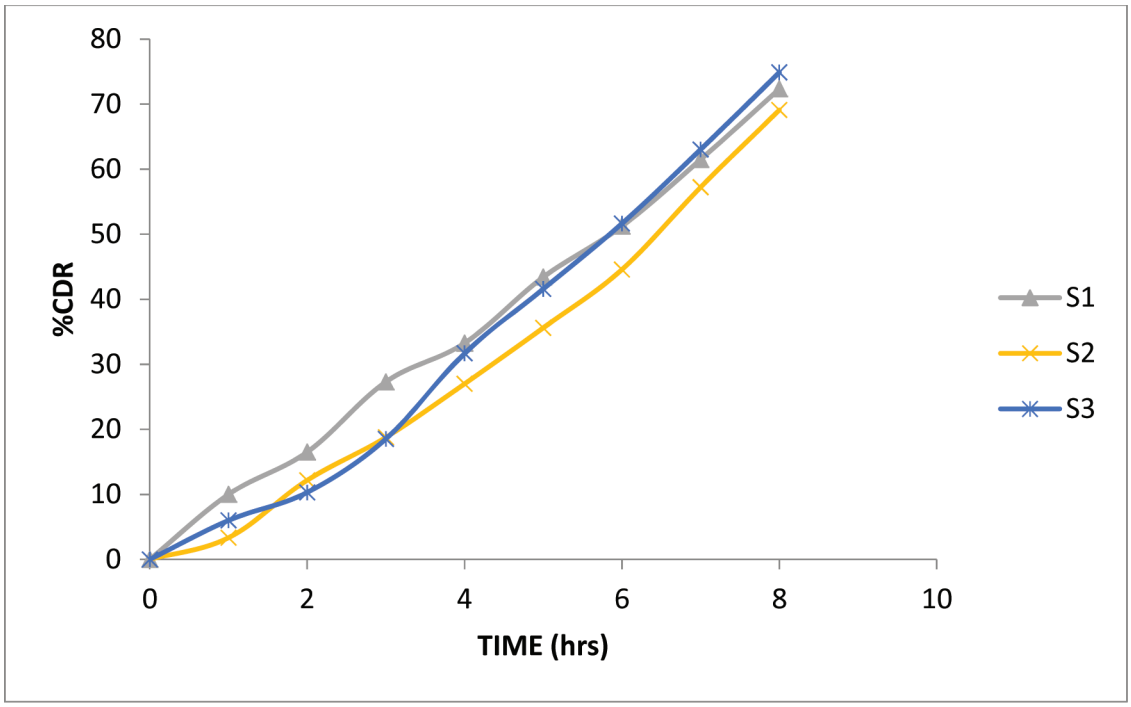

Figure 2. Comparative drug release profile of the formulations

Release kinetics:

The examination of the correlation coefficient 'r' indicated that the drug release followed diffusion controlled mechanism from the in situ gels and the results obtained is tabulated in Table 5. As the values of 'r' for first order (ranged from 0.910 to 0.95) found to be less in comparison to zero order (ranged from 0.997 to 0.983) and Higuchi's square root of time (ranged from 0.89 to 0.96). 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 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 0.82 to 1.1. The formulations S3 following fickian release mechanism ('n' values are less than 0.45), S2 following non-fickian release mechanism ('n' values are between 0.45-0.89) and S1 following super case II release ('n' values are more than 0.89).

Table 5. 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
S1	0.997	0.950	0.965	0.96	0.993
S2	0.993	0.919	0.948	0.86	0.991
S3	0.984	0.921	0.950	0.38	0.910

7. *Effect of sterilization*

The autoclaving exerted insignificant effect on the drug content, viscosity and pH of the formulations. However, haziness was observed in all the formulations after autoclaving due to precipitation of the polymers. But, it was found to be disappeared and the original clarity was regained after overnight storage at ambient conditions.

8. *Microbiological Studies of Sparfloxacin:*

The antibacterial activity of the best formulations S1 and S2 was compared with the reference standard (pure drug). It was found that the zone of inhibition (in diameters) of formulation S1 was equal to that of the reference standard (Figure 3 and 4). Results which are tabulated in Table 6 have showed that sparfloxacin is more active against microorganisms like *S.aureus* and least active against *Kelebsiella* sp.

9. *In vivo gelation and irritation studies:*

The *in vivo* gelation studies of the optimised *in situ* formulations were carried out on three male albino rats as shown in figure 5. The rats were visually observed for gelation and irritation studies like redness, swelling and watering of eye. The formulations were found to be non-irritant and gelation of *in situ* solution is observed visually after 10 sec of instillation.

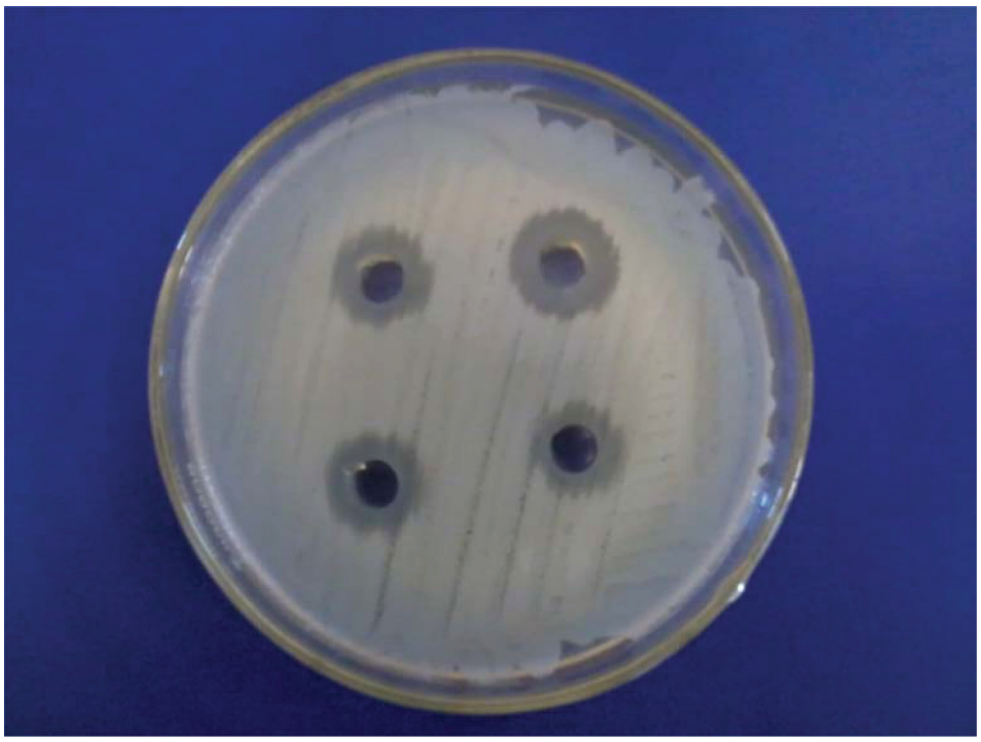

Figure 3. Zone of inhibition of sparfloxacin (pure drug) against *S. aureus*

Table 6. Anti bacterial activity of Sparfloxacin on different microorganisms

Name of the Microorganisms		Zone of inhibition (Diameter(mm))			
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)
Staphylococcus aureus	Pure drug	14	18	24	28
	S1	10	15	22	26
Kelebsiella	Pure drug	-	-	-	-
	S1	-	-	-	-

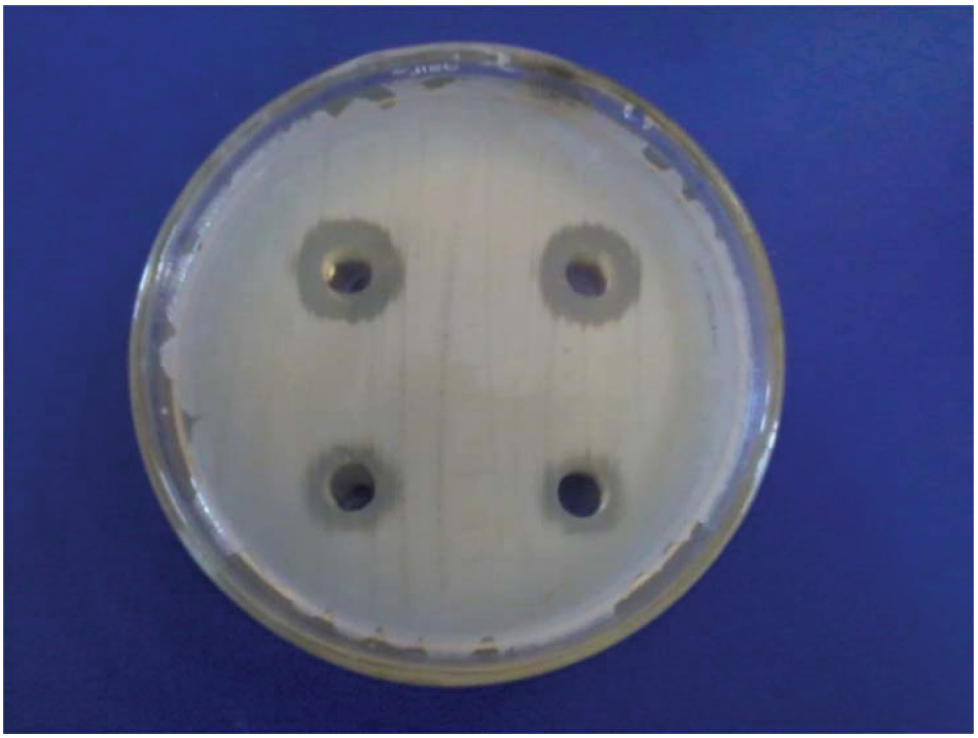

Figure 4. Zone of inhibition of formulation S1 against *S. aureus*

Figure 5. *In vivo* gelation and irritation studies

Table 7. stability studies of formulations stored at 40 ±1°C/ ambient humidity

No. of weeks	Drug content%		%CDR	
	S1	S2	S1	S2
1	94.56±0.021	96.62±0.012	72.30±0.012	69.1±0.021
2	94.54±0.036	96.64±0.015	72.29±0.032	69. ±0.015
3	94.52±0.056	96.60±0.025	72.26±0.021	69.3±0.019
4	94.50±0.066	96.59±0.028	72.24±0.034	68.9±0.023
5	94.52±0.075	96.57±0.032	72.21±0.037	68.8±0.019
6	94.49±0.084	96.58±0.039	72.23±0.036	68.8±0.026
7	94.46±0.083	96.54±0.043	72.18±0.039	68.7±0.032
8	94.46±0.085	96.55±0.056	72.19±0.032	68.7±0.031

10. Stability studies:

The stability studies were carried out for prepared *in situ* gelling systems. All the formulations were analysed for visual appearance, clarity, pH, gelling capacity, drug content and *in vitro* release studies. Eight weeks of stability studies revealed that there was no change in visual appearance and clarity. All the formulations have shown slight changes in pH which was in acceptable limits (±0.3). Study of drug content and *in vitro* drug release revealed that there were no definite changes observed to justify for drug degradation.

CONCLUSION

The present work is an attempt to develop sustained ocular delivery of sparfloxacin from pH-triggered *in situ* gelling system. The study has demonstrated various aspects and from the results obtained, it was concluded that Sparfloxacin shows broad antibacterial activity against Gram-positive bacteria. *In situ* gel formulation of Sparfloxacin with mucoadhesive properties is useful to prolonging pre-corneal residence time in eye. The developed formulation can release the drug at controlled rate for prolonged duration. Local drug delivery may be an advantageous in treatment, since it would probably eliminate side effects, which occur with systemic dosing. Effective and prolonged local levels of an anti-bacterial activity could be achieved without much systemic load with comparatively less frequency of administration. This type of drug delivery system can serve as a novel approach for treating ophthalmic infections with better patient compliance. The optimized formulations S1 and S2 were liquid before instillation into eye and underwent rapid gelation upon instillation into eye. The formulations were found to be clear, having good *in situ* gelling capacity and a drug content 94.54-97.24%. Optimised formulations were sterile and showed sustained drug release over 8 hrs period and have good antibacterial activity. From the *in vivo* studies, formulations were found to be non-irritant and gelation of *in situ* solution is observed after 10 sec of instillation.

Hence from the above results we can conclude that it is possible to formulate *in situ* ophthalmic gels of Sparfloxacin using HPMC and Carbopol for treating various bacterial infections.

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