Original Research Article

Thermosensitive periodontal sol of ciprofloxacin hydrochloride and serratiopeptidase: Pharmaceutical and mechanical analysis

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Abstract

Aim: The aim of the present work was to explore the development of a dual-controlled release periodontal system of a potent broad spectrum first-generation fluoroquinolone, ciprofloxacin, and the anti-inflammatory enzyme serratiopeptidase (STP). **Materials and Methods:** Based on 3^2 full factorial design, thermoreversible periodontal sols capable of controlled dual delivery of ciprofloxacin hydrochloride and STP were designed using pluronic F127 and carbopol 934P as thermosensitive gelling polymers. Sol gel transition characteristics, %cumulative drug release at 48th h and *exvivo* mucoadhesive strength were designated as dependent responses. The sols were mucoadhesive, syringeable, and inverted into gels at simulated periodontal cavity temperature. **Results:** F9 with optimal drug release was identified as the best formulation. Extra design check point generated using Design Expert software 8.02 (Stat-Ease, USA) validated the experimental design. Textural analysis revealed that the developed sols were syringeable and spreadable enough for periodontal treatment so it can be expected that hardness and compressibility of sols would pose no problem during clinical application. The *in vitro* release behavior exhibited controlled release of both cipro HCl and STP (>90% release). **Conclusion:** A dual-controlled release thermoreversible periodontal sol of ciproflaxin and STP was successfully developed. Incorporation of STP as anti-inflammatory agent has the potential of developing a therapeutically efficacious system of cipro HCl for treatment of periodontal inflammatory anaerobic infections.

Key words: Controlled delivery, ciprofloxacin HCl, mechanical properties, serratiopeptidase, thermoreversible sol

INTRODUCTION

Periodontal diseases are group of several pathological conditions which affect the teeth and its supporting structure. It causes inflammation of gums, periodontal ligaments, and dental cementum, and if not treated it may lead to tooth loss.[1] Healthy gingival sulcus is associated with gram-positive species which maintains normal microflora, because these bacteria are compatible with periodontal tissue and maintain equilibrium with the host defense.[2-4] During disease progression the number of subgingival bacteria increase and the bacterial composition shifts toward gram-negative facultative or obligate anaerobes,[5,6] including *Bacteroide spp, B. intermdius, B. gingivalis, Actinobacillus actinomycetecomitans, Eikenella corrodens, Fusobacterium nucleatum,* and so on.[7, 8]

Conventional methods for the treatment of periodontitis include mechanical debridement of plaque along with topically and systemically administration of antibacterial agents. The modality is limited by lack of accessibility to bacteria at the affected site, development of bacterial resistance, side effects associated with multiple systemic dose, and rapid decline in concentration of antibacterial agents to subtherapeutic level.[9,10] Southard and Godowaski[11] reported resistance of periodontitis causing anaerobic bacteria, against many first-line drugs like metronidazole, doxycycline, and tetracycline, and in such case broad-spectrum antibiotics seems to be effective against the resistance.

Ciprofloxacin hydrochloride is a potent broad-spectrum first-generation fluoroquinolone highly potent against gram-negative bacilli, confer long post antibiotic effect, low frequency of resistance against microbes, and high tissue

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Access this article online

Quick Response Code:	Website: www.jpionline.org
	DOI: 10.4103/2230-973X.127734

penetrability along with high water solubility which allows it to reach every site in the periodontal cavity.[12]Ahmed *et al.*,[13] developed periodontal film of ciprofloxacin hydrochloride and diclofenac but incorporation of higher amount of drug and difficulty in its administration into periodontal pocket led to poor patient compliance. In another report, Rao *et al.*,[14] developed medicated gel of ciprofloxacin hydrochloride but short residence time of gel reduced efficacy of the system.

To overcome inefficiency of the reported system thermoreversible *in situ* periodontal gel is proposed that has the advantages of ease of preparation, easy administration, biocompatibility, and sufficient residence time in cavity that can potentially increase patient compliance.[15] Maheswari *et al.*,[16] used serratiopeptidase (STP), a proteolytic enzyme with anti-inflammatory action in development of tetracycline *in situ* gel and documented an increased concentration of drug at the target site and reduced rate of infection due to removal of metabolic by product of microbes. Hence, inclusion of STP would enhance the efficacy of periodontal therapeutic system. The present study deals with preparation and evaluation of thermoreversible syringeable periodontal sols (*in situ* gels) for controlled delivery of ciprofloxacin hydrochloride and STP.

MATERIALS AND METHODS

Materials

Pluronic F127 was obtained from BASF (USA). Ciprofloxacin HCl (CIPRO HCl) was obtained from Prolab Laboratory Limited (Roorkee, India). STP (MW 52 kDa) was supplied by Advanced Biochemicals Limited (Thane, India). Carbopol 934 P, agar, and peptone were obtained from Qualigens Fine Chemicals, Mumbai, India all chemicals were of analytical grade.

Experimental design

A 3^2 full factorial design [Table 1] was used to develop and optimize thermoreversible syringeable periodontal sol formulations. The amount of pluronic F127 and carbopol 934P was taken as independent variables. Each variable was set at a low level, middle level, and high level. The solgel transition temperature (°C), *ex vivo* mucoadhesive strength (g) and % cumulative drug release (CDR) at 48th h were taken as dependent variables. Nine different formulations of sols (F1-F9) were developed using the amount of drug and excipients as listed in the experimental design. Design Expert version 8.0.7 (Stat-Ease, Inc, USA) was used to analyze the effect of each variable.[17]

Veirodt's method

For simultaneous estimation of CIPRO HCl and STP Veirodt's method was adopted. The absorption maxima's of CIPRO HCl and STP in phosphate buffer, pH 7.6 were selected from the overlay spectra using Shimadzu 1700S, spectrophotometer (Kyoto, Japan). The absorptivities ($A^{1\%, 1\,cm}$) for both the drugs at both wavelengths were determined and equations 1 and 2 were generated based on Beer's Lambert law[18]:

$$C_{X} = A_2 \varepsilon y_1 - A_1 \varepsilon y_2 / \varepsilon x_2 \varepsilon y_1 - \varepsilon x_1 \varepsilon y_2 \quad \ldots (1)$$

$$C_{Y} = A_1 \varepsilon x_2 - A_2 \varepsilon x_1 / \varepsilon_{x2} \varepsilon_{y1} - \varepsilon_{x1} \varepsilon_{y2} \quad \ldots (2)$$

εx_1 and ε_{x2} are the molar absorptivities of X at λ_1 and λ_2, respectively, εy_1 and εy_2 are the molar absorptivities of Y at λ_1 and λ_2, respectively, $A_1$and A_2 are the absorbance(s) of the diluted sample at λ_1 and λ_2, respectively.

Assay of serratiopeptidase

The enzyme activity was determined according to the *Food Chemical Codex 2003*. It was based on proteolytic hydrolysis of casein at 37°C for 30 min at pH 7.0. Unhydrolyzed casein was filtered and the filtrate was analyzed spectrophotometrically for the amount of solubilized casein, at 275 nm. The protease activity was expressed as protease unit (PC) unit of preparation where one bacterial protease unit (PC) was that quantity of enzyme that produces 1.5 µg/mL equivalent of L-tyrosine per min under the condition of enzymatic assay. The activity of enzyme was calculated by the following equation:

$$PC/g = (A_u/A_s)\ (0.75/30w) \quad \ldots (3)$$

Table 1: 3^2 Full factorial design of the periodontal sols of Ciprofloxacin HCl and serratiopeptidase

Formulation Code	CIPRO HCl (mg)	(STP) (mg)	Pluronic F127 (mg)	Carbopol 934P (mg)	Response
F1	30	10	900 (-1)	25 (-1)	sol-gel transition temperature mucoadhesive strength % CDR at 48th hr
F2	30	10	900 (-1)	50 (0)	
F3	30	10	900 (-1)	75 (+1)	
F4	30	10	1000 (0)	25 (-1)	
F5	30	10	1000 (0)	50 (0)	
F6	30	10	1000 (0)	75 (+1)	
F7	30	10	1100 (+1)	25 (-1)	
F8	30	10	1100 (+1)	50 (0)	
F9	30	10	1100 (+1)	75 (+1)	
F10*	30	10	1050	37.5	

*Extra design check point formulation, -1=Low level, 0=Intermediate level and +1=High level

where A_u is the value obtained by subtracting blank reading from test reading, A_s is the absorption of the standard solution, 0.75 is the final volume in mL of reaction mixture, 30 is the time of the reaction in min, and w is the weight of the original sample in grams.[19]

Preparation of periodontal sols

Sols were prepared on weight basis using the cold method. Briefly, an amount of pluronic F127 sufficient to yield thermoreversible periodontal sol was slowly added to cold water (4-5°C), and constant stirring was maintained to get homogenous dispersion. The dispersion was refrigerated for 4-5 huntil a clear solution was obtained. Carbopol 934P, STP, and CIPRO HCl were incorporated into the sol, with constant stirring and finally stored in refrigerator (10°C) in well-closed container.

Evaluation of sols

Estimation of drug content and pH

A specified quantified (100 mg) of sol was placed in 10 mL volumetric flask and the volume was made up to 10 mL with phosphate buffer, pH 7.6. After vortexing for 1 min, the solution was filtered and the filtrate was appropriately diluted with phosphate buffer, pH 7.6. The concentrations of CIPRO HCl and STP were determined spectrophotometrically at 203 and 273 nm, respectively. For pH determination, 1 g of each formulation was dissolved in the 100 mL of distilled water and stored at 4°C for 2 h. The pH system was determined using digital pH meter (Hicon Enterprises, New Delhi, India).

Viscosity

The viscosity of formulations was determined using a programmable Brookfield viscometer (Brookfield Engineering Laboratories, INC, USA). Brookfield viscometer attached with T-bar spindle (S-94) was used for determination of viscosity. Five gram of sol was filled in a 10 mL beaker and the spindle was lowered perpendicularly taking care that the spindle did not touch the bottom of the beaker. The spindle was rotated at a speed of 100 rpm so as to generate torque greater than 50%. Readings were recorded 60 s after the measurement was made.

Spreadability

Spreadability of periodontal gel was determined by laboratory fabricated apparatus [Figure 1] that has two glass slides containing lower slide fixed to a wooden plate and the upper one attached to a balance by a hook. One gram of gel was placed on lower slide and weight was applied to the upper slide. On applying weight, the upper slide moved linearly in the direction of applied weight and the time required for complete displacement of the upper slide was recorded. Using the weight required for displacement, spreadability was calculated by using Equation 4:

$$S = m \times l/t \text{ (gram cm/sec)} \quad \ldots (4)$$

S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide, and t is the time taken for complete displacement of upper slide.

Syringeability

Syringeability study was carried out by filling 1 g of periodontal sol in a plastic syringe (Dispo Van) of 1 mL fill capacity, bearing 22 gauge needles. The filled syringe was stored in a refrigerator at 4°C to maintain the formulations in "sol;' state. Gentle force was applied manually by pressing the injector so that sol can eject out from the syringe. The ease of ejection was assessed qualitatively.

Sol-gel transition temperature and time

The temperature at which the sol was converted into gel (sol-gel transition temperature) and time taken for complete transition by periodontal sols was determined using "test tube inverting method." Five milliliters of sol was transferred to coded test tubes (F1-F9) and the mouth of each test tube was covered with aluminium foil. The test tubes were then immersed in water bath maintained at 4°C. The temperature of water bath was increased in increments of 0.5°C and left to equilibrate for 1 min each new setting. The samples were examined for gelation, which is said to have occurred when the meniscus would no longer move upon tilting at 90° angle. The temperature and time, at which gelation occurred, was recorded.

Ex vivo mucoadhesive strength

A lab fabricated apparatus was used for determining the *ex vivo* mucoadhesive strength. Fresh goat buccal mucosa was obtained from a local slaughterhouse and used within 2 h of slaughtering. The mucosal membrane was washed with distilled water followed by washings with simulated gingival crevicular fluid (SGCF: phosphate buffer, pH 7.6) at 37°C. A lab fabricated apparatus, consisting of two sides of the balance were balanced with a 5 g weight on right-hand side. A piece (1 × 1 cm^2) of goat buccal mucosa was fixed with the mucosal side upward with cyanoacrylate glue over the opening of wide mouth bottle which is covered with inert aluminium surface. The bottle was lowered into the glass container, which was filled with 100 mL of SGCF. One gram of the syringeable periodontal gel was spread as a thin film on the lower surface of the left-hand pan of the balance. A weight of 5 g was removed from the right-hand pan, which lowered the left hand pan along with the periodontal gel. The balance was kept in this position for 2 min contact time. Water from 50 cc glass burette was gradually added at the rate of 100 drops/ min to the right-hand pan until the mucosal membrane detached from the gel surface. The detachment force was used for bioadhesive strength determination by the following equation:

$$\text{Force of adhesion (N)} = \text{Bioadhesive strength (g)} \times 9.81/1000 \quad \ldots (5)$$

Texture analysis

Texture analysis was done for the mechanical characterization of periodontal *in situ* gelsusing Stable Micro Systems texture analyzer. In texture profile analysis (TPA) mode, the hemispherical analytical probe (diameter 1 cm) was twice compressed into each sample at a defined rate (2 mm s^{-1}) to depth of 15 mm. The force was 3 g. A delay period (15 s) was allowed between the end of the first and the beginning of the second compression. Mechanical

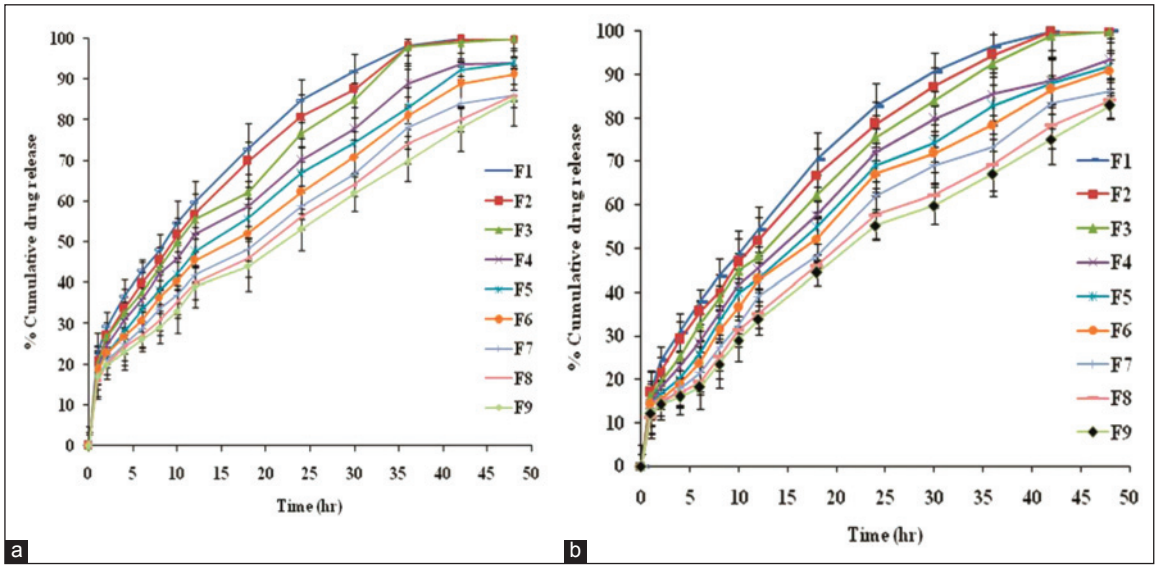

Figure 1: Comparative in-vitro drug release profiles of (a) Ciprofloxacin hydrochloride and (b) serratiopeptidase periodontal sols F1-F9; in phosphate buffer, pH 7.6 across the dialysis membrane

parameters determined were: (a) Hardness (the force required to attain a given deformation); (b) Compressibility (the work required to deform the sample during the first compression of the probe);(c) Adhesiveness (the work required to overcome the attractive forces between the surface of the sample and the surface of the probe); (d) Cohesiveness (the ratio of the area under the fitted force–time curve produced on the second compression cycle to that on the first compression cycle, where successive compressions are separated by a defined recovery period); and (e) Mucoadhesive strength (effect of time of contact between mucin disc and gel).

In-vitro drug release

In-vitro drug release of the developed periodontal sols was performed using dialysis bag method. Typically, 1 g of periodontal sol was placed in dialysis membrane (MWCO 12000). The membrane was then placed in a vessel containing 100 mL of SGCF maintained at 37°C and stirred at 100 rpm. Samples were collected periodically and replaced with fresh dissolution medium. The concentrations of CIPRO HCl and STP were determined spectrophotometrically using simultaneous equations.

Model fitting of release data

The quantitative interpretation of the value obtained in the *in vitro* drug release study was facilitated by use of generic equations that mathematically translate the dissolution curve in function of some parameters related with pharmaceutical dosage forms.[20] Release kinetics of each formulation was studied by determining model dependent parameters for zero order, first order, Higuchi, Peppas, and regression coefficients were determined.

Validation of experimental design

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_1^2 X_2 + \beta_8 X_1^2 X_2^2,$$

Where β_0, the intercept is the arithmetic mean of all quantities outcomes from nine runs

β_1-β_8 are the coefficients computed from the observed experimental values of Y, and

X_1 and X_2 are the coded levels of the independent variables and X_1X_2 and X_i^2 ($i = 1, 2$) are the interaction and polynomial terms, respectively. Validation of design was done by extra design check point formulation (F10) for which center point of each variable was selected. This coded level was transformed to actual value of each variable using principle of transformation.[17] Experimental value and predicted value of each response was determined by performing experiment and using Design expert software respectively.

In vitro antibacterial activity

The sterilized solidified nutrient agar medium was seeded by facultative anaerobic bacteria *Streptococcus mutans* by well diffusion method. Anaerobic conditions were maintained by placing agar plates in vacuum desiccator consisting of moistened calcium chloride that maintained anaerobic environment by slowly releasing the CO_2.[21] Specified quantity of optimized formulation was filled in the sterile cavity prepared by using sterile cork borer. Negative control (gel without drug and enzyme) and positive control (solution of ciprofloxacin hydrochloride, 100 μg/mL) were also taken to complete the study.[14] The plates were incubated at 37°C for 48 h and observed for zone of inhibition around the wells that was measured by antibiotic zone reader and compared against the negative and positive control(s). The readings were taken in three different fixed directions in all three replicates.[22]

Stability

The stability study of optimized formulation was performed according to International Conference on Harmonization guidelines at 25°C/60% RH and at 40°C/75% RH. Samples were taken at 0, 30, 60, and 90 days interval and assayed for content uniformity, color, and odor. Additionally, the samples were also subjected to enzymatic assay for STP in order to determine the enzymatic content of STP in sol according to Food Chemical Codex 2003.

RESULTS AND DISCUSSION

Development of simultaneous equations

According to Vogel (2001), the λ max obtained from the overlay spectra of two drugs, where wavelength maxima are separated by at least 50 n, can be used to develop simultaneous equations. Consequently, using Veirordt's method simultaneous equationswere developed as detailed below:

$$C_{stp} = A_{273}\ 4244.3 - A_{203}\ 17.92/\ 96.93 \quad \text{... (6)}$$

$$C_{cipro} = A_{203}10016.75\text{-}A_{273}147.8/96.93 \quad \text{... (7)}$$

Enzymatic activity of serratiopeptidase

The equation used for determination of enzymatic activity was: PC/g = (Au/As) × (22/30w) where A_u = 0.009; A_s = 0.018; 22 = Final volume in mL; 30 = Time in min; W = wt in g of original sample taken. Thus, PC/mg = (0.009/0.018) × (22/30 × 10) = 0.0366 and PC/g = 36.6 PC.

Drug content and pH

All the formulations exhibited fairly uniform drug content of CIPRO HCl and STP [Table 2]. The drug content was found in the range of 89.02 ± 0.06-98.02 ± 0.5 for CIPRO HCl and 88.2 ± 2.2-99.0 ± 0.5 for STP. The pH of thermoreversible injectable periodontal sols was extended over neutral pH range of 6.1 ± 0.04-7.1 ± 0.12, which is in the range (6.8-8.2) of pH of periodontal cavity during inflammation which indicate that the periodontal sols can be used in the periodontal cavity without any potential irritation. The uniformity in pH and drug content is attributable to single step preparation of sol, that is, addition of drug to the polymer dispersion that accounted for negligible drug loss. It also showed the superiority of cold method as compared to "Hot Method" which may cause inactivation of drug by high temperature and clump formations in formulation.[23]

Rheological studies

Viscosity is an important parameter for characterizing the sols as it affects its syringe ability, spread ability, and release of drug. Viscosity of developed formulations (F1-F9) was found to vary from 13 ± 0.002-18.21 ± 0.003-10^3 cps [Table 2]. Analysis of results indicated that viscosity of the developed sols was chiefly dependent on concentration of pluronic F127. Formulations containing higher amount of pluronic F127 (F7-F9) were more viscous than rest of the formulations. F9 exhibited highest viscosity of 18.21 ± 0.003 × 10^3 cps, while F1 showed least viscosity of 13 ± 0.002 × 10^3 cps. This is because at low concentration pluronic F127 forms monomolecular micelles in aqueous media and at higher concentrations (>18% w/w) multimolecular aggregates are formed consisting of a hydrophobic central polyoxyethylene polypropylene core with their hydrophilic polyoxyethylene chains facing external medium.[24] This results in formation of low viscosity transparent solutions at low temperature but gradually transforms to solid gels at body temperature due to increased entanglement of micelles which increase the size of micelles within the gel structure increasing the viscosity.[25] According to a patent report,[26] the desirable viscosity of periodontal gel should be 1 × 10^6-5 × 10^6 poise (0.1 radian/s); thus, the F9 formulation with viscosity value of 18.21 ± 0.03 × 10^3 cps can be considered suitable for periodontal application.

Spread ability represents the compressibility of formulation and affects the removal of product from the container and its application into the periodontal cavity. Spread ability of the formulations ranged from 28.81 ± 0.93 to 34.21 ± 0.25 g cm/s [Table 2] indicating decrease in spread ability with increased

Table 2: Pharmacotechnical characteristics of periodontal sols (F1-F9) and the response parameters (*n*=3)

Formulation Code	pH	Drug content (%)		Viscosity (x 10^3 cps)	Syringeability	Spreadability (g.cm/sec)	Sol-gel Transition		*Ex-vivo* Mucoadhesive Strength (g)
		CIPRO HCl	STP				Gelling Temp (°C)	Gelling Time (Sec)	
F1	6.63±0.03	94.12±0.21	96.12±1.54	13.13±0.02	PASS	34.21±0.25	31.20±0.57	33.20±1.57	18.02±0.42
F2	6.94±0.03	89.02±2.22	95.75±0.03	14.11±0.01	PASS	33.67±0.53	27.40±0.62	28.80±0.04	20.98±0.05
F3	6.52±0.54	94.12±1.71	97.81±0.09	14.84±0.13	PASS	32.21±0.07	26.80±1.07	26.60±0.57	21.20±2.78
F4	7.11±0.12	97.82±0.12	90.1.2±0.58	14.02±1.09	PASS	32.11±0.07	30.80±0.02	31.90±0.31	20.05±1.21
F5	6.14±0.04	96.12±0.50	97.11±0.02	15.11±1.02	PASS	31.87±0.51	28.90±0.12	29.20±0.32	23.67±0.02
F6	6.12±1.22	90.14±1.21	88.21±2.22	15.01±0.10	PASS	31.28±0.93	27.00±0.15	26.10±0.10	25.48±0.76
F7	6.37±0.12	91.31±0.04	95.11±2.54	15.80±0.19	PASS	29.52±0.34	29.20±0.52	32.00±1.43	20.18±0.64
F8	6.63±0.04	95.89±0.92	98.92±0.07	17.98±0.14	PASS	29.13±0.45	28.20±0.27	28.10±2.02	22.82±0.03
F9	6.68±0.12	98.71±1.89	98.18±0.32	18.21±0.03	PASS	28.81±0.93	26.00±1.06	25.40±0.57	25.92±1.52

concentration of pluronic F127. Consequently, formulation F9 with highest viscosity was least spreadable. Similar interpretation was revealed by Pandey *et al*.,[27] for the gel formulations. Grossly, all the formulations (F1-F9) had acceptable spread ability suitable for periodontal treatment.

As the gels were aimed to be applied locally, syringe ability studies were also carried out and results indicate that all formulations were syringeable through 22 gauge needle at room temperature and it was not affected by concentration of pluronic F127 and carbopol 934P.[28] The formulations are expected to be in sol state at room temperature and transform to gel state at the temperature of inflamed periodontal cavity. *In vitro* assessment of this property was done by determination of sol-gel transition temperature and time by using test tube inversion method which displayed that transition temperature ranged between 26.0 ± 1.06-31.0 ± 0.57°C and transition time was found to vary from 26.0 ± 0.02-33 ± 1.57 s [Table 2]. Sol-gel transition temperature and time lowered with higher concentration of pluronic F127. Consequently, formulations containing high concentration of pluronic F127 (F7-F9) exhibited low sol-gel transition temperature and less time as compared with formulation containing low concentration of pluronic F127 (F1-F3). Gelation was due to the formation of thermo-induced spherical micelle by pluronic F127 which shows transition from unimer to micelle state at higher temperature. The micelle formation is dependent on micellar volume and symmetry and it is reported that when the micellar volume fraction is increases to a critical value of ~0.53 which is equivalent to 18% concentration of pluronic F127, micelle formation will take place and on further increasing the concentration spherical micelle will be formed due to faster aggregation.[24,29,30] Hence, as the concentration of pluronic F127 was increased, strong gelation occurred that accounts for low gelation temperature and time for F7-F9. However formulations F3, F6, and F9 having high concentration of carbopol 934P lowered the sol-gel transition temperature of sols independent of concentration of pluronic F127 suggesting the influence of carbopol 934P on gelation mechanism.[31] The lowering of gelation temperature is attributed to the presence of -COOH group in carbopol which supplemented hydrogen bonding to the pluronic F127 etheral oxygen atom and enhanced the hydrophobicity of polypropylene polyoxyethylene group of pluronic F127.[32] It has been reported that gelation temperature can be considered to be suitable if it ranges from 25-37°C.[26,27] If the gelation temperature of a thermoreversible formulation is lower than 25°C, gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administration. If it is beyond 37°C, then it is not suitable for pharmaceutical drug delivery. Low gelation temperature and time is useful against the high gingival cervical fluid (GCF) flow rate observed during inflammatory condition,[33] which is enhanced up to 40 times of the normal GCF flow rate. In such case, low gelation temperature and time can prevent dilution of gel by high turnover rate of GCF which ultimately determines the clinical efficacy of periodontal sol.[34] Pluronic F127 formulations are known to exhibit thermoreversible gelation, depending on the polymer grade, concentration and other included formulation components.[26,24] So by altering the composition of sols, the temperature range of sol-gel phase transition could be narrowed to a certain extent, which might be very useful for their further application as injectable in *in situ* gel forming drug delivery system.

Ex vivo mucoadhesive strength

Assessment of mucoadhesive strength in terms of detachment stress showed that the adhesive property increased with increasing concentration of carbopol 934P [Table 2]. Mucoadhesive strength of the formulations ranged from 18.02 ± 0.42 (F1)-25.92 ± 1.52 g (F9). Consequently, F6 and F9 with high levels of carbopol 934P exhibited higher mucoadhesive strength as compared with F1, F4, and F7 having low level of carbopol 934P. These results are in accordance with the findings of Jones *et al*.,[35] and are attributable to the higher concentration of polymer chains at the mucin layer which subsequently increased the number of entanglements/interactions with mucus glycoproteins. It is also reported that carbopol 934P has COOH group which binds with oligosaccharide chain of mucin by hydrogen bonding.[36] Pluronic F127 is also known to be moderately adhesive with short retention time[15] and its adhesive property is increased in the presence of carbopol 934P. As an additional feature, carbopol 934P also protects the formulation from the mucolytic action of STP, thus prolonging the residence time of gel in periodontal cavity against the high GCF flow rate in periodontitis.[37] Results of *ex vivo* mucoadhesive strength in terms of detachment stress suggested that the formulations with high concentration of carbopol 934P would be suitable for periodontal treatment.

TPA

The mechanical parameters derived from TPA can be directly correlated with sensory parameters *in vivo, that is,* removal of product from the container and its application characteristics.[35] As the concentration of pluronic F127 and carbopol 934P was increased, the mechanical properties altered, resulting in increased gel hardness, compressibility and adhesion of gel to the analytical probe but cohesion was decreased. Adhesion ranged from 2.83 ± 0.07 Nmm-6.15 ± 2.17 Nmm [Table 3]. This is an important parameter in the development of gels, since a desirable retention time of gels at the mucosal surface would ensure better clinical efficacy. Formulation F7-F9 having higher adhesive characteristics due to high level of pluronic F127 and carbopol 934P can be considered optimum for periodontal treatment. Gel hardness and compressibility used for validation of syringeability and spreadability characteristics of gel respectively. Gel hardness and compressibility ranged from 0.67 ± 0.01 N to 3.01 ± 1.52 N and from 6.05 ± 0.08 Nmm-20.21 ± 1.43 Nmm, respectively [Table 3]. The findings suggested that on increasing the concentration of pluronic F127 and carbopol 934P, hardness and compressibility of formulation were also increased. Hardness and compressibility are the rheological parameters that quantify product deformation both under compression and shear, and also the resistance of formulation to compression, therefore reflects product viscosity. According to Jones *et al*., hardness

Table 3: Mechanical Properties of Formulation F1-F9 determined by Texture profile analysis

Formulation Code	Adhesiveness (Nmm)	Compressibility (Nmm)	Hardness (N)	Cohesiveness (Nmm)	Mucoadhesive strength (g)	
					T (120 sec)	T (240 sec)
F1	2.83±0.07	06.05±1.08	0.67±1.54	1.11±0.12	19.25±1.21	20.22±2.21
F2	3.04±0.13	07.24±2.18	0.70±2.34	0.92±0.01	18.11±2.11	22.98±0.15
F3	3.12±0.22	07.91±0.12	0.93±0.02	0.87±0.01	19.21±0.15	24.18±0.16
F4	4.12±0.31	11.52±0.32	1.26±1.24	0.82±0.23	20.22±1.23	25.55±1.51
F5	4.40±0.21	11.66±0.23	1.30±1.02	0.81±0.07	24.12±0.12	26.11±0.32
F6	4.68 ±1.25	13.55±0.45	1.63±0.33	0.81±0.05	29.22±1.15	32.12±0.19
F7	4.91±0.18	17.46±0.76	2.55±0.09	0.79±0.12	32.12±0.82	35.11±0.15
F8	5.22±1.23	18.22±1.23	2.81±0.12	0.76±2.98	34.10±0.11	36.21±0.11
F9	6.15±2.17	20.21±1.43	3.01±1.52	0.75±1.23	37.25±1.11	39.21±2.18

and compressibility represents syringeability and spread ability of the formulations. Cohesiveness is a parameter related to the structural reformation following successive shearing stress during application. As the concentration of pluronic F127 and carbopol 934P was increased, cohesiveness decreased from 1.11 ± 0.12 Nmm-0.75 ± 1.23 Nmm. Product cohesiveness has been reported to describe spatial aspects of structural reformation following product compression. The decrease in cohesiveness was due to increase in polymer concentration which increased the semisolid nature of product.[34] Mucoadhesive strength of polymeric gels was dependent on contact time between mucin and gel, and also on the concentration of each polymeric component. At high concentration of polymers, the amount of free water present in the polymeric gels cause movement of water from the gel to the mucin which ensured swelling of mucin and enable interpenetration of mucin polymeric chains with the polymers in the gels.[35,38] However, ideally, formulations designed for periodontal drug delivery should have low hardness and compressibility, along with high mucoadhesive strength, adhesiveness and cohesiveness. Low gel hardness and compressibility ensure that minimum work will be required for gel removal from the container and administration, while high gel adhesiveness and cohesiveness will ensure prolonged adhesion of the gel onto the mucosa and a complete structural recovery of the gel following application.[39]

Since all the developed sols were syringeable and spreadable enough for periodontal treatment, so it can be expected that hardness and compressibility of sols would pose no problem during clinical application. According to these findings, it can be stated that if the total polymer concentration is increased further than high level of polymers, there might be the problem in administration of sols into periodontal cavity. Formulation F7-F9 with high adhesiveness and mucoadhesive strength seems promising for the treatment but compromise can be made between cohesiveness and mucoadhesiveness.[34] Ease of administration and residence time is important for deciding the efficacy of periodontal sol in treatment of periodontitis. Hence, according to these findings, F9 seems to be promising.

In vitro drug release

The comparative *in vitro* drug release profiles of CIPRO HCl and STP are depicted in Figure 1. The % CDR of CIPRO HCl (Figure 1a) ranged from 85.15 ± 1.2-99.98 ± 1.6% and that of STP (Figure 1b) was ranged from 82.91 ± 0.6-82.91 ± 0.6%. It can be observed from the experimental design that formulations containing high level of pluronic F127 (F7-F9) exhibited lesser percent CDR of both CIPRO HCl and STP than the formulations containing low and intermediate level of pluronic F127 (F1-F6). Formulations F1-F3 containing low level of pluronic F127 exhibited highest % CDR of 99.99 ± 0.02% for CIPRO HCl and 99.9 ± 0.9% for STP even in less than 48 h. Additionally, carbopol 934P also had similar effect on % CDR as shown by pluronic F127. Formulations F1, F4, and F7 with low level of carbopol 934P exhibited higher drug release as compared to F2, F4, and F6 with intermediate level of carbopol 934P, and F3, F6, and F9 containing high level of carbopol 934P among their respective groups. Carbopol 934P supplemented hydrogen bonding, which enhanced the dehydration of the PPO block of the micelle and micelle entanglement. As a result of these micelle entanglements, micelles could not separate easily from each other, which accounts for the rigidity and slow dissolution of these gels.[16]

Formulations F1-F3 exhibited up to 99% drug release in less than 48 h which is not desirable for periodontitis treatment. However, early drug release behavior of F1-F3 can be attributed to low level of pluronic F127 which possibly results in low viscosity. This may influence the process of micellar aggregation and possibly prevent the formation of multimolecular spherical micelle cross-linked structure. As it was reported that multimolecular spherical micelles due to micellar volume are responsible for formation of hard sphere crystals which prolonged the release of drugs.[24] So, it seems that in F1-F3, micellar volume could not reach to level of formation of multimolecular spherical micelle core and strong barrier to drug release was not formed. Hence, formulation F1-F3 can be rejected on the basis of insufficient gelation and early release behavior.

In contrast, formulation F4-F9 with intermediate and high level of pluronic F127, drug release was decreased due to increased micellar volume fraction and aggregation which results in prolonged release behavior. On the basis of above findings, it can be concluded that thermoreversible gelation behavior of pluronic F127 gel was dependent upon its concentration, and it should be more than 18% w/v in formulations for effective gelation and release behavior.[16] Though the formulations F4-F9, exhibited drug release till 48th h which is suitable for periodontitis treatment; however, very high GCF flow rate can pose threat to whole treatment so residence time of formulations would be the one of most important parameter which depends upon mucoadhesive strength and sol-gel transition temperature and time. Mucoadhesive strength was high enough for F6 and F9 which can be considered optimum for the treatment purpose, however sol-gel transition temperature and time was least for F9 that is essential to prevent the dilution of sols from high flow rate of GCF during administration. Hence, F9 with least sol-gel transition temperature and time and highest mucoadhesive strength seems to be most suitable formulation for the treatment of periodontitis. To investigate the release kinetics of drug from periodontal sols, the release data were subjected to fit various kinetics models (such as zero order, first order, Higuchi, and Peppas model) and the best fit model was found to be zero order with r^2 value 0.9998 The release behavior indicated release of drug was controlled and drug releases from the proposed system up to desired time, that is, till the 48th h and indicated the efficacy of proposed system that periodontal sols can be used for delivery of drug till the desired time [Table 4].

Statistical analysis of experimental design by design expert 8.0.7

Statistical analysis of the experimental design was done by Design Expert software version 8.0.7 (Stat-Ease, Inc., Minneapolis, USA) and the second order polynomial equations were derived. The transformed equations obtained after removal of non-significant coefficients are as follows:

$$Y_1 = 27.19 + 1.78\ X_1 - 0.089\ X_1^2 + 1.52\ X_1X_2 - 0.81\ X_1^2X_2 - 1.95X_1X_2^2 + 0.87\ X_1^2X_2^2 \quad \ldots (8)$$

$$Y_2 = 26.02 + 4.10\ X_2 - 4.19\ X_1^2 - 1.37X_1X_2 - 3.32X_1^2X_2 + 2.08\ X_1X_2^2 - 1.98X_1^2X_2^2 \quad \ldots (9)$$

$$Y_3 = 77.11 + 6.11\ X_1 + 0.21\ X_1^2 + 0.041\ X_2 + 0.39\ X_1\ X_2 - 0.14\ X_2\ X_1 - 0.028\ X_1X_2^2 - 1.16\ X_1^2X_2^2 \quad \ldots (10)$$

where X_1 = Concentration of pluronic F127 and X_2 = Concentration of carbopol 934P, Y_1 = Sol-gel transition temperature, Y_2 = Mucoadhesive strength and Y_3 = % CDR at 48th h. From the polynomial equations three-dimensional response surface plots were generated which were used to predict the effect of independent variables on response variables. Response surface plots explained that factors have significant effect on dependent variables (sol-gel transition temperature, mucoadhesive strength and % CDR at 48th h). On increasing the concentration of both pluronic F127 and carbopol 934P, sol-gel transition temperature decreased [Figure 2a], whereas the mucoadhesive strength was increased by increasing the concentrations of carbopol934P and pluronic F127 [Figure 2b]. % CDR at 48th h decreased on increasing the concentrations of both pluronic F127 and carbopol 934P [Figure 2c]. The results clearly emphasize the effect of selected polymers on the performance characteristics of periodontal sols.

Validation of the experimental design

For the validation of 3^2 full factorial design extra check point formulation (F10) was formulated and evaluated for sol-gel transition temperature, mucoadhesive strength and % CDRat 48th h and compared with the results of optimized formulation. The results obtained suggested that the experimental values were close to the predictedvalues and percentage error was calculated by the following equation:

$$\text{Percentage error} = (\text{Predicted value} - \text{Actual value}/\text{Predicted value}) \times 100 \quad \ldots (11)$$

Table 4: Model fitting data for Ciprofloxacin HCl and Serratiopeptidase

Formulation code	Zero order		First order		Higuchi		Peppas	
	CIPRO HCl	STP	CIPRO HCl	STP	CIPRO HCl	STP	CIPRO HCl	STP
F1	0.9989	0.9778	0.9998	0.9991	0.9123	0.8162	0.9870	0.8791
F2	0.9997	0.9800	0.9996	0.9982	0.9238	0.9652	0.9166	0.8802
F3	0.9802	0.9839	0.9989	0.9992	0.9115	0.9786	0.8899	0.9829
F4	0.9982	0.9855	0.9705	0.9822	0.8833	0.8763	0.9605	0.9822
F5	0.9990	0.9995	0.9815	0.9834	0.9726	0.8876	0.9715	0.8734
F6	0.9992	0.9998	0.9930	0.9850	0.9765	0.9875	0.9830	0.9850
F7	0.9991	0.9996	0.9865	0.9872	0.9665	0.9942	0.8865	0.8872
F8	0.9902	0.9998	0.9871	0.9680	0.8987	0.8898	0.8971	0.8980
F9	0.9998	0.9997	09860	0.9795	0.8997	0.9871	08880	0.9795

The percentage error varied from 0.38-3.37%. The low magnitude of error in the current study indicates that 3^2 full factorial design was validated [Table 5].

Antibacterial activity

The zone of inhibition of optimized formulation (F9) against *S. mutans* was found to be 25±0.87 mm and of positive control it was 17.92±1.43 mm. No zone of inhibition was observed in negative control. The *in vitro* antibacterial activity revealed that the gel was able to inhibit the growth of microbes more effectively rather than pure drug solution. Incorporation of CIPRO HCl in the copolymer matrix provided a system that ensured continuous slow delivery of dissolved drug from the matrix to exert the antibacterial effect that was not possible with pure solution of the drug. No zone of inhibition was recorded in the negative control.

Stability studies

Stability study results revealed that optimized formulation F9 was stable when stored at 25ºC/60% RH and at 40ºC/75% RH for period of 90 days as the drug content was found to be greater than 95% till the end of the study period. The enzymatic content (PC) decreased slightly but was in the range of 10-44 PC. As per Food chemical codex 2003, this value indicates stability of periodontal sol in terms of its enzymatic activity [Table 6]. The organoleptic attributes of the gel remained unchanged during the study.

CONCLUSION

A thermoreversible, syringeable periodontal sol that offered controlled release of ciprofloxacin hydrochloride and STP was developed for application into the periodontal pocket. The developed optimized sol was satisfactory in terms of syringeability, mucoadhesiveness, and incorporation of STP as an anti-inflammatory agent, has the potential of developing a therapeutically efficacious system for treatment of periodontal inflammatory anaerobic infections.

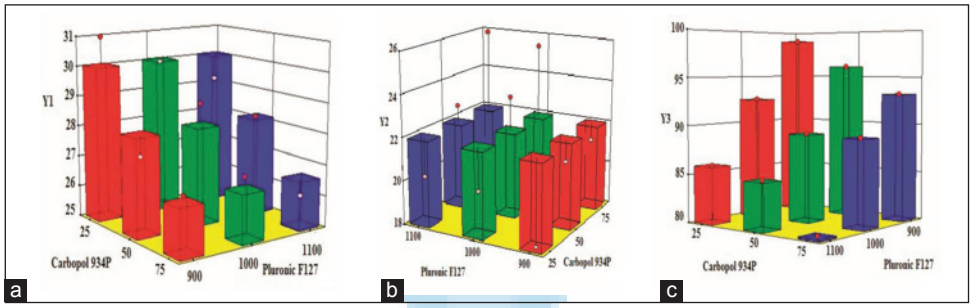

Figure 2: Response surface plots depicting simultaneous effect of Pluronic F127 and carbopol 934 P on (a) sol-gel transition temperature; (b) mucoadhesive strength and (c) % CDR at 48th hr

Table 5: Evaluation of extra design checkpoint formulation (F10)

Formulation code	Response parameter	Predicted value	Experimental value	Percentage Error (%)
F10	Sol-gel transition temperature (ºC)	27.19	26.00±1.06	3.37
	Mucoadhesive strength (g)	26.02	25.92±1.50	0.38
	% CDR at the 48th hr	77.08	76.11±2.51	1.25

Table 6: Stability study data of optimized formulation (F9)

Time (Days)	Color	Odor	Test conditions			
			(25º±2ºC/60% RH)		(40º±2ºC/75%RH)	
			CIPRO HCl (%Drug content)	STP (Enzymatic content PC)	CIPRO HCl (%Drug content)	STP (Enzymatic content PC)
0	—	—	97.82±1.06	36.82±2.34	97.33±1.16	36.11±1.98
30	—	—	97.42±1.43	36.21±1.22	97.08±1.89	36.15±1.27
60	—	—	97.40±2.06	36.19±2.89	97.80±1.44	36.02±2.89
90	—	—	97.19±1.66	35.92±1.67	97.13±2.16	35.90±1.19

— No change

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How to cite this article: Singh KP, Chhabra G, Sharma V, Pathak K. Thermosensitive periodontal sol of ciprofloxacin hydrochloride and serratiopeptidase: Pharmaceutical and mechanical analysis. Int J Pharma Investig 2014;4:5-14.
Source of Support: Nil. **Conflict of Interest:** None declared.