Development of Tetracycline-Serratiopeptidase-Containing Periodontal Gel: Formulation and Preliminary Clinical Study

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ABSTRACT

The purpose of this research was to reduce the polymer concentration and to obtain reasonable viscosity at a lower concentration of pluronic by the addition of a viscosity modifier. A 20% wt/wt pluronic gel was prepared on a weight basis using the cold method. The effect of the amount of tetracycline and Aerosil on gel properties was studied. The gel was evaluated using different parameters: polarizing microscopy, gelation, gel melting, bioadhesivity, viscosity, drug release, and stability of enzyme. An in vivo study was performed to evaluate the clinical efficiency of the liquid crystalline gel. Addition of Aerosil to the gel favored hexagonal phase formation. Viscosity and bioadhesivity increased with an increase in the concentration of Aerosil. Release of tetracycline was sustained as the concentration of Aerosil increased. Various clinical parameters confirmed the acceptability and efficiency of this gel system.

INTRODUCTION

Block copolymers exhibiting a cubic phase have attracted significant attention because they offer such advantages as controlled drug release, bioadhesivity, and protection of sensitive drug molecules. Pluronic F 127 exhibits a thermoreversible gelation property in water, with a high solubilizing capacity. It can form liquid crystalline mesophases with high viscosity, which enhance protein stability and provide controlled drug release. Hence, this polymer is of special interest to the pharmaceutical industry.[1-5] Drugs and excipients in the gel significantly affect the properties of the system. Pisal et al reported the effect of the addition of vitamin B_{12} and other excipients.[5] Pandit et al reported the effect of salt on the gelation behavior of pluronic F 127.[6]

The major limitation of the pluronic gels is the high concentration of polymer required to obtain the optimum viscosity and achieve the desired pharmaceutical performance. Therefore, attempts have been made to reduce the polymer concentration by adding carbopol, hydroxypropylmethylcellulose and carboxymethylcellulose.[7,8] These polymers are added in the concentration range of 1% to 5% wt/wt. Colloidal silicon dioxide (Aerosil 200) is a popular gelling agent that has been shown to gel in a wide range of solvents.[9] Silicon dioxide may cause significant changes in the liquid crystalline phases and modify the rheological properties. Shah and Paradkar have reported the use of magnesium trisilicate for modification of viscosity and release from glyceryl monooleate liquid crystal phases.[10]

Cubic phase systems are widely used for depot injections and for topical and periodontal delivery of drugs. Periodontal treatment is routinely based on mechanical debridement of the tooth surface and appropriate maintenance of oral hygiene. However, comprehensive mechanical debridement of sites with deep periodontal pockets is difficult to accomplish. Mechanical therapy alone may fail to eliminate the pathogenic microflora because of their location within the gingival and dental tissues or in other areas inaccessible to periodontal instruments.[11] As an adjunctive approach, systemic or local administration of antibiotics is used because of the microbial etiology of periodontitis. Antibiotics also aid in pocket elimination with nonsurgical periodontal therapy, where surgery is contraindicated. However, to obtain an effective concentration of the antimicrobial drug in the periodontal pocket after systemic administration, repeated intakes over a prolonged period of time are required. Furthermore, when broad spectrum antibiotics are used, there is always a risk of inducing bacterial resistance and distortion of commensal flora. Therefore, a more satisfactory approach to administering antimicrobial drugs directly into the pocket involves use of a controlled release device. Using such a device not only sustains an effective dose for the required length of time but also bypasses systemic complications and

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targets localized areas of periodontal destruction.[12] Local drug delivery limits the drug to its target site, with little or no systemic uptake, so a much smaller dose is required for effective therapy and harmful side effects can be reduced or eliminated.

A number of local drug delivery devices have been proposed, including fibers, strips, films, gels, sponges, and microparticles.[13,14] Goodson et al[15] developed ethyl vinyl-acetate hollow fibers loaded with tetracycline HCl, but the fibers, being nonresorbable, have to be removed after 10 days. Periochip is a biodegradable matrix containing chlorhexidine gluconate reported by Killoy.[16] Another commercially available product is metronidazole in glyceryl monooleate and sesame oil, formulated as a suspension, which turns into a controlled release semisolid at body temperature.[17] These commercially available delivery systems have a number of shortcomings, including limited duration of drug release, difficulty in application, and poor retention in the periodontal pocket.

The use of pluronic gels for delivery of tetracycline in the periodontal pocket has been well documented. Serratiopeptidase, a proteolytic enzyme with anti-inflammatory activity, is widely used in dental treatment. Serratiopeptidase improves microcirculation and reduces pain by blocking the release of pain-inducing amines from inflamed tissues. The topical use of enzymes is also associated with a significant increase in the concentration of antibiotic at the wound and decrease in the rate of infection.[18] Therefore, localized delivery of the enzyme along with antibiotic may provide better relief than antibiotic alone. But proteolytic enzymes may reduce bioadhesivity because of their mucolytic action.

The amount of gel that can be introduced in the periodontal pockets is extremely low, and it is necessary to raise the viscosity for better retention in the periodontal pocket and better control of drug release. Gelation of the thermoreversible gel is affected by a range of factors, such as temperature, polymer concentration, concentration of active ingredients, and electrolytes.[6,19] So, in the present study, to reduce polymer concentration and to obtain reasonable viscosity at a lower concentration of pluronic, the effect of Aerosil and tetracycline on gel properties was studied. Liquid crystalline gel was characterized using polarizing microscopy. The effect of variables on the gelation and gel melting, bioadhesivity, viscosity, stability, and drug release was evaluated. An in vivo study was performed to evaluate the clinical efficiency of the developed system.

MATERIALS AND METHODS

Materials

Pluronic F 127 was a gift sample obtained from BASF Svenska (Goeteborg, Sweden). Tetracycline HCl was obtained from Mercury Laboratory Limited (Varodara, India). Serratiopepti-

dase (MW 52 kDa) was supplied by Advanced Biochemicals Limited (Thane, India). Aerosil 200 was obtained from Get Rid Pharmaceuticals (Pune, India). Ammonium sulfate, disodium borate, sodium carbonate, glacial acetic acid, trichloroacetic acid, tyrosine, Hammerstein casein, potassium dihydrogen phosphate, and sodium hydroxide were purchased from Merck (Mumbai, India) and were of analytical grade.

Preparation of Gel

Gels were prepared on a weight basis using the cold method.[20] An amount of pluronic F 127 sufficient to yield 20% gel was slowly added to cold water (5°C); constant stirring was maintained. Each dispersion was refrigerated until a clear solution was formed (5 hours). To manipulate the various formulation properties, silicon dioxide (Aerosil 200) was added to the gel. The effect of different concentrations of Aerosil and drug on various properties of the gel was also studied. The concentration of serratiopeptidase (0.5% wt/wt) was kept constant in all the batches.

Characterization

All characterization studies were performed in triplicate.

Polarizing Light Microscopy

Gel samples were examined under a polarizing light microscope (Nikon, Melville, NY) using an $\lambda\frac{1}{4}$ compensator to study the existence of birefringence under crossed polarized light, employing a magnification of ×100. The lamellar, cubic, and hexagonal phases were identified according to the classification established by Rosevear.[21]

Gelation and Gel Melting

Gelation and gel melting were assessed using a modification of the Miller and Donavan technique.[22] A 5-mL aliquot of gel was transferred to test tubes, immersed in a water bath at 4°C, and sealed with aluminum foil. The temperature of the water bath (Haake Phoenix c25P, Karlsruhe, Germany) was increased in increments of 0.5°C and left to equilibrate for 1 minute at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°. The gel melting temperature, the temperature at which a gel starts flowing upon tilting through 90°, was recorded.

Viscosity Studies

The viscosity of gels was measured using Brookfield Cap 2000+ Version 1.10 programmable viscometer (Brookfield Engineering Laboratories Inc, Middleboro, MA). The

measurements were performed in a cone and plate geometry with a diameter of 24 mm (cone angle 0.8°). Viscosity parameters were collected at different rpm with 1-minute equilibration time at every rpm. Samples were applied to the lower plate using a spatula to ensure that formulation shearing did not occur. To test the effect of temperature, the measurements were made at 25°C and 35°C. Each data point is the mean of triplicate analysis. Error bars have been omitted to retain clarity.

Bioadhesivity

Bioadhesion was determined by the tensiometric method.[23] Freshly excised sheep intestinal tissue was stored in Tyrode's solution at 4°C and thawed to room temperature before use. The sheep intestinal tissue was cut into pieces (4 cm), washed with cold Tyrode's solution, and blotted with tissue paper. The intestinal tissue was mounted on the stainless steel base of the apparatus with double-sided adhesive. The liquid crystalline gel was placed on the lower side of the moving instrument probe of the advanced force gauge (Mecmesin, West Sussex, England), which was then lowered onto the tissue at a test speed of 0.5 mm/s so as to bring the tissue and sample into contact with each other. The experiments were performed at room temperature. The contact force was 0.2 N, the contact time was 1 minute, and the probe was withdrawn at a rate of 0.1 mm/s. The peak detachment force was considered to be the bioadhesive force.

Dissolution Studies

The dissolution studies were performed using the dialysis method. Typically, 1 g of pluronic gel was placed in a dialysis tube (MW 12 000 cutoff). The dialysis tube was then placed in a vessel containing 100 mL of phosphate buffer pH 7.2, maintained at 37.5°C, and stirred at 100 rpm. Samples were collected periodically and replaced with fresh dissolution medium. After filtration through Whatman filter paper 41, the concentration of tetracycline was determined spectrophotometrically at 353 nm. The kinetic analysis of the release data was done using PCP-Disso v2.08 software (Poona College of Pharmacy, Pune, India) using the Korsmeyer equation.[24]

Stability Study

A stability study was performed to determine the effect of temperature and humidity on the proteolytic activity of serratiopeptidase. The formulation containing 3% wt/wt tetracycline, 1.0% wt/wt Aerosil, and 0.5% wt/wt serratiopeptidase was selected for the stability study. The stability study was performed according to International Conference on Harmonization (ICH) guidelines at 25°C/60% relative humidity (RH) and 40°C/75% RH. Samples were withdrawn at 0, 7, 15, 30, 60, and 90 days.

The enzyme activity was determined as per the method reported in the *Food Chemical Codex*.[25] The assay was based on a 30-minute proteolytic hydrolysis of casein at 37°C and pH 7.0. Unhydrolyzed casein was removed by filtration, and the solubilized casein was determined spectrophotometrically at a wavelength of 275 nm. In this method, the protease activity is expressed as protease unit (PC) of preparation derived from *Bacillus subtilis var* and *Bacillus licheniformis var*. One bacterial protease unit (PC) is defined as the quantity of enzyme that produces 1.5 μg/mL equivalent of L-tyrosine per minute under the condition of the assay.

Clinical Evaluation

A total of 30 subjects of both sexes who were more than 21 years old and had been diagnosed as suffering from chronic localized or generalized periodontitis were considered for the study. The patients were receiving outpatient treatment in the Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital (Pune, India). Subjects needed to have been diagnosed as suffering from chronic periodontitis with a pocket depth of 5 mm to 8 mm in at least 2 nonadjacent sites in different quadrants of the mouth and to have at least 20 remaining teeth.

Selected sites were randomly divided into control sites and experimental sites and treated by using a split-mouth design. The 30 control sites were treated by scaling and root planing alone. The 30 experimental sites were treated with scaling and root planing followed by placement of the pluronic gel (3% wt/wt tetracycline, 0.5% wt/wt serratiopeptidase, and 1.0% wt/wt Aerosil) in the periodontal pocket.

The recorded parameters were plaque index, gingival index, sulcus bleeding index, pocket depth, relative distance between base of pocket and fixed reference point on the stent for assessing clinical attachment gain or loss, and microbiological study of collected plaque sample for spirochetes and coccoids under dark field microscopy.[26-29] The above clinical and microbiological parameters were recorded on days 0, 21, and 45.

The clinical and microbiological parameters were assessed at the baseline at selected sites followed by thorough scaling and root planing. On completion of scaling and root planing, control sites were covered with Coe-Pak (Coe Laboratories, Inc, Chicago, IL). The experimental sites were completely dried using an air syringe, and then the sites were isolated with cotton rolls to prevent contamination from saliva. The gel formulation stored at 4°C (before application) was carried in a 1-mL syringe with a needle (21) attached to it. The liquid (0.2 mL) was placed in the periodontal pocket,

and as the temperature increased, gel formation occurred. Gentle force was applied so that the material filled the depths of the pocket. The pocket opening was covered with Coe-Pak to hold the material in the pocket as well as to prevent the ingress of oral fluids. Each patient was instructed not to brush in the area where the periodontal dressing had been placed, not to chew hard or sticky food, not to floss on the treated site, and not to probe the area with the tongue, a finger, or a toothpick. Each patient was also told to report immediately if the material or pack was dislodged before the scheduled recall visit or if discomfort, a burning sensation, or any other allergic reaction occurred. Subjects were recalled after 7 days for removal of the periodontal dressing, oral hygiene maintenance instructions, and recording of subjective and objective criteria. Recall visits were again scheduled 21 days and 45 days after placement of the local experimental drug, to measure the clinical parameters, perform the microbiological study, and assess subjective and objective criteria as per the study design.

Posttreatment changes from baseline to different time intervals in various clinical parameters were analyzed by paired t test (intragroup). Intergroup comparisons of posttreatment changes were analyzed by unpaired t test. A P value less than .05 was considered a significant difference.

The research followed the tenets of the Declaration of Helsinki, promulgated in 1964, and was approved by the institutional human experimentation committee.

RESULTS AND DISCUSSION

Pluronic gel (20% wt/wt) was equilibrated with Aerosil 0.5% to 1.5% and tetracycline 3% to 9% wt/wt. It was observed that during the 24-hour equilibration period, Aerosil that was more than 1.5% wt/wt caused phase separation; and at a 9% wt/wt concentration of tetracycline HCl, undissolved drug crystals were observed in the gel. Therefore, the concentrations of Aerosil and tetracycline were kept in the above-mentioned ranges.
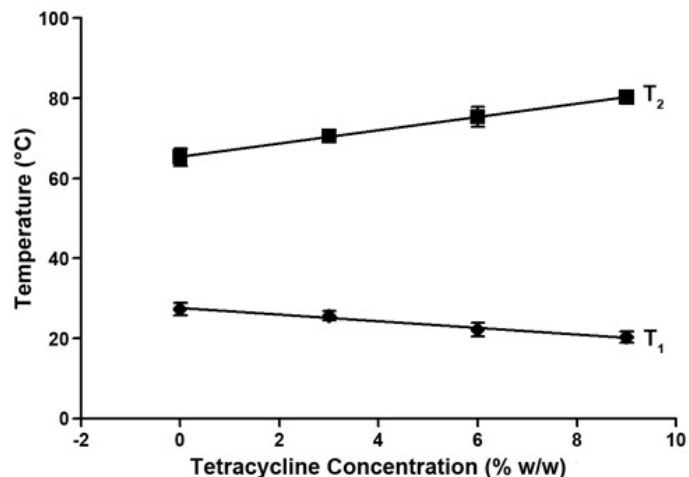

Figure 2. Effect of tetracycline concentration on gelation point (T_1) and gel melting point (T_2).

Polarizing photographs of plain 20% wt/wt gel and a gel containing 1% Aerosil are shown in Figure 1. Photographs showed a dark background in the case of plain gel, whereas some fan-like structures were observed in the polarizing photograph of the Aerosil-containing gel. Incorporation of tetracycline did not affect the liquid crystalline phase of gel, it remained in the cubic phase. Incorporation of Aerosil did affect the phase structure where it converts from the cubic phase into the hexagonal phase. However, an increase in the concentration of Aerosil did not produce any change in the phase structure. It was revealed that plain gel is in the cubic phase, which transforms to the hexagonal phase after the addition of Aerosil. For a poly oxyethylene-poly oxypropylene (PEO-PPO-PEO) block copolymer of a given composition and molecular weight, the type of structures obtained in the presence of selective solvent appears to be a function of the volume fraction of the polar/apolar component. This is attributed to the ability of the macromolecule blocks to swell to a different extent (based on the amount of solvent available) with the respective solvents and thus to modulate the interfacial curvature and resulting structure. The interfacial
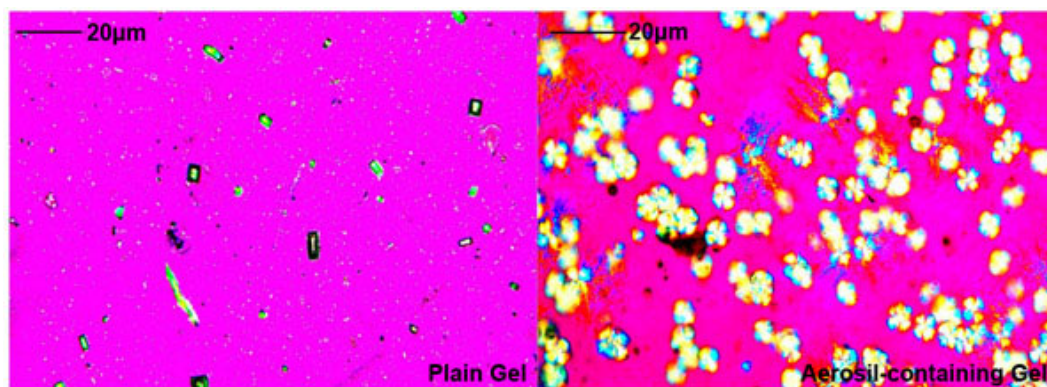
Figure 1. Polarizing light microphotographs of pluronic gel showing different phases: cubic phase (plain gel), hexagonal phase (Aerosil-containing gel).
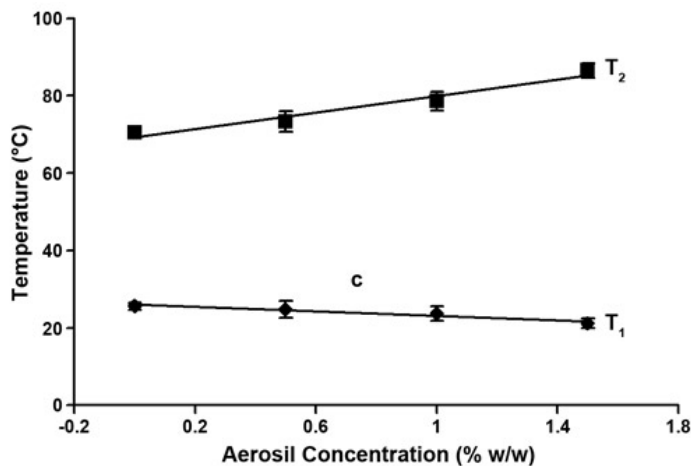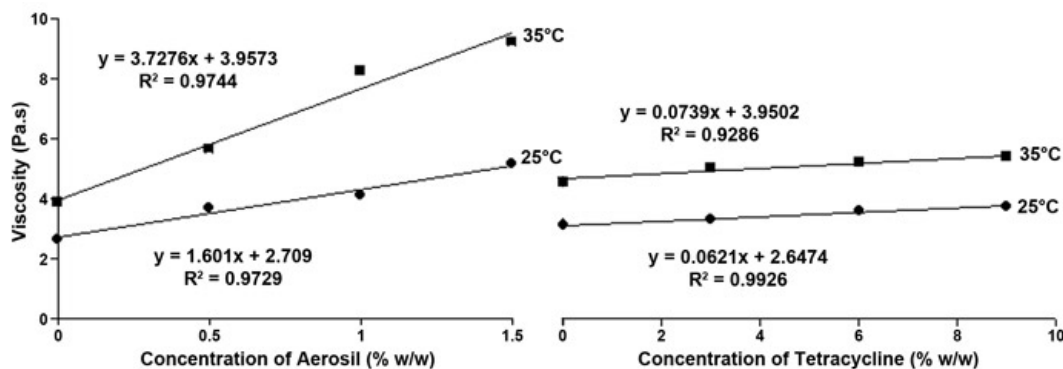
Figure 3. Effect of Aerosil concentration on gelation point (T_1) and gel melting point (T_2).

curvature is defined as positive when the interface bends toward the apolar domains—that is, the micelles are surrounded by the polar domains confining the apolar domains inside them, and vice versa.[30,31] In the cubic phase, interfacial curvature is highly positive because of the spherically shaped micelles. The normal hexagonal phase has been obtained at a high content of pluronic F 127 because of decreased solvation of the PEO blocks. Aerosil, being highly porous, absorbed water from the system; as a result, less water was available for pluronic, which caused the transformation of the cubic phase to the hexagonal phase.

Pluronic, a block copolymer, shows a thermoreversible gel at concentrations above 18% wt/wt. The effect of tetracycline concentration on gelation and gel melting is shown in Figure 2. The gelation temperature was lowered in the presence of tetracycline and decreased linearly with its increasing concentration, whereas the melting temperature increased with the concentration of tetracycline. Physically, gel formation is related to micellar packing and volume fraction. Researchers have attributed gelation to the dehydration of PPO groups in the micelle core,[32] a change in the micellar volume,[33] or a decrease in the critical micelle concentration and an increase in the aggregation number.[34] The finding that tetracycline lowered the gelation temperature was similar to the result reported by Esposito et al.[35] This feature was tentatively explained by a facilitation of the interaction between the hydrophobic portion of the polymer molecules, which could disrupt the micellar structure and increase the entanglement of micelles. Pisal et al have also reported a significant decrease in gelation temperature by vitamin B_{12} at all concentrations of pluronic F 127, attributed to the higher water solubility of vitamin B_{12}. The presence of vitamin B_{12} predominantly decreased the critical micellar concentration and increased the micellar aggregation.[5] Tetracycline has also been shown to increase the gel melting temperature. The entanglement of the large molecule in the outer PEO chain favoring hydration may be responsible for the increase in melting temperature. At higher concentrations of tetracycline, lowering of the critical micellar concentration facilitates closer packing of micelles, which means that more energy is needed to break the gel structure.

The effect of Aerosil concentration on sol-gel and gel-sol transition is shown in Figure 3. Incorporation of Aerosil shifted sol-gel transition to a lower temperature but gel-sol to a higher temperature. Thus, the gelation range broadens with the concentration of the Aerosil. Block copolymer pluronic F 127 gel is thought to be formed by H-bonding in the aqueous system, caused by the attraction of the pluronic ether oxygen atom to a proton of water. If the hydrogen bonding is supplemented by adding compounds with a hydroxyl group, the gelation point decreases.[19] The gel structure was thought to remain unaltered with temperature until an excessively high temperature caused the destruction of the gel structure. At higher temperatures, the gel underwent dehydration, but excessive hydrogen bonding and closely packed micelles restricted the destruction of the gel structure. As the concentration of the Aerosil increased, the gel structure became more closely packed, with the arrangement in a lattice pattern. In turn, the disruption of the lattice melting of the gel occurs at higher temperatures.

Figure 4. Effect of different concentrations of Aerosil and tetracycline on the viscosity of pluronic gel at different temperatures.
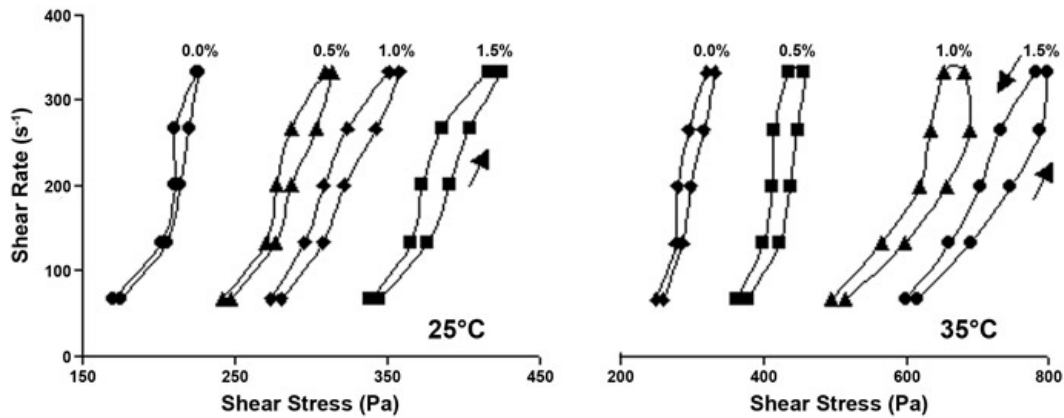
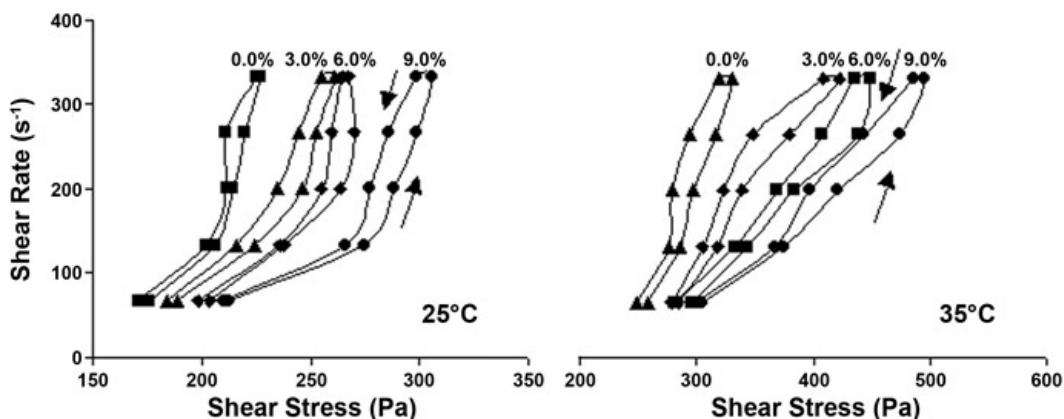**Figure 5.** Flow curve of pluronic gel containing different concentrations of Aerosil at 25°C and at 35°C.

The effect of varying amounts of Aerosil and tetracycline on viscosity is shown in Figure 4. All gel formulations exhibited an increase in viscosity with increasing temperature. The effect of Aerosil concentration on viscosity as shown by the regression equation is much greater than tetracycline's. An increase in the viscosity with temperature was found to be reflected by an increase in the number of interactions among the chains during the desolvation process. Figures 5 and 6 show the flow curves of formulations with different concentrations of Aerosil and tetracycline at different temperatures, respectively. The formulations showed non-Newtonian behavior: the up curve did not coincide with the down curve, indicating the presence of thixotropy, with a wide hysteresis loop. The area of the hysteresis loop increased with the concentration of Aerosil and tetracycline and moved to a higher shear stress value, indicating compact structure of the gels. But the recovery of the consistency was slow when there were higher amounts of Aerosil and tetracycline.

It was assumed that if the rate of shear were reduced once the desired maximum rate had been reached, the down curve would be identical to the superimposed up curve in the case of Newtonian systems, whereas the down curve for non-Newtonian systems could be displaced with regard to the up curve. This indicates a breakdown of structure (and hence shear thinning) that does not reform immediately when the stress is removed or reduced. The recovery process is not instantaneous; rather, there is progressive restoration of consistency.

Flow of the hexagonal liquid crystalline systems is presumably a function of the alignment of the rod-like aggregates along their long axis in the direction of the flow. The shear thinning effect is ascribed to orientation of the liquid crystalline domains in the direction of the shear stress vector and structural breakdown. This results in reduced resistance to flow and hence a decrease in viscosity.

The retention of the product in the periodontal pocket was the major concern, as it was necessary to ensure that the product would remain there for the intended period of drug release. Figure 7 shows the mucoadhesive behavior of the different formulations. The detachment force increased significantly with the concentration of Aerosil. The results of the detachment force study support the hypothesis that the possible mechanism of the mucoadhesion exhibited by the
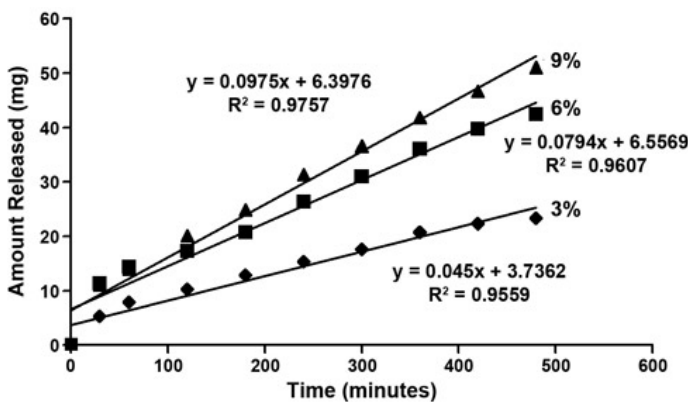

Figure 6. Flow curve of pluronic gel containing different concentrations of tetracycline at 25°C and at 35°C.
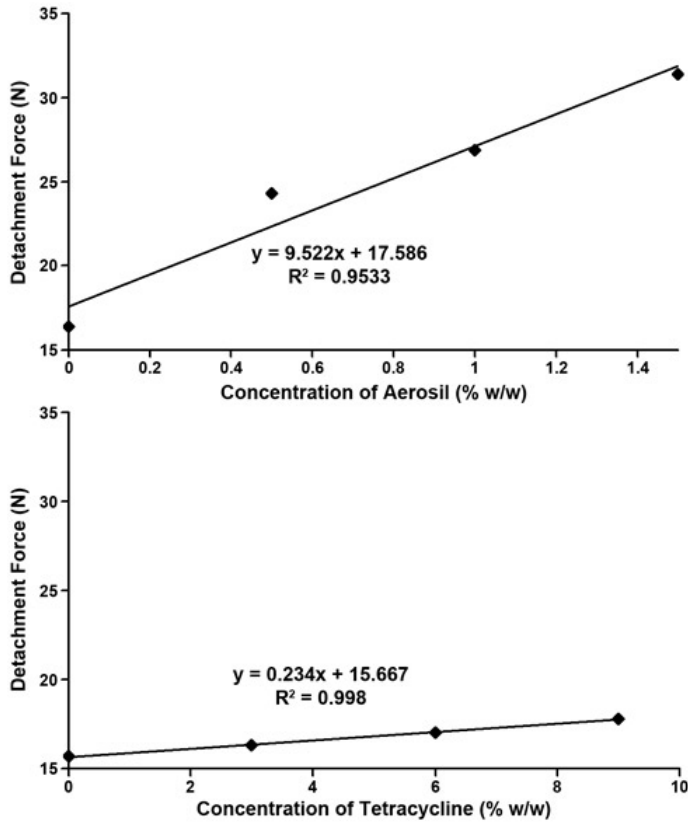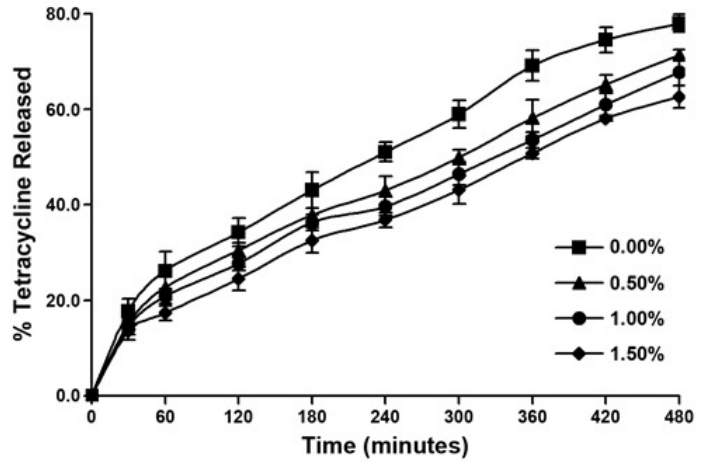
Figure 7. Effect of different concentrations of Aerosil and tetracycline on the detachment force of pluronic gel.

liquid crystalline pluronic gels is the dehydration of the mucosa (ie, water uptake by the mucoadhesive material). The amount of water taken up by the liquid crystalline gels governed the mucoadhesive force; the gel having greater water uptake capacity showed greater mucoadhesion.

The in vitro release profile provides insight into the efficiency of the drug delivery system proposed for the controlled release of the drug. To optimize the formulation, the effect of different concentrations of tetracycline and Aerosil


Figure 9. Release profile of gel containing different concentrations of aerosil.

was studied. The effect of the tetracycline concentration on the amount released is shown in Figure 8. The amount of tetracycline release increased with an increase in the concentration of the tetracycline in the gel. The following aspects affected the release of tetracycline from the pluronic formulation: solubility of the drug in the pluronic formulation, diffusion rate of the drug in the gel, and diffusion rate of water into the gel. The rate of release increased with greater concentrations of tetracycline because of higher solubility in water. The effect of the Aerosil concentration on tetracycline release is shown in Figure 9. The results of tetracycline release kinetics suggested that all the formulations followed an anomalous (non-Fickian) release pattern. The release of tetracycline decreased as the concentration of Aerosil increased. These results indicated that the structure of the gel functioned as a barrier to drug release. Such enhanced resistance may be due to the increase in the size of micelles within the gel structure, which led to higher viscosity and lower drug release. Aerosil supplemented hydrogen bonding, which enhanced the dehydration of the

Figure 8. Amount of tetracycline released from gel containing different concentrations of tetracycline.
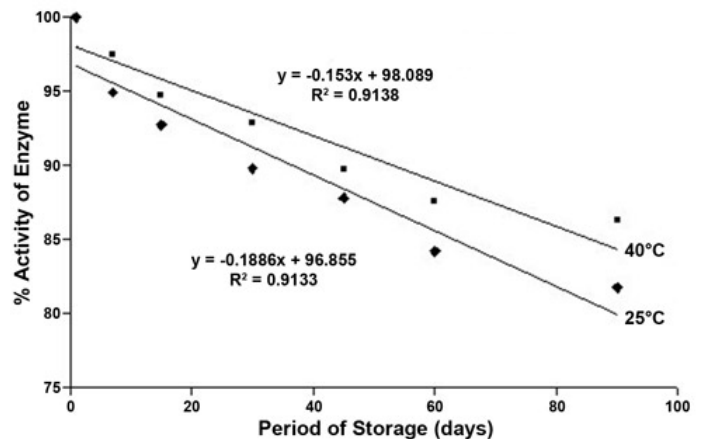
Figure 10. Stability profile of pluronic gel at different temperatures.

Table 1. Comparative Analysis of Clinical Parameters in the Control and Experimental Groups*

Parameters	Baseline Mean (SD)	21 Days Mean (SD)	21 Days Change (SD)	t	P	45 Days Mean (SD)	45 Days Change (SD)	t	P
Plaque Index									
Group A[†]	2.76 (0.34)	1.63 (0.45)	1.13 (0.39)	15.82	< .01	1.31 (0.35)	1.45 (0.40)	19.78	< .01
Group B[‡]	2.73 (0.41)	1.50 (0.32)	1.23 (0.45)	15.02	< .01	1.01 (0.33)	1.71 (0.48)	19.36	< .01
Gingival Index									
Group A	2.51 (0.31)	1.91 (0.28)	0.60 (0.33)	9.89	< .01	1.53 (0.24)	0.98 (0.30)	17.93	< .01
Group B	2.55 (0.33)	1.64 (0.34)	0.91 (0.37)	13.45	< .01	0.89 (0.44)	1.66 (0.47)	19.22	< .01
Sulcus Bleeding Index									
Group A	2.90 (0.48)	1.85 (0.46)	1.05 (0.39)	14.58	< .01	1.50 (0.41)	1.40 (0.39)	19.72	< .01
Group B	2.88 (0.34)	1.23 (0.32)	1.65 (0.39)	22.50	< .01	0.67 (0.25)	2.20 (0.41)	29.10	< .01
Pocket Probing Depth									
Group A	5.73 (0.74)	4.76 (0.72)	0.96 (0.32)	16.55	< .01	4.31 (0.70)	1.41 (0.41)	18.61	< .01
Group B	5.76 (0.62)	3.96 (0.68)	1.80 (0.75)	13.15	< .01	3.38 (0.70)	2.38 (0.81)	15.98	< .01
Relative Clinical Attachment									
Group A	14.60 (1.07)	13.70 (1.21)	0.93 (0.52)	9.82	< .01	13.10 (1.11)	1.50 (0.57)	14.35	< .01
Group B	15.30 (1.39)	13.50 (1.39)	1.80 (0.81)	12.09	< .01	12.90 (1.36)	2.37 (0.85)	15.25	< .01

*Paired *t* test; *P* > .05 is nonsignificant.
†Control group.
‡Experimental group.

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.

Most of the enzymes are only marginally stable at neutral pH and room temperature and hence are readily denatured by an increase in the temperature, pressure, and pH. In the present study, stability was examined at 25°C/60% RH and 40°C/75% RH. Figure 10 shows the stability profile at the different temperatures. The rate of degradation at room temperature was found to be higher than the rate at 40°C. This degradation was due to low viscosity at the lower temperature, which provided mobility to water molecules and physical inclusion of oxygen. In earlier reports the liquid crystalline phase was successfully used to enhance the stability of biomolecules because of the viscoelastic properties of the liquid crystalline system.[36] Higher viscosity reduces the diffusion coefficient of water as well as the physical inclusion of oxygen. Stratton et al reported enhancement of the stability of protein loaded in the gel matrix, with complete recovery of full activity when the gel was dissolved in excess buffer.[37]

A total of 60 sites in 30 patients were treated: 30 experimental sites received the pluronic gel, and 30 sites did not receive the local drug. At the end of 45 days of observation, all the sites had healed uneventfully. Neither complications nor allergic reactions that could be related to the experimental treatment modalities were observed.

In the present study, a statistically significant reduction in mean plaque index, gingival index, sulcus bleeding index, and probing pocket depth, and a statistically significant gain in clinical attachment, were observed in both the groups from the baseline. A significant reduction in the percentage of spirochete count and a proportionate increase of coccoid

Table 2. Comparative Analysis of Microbiological Parameters in the Control and Experimental Groups*

Parameters	Baseline Mean (SD)	21 Days Mean (SD)	21 Days Change (SD)	t	P	45 Days Mean (SD)	45 Days Change (SD)	t	P
Spirochetes									
Group A[†]	41.10 (13.65)	22.40 (10.69)	18.70 (8.40)	12.08	< .01	18.90 (9.75)	22.20 (10.6)	11.42	< .01
Group B[‡]	39.60 (14.40)	10.57 (5.54)	29.03 (15.10)	10.48	< .01	10.53 (5.72)	29.07 (12.4)	12.77	< .01
Coccoid Cells									
Group A	20.67 (9.69)	52.57 (18.96)	31.90 (18.86)	9.26	< .01	58.61 (17.63)	37.95 (19.13)	10.86	< .01
Group B	22.17 (7.58)	65.17 (9.26)	43.0 (13.90)	16.88	< .01	73.17 (14.32)	51.00 (14.9)	18.63	< .01

*Paired *t* test; *P* > .05 is insignificant.
†Control group.
‡Experimental group.

cell count was observed for both the groups in comparisons against the baseline.

However, a statistically significant reduction in mean plaque index, gingival index, sulcus bleeding index, and probing pocket depth, and a statistically significant gain in clinical attachment, were observed in experimental sites when compared with control sites (Table 1). A significant reduction in the percentage of spirochete count and a proportionate increase of coccoid cell count was observed in the experimental group when compared with the control group (Table 2). Similar results have been reported by Heijl et al[38] using tetracycline-containing fibers.

The acceptability of the experimental material was observed in terms of subjects' taste and comfort. Good biological acceptability was seen as no evidence of burning sensation, dryness/soreness, ulcer formation, or staining of teeth. Thus, the gel formulation along with scaling and root planing was effective in removing the local irritants, reducing gingival inflammation, reducing pocket depth, and increasing clinical attachment. It also controlled the localized infection and prevented new lesion formation.

The local drug delivery system used in the present study is simple and easy to use. Its syringeability allows easy insertion into the pocket. Also, the fact that the experimental drug delivery system is bioadhesive allows better retention. The system is biologically accepted without any side effects.

CONCLUSIONS

It may be concluded from the present study that addition of Aerosil to pluronic gel caused significant changes in the pharmaceutical properties of the gel. Although Aerosil and tetracycline caused broadening of the gel boundaries, only Aerosil caused a significant increase in viscosity and bioadhesivity. The gel improved clinical attachment as well as patient compliance. Local delivery of the tetracycline along with serratiopeptidase in the form of a thermoreversible gel was clinically effective along with scaling and root planing.

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