Original Article

Periodontal thermoresponsive, mucoadhesive dual antimicrobial loaded *in-situ* gel for the treatment of periodontal disease: Preparation, *in-vitro* characterization and antimicrobial study

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ABSTRACT

Background: This study aimed to formulate and characterize *in-situ* gel containing levofloxacin and metronidazole to release drugs in controlled manner for treatment of periodontitis.

Material and Methods: Medicated *in-situ* gel with levofloxacin (10% w/v), metronidazole (25% w/v) and vehicle *in-situ* gel without drugs having poloxamer 407 (20% w/v) and chitosan (0.5%, 1%, 1.5%, 2.0% 2.5% w/v) were prepared and characterized for physicochemical, mechanical properties, stability and *in-vitro* drug release. Fourier transform infrared spectroscopy and differential scanning calorimetry studies were done. Optimized formulation was evaluated by scanning electron microscope (SEM) and *in-vitro* antimicrobial activity against 5 bacterial strains.

Results: The results revealed that drugs and polymers were compatible to formulate. All formulations were light yellow, clear and syringeable except formulation having 2.5% w/v chitosan. pH was in the range of 6.20 to 6.74. 1.0% w/v and 1.5% w/v chitosan formulations showed gelation temperature $37 \pm 0.32^\circ\text{C}$ and $34 \pm 0.21^\circ\text{C}$. Further, mucoadhesive strength indicated mucoadhesivity of gel. *In-vitro* release study of 1.5% w/v chitosan formulation showed initial burst where about 55–60% MZ and 60–70% LVF got released within 6–7 hrs followed by sustained release upto 48 hrs. SEM images of 1.5% w/v chitosan optimized medicated *in-situ* and vehicle *in-situ* gel appeared similar indicating homogeneous mixing of polymers with drugs. *In-vitro* antimicrobial study showed that medicated *in-situ* gel was more effective than vehicle.

Conclusions: In conclusion, optimized 1.5% w/v chitosan *in-situ* gel was thermoresponsive, mucoadhesive, syringeable, and released drugs in slow and controlled manner with effectiveness against broad range of microbes.

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1. Introduction

Periodontitis is primarily caused by microorganisms and their products that reside within periodontal pocket and may reach up to the connective tissue, radicular dentin, exposed cementum and alveolar bone.^{1,2} Antimicrobial therapy acts as adjunct to mechanical therapy which has been attempted systemically or locally. Local drug delivery including mouth wash, gel, toothpaste,

supragingival and subgingival irrigation fail to maintain antimicrobial concentration in subgingival area.^{3,4} Controlled drug delivery devices are developed to maintain drug concentration for more than 24 hours.^{5,6}

Controlled drug delivery requires carrier that can deliver antimicrobial agent in a controlled manner and increase the substantivity of agent at site of action. Chitosan is a natural mucopolysaccharide polymer which is the main component of the exoskeleton of crustaceans such as lobsters, shrimps, crabs that are second abundant source in nature. Chitosan is being used in various biomedical preparations due to its known properties such as biocompatibility, mucoadhesive, biodegradability, antimicrobial and wound healing.⁷ Poloxamer 407 (Pluronic F-127) is a triblock copolymers consisting of poly (ethylene oxide)-poly (propylene

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oxide)-poly (ethylene oxide) (PEO-PPO-PEO) which has thermoresponsive property. It is a thermoreversible polymer that undergoes *in-situ* gelation with increase in temperature.⁸

Controlled delivery devices containing several antimicrobials agents have been examined in the treatment of periodontal disease.⁹ Levofloxacin, an oral fluoroquinolone, is optical S(–) isomer of ofloxacin. Levofloxacin has antibacterial spectrum against most aerobic gram-positive and gram-negative organisms and demonstrates moderate activity against facultative anaerobes.¹⁰ Metronidazole is active against anaerobic bacteria. Therefore, objective of the present study was to formulate and characterize controlled drug releasing *in-situ* gel containing levofloxacin and metronidazole to release the drug slowly in periodontal pocket and cover the most of the microorganisms present in deep pocket.

2. Material and methods

Levofloxacin (LVF) and metronidazole (MZ) were obtained as a gift sample from Zydus Cadila Healthcare, Ltd. Ahmedabad (India), and Nicholas Piramal, Mumbai (India) respectively. Chitosan (Cs, medium molecular weight) was purchased from Hi-Media labs. Poloxamer 407 (PI407) was purchased from Sigma Chemicals (USA). All other chemicals used in this study were of analytical grade.

3. Preparation of the vehicle and medicated *in-situ* gels

Vehicle *in-situ* gels were prepared by dissolving weighed amount of Cs (0.5%, 1%, 1.5%, 2.0%, 2.5% w/v) into 0.5% v/v acetic acid under constant stirring. On the other side, poloxamer gel was prepared by cold method as described by Schmolka 1972¹¹ with slight modifications. Briefly, weighed amount of PI407 sufficient to yield 20% w/v was slowly added to cold distilled water (maintained at 4 °C in ice bath) under constant stirring and solution was kept in refrigerator overnight. Then prepared cold PI407 and Cs solution were mixed homogeneously under constant stirring in an ice bath. Benzalkonium chloride (0.01% w/v) was added as preservative to *in-situ* gel. In medicated formulation, weighed amount of drugs LVF (10% w/v) and MZ (25% w/v) were uniformly dispersed in Cs solution overnight under magnetic stirring. Further, medicated *in-situ* gels were prepared using similar method as vehicle *in-situ* gels. The compositions of different batches are shown in Table 1.

4. Fourier Transform Infrared (FTIR) spectroscopy

FTIR (Shimadzu, Model 8400S, Tokyo, Japan) spectroscopy of LVF, MZ, Cs and PI407 powders and their physical mixture was done by KBr pellet method. These pellets were scanned at wave number from 4000 to 400 cm^{–1} and characteristic peaks were noted.

5. Differential scanning calorimetry (DSC)

DSC (Mettler, DSC 4000) of individual drugs including LVF & MZ and polymers i.e. Cs and PI407 and physical mixture was also done to detect possible chemical interactions between drugs and polymers. The heating rate was kept at 10 °C min^{–1} under a constant flow of nitrogen gas.

6. Characterization of *in-situ* gel

The characterization of formulations was done under the following parameters in triplicate:

6.1. Appearance, clarity, syringeability and pH measurement

Appearance and clarity were recorded visually against white and black background in light before and after gelling. Syringeability was examined with 21 gauge needle. pH of *in-situ* gel was calibrated with the help of digital pH meter (Perfit India, Deluxe pH meter) by bringing glass electrode and reference electrode near surface of formulations at room temperature. The pH meter was calibrated before each use with standard.

6.2. Drug content

1 ml of prepared *in-situ* gel was transferred to 50 ml of pH 6.6 mcllvaine buffer in 100 ml of volumetric flask followed by continuous shaking to give a clear solution. Final volume was adjusted to 100 ml with the help of buffer. After suitable dilutions, the concentration was calculated by the method developed by Khan et al.¹² at 288 nm and 319 nm wavelength for LVF and MZ respectively through UV spectrophotometer (Shimadzu1700, Double beam, Japan).

6.3. Gelation temperature and gelation time

Gelation temperature was measured by using method described by Baloglu et al.¹³ 5 ml of formulation was taken in 20 ml of transparent vial having a magnetic bar that was placed in a water bath (maintained at 4 °C). The temperature was increased gradually under a constant stirring at 200 rpm. The displayed temperature on the immersed thermometer was noted as gelation temperature when the magnetic bar stopped moving due to gelation. To find out the gelling time, 5 ml of *in-situ* gel was taken in 20 ml test tube and placed in a water bath maintained at 37 °C and the time taken for solidification was noted.

6.4. Viscosity

Viscosity of all formulations was examined by programmable Brookfield's viscometer (DVII) at 5 °C and 25 °C temperatures. 10 ml of formulation was taken in 50 ml beaker. The viscosity

Table 1
Composition of prepared different formulations.

Formulation Codes	Concentration of chitosan (%w/v ^a)	Concentration of poloxamer 407 (%w/v)	Concentration of LVF ^b (%w/v)	Concentration of MZ ^c (%w/v)
LM1	0.5	20	10	25
LM2	1.0	20	10	25
LM3	1.5	20	10	25
LM4	2.0	20	10	25
LM5	2.5	20	10	25

^a weight/volume.

^b Levofloxacin.

^c Metronidazole.

measurements were recorded after 10 min from the rotation of sample by using spindle no 4 at the speed of 100 rpm.

6.5. Mucoadhesive strength

It was determined by the modified physical balance method developed by Yong et al.¹⁴ with minor modifications. The apparatus comprised of two balance arm. On one arm, two glass plates were attached: one was fixed to base of the apparatus and other one was fixed to the arm of balance. The second arm contained the container. Bovine cheek pouch was obtained from local slaughter house and cut into the $1 \times 1 \text{ cm}^2$. The mucous membrane was washed with distilled water and then McIlvaine buffer pH 6.6. Fresh tissue was stuck to the upper and lower glass plate facing each other with the help of cyanoacrylate adhesive. 0.5 ml of formulation was placed over the tissue as a thin film on the lower plate. After that upper plate was lowered on lower plate and the contact pressure (preload force) of 0.2 N for 2 min was applied over the upper plate. The water was dropped into the container of second arm of balance at a rate of 2 ml/minute from burette until the detachment of upper and lower plate had taken place. The weight of water dropped into container for detachment of plates was noted to determine mucoadhesive strength.

6.6. Texture profile analysis

Mechanical properties like firmness, cohesiveness and consistency of formulated *in-situ* gels were evaluated with the help of Texture Analyzer (TA-XT, Haslemere, Surrey, USA). The experiment was carried out at room temperature. Temperature of the formulation was maintained at room temperature before starting the experiment. The formulation was transferred in the beaker to a fixed height. The analytical probe of 10 mm diameter was inserted twice in each sample to a depth of 15 mm at a definite rate of 2.0 mm/s. In this method, the resultant force-time area was used for the calculation of the above mentioned mechanical parameters.

6.7. In-vitro drug release study

In-vitro drug release study of formulated *in-situ* gel was done by using the dialysis method.¹⁵ Prior to diffusion studies, dialysis membrane (Himedia labs, cutoff weight 12,000–14,000 Da) was soaked overnight in 1:1 mixture of McIlvaine buffer at pH 6.6 and glycerol. In this technique, each bag was loaded with 2 ml of formulation, hermetically sealed and dialyzed against 200 ml McIlvaine buffer at pH 6.6 contained in 500 ml beaker kept on a thermostatically controlled magnetic stirrer maintaining temperature of $37 \pm 0.5^\circ\text{C}$ and stirring at 100 rpm. The samples were withdrawn at predetermined time intervals 0, 0.5, 1, 2, 3, 4, 5, 6, 24 and 48 h and replaced with equivalent amount of buffer to maintain sink condition. The amount of drug released was calculated using UV spectroscopy as discussed earlier. Kinetic models (Zero-order, First order, Higuchi and Korsmeyer-Peppas) were applied to determine mechanism of release.

6.8. Stability study

Stability study was performed by storing samples at two different temperatures at $5 \pm 3^\circ\text{C}$ (in refrigerator) and $40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ RH (in humidity controlled oven) for 90 days as per ICH guidelines Q1A (R2). The samples were examined at 0 and 90 days for appearance, clarity, pH, % drug content and viscosity. *In-vitro* release study of optimized batch was also performed.

6.9. Morphological characterization through Scanning electron microscope (SEM)

Surface morphology of optimized formulation was investigated by SEM. Optimized formulation was freeze dried under vacuum for 2 days. The freeze dried sample was sputter coated with gold to make it conducting under vacuum and evaluated under SEM.

6.10. In-vitro antimicrobial Test

Optimized medicated and vehicle *in-situ* gels were tested for their antibacterial activity using disc diffusion method with slight modification.¹⁶ A total of 5 bacterial strains were used to carry out the antimicrobial assay. These were *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Vibrio cholera* (ATCC39315), *Klebsiella pneumonia* (ATCC700603) and *Proteus vulgaris* (ATCC29905). *S.aureus* is gram positive cocci whereas others are gram negative rods. All microbial cultures were preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India which were obtained from American Type Culture Collection (ATCC). Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates. A drop of 10 μl of optimized and vehicle formulation was placed on surface of medium and incubated at 37°C for 24 h. At the end of incubation, inhibition zones were measured around drop with transparent ruler in mm. This study was performed in triplicate.

6.11. Statistical analysis

Mean $\pm$ SD was calculated for the data obtained from different experiments and was presented in the form of tables and figures. Drug release data was analysed from release exponent 'n' as mentioned in Korsmeyer and Peppas equation¹⁷:

$$Q(t) = K_p \cdot t^n$$

Where, $Q(t)$ is the percent drug release at time 't', K_p is a release rate constant (h^{-n}), and n is release exponent indicative of the mechanism of drug release. $n < 0.5$ indicates Fickian (case I) release, 0.89–0.5 shows non-Fickian (anomalous) release, $n = 1$ indicates case-II transport (zero-order release), and > 1 indicates super case II type of release.

7. Results

Five formulations of LVF and MZ in the form of *in-situ* gel were prepared by varying the concentration of Cs to see the effects on physicochemical, mechanical properties and drug release of *in-situ* gel. PI407 was used as *in-situ* gelling agent and all formulations have similar drug concentration i.e. LVF (10%w/v) and MZ (25%w/v).

FTIR studies (Fig. 1a) showed the MZ peaks at 1265.35 cm^{-1} , 1369.50 cm^{-1} , 1539.25 cm^{-1} , 3221.23 cm^{-1} for C—O, N—O, C=N stretching and O—H group respectively. Characteristic peaks at 1724.42 cm^{-1} , 2935.76 cm^{-1} , and 3267.52 cm^{-1} were observed for lactum ring, aromatic C—H stretching, O—H group of carboxyl moiety in LVF. Cs showed characteristic band at 3414.12 cm^{-1} which is attributed to amide group. Peaks at 1153.47 cm^{-1} , 1087.89 cm^{-1} , 1022.31 cm^{-1} and 898.86 cm^{-1} showed its saccharide structure. PI407 is characterized by principal peaks at 3450.77 cm^{-1} , 2877.89 cm^{-1} , 1352.14 cm^{-1} & 1105.25 cm^{-1} due to stretching of terminal hydroxyl group, C—H, O—H bending and C—O stretch. DSC results (Fig. 1b) showed the two endothermic peaks between 60°C – 80°C (broad) and 233.06°C (sharp) of LVF. MZ showed sharp endothermic peak at $159.53.5^\circ\text{C}$. Thermogram of Cs

Fig. 1. a) FTIR overlap spectra, b) DSC overlap graph of levofloxacin, metronidazole, poloxamer 407, chitosan, physical mixture of polymers and drugs.

showed a broad peak at 92.46 °C over a large temperature range. One endothermic peak at 57.73 °C was shown for PI407.

All formulations were light yellow, clear and syringeable through 21 gauge needle except LM5 that was yellow, hazy and not syringeable. The results of the physicochemical and mechanical properties of *in-situ* gels were shown in the Table 2. The pH of all formulations was found in the range of 6.20 to 6.74. Mean % drug content for LM1, LM2, LM3, LM4, & LM5 batches was 94.66, 95.55, 98.54, 93.22, & 94.37 for LVF and 96.46, 96.25, 98.28, 95.22 & 96.07 for MZ respectively. The gelation temperature of LM1 to LM5 formulations was observed from 40 ± 0.11 °C to 25 ± 0.30 °C and gelation time ranged from 39 ± 0.08 to 26 ± 0.19 seconds respectively. The average viscosity was 302, 355, 388, 446, & 578 cps at 5 °C and 952, 1257, 1554, 1774 & 1988 cps at 25 °C for LM1, LM2, LM3, LM4, & LM5 batches respectively. Mucoadhesive strength was ranged between 15.71 ± 1.39 gm to 46.68 ± 1.94 gm. Firmness was found within 41.292 ± 2.69 to 61.802 ± 1.56 gm and consistency ranged from 98.127 ± 3.89 to 212.205 ± 4.91 gm/sec from LM1 to LM5. Cohesiveness was ranged from 24.395 ± 2.79 to 9.201 ± 1.23 gm.

In-vitro release studies showed that drugs were released upto 2 days in which both drugs about 70–80% from LM1 and 40–60% from LM5 were released within 6 hrs (Fig. 2a,b). Table 3 showed the release kinetics of LVF and MZ from the formulations. Appearance, clarity, % drug content and viscosity were similar in LM1, LM2 &

LM3 formulations except LM4 and LM5 after storage of 3 months. *In-vitro* drug release of LM3 formulation after storage was approximately similar to the baseline value (Fig. 2c,d). SEM images of vehicle and medicated *in-situ* gel of LM3 at different magnifications 1, 5 and 10 KX showed the similar morphology (Fig. 3i). SEM images of freeze dried 1.5%w/v Cs *in-situ* gel at different magnifications 2, 5 and 10 KX exhibited cracks, pits, and lumps (Fig. 3ii). *In-vitro* antimicrobial study results revealed that the observed mean zone of inhibition with optimized 1.5% w/v Cs medicated *in-situ* gel formulation was 20, 7, 24, 24 and 23 mm for *S. aureus*, *E. coli*, *V. cholera*, *K. pneumonia*, *P. vulgaris* respectively. Whereas, the vehicle *in-situ* gel showed 2, 2, 4 and 2 mm for *S. aureus*, *E. coli*, *V. cholera*, *K. pneumonia* (Table 4, Fig. 4).

8. Discussion

In the present study, *in-situ* gel containing LVF and MZ was formulated and characterized. PI 407 was added to make it thermoreversible. Influence of changing in concentration of Cs was observed on physicochemical, mechanical properties and *in-vitro* drug release. Drug compatibility studies like FTIR and DSC demonstrated that physical mixture of CS, PI407, MZ and LVF showed characteristic peaks of drugs and polymers and no new bands or shifts in peaks appeared which indicates absence of any interaction between drug and polymer.

Table 2

pH drug content, Gelation temperature, gelation time, viscosity, mucoadhesive strength, firmness, consistency and cohesiveness of different formulations.

Parameters		LM1	LM2	LM3	LM4	LM5
pH measurement ± SD		6.20 ± 0.12	6.34 ± 0.09	6.52 ± 0.20	6.65 ± 0.15	6.74 ± 0.07
% drug content ± SD	LVF [†]	96.46 ± 1.03	95.55 ± 2.17	98.54 ± 1.29	93.22 ± 1.09	94.37 ± 0.95
	MZ [#]	94.66 ± 0.83	96.25 ± 0.93	98.28 ± 1.01	95.22 ± 1.19	96.07 ± 0.95
Gelation temperature(°C) ± SD		40 ± 0.11	37 ± 0.32	34 ± 0.21	30 ± 0.34	25 ± 0.30
Gelation time (seconds) ± SD		39 ± 0.08	34 ± 0.15	32 ± 0.11	29 ± 0.06	26 ± 0.19
Viscosity (cps) ± SD	5 °C	302 ± 54	355 ± 72	388 ± 34	446 ± 66	578 ± 21
	25 °C	952 ± 49	1257 ± 77	1554 ± 51	1774 ± 44	1988 ± 37
Mucoadhesive strength (gm) ± SD		15.71 ± 1.39	30.15 ± 1.89	39.71 ± 1.23	42.75 ± 1.01	46.68 ± 1.94
Firmness (gm) ± SD		41.292 ± 2.69	46.137 ± 1.99	56.966 ± 2.03	58.072 ± 2.70	61.80 ± 1.56
Consistency (gm/sec) ± SD		98.127 ± 3.89	149.955 ± 4.02	173.008 ± 3.01	190.31 ± 4.10	212.21 ± 4.91
Cohesiveness (gm) ± SD		24.395 ± 2.79	22.283 ± 1.90	16.561 ± 1.01	13.095 ± 0.89	9.201 ± 1.23

[†] Levofloxacin.

[#] Metronidazole.

Fig. 2. Graph showing the cumulative percentage release of a) Metronidazole and b) Levofloxacin at predetermined time period from different formulations c) Levofloxacin & metronidazole at predetermined time period from the optimized 1.5%w/v chitosan formulation before storage, after storage at 40 °C and at 5 °C.

Table 3
Release kinetics of levofloxacin (LVF) and metronidazole (MZ) from the formulations.

		Zero order		First order		Higuchi		Korsmeyer –Peppas		
		r^{2a}	k^b	r^2	K	r^2	k	r^2	k	n^c
LM1	LVF	0.6276	4.183	0.237	0.074	0.8717	20.056	0.9929	0.4456	0.2271
	MZ	0.5411	2.737	0.2526	0.078	0.8921	23.124	0.9648	0.467	0.2521
LM2	LVF	0.7167	4.798	0.278	0.077	0.9277	19.3	0.9859	0.3657	0.2677
	MZ	0.5878	2.768	0.2684	0.079	0.9104	22.054	0.9555	0.4232	0.263
LM3	LVF	0.8041	4.787	0.328	0.082	0.9677	18.567	0.9788	0.2974	0.3153
	MZ	0.6213	2.7068	0.28	0.0791	0.9196	20.743	0.952	0.3852	0.2713
LM4	LVF	0.8356	4.434	0.386	0.081	0.9746	16.929	0.9812	0.2631	0.3224
	MZ	0.6523	2.6089	0.2948	0.0795	0.9362	19.439	0.9622	0.3466	0.2825
LM5	LVF	0.869	4.205	0.38	0.0836	0.9837	15.816	0.9718	0.2252	0.345
	MZ	0.6745	2.4673	0.3046	0.079	0.9743	15.578	0.9583	0.3152	0.2847

^a regression coefficient.

^b release rate constant.

^c release exponent.

Formulations LM3, LM4 and LM5 have the pH around 6.6 that was closer to the pH of gingival crevicular fluid (GCF) which indicates that these formulations can be used in periodontal pocket without any potential irritation. The results have shown uniform drug content of formulations. LM2 and LM3 formulations showed the gelation temperature $37 \pm 0.32^\circ\text{C}$ and $34 \pm 0.21^\circ\text{C}$ that was

close to body temperature. Gelation temperature was influenced negatively by increasing the concentration of Cs. During gelation, hydrophobic domains in PI407 were aggregated to minimize the hydrophilic surfaces and reduce the amount of water surrounding the hydrophobic domains.¹⁸ Viscosity results indicated that the formulations containing higher amount of Cs were more viscous

Fig. 3. i) Scanning electron microscopic images of liquid vehicle gel (P1, P2, P3) and 1.5%w/v chitosan (S1, S2, S3) at different magnifications 1, 5 and 10 KX, ii) Scanning electron microscopic images of dried 1.5%w/v chitosan *in situ* gel at different magnifications 2, 5 and 10 KX.

Table 4

In-vitro antibacterial activities of optimized 1.5%w/v chitosan medicated and vehicle *in-situ* gel formulation.

S. no.	Microorganisms	Duration in hrs ^a	Zone of inhibition in mm $\pm$ SD ^b	
			1.5%w/v chitosan medicated <i>in-situ</i> gel	1.5% vehicle <i>in-situ</i> gel
1.	<i>Staphylococcus aureus</i>	24	20 $\pm$ 0.45	2 $\pm$ 0.29
2.	<i>Escherichia coli</i>	24	7 $\pm$ 0.91	2 $\pm$ 0.98
3.	<i>Vibrio cholerae</i>	24	24 $\pm$ 0.36	4 $\pm$ 0.82
4.	<i>Klebsiella pneumoniae</i>	24	24 $\pm$ 0.87	2 $\pm$ 0.53
5.	<i>Proteus vulgaris</i>	24	23 $\pm$ 0.76	NIL

^a hours.

^b Standard deviation.

than the formulations containing lesser amount. Consequently, LM5 exhibited highest viscosity of 578 ± 21 cps at 5 °C and 1988 ± 37 cps at 25 °C. LM1 showed lowest viscosity of 302 ± 54 at 5 °C and 952 ± 49 at 25 °C. Lower viscosity is desirable at lower temperature to easily inject the *in-situ* gel into pockets, while higher viscosity is desirable for gels to remain for longer time into pockets.

The mucoadhesive strength required to detach the plates is lowest for the LM1 and highest for the LM5 formulation. The PI407 concentration was constant in all formulation but Cs concentration varied. Among all formulations, LM5 has more concentration of Cs

therefore; and has the highest value of mucoadhesive strength. The results suggested that concentration of Cs influenced positively the mucoadhesive strength of the formulations. Mechanical properties like firmness, consistency and cohesiveness of studied formulations were assessed using texture profile analyzer. Increasing concentration of Cs increased formulation firmness and consistency. In the case of cohesiveness, the findings were reversed. The sequence of decreased firmness and consistency is as follows: LM5 > LM4 > LM3 > LM2 > LM1 (Table 3). LM5 formulation showed the highest firmness and consistency and lowest cohesiveness. For better syringeability, the *in-situ* gel should have

Fig. 4. *In-vitro* antimicrobial study showing zone of inhibition in different microorganisms a. *Klebsiella pneumoniae* b. *Escheria coli* c. *Staphylococcs aureus* d. *Vibrio cholarie* e. *Proteus vulgaris*.

less firmness and consistency value. The formulation should be more cohesive that increases the full structural recovery following gel application.¹³

In-vitro release studies performed in McIlvaine buffer 6.6 pH revealed biphasic pattern of drug release i.e. initial burst release in the first six to seven hours followed by slow and sustained release till 48 h. About 70–80% of both drugs were released from LM1 within 6 hours, whereas only 40–60% of both drugs were released from LM5. These observations indicated that the burst effect is inversely related to Cs concentration (Fig. 2a,b). Slow and more prolonged release was obtained with batches containing higher concentration of Cs polymer as the concentration of Pl407 was kept constant in all batches. The results indicated that higher amount of Cs causes the more cross-linking with pl407 that influences the release of drug from the *in-situ* gel. The mechanism of drug release was identified as Korsmeyer-peppas model. The value of coefficient release 'n' was obtained less than 0.5 indicating Fickian diffusion release pattern (Table 3). The results were similar as obtained by Esposito et al.¹⁹

Stability study of all formulated batches was carried out. Formulations LM1, LM2 & LM3 remained unchanged in respect of appearance, clarity, % drug content and viscosity. Thus, these formulations were stable at different conditions of temperature and humidity. However, LM4 formulation was not stable and LM5 was not clear at the time of preparation. Thus, LM4 and LM5 formulations were not considered for further evaluations and LM3 was optimized after considering the observations of all experiments and further evaluated for its drug release after storage of 3 months that was approximately similar to the baseline release (Fig. 2c). SEM images of vehicle (P1, P2, P3) and medicated *in-situ* gel of LM3 optimized batch (S1, S2, S3) at different magnifications 1, 5 and 10 KX showed that the morphology of vehicle and medicated *in-situ* gels appeared similar indicating homogeneous mixing of polymers with drugs as no drug particles were observed on the surface of sample gels. Further, the rough appearance of liquid gels could be attributed to Pl407 as it is present in large amount (Fig. 3i). Fig. 3ii showed the SEM images of freeze dried LM3 batch at different magnifications 2, 5 and 10 KX which

exhibited cracks, pits, lumps formed due to loss of water during drying process concluding towards large water content of gel and supporting their hydrogel nature.

In-vitro antimicrobial study revealed that medicated *in-situ* gel showed larger zone of inhibition (20–24 mm) as compared to vehicle formulation which supports activeness of prepared drug containing *in-situ* gel against gram –ve and gram +ve. However, in case of *E. coli* only 7 mm of zone of inhibition was observed. Moreover, vehicle gel showed 2 to 4 mm zone of inhibition for all bacteria except *P. vulgaris*. That might be due to the antimicrobial activity of Cs present in vehicle gel. Difference in zone of inhibition in vehicle and medicated *in-situ* gels obtained from the *in-vitro* antimicrobial study indicated that device having drugs is active and releases drugs.

9. Conclusion

In the present study, 1.5% w/v Cs formulation was thermoreversible, mucoadhesive, syringeable and released the drug slowly upto 48 h. Further, antimicrobial studies revealed the efficacy of medicated *in-situ* gel on broad range of microorganisms including gram+ve, gram-ve, cocci and rods as compared to vehicle. Conclusively, the studies conducted so far indicate that this thermoreversible, mucoadhesive, controlled release intrapocket *in-situ* gel might be used in eradication of deep periodontal pocket infections and could enhance the therapeutic outcomes of scaling and root planing which can be proved by clinical studies in the future.

Conflict of interests

None.

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