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Mucoadhesive *in situ* gels of local anaesthetic for periodontia

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Abstract

The present study was carried out to formulate mucoadhesive in situ gels of local anaesthetics for administering into periodontal pocket that gives fast onset of anaesthesia lasting throughout the dental procedure, which is painless. Temperature sensitive mucoadhesive in situ gelling system containing 5% w/v lidocaine hydrochloride (Lidocaine HCl) was formulated by cold method using combination of pluronic F 127 (PF 127) and carbopol 934 (C 934). The gels were evaluated for drug content, gelation temperature, viscosity, mucoadhesive strength, sterility, in vitro release and clinical studies. The drug content in all the formulations was found to be satisfactory. The formulations containing 18% w/v of PF 127 showed gelation near to body temperature. Viscosity studies showed a marked increase in viscosity of the gels at 37^0C due to sol-gel conversion with increase in temperature. The viscosity and mucoadhesiveness increased with increase in C 934. In vitro release studies showed that as the concentration of PF 127 increased the rate of drug release decreased. The drug release from the formulations was diffusion controlled without swelling. A placebo-controlled, double-blind clinical trial was conducted to assess the safety and efficacy of the Lidocaine HCl gel during scaling and root planing (SRP). In the study population, local anaesthetic gel 5% was statistically significant and more effective than the placebo. The results suggested that Lidocaine HCl 5% in situ gel may be a suitable formulation as it was clinically effective in reducing SRP pain for those patients who perceive the procedure to be painful.

Keywords: Lidocaine hydrochloride; *in situ* gels; pluronic F 127; carbopol 934; periodontitis.

INTRODUCTION

Periodontitis is a common and widespread disease, which occurs due to the pathogenic bacterial infections established within the gingival sulcus. This condition, when not arrested, will cause formation of periodontal pocket.

It is estimated that approximately 10-30% of the human population suffers from periodontal diseases with pathological periodontal pockets. In order to eliminate or control the disease and arrest further periodontal tissue destruction, periodontal pockets need repeated sub gingival mechanical debriment/cleansing. The number of periodontal pockets and the pocket depth vary from patient to patient. Approximately 40% of all periodontal scaling procedures performed involve the use of anaesthesia. The scaling procedure is unpleasant and painful and anaesthetic techniques used for this are nerve block/infiltration anaesthesia in combination with topical anaesthesia **[**1-3].

Mathews D C reported that majority of patients wanted some form of anaesthetic, in the form of gel or local infiltration. Of those patients, who choose gel, were willing to return for recall visits, indicating the preference of gel over local infiltration [4].

Numbers of topical anaesthetics are used in dentistry, but the short comings with most of these formulations include their low degree of efficacy, tendency to spread in other areas causing numbness of lips and tongue, bitter taste, difficulty in administration and short duration of action.

Considering the above shortcomings, this research work was planned with the objectives to develop a mucoadhesive local anaesthetic *in situ* gelling system suitable for periodontal pocket administration, which would enable a patient to have painless treatment without distress of injection. Such formulation stays at application site due to increased viscosity and mucoadhesiveness, and gives a fast onset of anaesthesia lasting throughout the dental procedure. The gel can be easily rinsed out with water after the treatment causing a fast decline in anaesthetic effect.

To accomplish the objectives, gel forming solutions by phase transition (sol-gel transition), mediated by temperature were formulated using PF 127 in which C 934 was used as bioadhesive polymer. Poloxamer is a triblock polymer consisting of polyoxyethylene–polyoxypropylene–polyoxyethylene units, and is used both internally and externally in various products that are designed for animal and human uses. Carbopol is a very high molecular weight polymers of acrylic acid cross-linked with polyalkenyl ethers, and they have been used for development of bioadhesive controlled drug delivery systems owing to their bioadhesive properties **[**5].

Sang–Chul Shin studied the mucoadhesive and physicochemical properties of carbopol-poloxamer gels containing triamcinolone acetonide. The viscosity and bioadhesive property of carbopol–poloxamer gels containing triamcinolone acetonide were tested with various concentrations of carbopol gels of various pH **[**5].

Hong–Ru Lin studied carbopol/ pluronic phase change solutions for ophthalmic drug delivery. The major purpose of this study was to develop and characterize a series of carbopol and pluronic based solutions as the *in situ* gelling vehicles for ophthalmic drug delivery [6].

Chunjie Wu studied a thermosensitive *in situ* gelling and mucoadhesive ophthalmic drug delivery system containing puerarin based on poloxamers analogs (21% w/v poloxamer 407/5% w/v poloxamer 188) and carbopol (0.1% w/v or 0.3% w/v carbopol 1342P). The combined solutions would convert to a firm gels under physiological condition and attached to ocular mucosal surface for a long time **[**7].

MATERIALS AND METHODS

Lidocaine hydrochloride (Lidocaine HCl) was obtained as a gift sample from Astra Zeneca pharma Ltd., Bangalore, India. Pluronic F 127 (PF 127) was purchased from Sigma Aldrich, USA. Carbpol 934 (C 934) and benzalkonium chloride were procured from Loba Chemie, Mumbai, India. Triethanolamine was procured from Reachem Labs, Chennai, India. All other reagents used were of analytical grade and were used as procured.

Formulation of in situ gels

Mucoadhesive in situ gel containing 5% Lidocaine HCl was prepared using different concentrations of C 934 and PF 127 by cold method (Table 1)[5]. Cold PF 127 solution containing drug was added to C 934 solution with continous stirring and left overnight at 5°C to complete polymer desolvation. Benzalkonium chloride (0.001% w/v) was added as preservative and formulation was adjusted to neutral pH with triethanolamine (quantity sufficient).

Table 1. Composition of formulations

Formulation code	Ingredients (%w/v)		
	Lidocaine HCl	**PF 127**	**C 934**
F1	5	18	0.1
F2	5	18	0.3
F3	5	18	0.5
F4	5	20	0.1
F5	5	20	0.3
F6	5	20	0.5
G1	5	18	-
G2	5	20	-

FTIR spectrophotometry

In order to evaluate the integrity and compatibility of the drug in the formulations, IR spectra of the drugs and its formulations were obtained by FTIR spectrophotometer (Shimadzu 8400S, Japan).

***In vitro* Studies**

Determination of drug content

The prepared formulations (F1 to F6) were analyzed for drug content by taking 1 mL of gel in 100 mL volumetric flask, dissolved and the volume was made upto 100 mL with 6.4 phosphate buffer. From the above solution 4 mL was pipetted out into a 10 mL volumetric flask and volume was adjusted with 6.4 phosphate buffer. Absorbance was measured at 263 nm [8].

Determination of gelation temperature

The gelation temperature of formulations (F1 to F6) was estimated by heating the solution (about 1-2 °C/min) in a test tube with gentle shaking until gel formed. Gel formation was taken as the point where there was no flow when the test tube was overturned [8].

Determination of mucoadhesive strength based on shear stress

The mucoadhesive strength of formulations was determined by method described by Konde A *et al.* using polished flexi glass blocks [9]. Two smooth polished flexi glass blocks of size 10 cm^2 were selected. One block was fixed with adhesive on a glass plate, which was fixed on leveled table. To the upper block a thread was tied and was passed through a pulley. A pan was attached at the end. The length of the thread from the pulley to the pan was fixed. A fixed amount of formulation was (0.1 mL) kept on the centre of the first block and then the second block was placed over it and pressed by applying 100 g weight for uniform spreading of the polymer solution as film. After keeping the weight for fixed time intervals of 5, 10 and 15 min, the weight was removed and weights were added into the pan. The weight just sufficient to pull the upper block to a distance of 2 cm was expressed as adhesive strength, which is the shear stress. The mucoadhesive strength of the formulation containing only PF 127 with drug (G1 and G2) compared with the mucoadhesive strength of the formulations containing both PF 127 and C 934 with drug (F1 to F6), to assess the change in the mucoadhesive strength.

Determination of viscosity

The viscosity studies of all the formulations (F1 to F6) were measured by using Brookfield digital viscometer (Brookfield DV II+, USA) with spindle number 94 at 50 rpm. Viscosity was measured at 10±1°C and at 37±1°C using a thermostated water jacket.

Microbiological evaluation

Sterility testing was done for aerobic bacteria using nutrient broth media and for anaerobic bacteria by using fluid thioglycollate media at 37±1°C for 14 days in incubator (Thermocon, India).

In vitro release studies

The *in vitro* release studies of formulation (F1 to F6) were performed. The dialysis membrane 50, having an average flat width of 24.26 mm, average diameter of 14.3 mm and capacity of approximately of 1.61 mL/cm was utilized for diffusion. Prior to diffusion studies, the dialysis membrane was soaked overnight in pH 6.8 phosphate buffer solution. 1 mL of gel was placed in dialysis membrane, which was sealed on both sides. The dialysis tube was placed in a glass beaker containing 20 mL of pH 6.8 phosphate buffer solution. The release studies were performed at 37±0.5°C for different time intervals. 5 mL of sample was pipetted out after every 15 min for the first hour and then every 30 min up to 4 hrs and was replaced with same volume

of pH 6.8 phosphate buffer to maintain the sink condition. After suitable dilutions, samples were analyzed spectrophotometrically at 263 nm for Lidocaine HCl.

Clinical Evaluation

Clinical study was designed and conducted as per Helsinki agreement. A placebo-controlled, double- blind clinical trial was conducted to assess the safety and efficacy of the Lidocaine HCl gel. A total of 30 patients, 15 for the test formulation and 15 for placebo group, were selected from the outpatient department of periodontics, J.S.S. Dental College & Hospital, Mysore, India. The ethical approval was obtained for the study. Inclusion criteria included, patients having at least one quadrant that had not been scaled within the last 12 months were selected for treatment. This quadrant should contain a minimum of 5 natural teeth, of which at least 3 teeth should have a pocket depth of 5 mm or greater; patients who elicited pain when probing the pocket utilizing a pressure sensitive probe and patients who reported pain score ≥ 30 mm upon probing on a 100 mm visual analog scale (VAS); aged at least 18 years; hypersensitive teeth.

Exclusion criteria: Patients having history of allergy, sensitivity, or any form of reaction to local anaesthetics of amide type; patients who had been treated with an anaesthetic or sedative within 12 hours prior to scaling and root planning (SRP); patients having significant disease and/or abnormalities; patients requiring tooth extraction in study quadrant; patients with dental implants in the study quadrant; patients having ulcerative lesions in oral cavity; patients having abscess or acute infections in oral cavity.

Baseline was defined as prior to application of investigational product. The following baseline assessments were done before SRP [10].

1. Periodontal probing depths- using a pressure sensitive probe (6 sites per tooth).
2. Presence or absence of bleeding on probing to the base of the pocket (6 sites per tooth).
3. Hypersensitive teeth – identified by isolating the teeth with a gauze square and using a stream of 2 seconds of compressed air.

Selection and dosing schedule

Thirty patients based on purposive sampling were screened for eligibility based on inclusion and exclusion criteria. During the treatment, the chosen quadrant had undergone SRP along with either anaesthetic/placebo gel. The anaesthetic/placebo gel was applied into the periodontal pocket using a blunt applicator. Following a wait period of 2 min SRP was performed. If the patient had any discomfort, a second application of the gel was done. A maximum of two applications of either anaesthetic/placebo gel was done per tooth and later SRP was carried out. If the SRP procedure was still painful after reapplication, no further application of the gel was done and the subject was classified as patient requiring rescue anaesthesia, which was an efficacy parameter in the study. At the end of the SRP the patient was asked to rate the over all pain perception on a visual analog scale (VAS) and verbal rating scale (VRS). Possible adverse events were monitored throughout the treatment period and at follow up visit one week after the treatment visit [10].

Efficacy parameters

The main efficacy parameters were [10]

VAS

After the SRP the overall pain was assessed by the subject using a 100 mm (10 cm) horizontal scale, with the left end point marked "no pain" and the right end point marked "worst pain imaginable" as the primary efficacy parameter.

VRS

As the secondary efficacy parameter the overall pain from the SRP was assessed using a 5-point verbal rating scale: no, mild, moderate, severe and very severe pain. After the SRP pain had been assessed by VAS, the patient was asked to rate the overall pain on the VRS in response to the question, "How much pain did you feel during the SRP procedure? The alternative that best describes the pain was chosen."

The assessment of the VAS pain score (primary efficacy parameter) was always made before the VRS pain score (secondary efficacy parameter) to avoid influence of an already expressed verbal statement.

Need for rescue anaesthesia

Patients were considered to have rescue anaesthesia, if any, was given upon patient request or SRP was terminated because of pain due to insufficient anaesthesia after a second application of the gel to the same tooth [10].

Safety

Adverse events during the procedure and through 1 week comprised the safety variables.

Statistical methods

Descriptive statistics were performed on demographic characteristics; age, gender. t–Test was performed on baseline scores, VAS and VRS scores. The test was 2- tailed, with a confidence interval of 5%.

RESULTS

***In vitro* Evaluation**

FTIR spectra of Lidocaine HCl pure sample and formulation were found to be identical (Figure 1). The characteristic IR absorption peaks of Lidocaine HCl viz., 3460, 3387 (amide N-H), 2963 (C-H stretch), 1662 (C=O), 2476 (NH^+), 1543 (amide II) and 779 cm^{-1} (aromatic C-H) were obtained [11].

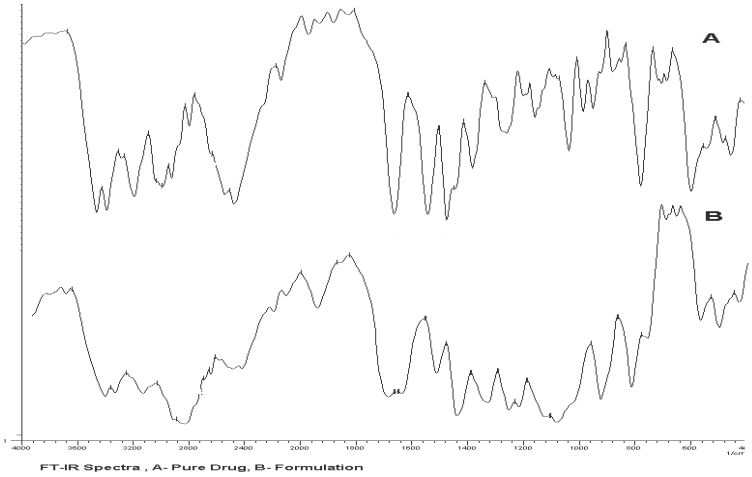

Figure 1. FTIR Spectra of Lidocaine HCl and Formulation

Drug content of formulations (F1 to F6) was in the range of 49.64 mg/mL to 49.81 mg/mL. Gelation temperature of formulations (F1 to F6) was in the range of 20ºC to 37ºC. Mucoadhesive strength of formulations (F1 to F6) with C 934 was in the range of 93g to 198g. Mucoadhesive strength of formulations G1 and G2 (without C 934) was 32g and 47g respectively. Viscosity of the formulations (F1 to F6) was in the range of 2798 cps to 38248 cps (Table 2).

Table 2. Drug content, gelation temperature, mucoadhesive strength and viscosity of formulations

Formulation code	Drug content (mg/mL)	Gelation temperature (°C)	Mucoadhesive strength (g)		Viscosity (cps)
			With C 934	Without C 934	
F1	49.81±0.244	32±0.577	93±1.124	-	27982±1.547
F2	49.81±0.389	36±0.577	125±1.905	-	28356±2.589
F3	49.70±0.207	37±1.130	185±1.172	-	28924±2.415
F4	49.64±0.192	20±1.450	110±1.154	-	37623±1.534
F5	49.77±0.673	21±0.816	153±1.167	-	37992±1.842
F6	49.68±0.067	23±1.396	198±1.154	-	38248±1.769
G1	-	-	-	37±2.013	-
G2	-	-	-	42±1.082	-

After 14 days of incubation period there was no colony growth. The results of *in vitro* release studies showed that with increase in concentration of PF 127 and C 934, the rate of drug release decreased (Figure 2).

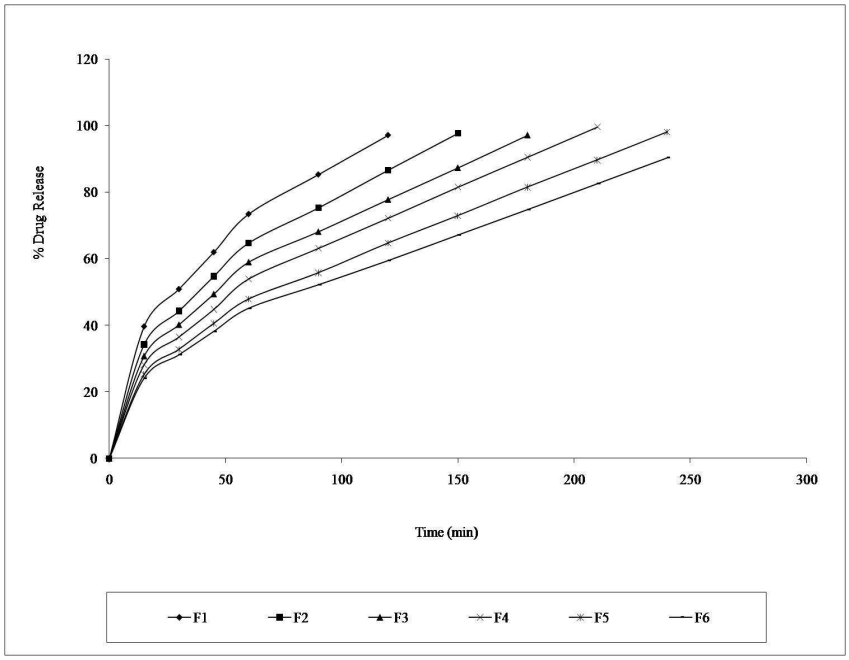

Figure 2. Drug release profile of formulations (F1-F6)

To determine whether the drug release was diffusion controlled or not the dissolution data was fit into Higuchi's diffusion model. All the cumulative percent release vs square root of time plots were straight lines with correlation coefficients ranging from 0.9968 to 0.9988 (Figure 3, Table 3). To ascertain the mechanism of drug release, the release data was plotted as per Peppas's model. The log percent drug release vs log time plots were also straight lines with correlation coefficient between 0.9971 to 0.9984, indicating a perfect correlation (Figure 4, Table 3). The value of diffusion coefficient "n" was determined and all the "n" values were less than 0.5 (Table 3).

Table 3. Correlation coefficients of Higuchi's and Peppas model, and "n" values

Formulation Code	Correlation Coefficient (R)			n value
	Zero Order	Higuchi's model	Peppas's model	
F1	0.9862	0.9981	0.9976	0.4396
F2	0.9888	0.9988	0.9981	0.4595
F3	0.9888	0.9987	0.9984	0.4634
F4	0.9920	0.9984	0.9980	0.4815
F5	0.9946	0.9973	0.9975	0.4899
F6	0.9943	0.9968	0.9971	0.4739

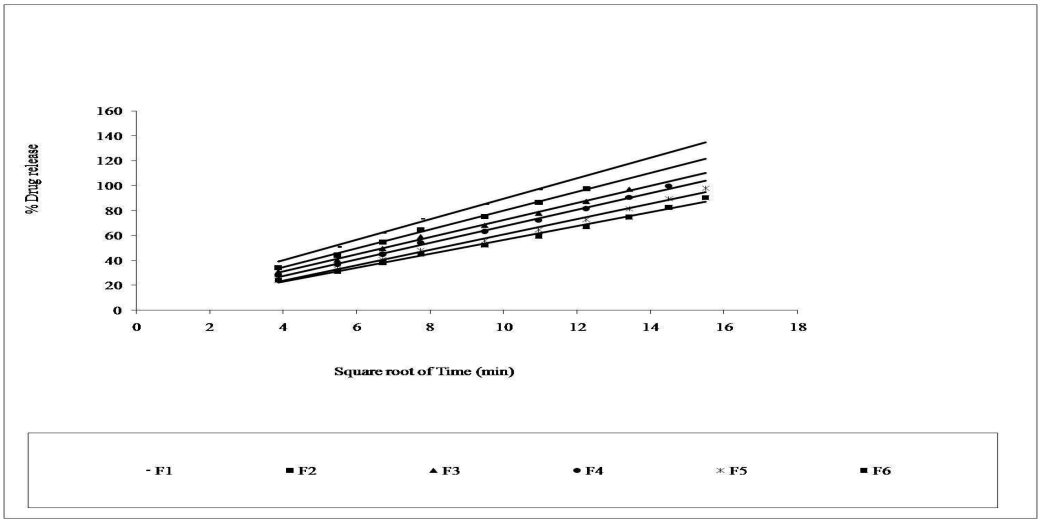

Figure 3. Higuchi's plot of formulations (F1-F6)

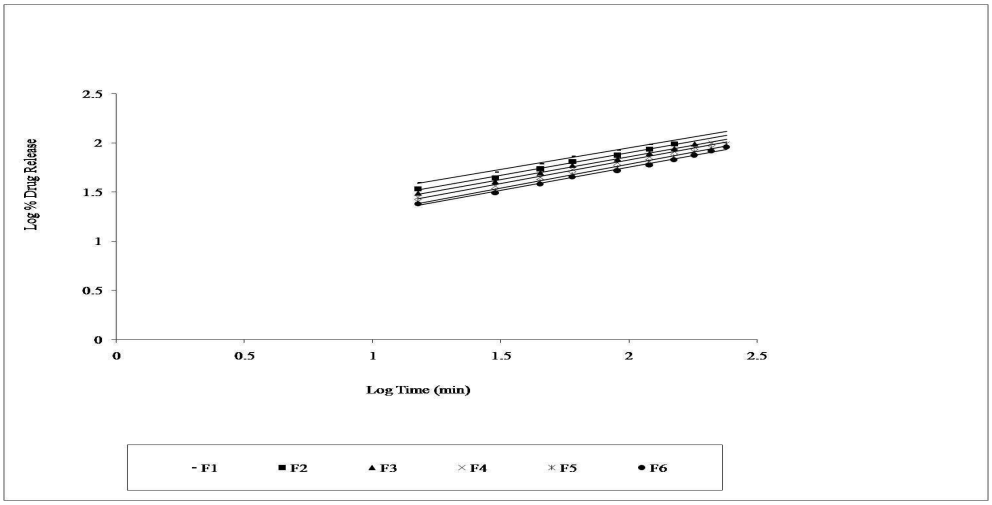

Figure 4. Peppas's plot of formulations (F1-F6)

Clinical Evaluation

The mean age ± standard deviation for the group receiving the active gel was 36.6 ± 12.0 yrs and that of receiving the placebo gel was 44.5 ± 14.1 yrs. The median age (range) for active was 34 (20-40) yrs and for placebo was 40 (20-45) yrs (Table 4). Baseline findings are summarized in Table 4. The patients in the active gel group had 5 to 8 teeth in the treated quadrant with a mean pocket depth of 3.53 ± 0.5 mm. In the placebo group, the patient had 5 to 8 teeth in the treated quadrant with a mean probing depth of 3.82 ± 0.6 mm. The mean percentage of pockets with bleeding on probing was 79.16% in active gel group and 80.83% in placebo group. The percentage of teeth with hypersensitivity was 18.33% in active gel group and 19.17% in Placebo group.

Table 4. Distribution of subjects by gender and age for treatment group and baseline scores

	Active gel (N = 15)	Placebo gel (N = 15)
Median age (years)	34 (20-60)	40 (20-65)
Gender (Numbers)		
Male	8	8
Female	7	7
*** Mean pocket depth (mm)**	3.53±0.5	3.82±0.6
Percent pockets with bleeding	79.16±19.3	80.83±27.8
Percent hypersensitive teeth	18.33±24.4	19.17±19.9

*For each patient, the mean probing depth in the treated quadrant was calculated. The values are mean of the individual probing depths.

In the active gel group 3 patients complained for headache after SRP. One patient complained for burning sensation and one patient complained for altered taste sensation in active gel group. In the placebo gel group only one patient was reported for edema at the treatment site. The mean VAS score in active gel group and placebo gel group was found to be 2.96 ± 1.239 and 4.80 ± 2.001 cm respectively. The mean VRS score in the active gel group and placebo gel group was found to be 1.53 ± 0.516 and 2.40 ± 0.828 respectively. In active gel group, 47% of the patients reported no pain and 53% of the patients reported mild pain. In placebo group, the corresponding figures were 13% and 40% respectively. In placebo group, 40% of the patients reported moderate pain and only one patient reported severe pain whereas in active gel group, no patient reported moderate pain or severe pain. The statistical analysis indicated a trend towards higher VAS pain scores in placebo group when the probing depth was deeper or bleeding on probing or presence of hypersensitivity. But, no such trend was observed in active gel group and no patient required rescue anaesthesia whereas in placebo gel group, 2 out of 15 (13%) patients required rescue anaesthesia.

DISCUSSIONS

The FTIR spectra of the pure drug as well as formulations indicated that no chemical interaction occurred between the Lidocaine HCl and the polymers used. But, a slight shift in absorption peaks position was noticed which may be due to physical interaction between drug and the polymer. Content uniformity studies showed that the drug was distributed uniformly in all formulations. Gelation temperature studies showed that gels with 18% PF 127 were found to be most suitable with gelation temperature near to body temperature. It was reported that gelation temperature decreased with increase in concentration of PF 127. Gelation temperature increased slightly with higher concentration of C 934, which may be due to the decrease in the average polyoxypropylene content of PF 127**.** Mucoadhesive strength was found to increase with the increase in concentration of PF 127 and C 934 when measured after 15 min contact time. Viscosity studies showed a marked increase in viscosity of the gels at 37°C due to sol-gel conversion due to increase in temperature. Viscosity of the gel was found to be optimum to pass through 23 gauge blunt needle. Sterility studies proved that formulations were free from microbes. The results of *in vitro* release studies showed that with increase in concentration of PF 127, the rate of drug release decreased. The reason for this may be the reduction in number and

dimensions of water channels of PF 127 through which drug diffuses. It was further reported that, as the concentration of C 934 increased, drug release rate decreased. The retardation of drug release with C 934 could be explained by the ability of C 934 to increase the overall gel viscosity. The release of drug from the formulated gels was following diffusion controlled without swelling. As the concentration of C 934 was increased, the "n" values were nearing 0.5, which indicates a tendency to swell at higher concentration of C 934.

One of the important objectives of this research was that anaesthetic effect should not last for longer duration after the SRP and gelation temperature should be near to body temperature. So the formulation F1 was selected as optimized formulation for clinical evaluation.

The gender distribution was similar between the active group and placebo group. The statistical analysis of baseline characteristics in both the groups (active gel group and placebo group), indicated that both the groups were almost similar in all the parameters. The majority of the adverse events were local events in oral cavity during SRP. None of the adverse events was of major clinical significance. The statistical analysis showed that VAS and VRS pain scores in the active gel group were significantly lower than those in the placebo group. So, clinical studies of formulation F1 showed that, local anaesthetic gel 5% was overall significant and more effective than placebo. The results suggested that Lidocaine HCl gel 5% was clinically effective in reducing SRP pain for those patients who perceive the procedure to be painful.

CONCLUSION

The study revealed that the temperature mediated *in situ* mucoadhesive gelling system can be formulated using optimum concentration of PF 127 and carbopol 934 with good mucoadhesive strength. From FTIR analysis, it was observed that there was no chemical interaction between the drug and the polymers. Formulation F1 containing 18% w/v PF 127 and 0.1% w/v carbopol 934 may be helpful to provide fast onset of anaesthesia for cleansing of periodontal pockets. Gels containing higher concentration of PF 127 may be helpful for periodontal anaesthesia where lengthy procedure is required. It may be concluded that thermosensitive *in situ* mucoadhesive gel delivery system is a novel approach for the treatment of periodontitis.

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