Formulation and evaluation of periodontal in situ gel

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Abstract

Background: The present study was aimed to develop and optimize in situ gel for the treatment of periodontal disease. Materials and Methods: Temperature-sensitive in situ gel containing 0.1% w/v Chlorhexidine hydrochloride was formulated by cold method using different polymers. Preliminary study was carried out to optimize different types and concentration of polymers such as Poloxamer 188, Poloxamer 407, Gellan gum, and Carbopol 934P. Central composite design was employed for optimization of the effect of independent variables such as Poloxamer 407 and Carbopol 934P on responses such as gelation temperature, spreadability, cumulative percentage release at 2 h, and time for 50% drug release ($t_{50}$%). Each formulation was evaluated for clarity, pH, gelation temperature, spreadability, cumulative percentage drug release at 2 h, and time for 50% drug release ($t_{50}$%). The desirability function was utilized to find out optimized formulation of the factorial design. Formulation F6 showed the highest overall desirability of 0.6283 and, therefore, this formulation was considered to be the optimized formulation. The % relative error was calculated, which showed that observed responses were in close agreement with the predicted values calculated from the generated regression equations. Conclusion: The clarity, pH, drug content of all formulations was found to be satisfactory. Further, all the formulations showed sustained drug release for a period of 6 h, which satisfied to treat periodontal disease.

Key words: Carbopol 934P, chlorhexidine hydrochloride, in situ gel, periodontal disease, poloxamer 407

INTRODUCTION

Periodontal disease is term used to ascribe to some pathological conditions characterized by degeneration and inflammation of gums, periodontal ligaments, alveolar bone, and dental cementum. It is a localized inflammatory reaction caused by bacterial infection of a periodontal pocket accompanying with sub-gingival plaque. Although bacteria are the principal cause of periodontal disease, the appearance of microbial pathogenic factors alone may not be enough to cause periodontitis. Periodontal pathogens generate destructive by-products and enzymes that break extracellular matrices as well as host cell membranes to produce nutrients for their growth. In doing so, they start damage directly or indirectly by triggering host-mediated responses that lead to self-injury. In the early phase of the disease, inflammation is localized to the gingiva called gingivitis but extends to deeper tissues in periodontitis, leading to gingival swelling, bleeding, and bad breath. In the late phase of the disease, the supporting collagen of the periodontium is disintegrated, alveolar bone begins to resorb, and gingival epithelium migrates along the tooth surface forming a ‘periodontal pocket.’

In situ gel forming formulations current a novel idea of deliver drugs to patients as a liquid dosage form, yet achieve sustained release of drug for the desired period. Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs. In recent years, there has been an increasing interest in water-soluble polymers that are able to form gels after application to delivery site. These so-called in situ gelling polymers are highly advantageous compared with other polymers because, in contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption, they swell to form a strong gel that is capable of prolonging the residence time of the active substance.

Chlorhexidine, a bisbiguanide compound, has been shown to possess a broad-spectrum of topical anti-microbial activity.
has been used by dental professionals for plaque control and for the treatment of gingival inflammation. Chlorhexidine was primarily used in mouth-rinses and was recommended in the hygiene phase of treatment as an adjunct to tooth-brushing. Most attention, however, has been focused on the use of chlorhexidine during the operative and immediate post-operative phases of non-surgical and surgical periodontal treatment.

The Poloxamer consist of more than 30 different non-ionic surface-active agents. These polymers are ABA-type triblock co-polymers composed of PEO (A) and PPO units (B). Pluronic are commercially available in a range of molecular weights, composition ratios, and forms, it would be useful to mention the nomenclature rules for these copolymers. The letter in the notation stands for liquid (L), paste (P), or flakes (F), whereas the first two numbers indicate the molecular weight of the PPO block and the last number the weight fraction of the PEO block. For example, the commonly used in biomedical applications F127 has a weight percentage of 70% PEO and a molecular weight of PPO around 4000.

Carbopol is a well-known pH-dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. Carbopols, which are very high molecular weight polymers of acrylic acid, have been used mainly in liquid or semi-solid pharmaceutical formulations, such as gels, suspensions and emulsions, as a thickening agent, in order to modify the flow characteristics.

**MATERIALS AND METHODS**

**Materials**

Chlorhexidine hydrochloride was obtained as a gift sample from Cadila Healthcare Pvt. Ltd. Ankleshwar, Gujarat. Poloxamer 188 was purchased from Hi media Laboratories Pvt. Ltd. Mumbai, India. Poloxamer 407 was purchased from Zeel pharmaceutical Pvt. Ltd. Mumbai, India.

Gellan gum was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Carbopol 934P were obtained from Noveon Ltd. Mumbai, India. Triethanolamine were obtained from Burgoyne burbridge and co. Mumbai, India.

**Optimization of types and concentration of polymers**

Different grades of Poloxamer i.e., Poloxamer 188, Poloxamer 407, and Gellan gum was used for the preparation of in situ gel formulation. The periodontal in situ gel was prepared using different Poloxamer 188, Poloxamer 407 concentration by cold method. This method involved slow addition of polymer in cold water with continuous stir. The formed mixtures were stored overnight at 4°C and studied for their gelation temperature to select optimum concentration of Poloxamer grades for effective in situ gel formulation.

**Method of preparation of in situ gel**

The method of preparation of in situ gel involved slow addition of polymers (i.e., Poloxamer 188, Poloxamer 407), and methyl paraben were solubilized in required quantity of cold deionized water. An appropriate amount of (0.1% w/v) Chlorhexidine HCl was solubilized in polymer solution with continuous stirring until uniform drug solution obtained. 0.1% w/v Chlorhexidine thermosensitive hydrogel is a strong candidate as a local drug delivery system for periodontal treatment. Required quantities of Carbopol 934P were added into the solution with continuous stirring by mechanical stirrer until uniform solution obtained. The final solution was kept overnight in refrigerator at 5°C to completely dissolve polymers in solution, and then addition of small amount of triethanolamine (TEA) was added to adjust the pH.

**Experimental design**

Central composite design (CCD) was employed for systematic study of joint influence of the effect of independent variables [Poloxamer 407 (X1) and Carbopol 934P (X2)] on responses such as gelation temperature (°C), spreadability (gm·cm/sec), cumulative percentage release (CPR) at 2 h and time for 50% drug release (t50%). Based on preliminary trials, two factors were determined as follows: Poloxamer 407 (X1): 15–19% w/v and Carbopol 934P (X2): 0.20–0.40% w/v. In this design, two factors with five levels were probed to investigate the main effects and interaction of the two factors on two responses [Table 1]. The design consists of nine runs (4 factorial points, 4 star points, and 1 center point) and four replicated runs (center points) yielding 13 experiments in total. The main purpose of the replication runs was to increase the precision and to minimize experimental error. The data obtained for the four responses in each trial were fitted to classical second order polynomial model. The mathematical model was expressed as follow, second order polynomial model:

\[ Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2 \]  

Where \( Y \) is the measured response, \( b_0 \) is an intercept, and \( b_1, b_2, b_3, b_4, b_5 \) is the regression co-efficients, \( X_1 \) represents the main effect, the quadratic effect, and \( X_1 X_2 \) is the interaction effect. The effects were evaluated statistically at 0.1 level (\( \alpha = 1.414 \)). Data were

| Table 1: Layout of Experimental design batches |
|-----------------------------------------------|
| Formulations | X1 (% w/v) | X2 (% w/v) |
| F1 | −α | 0 |
| F2 | −1 | −1 |
| F3 | −1 | +1 |
| F4 | 0 | −α |
| F5 | 0 | +α |
| F6 | +1 | −1 |
| F7 | +1 | +1 |
| F8 | +α | 0 |
| F9-F13 | 0 | 0 |

| Factor | −α | +α |
|--------|----|----|
| Level  | −1 | 0  | +1 | +α |

| X1: Poloxamer 407 (% w/v) |
|---------------------------|
| Poloxamer 407 | 14.17 | 15 | 17 | 19 | 19.83 |

| X2: Carbopol 934P (% w/v) |
|---------------------------|
| Carbopol 934P | 0.16 | 0.2 | 0.3 | 0.4 | 0.44 |

\( (\alpha = 1.414) \)
further analyzed by Microsoft Excel® 2007 for regression analysis. Analysis of variance (ANOVA) was implemented to assure that there was no significant difference between the developed full model and the reduced model. Response surface plots were plotted to study response variations against two independent variables using Design Expert® Version 8 software.\[15,16\]

**Desirability function**

The desirability value is a satisfaction index ranging between 0 and 1, characterizing the level of a response considering a particular objective. For the optimization of a process involving multiple responses, the individual responses have to be combined to define a product with the desired characteristics. The desirability value\[17,18\] is used to classify experiments according to the degree of satisfaction. For each response (Yi), the desirability function D (Yi) assigns values from 0 and 1 to the possible values of Yi; D (Yi) = 0 corresponding to a completely undesirable value of Yi, and D (Yi) = 1 corresponding to a completely desirable or ideal response value. Two desirability functions D (Yi) were used depending on whether the response Yi had to be maximized or minimized up to a target value (T).

**Evaluation of trans-dermal films**

**clarity**

The in situ gel solutions was prepared and evaluated visually for clarity against white and black backgrounds in light.\[19\]

**pH measurement**

The two areas of critical importance are the effect of pH on solubility and stability. The pH of in situ gel formulation should be such that the formulation be stable at that pH and at the same time, there would be no irritation to the patient upon administration of the formulation. In situ gel formulations should have pH range in between 6.2 and 7.4. The developed in situ gel formulations were evaluated for pH by using calibrated digital pH meter. The pH meter was calibrated before each use with standard pH 4, 7, and 9.2 buffer solutions. The formulation temperature was maintained at 25°C.\[20,21\]

**Gelation temperature**

Gelation temperature was assessed using a modified Miller and Donovan technique.\[22\] Two mL aliquots of the gel were transferred to test tubes sealed with parafilm and immersed in a water bath at 4°C. The temperature of the bath was increased in increments of 1°C and left to equilibrate for 15 min at each new setting. The samples were examined for gelation, which was deemed to have occurred when the meniscus would no longer move upon tilting through 90°C. All measurements were performed in triplicate (n = 3).\[23\]

**Drug content**

The prepared in situ gel formulations were analyzed for drug content by transferring 1 mL of formulation in 100 mL volumetric flask. In this volumetric flask, 50 mL of phosphate buffer with pH 6.8 was added, followed by continuous shaking until the gel was totally dispersed to give a clear solution. Final volume was adjusted to 100 ml with the help of phosphate buffer pH 6.8 and filtered the solution. Drug concentration in filtrated solution was determined spectrophotometrically at 257 nm using UV-Visible spectrophotometer (Shimadzu 1700, Japan).\[24\]

**Instrumental analysis**

**Fourier transform infrared spectroscopy**

Pre-formulation studies regarding the drug-polymer interaction are, therefore, very critical in selecting appropriate polymers. The FTIR spectra of the samples were recorded using KBr pellets method. The samples were then placed in the sample holder of the instrument. These were analyzed by FTIR to study the interference of polymer for drug analysis. The integrity and compatibility of the pure drug and polymer were evaluated with the help of IR spectra of the pure drug, and polymer was carried out using FTIR spectrophotometer.\[24\]

**Differential scanning calorimetry**

DSC study was carried out using DSC-60 instrument (Shimadzu corporation, Japan) to check the formation as well as the compatibility of ingredients. Thermogram of pure drug (Chlorhexidine hydrochloride) and optimized formulation was scanned for DSC. Accurately weighed samples were placed on aluminum plates sealed with aluminum seals and heated at constant temperature of 10°C/min over a temperature range of 0-300°C.\[25,26\]

**Viscosity**

The viscosity determination of the prepared optimized in situ gel formulation was determined using Brookfield Digital Viscometer (LVDV III U, Brookfield Engineering Labs. USA). The optimized in situ gel formulation was taken in a beaker and maintained at room temperature. The measurements were carried out using spindle no. 62 at the speed of 50 rpm in the sample, and the viscosity was measured at 10 min after the rotation of the spindle.\[20,26\]

**Spreadability test**

Spreadability was measured by apparatus, which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A ground glass slide was fixed on this block. An excess of gel (about 1 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. One kg weight was placed on the top of the two slides for five minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. The time required to separate the two slides and spreadability measured by using following equation\[27,28\]

\[
S = \frac{M \times L}{T} \quad \text{(2)}
\]
Where,
M = weight tide to upper slide
L = length moved on the glass slide
T = time taken to separate two slides.

In vitro drug release study
The in vitro drug release study of Chlorhexidine HCl from in situ gel was determined using a Franz-diffusion cell. The cellophane membrane was fixed on the receptor cell.[20] The donor cell was filled with 1 g of in situ gel formulation. The receptor compartment was filled with phosphate buffer pH 6.8 and constantly stirred with a small magnetic bar at a speed of 200–250 rpm during the experiments to confirm homogeneity, and temperature was maintained at 37 ± 1°C by circulating hot water through the jacket of Franz-diffusion cell. The 0.5 mL samples were withdrawn at scheduled time intervals (0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 min) and were replaced with same volume of pH 6.8 phosphate buffer to maintain the sink condition. Samples were analyzed at 257 nm on UV-visible spectrophotometer.[20]

Stability study
Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. Stability studies of periodontal in situ gel were carried out to determine the effect of contents on the stability of the drug. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at 40 + 2°C and 75 ± 5% RH for 1 month using stability chamber (Remi, India). The optimized formulation were evaluated for clarity, pH measurement, drug content, gelation temperature, spreadability, CPR at 6 h, CPR at 2 h, and t_{50%}.[20]

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy
FTIR study was performed to evaluate compactability between drug and polymer utilized in study. IR spectrum of Chlorhexidine HCl is characterized by principal absorption peaks at 2542, 2956.67 cm⁻¹ (N-H stretch, salt of secondary amine), 1519.80 cm⁻¹ (N-H bending, secondary aromatic amine), 1259.43 cm⁻¹ (C-N stretch, secondary aromatic amine), 1022.20, 1155.28 cm⁻¹ (C-N stretch) shown in Figure 1. IR spectrum of poloxamer 188 is characterized by principal absorption peaks at 2883.38 cm⁻¹ (C-H stretch aliphatic), 1348.15 cm⁻¹ (in-plane O-H bend), and 1107.06 cm⁻¹ (C-O stretch) shown in Figure 2. IR spectrum of poloxamer 407 is characterized by principal absorption peaks at 2893.02 cm⁻¹ (C-H stretch aliphatic), 1355.86 cm⁻¹ (in-plane O-H bend), and 1259.43 cm⁻¹ (C-O stretch) shown in Figure 3. IR spectrum of Carbopol 934P is characterized by principal absorption peaks at 3089.75 cm⁻¹ (O-H stretching), 1706.88 cm⁻¹ (carboxyl group) shown in Figure 4. The IR spectrum of physical mixture of drug and polymers is characterized by principal absorption peaks at 2935.45 cm⁻¹ (N-H stretch, salt of secondary amine), 1529.45 cm⁻¹ (N-H bending, secondary aromatic amine), 1155.28 cm⁻¹ (C-N stretch).

The interaction between the drug and the polymers often leads to identifiable changes in the FTIR profile of solid systems. FTIR spectra for pure drug, polymers, and physical mixture of drug and polymers have been depicted in Figures 1–5. The spectrum of physical mixture of drug and polymers was equivalent to the addition spectrum of pure drug, indicating no interaction occurring in the simple physical mixture of drug and polymer as shown in Figure 5.

Selection of polymers
Different grades of Poloxamer i.e. Poloxamer 188, Poloxamer 407, and Gellan gum were used for the preparation of in situ gel formulation. From all polymers, Poloxamer 188 and Poloxamer 407 were formed viscous clear solution and formed gel at elevated temperature. Gellan gum was not used to prepare formulation because of its gel formation during formulation shown in Table 2. So, Poloxamer 188 and Poloxamer 407 were selected as polymer for in situ gel formulation on bases of visual examination and gelation temperature.

Selection of polymers concentration
The gelation temperatures have been considered to be suitable if they are in the range of body temperature. As the temperature of the periodontal cavity is nearest 37°C, this study aimed at preparing the liquid formulations that may gel below 37°C. The gelation of Poloxamer vehicles was known to result from the change in micellar number with temperature. With increasing temperature, the number of micelles formed increases as a consequence of the negative co-efficient of solubility of block copolymer micelles. Eventually, the micelles become so tightly packed that the solution becomes immobile, and gel is formed. Conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon. Gelation temperatures for Poloxamer 188 and Poloxamer 407 gels were observed for the different concentration range of polymer, and it was found that the gelation temperature of formulation decreased with increasing concentration of polymer.

As the concentration of polymer increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern.[23] Result revealed that the formulation containing Poloxamer 188 have found to be higher gelation temperature compared to body temperature. So, gelation temperature of Poloxamer 188 in situ gel was not fulfilling the requirement of in situ gel formulation. Only 15% w/v and 20% w/v concentration

| Polymers       | Observation          |
|---------------|----------------------|
| Poloxamer 188 | Clear solution formed|
| Poloxamer 407 | Clear solution formed|
| Gellan gum    | Gel formed           |

Table 2: Selection of polymer
Figure 1: FTIR spectrum of chlorhexidine HCl

Figure 2: FTIR spectrum of poloxamer 188
of Poloxamer 407 in situ gel showed ability to form gel in the range of body temperature shown in Table 3. So, 15-20% w/v concentration of Poloxamer 407 was used for further studies. Potential drawbacks of Poloxamer gels include their weak mechanical strength, rapid erosion, and the non-biodegradability of PEO-PPO-PEO, which prevented the use of high molecular
weight polymers. Thus, it was necessary to formulate them with other bioadhesive polymers, including cellulose ethers polymers such as carboxymethylcellulose (CMC), HPMC, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), acrylic polymers.

Experimental design

Preliminary investigations of the process parameters exposed that factors such as Poloxamer 407 ($X_1$) and Carbopol 934P ($X_2$) exhibited significant influence on gelation temperature, spreadability, CPR at 2h, $t_{50\%}$; hence, they were utilized for further systematic studies. All selected dependent variables for all 13 batches showed a wide variation of data [Table 4]. The polynomial equations can be used to draw conclusions after considering the magnitude of co-efficients and the mathematical sign carried: Positive or negative.

Effect of formulation variable on gelation temperature ($Y_1$)

Concerning $Y_1$, the results of multiple linear regression analysis showed that both the co-efficients $b_1$ and $b_2$ bear a negative sign [Table 5]. The negative of both $X_1$ and $X_2$ co-efficient indicates that as the concentration of $X_1$ (Poloxamer 407) and $X_2$ (Carbopol 934P) increases, there is decrease in the gelation temperature. The fitted equation relating the response $Y_1$ to the transformed factor is shown in following equation,

$$
Y_1 = 33.33 - 3.769X_1 - 0.755X_2 - 0.590X_1^2
+ 0.6699X_2^2 + 0.5825X_1X_2 \\
...\text{(3)}
$$

The $Y_1$ for all batches F1 to F13 shows good correlation co-efficient of 0.910009. Variable $X_1$ has $P$ value 0.0000916 ($P < 0.05$), and variable $X_2$ has $P$ value 0.038169 ($P < 0.05$). Variables, which have $P$ value less than 0.05, significantly affect the gelation temperature. As the concentration of Poloxamer 407 increases, there is micelle formation, followed by micellar aggregation. The gel phase can only occur when the concentration is above the micellar concentration. When the material is in cold water, hydrogen bonding between POP chains and water keeps the hydrophobic portions of the pluronic separate. When the temperature is increased, the hydrogen bonding is disrupted, and hydrophobic interactions cause a gel to be formed. It is to be noted that the addition of increasing concentrations of Carbopol 934P from 0.2% to 0.4% further lowered the gelation temperature. As the concentration of mucoadhesive polymers (Carbopol 934P) increased, the gelation temperature of gel decreased. This ability of mucoadhesive polymers to lower.

### Table 3: Optimization of different polymer concentration

| Polymers      | Concentration (% w/v) | Gelation temperature (°C) |
|---------------|-----------------------|---------------------------|
| Poloxamer 188 | 5                     | 70                        |
|               | 10                    | 65                        |
|               | 15                    | 59                        |
|               | 20                    | 55                        |
|               | 25                    | 49                        |
| Poloxamer 407 | 5                     | 50                        |
|               | 10                    | 44                        |
|               | 15                    | 39                        |
|               | 20                    | 28                        |

Figure 5: FTIR spectrum of physical mixture
gelation temperature may be due to increased viscosity after dissolution of polymers. The ability of mucoadhesive polymers to lower gelation temperature could be explained by their ability to bind to the polyoxylene chains present in the Poloxamer 407 molecules. This would promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding, thus leading to gelation at lower temperature. Combination of bioadhesive agents and Poloxamer 407 on gelation temperature that the packing and entanglements of micelles were promoted by adding Carbopol 934P, which accordingly lead to decrease of gelation temperature.

The relationship between formulation variables ($X_1$ and $X_2$) and $Y_1$ was further elucidated using response surface and contour plot. The effects of $X_1$ and $X_2$ on $Y_1$ are shown in Figures 6a and b. Table 4 showed that with the increase of amount of Poloxamer 407 and Carbopol 934P, gelation temperature was reduced.

**Effect of formulation variable on spreadability ($Y_2$)**

Concerning $Y_2$, the results of multiple linear regression analysis showed that co-efficient $b_1$ bear positive sign and co-efficient $b_2$ bear negative sign [Table 5]. The positive $X_1$ co-efficient indicates that as the concentration of $X_1$ (Poloxamer 407) increases, there is increase in the spreadability of in situ gel. The negative $X_2$ co-efficient indicates that as the concentration of $X_2$ (Carbopol 934P) increases, spreadability of in situ gel decreases. The fitted equation relating the response $Y_2$ to the transformed factor is shown in following equation,

$$Y_2 = 21.573 + 0.9412X_1 - 2.677X_2 - 0.842X_1^2 - 0.681X_2^2 - 1.68X_1X_2$$  

...(4)

The $Y_2$ for all batches F1 to F13 shows good correlation co-efficient of 0.749198. Variable $X_1$ has $P$ value 0.003824 ($P < 0.05$), and variable $X_2$ has $P$ value 0.006702 ($P < 0.05$). Variables, which have $P$ value less than 0.05, significantly affect the spreadability.

The relationship between formulation variables ($X_1$ and $X_2$) and $Y_1$ was further elucidated using response surface and contour plot. The effects of $X_1$ and $X_2$ on $Y_1$ are shown in Figures 7a and b. As the concentration of $X_1$ (Poloxamer 407) increases, there is increase in the spreadability of in situ gel, and as the effect of concentration of $X_2$ (Carbopol 934P) increases, spreadability of in situ gel decreases.

Results of the spreadability testing are shown in Table 4. All the prepared in situ gels using different polymers concentrations were spreadable. Data in Table 4 revealed that increasing the concentration of any of the gelling agents was always associated with a decrease in the spreadability. One of the criteria for a gel to meet the ideal qualities is that it should possess good spreadability. It is the term expressed to denote the extent of area.

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**Table 4: Results of experimental design batches of variables**

| Formulation | $X_1$ (%) | $X_2$ (%) | $Y_1^*$ (°C) | $Y_2^*$ (gm·cm/sec) | $Y_3^*$ (%) | $Y_4^*$ (min) |
|-------------|-----------|-----------|--------------|------------------|------------|---------------|
| F1          | 14.17     | 0.3       | 39.33±0.58   | 21.73±1.22       | 52.48±0.36 | 153.54±0.69  |
| F2          | 15        | 0.2       | 37.33±1.53   | 17.33±0.77       | 47.57±0.35 | 175.54±0.75  |
| F3          | 15        | 0.4       | 34.33±0.58   | 15.80±0.42       | 47.60±0.43 | 180.79±0.37  |
| F4          | 17        | 0.16      | 35.67±1.15   | 25.42±1.68       | 50.19±0.51 | 178.19±0.49  |
| F5          | 17        | 0.44      | 34.00±1.00   | 17.21±1.35       | 37.53±0.35 | 224.19±0.90  |
| F6          | 19        | 0.2       | 31.00±1.00   | 25.48±2.16       | 37.44±0.55 | 236.96±0.84  |
| F7          | 19        | 0.4       | 30.33±1.53   | 17.23±1.48       | 36.31±0.56 | 288.23±0.57  |
| F8          | 19.83     | 0.3       | 25.33±0.58   | 20.26±1.06       | 39.32±0.27 | 211.09±0.38  |
| F9          | 17        | 0.3       | 33.33±0.58   | 21.20±0.88       | 46.47±0.30 | 186.59±0.51  |
| F10         | 17        | 0.3       | 32.67±0.58   | 22.24±0.83       | 48.10±0.60 | 175.21±0.40  |
| F11         | 17        | 0.3       | 33.67±1.15   | 21.73±1.22       | 47.66±0.24 | 179.25±0.59  |
| F12         | 17        | 0.3       | 33.33±0.58   | 21.22±1.13       | 47.42±0.32 | 180.77±0.53  |
| F13         | 17        | 0.3       | 33.67±0.58   | 21.45±0.77       | 46.33±0.36 | 186.28±0.41  |

*All the values are in mean±S.D. (n=3), $X_1$: Poloxamer 407 (% w/v), $X_2$: Carbopol 934P (%w/v), $Y_1$: Gelation temperature (°C), $Y_2$: Spreadability (gm·cm/sec), $Y_3$: CPR at 2 h, $Y_4$: 50% (min)

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**Table 5: Summary of regression analysis**

| Co-efficients | $b_0$  | $b_1$  | $b_2$   | $b_3^*$ | $b_4^*$ | $b_5^*$ |
|---------------|--------|--------|---------|---------|---------|---------|
| $Y_1^*$ Gelation temperature | FM | 33.3348 | -3.76997 | -0.75533 | -0.59003 | 0.669967 | 0.5825 |
| | RM | 33.3385 | -3.76997 | -0.75533 | - | - | - |
| $Y_2^*$ Spreadability | FM | 21.57322 | 0.941213 | -2.67748 | -0.84223 | -0.68128 | -1.68 |
| | RM | 20.63846 | 0.941213 | -2.67748 | - | - | - |
| CPR at 2h | FM | 47.26385 | -4.84002 | -2.40705 | -1.32433 | -2.32277 | -0.45 |
| | RM | 45.02615 | -4.84002 | -2.40705 | - | - | - |
| $t_{50\%}$ | FM | 181.9037 | 27.9006 | 11.43165 | 5.652703 | 15.20705 | 3.6725 |
| | RM | 194.7023 | 27.9006 | 11.43165 | - | 14.48642 | - |

FM: Full model, RM: Reduced model, $t_{50\%}$ time required for 50% drug release, *Response is insignificant at $P>0.05$
to which gel readily spreads on application site. Lesser the time taken for separation of two slides, better the spreadability. The spreadability of all prepared in situ gel formulations were found to be in range of 17.21-25.48 gm·cm/sec.

**Effect of formulation variable on CPR at 2 hr (Y₂)**

Concerning Y₂, the results of multiple linear regression analysis showed that both the co-efficients b₁ and b₂ bear a negative sign [Table 5]. The negative of both X₁ and X₂ co-efficients indicates that as the concentration of X₁ (Poloxamer 407) and X₂ (Carbopol 934P) increases, there is decrease in the cumulative percentage drug release. The fitted equation relating the response Y₂ to the transformed factor is shown in following equation,

\[
Y_2 = 47.263 - 4.840X_1 - 2.407X_2 - 1.324X_1^2 - 2.322X_2^2 - 0.45X_1X_2 \quad (5)
\]

The Y₂ for all batches F1 to F13 shows good correlation co-efficient of 0.852097. Variable X₁ has P value 0.001251 (P < 0.05), and variable X₂ has P value 0.036108 (P < 0.05). Variables, which have P value less than 0.05, significantly affect the release profile.

The relationship between formulation variables (X₁ and X₂) and Y₂ was further elucidated using response surface and contour plot. The effects of X₁ and X₂ on Y₂ are shown in Figures 8a and b. This finding was probably due to the increased strength of the formed gel structure. It is worthwhile that the drug diffusion is controlled by the penetration of liquid through the gel structure and retard release of drug into the medium.  

**Effect of formulation variable on t₅₀% (Y₄)**

Concerning Y₄, the results of multiple linear regression analysis showed that both the co-efficients b₃ and b₄ bear a positive sign [Table 5]. The positive of both X₁ and X₂ co-efficient indicates that as the concentration of X₁ (Poloxamer 407) and X₂ (Carbopol 934P) increases, there is increase in the time for the drug release from formulation. The fitted equation relating the response Y₄ to the transformed factor is shown in following equation,

\[
Y_4 = 181.90 + 27.90X_1 + 11.431X_2 + 5.6527X_1^2 + 15.207X_2^2 + 3.672X_1X_2 \quad \ldots (6)
\]

The Y₄ for all batches F1 to F13 shows good correlation co-efficient of 0.848547. Variable X₁ has P value 0.00125 (P < 0.05), and variable X₂ has P value 0.042157 (P < 0.05). Variables, which have P value less than 0.05, significantly affect the release profile. The relationship between formulation variables (X₁ and X₂) and Y₄ was further elucidated using response surface and contour plot. The effects of X₁ and X₂ on Y₄ are shown in Figures 9a and b. A strong influence of both independence variables (Poloxamer 407 and Carbopol 934P) was observed on in vitro drug release (t₅₀%). The highest t₅₀% value (258.23 ± 0.57) was observed with Batch 7 [Poloxamer 407 (19% w/v) and Carbopol 934P (0.4% w/v)]. It is possible that at higher polymers concentration, drug is trapped in smaller polymer cells, and it is structured by its close proximity to the polymer molecules. So, increasing the amount of the polymer in the formulations increased the time for the drug to leave the formulation and delayed release of drug into the medium.
Analysis of variance
The R² value for gelation temperature (Y₁), spreadability (Y₂), CPR at 2 h (Y₃), and t₉₀₈ (Y₄) are 0.910009, 0.749198, 0.852097, and 0.848547, respectively, indicating good correlation between dependent and independent variables [Table 6]. The reduced models were developed for response variables by omitting the insignificant terms with P > 0.05. For all selected dependent variables, co-efficients bᵢ, bᵢ, bᵢ, bᵢ, were found to be insignificant, as P values were more than 0.05 and hence removed from the full model.

Table 6 shows the results of analysis of variance (ANOVA) performed to justify the removal of insignificant factors. The high values of correlation co-efficients for gelation temperature, spreadability, CPR at 2 h, and t₉₀₈ indicate a good fit. The calculated values of F for gelation temperature, spreadability, CPR at 2 h, and t₉₀₈ was found to be 1.43, 1.55, 2.19, and 1.91, respectively, which were less than F critical value (4.35). These observations suggest no significant difference between the full and reduced model.

Clarity and pH
The clarity of first three formulations (F1-F3) was slightly hazy, and all other formulations (F4-F13) were found to be clear. The pH value of all prepared in situ gel formulations (F1-F13) were found in range 6.72 to 7.10 [Table 7]. The pH values of all prepared formulations were within the limit of neutral pH; this indicates formulations can be used without any irritation in the oral cavity.

Table 6: Calculation of testing the model in portions

| Model                      | DF | SS    | MS    | R²  |
|----------------------------|----|-------|-------|-----|
| Gelation temperature       |    |       |       |     |
| Regression                 |    |       |       |     |
| FM 5                       | 5  | 125.5653 | 25.11307 | 0.910009 |
| RM 2                       | 2  | 117.9135 | 58.95677 | 0.854554 |
| Error                      |    | 12.41718 | 1.77383 |    |
| Spreadability Regression   |    | 44.26465 | 32.123 | 0.749198 |
| RM 10                      | 10 | 20.06898 | 2.006898 |    |
| CPR at 2 h Regression      |    | 27.69737 | 3.956768 |    |
| Error                      |    | 46.18892 | 4.618892 |    |
| CPR at 2 h Error           |    | 233.0623 | 116.5311 | 0.713082 |
| Spreadability Error        |    | 48.34025 | 6.90575 |    |
| RM 10                      | 10 | 93.77561 | 9.377561 |    |
| t₉₀₈% Regression           |    | 8997.068 | 1799.414 | 0.848547 |
| Error                      |    | 7251.536 | 3625.686 | 0.683904 |
| RM 7                       | 7  | 1605.841 | 229.4058 |    |
| t₉₀₈% Error                |    | 3351.536 | 335.1536 |    |

DF: Degree of freedom, SS: Sum of squares, MS: Mean of squares, R²: Regression co-efficient

Drug content
Drug content was one of a significant requirement for any type of dosage form. Amount of the drug present in the formulation should not deviate beyond certain specified limits from the labeled amount. All formulations were found to having drug content in the range of 96.89-100.32%, representing homogenous drug distribution throughout gel shown in Table 7.

Viscosity
One essential necessity for a periodontal in situ gel was viscosity of the formulation. As indicated, a formulation suitable for application to the periodontal pocket should ideally have a low viscosity when apply and after instillation, should have a high viscosity in order to stay at the application site. The viscosity of the optimized in situ gel formulation measured at 37°C was found to be 30596.33 ± 1.52 cps.

Differential scanning calorimetry study
Drug excipients compatibility study was carried out by Shimadzu DSC-60 in dry N₂ atmosphere (flow rate 20 mL/min), and temperature scanning rate was 10°C/min up to 300°C. About 2 mg of each sample was weighed using closed aluminum pans. Thermogram of pure drug (Chlorhexidine HCl) is shown in Figure 10. Melting transition of Chlorhexidine HCl was observed from 266.65°C (Onset) to 275.46°C (Endset). Sharp melting transition of Chlorhexidine HCl was observed at 272.08°C. In the optimized formulation drug and excipients had two melting endotherm was observed first from 36.72°C (Onset) to 181.09°C (Endset), and second was observed at 265.69°C (Onset) to 276.4209°C (Endset). Sharp melting transition of Chlorhexidine HCl in optimized formulation was observed at 271.06°C, which is shown in Figure 10. There was no much difference in the melting point of the drug in all the thermogram, it was concluded that the drug is in the same state, even in the optimized formulation without interacting with polymers. Co-operation of thermogram of pure drug and optimized formulation is shown in Figure 10.

In vitro drug release
The effect of polymer concentration on in vitro drug release from in situ gels was shown in Figure 11. The cumulative percent of Chlorhexidine HCl released as a function of time is shown in Figure 11. The combination of Carbopol 934P with
Poloxamer 407 decreased the release rate for Chlorhexidine HCl from gel. The results indicated that combination of bioadhesive agents with Poloxamer 407 could prolong drug action time in periodontal cavity due to the increase of viscosity with addition of Carbopol 934P. The mechanism for resistant barrier of single Poloxamer 407 gels to drug release may be due to reduction in the number and dimension of water channels and the increase in the number and size of micelles within the gel structure. The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles, which result in higher viscosity and lower rate of drug release.

Optimization of the formulation using the desirability function

The desirability function was used for optimization of the formulation. During optimization of formulations, the responses have to be combined in order to produce a product of desired characteristics. The application of the desirability function combines all the responses in one measurement and gives the possibility of predicting optimum levels for the independent variables.

The combination of the responses in one desirability function requires the calculation of the individual functions. A suitable

\[ d_1 = 0 \text{ for } Y_i < Y_{\text{max}} \]
\[ d_1 = 1 \text{ for } Y_{\text{min}} < Y_i < Y_{\text{max}} \]
\[ d_1 = 0 \text{ for } Y_i > Y_{\text{max}} \]  ... (7)

Where \( d_1 \) is the individual desirability of the gelation temperature.

The spreadability values were maximized in the optimization procedure, as suitable in situ gel should have high spreadability. The desirability functions of these responses were calculated using the following equation:

\[ d_2 = \frac{Y_i - Y_{\text{min}}}{Y_{\text{target}} - Y_{\text{min}}} \text{ For } Y_i < Y_{\text{target}} \]
\[ d_2 = 1 \text{ for } Y_i > Y_{\text{target}} \]  ... (8)

Where \( d_2 \) is the individual desirability of Spreadability.

The CPR at 2 h value was minimized in the optimization procedure, as suitable in situ gel should have low CPR at 2 h. The desirability functions of this response were calculated using the following equation:

\[ d_3 = \frac{Y_{\text{max}} - Y_i}{Y_{\text{max}} - Y_{\text{target}}} \text{ For } Y_i < Y_{\text{target}} \]
\[ d_3 = 1 \text{ for } Y_i > Y_{\text{target}} \]  ... (9)

Where \( d_3 \) is the individual desirability of % drug release at 2 h (CPR at 2 h).

The \( t_{50\%} \) values were maximized in the optimization procedure, as suitable in situ gel should have high \( t_{50\%} \). The desirability functions of these responses were calculated using the following equation:

\[ d_4 = \frac{Y_i - Y_{\text{min}}}{Y_{\text{target}} - Y_{\text{min}}} \text{ For } Y_i < Y_{\text{target}} \]
\[ d_4 = 1 \text{ for } Y_i > Y_{\text{target}} \]  ... (10)

Where \( d_4 \) is the individual desirability of \( t_{50\%} \).
The overall desirability values were calculated from the individual values by using the following equation: [39]

\[ D = \left( d_1d_2d_3d_4 \right)^{1/4} \]  

... (11)

**Formulation of optimized formulation of in situ gel**

From the desirability function was utilized to find out the best batch out of 13 batches. Batch F6 showed the highest overall desirability of 0.6282 [Table 8]. Therefore, this batch was considered to be the best batch, and the values of independent variables of this batch were considered to be optimum values for the preparation of in situ gel. The final optimized formulation was prepared using 19% w/v Poloxamer 407 and 0.2% w/v Carbopol 934P.

**Comparison between observed and predicted results of checkpoint batches**

In order to assess the reliability of the equations that describes the influence of the factors on the gelation temperature, spreadability, CPR at 2 h, and t\textsubscript{50} of in situ gel. The experimental values and predicted values of each response are shown in Table 9. The % relative error between predicted values and experimental values of each response was calculated using the following equation: [38]

\[ \% \text{ Relative error} = \left( \frac{| \text{Predicted value} - \text{Experimental value} |}{\text{Predicted value}} \right) \times 100 \]  

... (12)

The % relative error obtained from checkpoint batch was in the range of 1.9876-4.6353. It can be seen that in all cases, there was a reasonable agreement of predicted values and experimental values, since low values of the relative error were found. This confirmed the role of a derived reduced polynomial equation, proved the validity of the model, and ascertained the effects of Poloxamer 407 and Carbopol 934P on dependent variables.

**Stability studies**

*In situ* gel formulation optimized from statistical design application and was selected for the stability studies. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at 40 ± 2°C and 75 ± 5% RH for 1 month using stability chamber (Remi, India). The optimized formulation were evaluated for clarity, pH measurement, drug content, gelation temperature, spreadability, CPR at 6 h, CPR at 2 h, and t\textsubscript{50}. The results after the stability period are given in Table 10. Results of the stability study show no remarkable change in the release profile, assay, and other evaluation parameters of the periodontal in situ gel after exposing to accelerated stability conditions.

**CONCLUSION**

Chlorhexidine hydrochloride, a broad-spectrum anti-microbial agent used in the treatment of periodontal disease, was successfully formulated as temperature-sensitive in situ gel by cold method using Poloxamer 407 and Carbopol 934P as gelling agent. The clarity, pH, and drug content of all formulations were found to be satisfactory. All the formulations showed sustained drug release for a period of 6 h. The desirability function was utilized in order to find out the best batch out of all 13 batches of the central composite design. Formulation F6 showed the highest overall desirability of 0.6282. Therefore, this formulation was considered to be the best formulation, and the values of independent variables of this formulation were considered to be optimum values for the preparation of in situ gel. The results of the stability study show no remarkable change in the release profile, assay, and other evaluation parameters of the periodontal in situ gel after exposing to accelerated stability conditions.

**REFERENCES**

1. Vyas SP, Sihorkar V, Mishra V. Controlled and targeted drug delivery strategies towards intraperiodontal pocket diseases. J Clin Pharm Ther 2000;25:21-42.
2. Haffajee AD, Socransky SS. Attachment level changes in destructive periodontal diseases. J Clin Periodontol 1986;13:461-75.
3. Iqbal Z, Jain N, Jain GK, Talegaonkar S, Ahuja A, Khar RK, et al. Dental therapeutic systems. Recent Pat Drug Deliv Formul 2008;2:58-67.

4. Nagarwal RC, Srinatha A, Pandit JK. In situ forming formulation: Development, evaluation and optimization using 3^3 factorial design. AAPS Pharm Sci Tech 2009;10:977-84.

5. Mahajan H, Shaikh H, Gattani S, Nerkar P. In situ gelling system based on thiolatedgelgum as new carrier for nasal administration of dimethyldihydroxide. Int J Pharm Sci Nanotech 2009;2:544-50.

6. Krishna MK, Ravindran SK, Vivekanandan G, Navasivayam A, Thiagarajan R, Mohan R. Effect of a single episode of subgingival irrigation with tetracycline HCI or chlorhexidine: A clinical and microbiological study. J Indian Soc Periodontol 2011;15:245-9.

7. Wannachaiyasit S, Phaechamud T. Development of chlorhexidine thermosensitive gels as a mouth antiseptic. J Miner Met Mater Soc 2010;20:165-8.

8. Schwach-Abdelwauia K, Vivien-Castillonib N, Gurny R. Local delivery of antimicrobial agents for the treatment of periodontal diseases. Eur J Pharm Biopharm 2000;50:83-99.

9. Ruel-Gariépy E, Leroux JC. In situ-forming hydrogels: Review of temperature-sensitive systems. Eur J Pharm Biopharm 2004;58:409-26.

10. Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. Eur J Pharm Biopharm 2008;68:34-45.

11. Nirmal HB, Bakliwal SR, Pawar SP. In situ gel: New trends in controlled and sustained drug delivery system. Int J Pharm Tech Res 2010;2:1398-408.

12. Jelvehgaria M, Rashidib MR, Samadia H. Mucoadhesive and drug release properties of benzocaine gel. Iranian J Pharm Sci Autumn 2006;2:185-94.

13. Ji QX, Zhao QS, Deng J. A novel injectable chlorhexidine-thermosensitive hydrogel for periodontal application: Preparation, antibacterial activity and toxicity evaluation. J Mater Sci Mater Med 2010;21:2435-42.

14. Jayaraj Kumar K, Jayachandran E, Srinivas GM, Girdhar B, Nair R, Jayakandan M. Formulation of thermoresponsive and buccal adhesive in situ gel for treatment of oral thrush containing itraconazole. J Pharm Sci Res 2010;2:116-22.

15. Singh G, Pai RS, Devi VK. Response surface methodology and process optimization of sustained release pellets using taguchi orthogonal array design and central composite design. J Adv Pharm Technol Res 2012;3:30-40.

16. Garala K, Patel J, Patel A, Dharamsi A. Enhanced encapsulation of metropol tranquil with carbon nanotubes as adsorbent. Appl Nanosci 2011;1:219-230.

17. Harrington J. The desirability function. Ind Qual Control 1965;21:494-8.

18. Derringer R, Suich R. Simultaneous optimization of several response variables. J Qual Technol 1980;12:214-9.

19. Gupta H, Sharma A, Shrivastava B. Pluronic and chitosan based in situ gel system for periodontal application. Asian J Pharm 2009;94-6.

20. Dabhi MR, Nagori SA, Gohel MC, Parikh RK, Sheth NR. Formulation development of smart gel periodontal drug delivery system for local delivery of chemotherapeutic agents with application of experimental design. Drug Deliv 2010;17:520-31.

21. Nanjawade BK, Manvi FV, Manjappa AS. In situ forming hydrogels for sustained ophthalmic drug delivery. J Control Release 2007;122:199-34.

22. Miller SC, Donovan MD. Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. Int J Pharm 1982;12:147-52.

23. Badgujar SD, Sontakke MA, Narute DR, Karmarkar RR, Tupkar SV, Barhate SD. Formulation and evaluation of sumatriptan succinate nasal in situ gel using fulvic acid as novel permeation enhancer. Int J Pharm Res Dev 2010;2:1-8.

24. Kumar P, Awasthi R, Kumar PR, Kumar M, Kumar MP. Mucoadhesive in situ gels of local anaesthetic for periodontia. Der Pharm Lettre 2010;2:28-39.

25. Patel RP, Baria AH, Pandya NB, Tank HM. Formulation evaluation and optimization of stomach specific in situ gel of ranitidine hydrochloride. Int J Pharm Sci Nanotech 2010;3:834-43.

26. Wamorkar V, Varma MM, Manjunath SY. Formulation and evaluation of stomach specific in situ gel of metoclopramide using natural, bio-degradable Polymers. Int J Res Pharm Biomed Sci 2011;2:193-201.

27. Harish NM, Prabhu P, Charyulu RN, Gulzar MA, Subrahmanyam EV. Formulation and evaluation of in situ gel containing clotrimazole for oral candidiasis. Indian J Pharm Sci 2009;71:421-7.

28. Kumar L, Verma R. In vitro evaluation of topical gel prepared using natural polymer. Int J Drug Del 2010;2:58-63.

29. Scherlund M, Welin-Berger K, Brodin A, Malmsten M. Local anaesthetic block copolymer system undergoing phase transition on dilution with water. Eur J Pharm Sci 2001;14:53-61.

30. Shams MS, Alam MI, Ali A, Sultana Y, Aqil M. Pharmacodynamics of a losartan transdermal system for the treatment of hypertension. Drug Dev Ind Pharm 2010;36:385-92.

31. Gui-Ling Li, Mei L. Preparation and evaluation of ophthalmic thermosensitive in situ gels of penciclovir. J Chinese Pharm Sci 2007;16:90-5.

32. Majithiya RJ, Ghosh PK, Umrethia ML, Murthy SR. Thermoreversible-mucoadhesive gel for nasal delivery of Sumatriptan. AAPS Pharm Sci Tech 2006;7:67.

33. Gonjari ID, Hosmani AH, Karmarkar AB, Godage AS, Kadam SB, Dhabane PN. Formulation and evaluation of in situ gelling thermoreversible mucoadhesive gel of fluconazole. Drug Discov Ther 2009;3:6-9.

34. Abdel-Mottaleb MM, Mortada ND, Elshamy AA, Awad GA. Preparation and evaluation of fluconazolegels. Egypt J Biomed Sci 2007;23:266-86.

35. Vijayaralkshmi A, Tripura A, Ravichandiran V. Development and evaluation of anti-acne products from terminaliaarjuna bark. Int J Chem Tech Res 2011;3:320-7.

36. Patel RP, Dadhani B, Ladani R, Baria AH, Patel J. Formulation, evaluation and optimization of stomach specific in situ gel of clarithromycin and metronidazole benzoate. Int J Pharm Sci 2009;71:421-7.

37. Bhardwaj R, Blanchard J. Controlled release delivery system for the α-MSH analog melanotan-1 using poloxamer 407. J Pharm Sci 1996;85:915-9.

38. Paterakis PG, Korakianiti ES, Dallas PP, Rekkas DM. Evaluation and simultaneous optimization of some pellets characteristics using 3 (3) factorial design and the desirability function. Int J Pharm 2002;248:51-60.

39. Mashru RC, Sutariya VB, Sankalia MG, Parikh PP. Development and evaluation of fast-dissolving film of salbutamol sulphate. Int J Drug Del 2010;2:141-53.

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