

Pluronic and Chitosan based *in situ* gel system for periodontal application

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The aim of this paper is to develop and evaluate a physiologically activated *in situ* gel for local periodontal application. The gel, when at formulation pH and temperature (pH 6, 25°C) will be at liquid form which will be converted to gel at body pH and temperature (pH 7.4, 37°C) showing ease of administration and prolonged duration of action. Chitosan which was both mucoadhesive as well as pH sensitive polymer was used in combination with pluronic F-127 which is a temperature simulated gelation polymer. Prilocaine hydrochloride was used as model drug to check the efficacy of the developed *in-situ* gel system. Different combination of Chitosan and pluronic F-127 were tested and final combination of 0.5% w/v and 10% w/v of Chitosan and pluronic F-127 respectively were selected and further evaluated for parameters like physicochemical properties, viscosity, gelation pH, gelation temperature, *in-vitro* release, sterility testing and stability testing. The system thus developed was found to be clear and have good viscosity with prolonged release at pH 7.4 and 37°C. The formulation can be easily packaged and sterilized with method employed. As per ICH guidelines gel was found to be stable and a shelf life of 2 years was assigned to the formulation.

Key words: Chitosan 150KD, *in situ* gel, pluronic F-127, periodontal anaesthesia, prilocaine hydrochloride

INTRODUCTION

It is estimated that approximately 10-30% of the population suffers from periodontal diseases with pathological periodontal pockets. Repeated sub gingival mechanical debriment/cleansing was done to arrest further periodontal tissue destruction. The number of periodontal pockets in a patient may vary as can the pocket depth. Approximately 40% of all periodontal scaling procedures performed involve some kind of anaesthesia^[1] and surgical procedures.

A number of topical anesthetics and antibiotics are used in dentistry through systemic route, which induces pain and have some shortcomings like their low degree of efficacy, tendency to spread in others area causing numbness of lips and tongue, bitter taste, difficulty in administration, and short duration of action. So taking into consideration the above shortcomings, this research work was planned to develop a topically applied *in situ* gelling system suitable for administering in periodontal

pocket, which would enable the patient to have painless treatment without distress of injection, stay at application site due to viscosity increase and give a fast onset of action lasting throughout the dental procedure and after that can be easily rinsed out with water causing a fast decline in their therapeutic effect.

In keeping above objective in mind, a novel *in situ* polymeric gel system for periodontal application was proposed which when at formulation pH and temperature (pH 6, 25°C) will be in liquid form which will be converted to gel at body pH and temperature (pH 7.4, 37°C) showing ease of administration and prolonged duration of action. Chitosan which was both mucoadhesive as well as pH sensitive polymer was used in combination with pluronic F-127 which is a temperature simulated gelation polymer. Prilocaine hydrochloride was used as model drug to check the efficacy of the developed *in-situ* gel system.

MATERIALS AND METHODS

Gufic biosciences Ltd generously gifted Prilocaine HCl IP. Pluronic F-127 and Chitosan (practical grade, 75-85% deacetylated, molecular weight 150 kDa) were purchased from Sigma Chemicals (St. Louis, mo,USA). All other chemicals used were of analytical grade.

Formulation of *in-situ* gels

Various combinations of placebo formulations were

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developed and evaluated for their characteristics. Chitosan was dissolved in phosphate buffer system and pH was adjusted to 5.5-6.0 by 1% acetic acid. Pluronic F-127 was dissolved in normal saline, pH 7.4. Viscosity was measured using Brookfield's viscometer (model DV II, spindle no. 02, at 20 rpm), while clarity was examined through visual inspection. Different combinations tested are shown in Table 1. Selection or rejection of the formulation is based on their clarity and viscosity of the formed gel at pH 7.4 and 37°C temperature.

From the above data formulation 5 consisting of Chitosan (0.5% w/v) and pluronic F-127 (10% w/v) was found to form a good clear gel and was used for further studies.

Preparation of medicated formulation

For periodontal anaesthetic activity prilocaine HCl was prescribed in a dose of 5%. Hence a dose of 5% was used during formulation. Complete formulae for developed formulation were shown in Table 2.

Physicochemical characteristics

Medicated formulation was tested for their physicochemical characteristics:

Clarity

The clarity of the formulations after and before gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

Gelation pH and temperature

Formulation was taken in a beaker and 1M NaOH was added dropwise with continuous stirring. Temperature was gradually increased and checked by thermometer. pH was checked using pH meter (Equitronics digital pH meter) and viscosity was determined at regular intervals. The pH and temperature at which sudden change in viscosity was observed were noted as gelation pH and gelation temperature respectively [Table 3].

Viscosity

Viscosity of formulations was determined by using Brookfield's viscometer (model DV II, spindle no. 02, at 20 rpm).

Refractive index

Refractive index of the formulation were determined by Abbe's refractometer. Data is shown in Table 3.

In-vitro drug release

Medicated formulation screened out from the rheological studies and physical characterizations were subjected to *in-vitro* release studies using the dialysis technique.^[2] 1 ml formulation was taken in the dialysis tubes (Sigma chemicals, USA), which was suspended in beaker at $37 \pm 0.5^\circ\text{C}$ containing 100 ml simulated fluid (sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dehydrate 0.008 g, and purified water q.s. 100g)

Table 1: Combinations of Chitosan/ Pluronic F-127 studied

| Chitosan (% w/v) | Pluronic F-127 (% w/v) | Gelling capacity |
|------------------|------------------------|------------------|
| 0.25 | 7 | + |
| 0.5 | 7 | ++ |
| 0.25 | 9 | ++ |
| 0.5 | 9 | ++ |
| 0.5 | 10 | +++ |
| 0.25 | 10 | ++ |
| 0.25 | 14 | +++ |
| 0.5 | 14 | +++ |

* Values are expressed as mean \pm S.D. (n = 5)

Table 2: Formula of the developed *in situ* formulation

| Ingredients | Concentration (w/v)% |
|----------------|----------------------|
| Prilocaine HCl | 5 |
| Chitosan | 0.5 |
| Pluronic F-127 | 10 |
| Methyl paraben | 0.1 |
| Water (q.s.) | 100 |

Table 3: Physicochemical properties of the developed *in situ* gel formulation

| Parameter | Inference |
|---------------------------------------|-------------------|
| Clarity | Clear solution |
| pH | 6.1-6.3 |
| Gelation temperature | 35-37°C |
| Gelation pH | 7.2-7.4 |
| Refractive index | 1.332-1.335 |
| Viscosity (at 25-27°C and pH 6.0-6.4) | 70 \pm 5 cps |
| Viscosity (at 35-37°C and pH 7.2-7.4) | 2000 \pm 10 cps |

* Values are expressed as mean \pm S.D. (n = 5)

pH 7.4 and 37°C under continuous stirring. Aliquots of medium were withdrawn at different time intervals and equal volumes of fresh media were added to replace the withdrawn samples. Withdrawn samples were diluted appropriately and absorbances of the samples were determined by U.V. spectrophotometrically at λ_{max} 230 nm. Cumulative percent drug released was calculated. Data is represented graphically in Figure 1.

Packaging, sterilization and test for sterility

The optimized formulations were packaged and sterilized. The tests for sterility were also conducted on these formulations as per IP 1996.^[3] The optimized *in-situ* gel system was packed in amber colored bottle each fitted with a teat. All packs were sterilized by autoclaving at 121°C for 20 min at 15 psi. The inoculated culture media for bacteria and fungi were incubated at $32 \pm 1^\circ\text{C}$ and $25 \pm 1^\circ\text{C}$ respectively in a BOD incubator. Results were based on visual observations for appearance of turbidity in culture media after every day for a period of 14 days.

Stability studies according to ICH guidelines

The international conference on harmonization (ICH) Tripartite Guidelines defines the stability testing requirements for a registration application. 3 packs of formulation were subjected

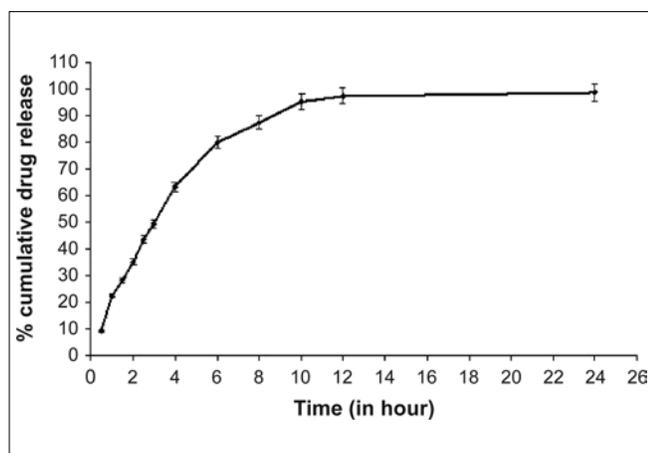


Figure 1: *In-vitro* drug release profile of developed formulations

to these stability studies. The samples were kept in Humidity and temperature control cabinets (Topsun made). The cabinets were then set at 40°C temperature and 75% relative humidity while keeping the samples in the cabinets. At 0, 30, 60 and 90 days 0.1 ml of samples were withdrawn, diluted with distill water to 10 ml and assayed by UV spectrophotometer. Samples were also tested for viscosity, gelation pH, gelation temperature and appearance after every one month till three months.

RESULTS AND DISCUSSION

Present study employed combination of Chitosan and pluronic F-127 for the development of *in situ* gel for periodontal application. Properties of Chitosan like bioadhesiveness, viscous nature and ability to convert into hydrogel at mucus pH (pH 7.4) make it best suitable candidate for the development of such type of delivery systems. Whereas pluronic F-127 is temperature activated polymer, hence combination of both polymers gives a better drug delivery system. Chitosan is insoluble at neutral or alkaline pH and converted into hydrogel when the pH of the formulation is raised and resulted in sudden increase in the viscosity.

Different combination of Chitosan and pluronic F-127 were tested for good gelling capacity. Gelling capacity was evaluated on visual basis against white and black ground on increasing the pH and temperature. Chitosan in 0.5% w/v in combination with 10% w/v pluronic F-127 was found to given good gel strength at low polymeric concentration then other combination. Table 1 Hence formulation with this combination was selected for further studies.

In-vitro drug release profile of the formulation was determined in simulated fluid (pH 7.4) and the formulation displayed a slow release profile [Figure 1]. Chitosan and Pluronic F-127 based formulation displayed a 35.2% cumulative drug release after 2 h, 79.9% after 6 h and 98.6% after 24 h.

For packing the formulations the amber colored bottle closed with rubber closure and dropper with teat were used and found to be appropriate packaging system for current formulation. The packaging material was tested for resistance for autoclaving, leakage and pourability. Packages passed all the tests and proved to be a good choice for packaging of present formulation.

Sterilization of the product was done by autoclaving at 121°C for 20 min at 15 psig and test for sterility was performed on autoclaved packaging according to IP 1996 standards. No microbial growth/ microbial contamination was observed up to 14 days of incubation. Hence the formulation passed the sterility test.

ICH guidelines are meant for rendering optimum conditions of temperature and humidity to carry out stability studies. According to ICH, New Delhi have been kept under climatic zone III, so 3 packs of formulation were kept for stability testing at 40°C and 75% RH for 90 days. Graph was plotted between time (in days) and drug remaining in pack. Degradation constant (K) was calculated from the slope of line, which was found to be $3 \times 10^{-4} \text{ days}^{-1}$. As the amount of drug degraded in 90 days was 3.243%; therefore arbitrary shelf life of 2 year was assigned to the optimized formulation as the amount of degradation was less then 5%.

CONCLUSION

Results revealed that the developed *in situ* gel system of Chitosan and pluronic F-127 can be a good vehicle/system for the delivery of drugs like anaesthetics, antibiotics etc., for periodontal application. Developed system thus gives a stiff gel at mucous pH and body temperature with prolong action that may be helpful against conventional painful periodontal application, where surgical procedure and systemic delivery is required. The formulation can be easily packaged and sterilized with method tested with shelf life of 2 years. Hence the developed system can further be studied for *in vivo* and clinical efficacy.

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