AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Evaluation of In Situ Gelling System for Treatment of Periodontitis

Khushbu Patel[1*], K. R.Vadalia[2], J. K. Patel[3]

1. Research Scholar, School of Pharmacy, RK University, Rajkot, India, Nootan Pharmacy College, Visnagar, Gujarat, India (Parent institute).

2. Department of Pharmaceutical Analysis, Shree H.N. Shukla institute of pharmaceutical Education & Research, Rajkot, Gujarat, India.

3. Department of Pharmaceutics, Nootan Pharmacy College, Visnagar, Gujarat, India

ABSTRACT

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms. The concept that localized problem sites may be treated by local drug delivery appears attractive as the antimicrobial agent is delivered within periodontal pockets and the therapy is targeted on specific pathogenic microorganisms. Local delivery of antimicrobial agents using controlled release systems should be considered as adjunctive to mechanical debridement for the treatment of localized forms of periodontal destruction. Local delivery of in situ gelling system to periodontal pockets has the benefit of putting more drugs at target site while minimizing exposure of the total body to the drug. In situ gelling system helps in maintaining effective levels of drug in gingival cervicular fluid to produce desirable clinical effects. In situ gel for controlled drug delivery system of periodontal pocket has received greater interest and appears to hold some promise in periodontal therapy. They are designed to release drug slowly with more prolonged drug availability and sustained drug action. Controlled release systems offer an advantage of decrease in frequency of administration, improving patient compliance. The dose of the drug can also be decreased and hence, the toxicity when compared to conventional therapy. In controlled drug delivery, the drug is released over an extended period of time by zero order kinetics and hence constant plasma drug concentration can be achieved.

Key words: Periodontitis, Periodontal pocket, In situ gel, Controlled drug delivery

*Corresponding Author Email: khushbus_patel@yahoo.com

Received 31 May 2012, Accepted 14 June 2012

Please cite this article in press as: Patel K *et al.*, Development and Evaluation of In Situ Gelling System for Treatment of Periodontitis. American Journal of PharmTech Research 2012.

INTRODUCTION

PERIODONTAL DISEASE

Periodontal diseases are prevalent worldwide, and while up to 15% of U.S. adults experience advanced periodontitis, the majority of adults suffer from gingivitis to moderate periodontal disease[1]. Subgingival biofilm can develop into a community where the bacterial population is able to migrate from the sulcus region to the periodontal tissues, forming a tissue biofilm. Three to twelve weeks after biofilm formation begins, the Subgingival biofilm becomes a well differentiated and structured community, containing mainly gram-negative anaerobic bacteria[2].

Although more than 300 species have been identified in periodontal pockets, it is generally agreed that between 30 - 40 species are associated with periodontitis, with three bacteria

– P. gingivalis, T. forsythensis and T. denticola

– Usually found in diseased sites[3, 4].

Disease risk can also be assessed by measurement of the bacterial count for these highly virulent bacteria while bacteria are causal for periodontal disease, their presence does not determine periodontal disease progression[5].

Progression of periodontal disease

The host response has been identified as the primary factor determining periodontal disease progression, & is influenced by systemic diseases, risk factors, hormones and local factors[6]. The host response to antigens and irritants released by bacteria includes the local release of antibodies, lymphocyte and neutrophil activation and their infiltration into the gingival tissue. The activation of lymphocytes and neutrophils is defensive and involves bacterial as well as possible tissue destruction. An example is Down's syndrome patients who have been found to have an altered immune response, with increased production of prostaglandins and MMP, supporting the importance of the genetic component in periodontal disease progression[7]. Systemic diseases, hormones and risk factors have also been found to influence the progression of periodontal disease. Diabetic patients are at increased risk for periodontal disease, and have vascular abnormalities, changes in the gingival cervicular fluid and abnormal collagen activity Changes in sex hormone levels may amplify the response to bacterial plaque and lead to gingivitis. Sex hormones are able to modify the host response, and experimental investigation suggests that increased levels of estrogen may influence the development of gingival inflammation[8]. Smokers account for the majority of cases of refractory periodontitis and

smoking is regarded as a strong risk factor for periodontal disease and for poor response to periodontal treatment[9, 10].

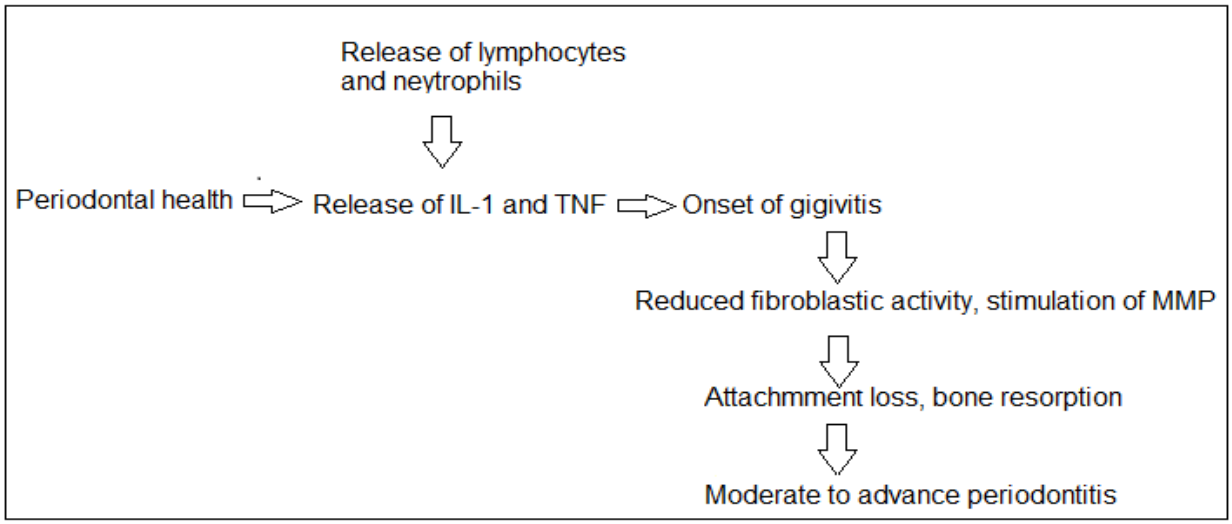

Figure 1: Progression of periodontal disease

Drug delivery devices

However, in recent years systemic antibiotics are only recommended for the treatment of rapidly progressing or refractory Periodontitis[11, 12]. Multiple systemic doses of antibiotics have shown several drawbacks including: inadequate antibiotic concentration at the site of the periodontal pocket[13]; a rapid decline of the plasma antibiotic concentration to sub therapeutic levels development of microbial resistance; and high peak-plasma antibiotic concentrations[14].

This may be associated with side effects. These obvious disadvantages have evoked an interest in the development of novel intra-pocket drug delivery systems for the treatment of periodontal diseases[15].

The periodontal pocket provides a natural reservoir, which is easily accessible for the insertion of a delivery device. The GCF provides a leaching medium for the release of a drug from the dosage form and for its distribution throughout the pocket. These features, together with the fact that the periodontal diseases are localized to the immediate environment of the pocket, make the periodontal pocket a natural site for treatment with local delivery systems.

Intra-pocket drug delivery systems are highly desirable due to the potentially lower incidence of undesirable side effects, improved efficacy and enhanced patient compliance. The attractiveness of treating periodontal diseases by the intra-pocket drug delivery systems is based on the prospects of maintaining effective high levels of drug in the GCF for a prolonged period of time to produce the desirable clinical benefits. For these systems, the delivery vehicles can be of natural origin or semi synthetic or synthetic nature. Recent developments in polymer sciences

have disclosed biocompatible and biodegradable synthetic polymers, which can be modified to meet pharmacological and biological requirements. Many polymer-based intra-pocket devices containing therapeutic agents for the treatment of periodontal disease have been studied and are listed in Table 1.

Table 1: Intra pocket delivery system

Systems	Polymer matrix	Drug incorporated	Ref
Fibers	Cellulose acetate	Tetracycline HCl, Chlorhexidine	66
	Ethylene vinyl acetate	Tetracycline HCl	67
	Poly (caprolactone)	Tetracycline HCl	68
Strips	Acrilic	Tetracycline HCl, Metronidazole	69
	HPC	Chlorhexidine, tetracycline,Doxycycline	70
	HPC+Methacrylic acid	Ofloxacin	71
	Polyhydroxybutyric acid	Tetracycline HCl	72
	(PLGA)	Chlorhexidine, Tetracycline HCl,	73
	Ethyl cellulose	Chlorhexidine	74
Films	Ethyl cellulose	Metronidazole, Tetracycline HCl, Minocycline	75
	Cross-linked atelocollagen	Tetracycline	76
	Gelatin(BycoW protein)	Chlorhexidine diacetate	77
	Cross-linked gelatin + glycerine	Chlorhexidine digluconate	78
	Chitosan	Taurine	79
	Chitosan + PLGA	Iproflavone	80
	Chitosan + PCL	Metronidazole	81
	PVA + carboxymethyl chitosan	Ornidazole	82
	PLGA	Tetracycline, Amoxycillin+ metronidazole	83
	Poly(ortho ester)	Metronidazole	84
	Eudragit LW and Eudragit SW	Clindamycin	85
	PCL	Minocycline	86
Gels	Chitosan	Metronidazole	87
	HEC + PVP	Tetracycline	88
	HEC + polycarbophil	Metronidazole	89

	Poloxamer 407+ Carbopol 934P	Propolis	90
	Poly(DL-lactide) + N-methyl 2-pyrrolidone	Saguinarium, Doxycycline hyclate	91
	Glycerol mono oleate + sesame oil	Metronidazole	92
	PLGA	Tetracycline	93
Microparticles	Pluronic F 127	Tetracycline	94
	PLGA	Tetracycline, Histatin peptides	95
	PLGA + PCL	Doxycycline	96
Nanoparticles	2-Hydroxyethyl methacrylate+ PEG dimethacrylate	___	97
	PLGA	Harungana madagascariensis leaf extract	98
	Chitosan	Antisense oligonucleotide	99
	Cellulose acetate phthalate	Triclosan	100
	PLGA	Triclosan	100
Vesicular system	Phosphatidylinositol	Triclosan	101
	Immunoliposomes	Anti-oralis	102
Other system	Poly(ethylene-co-vinyl acetate)	Acyclovir, Chlorhexidine	102

ROLE OF IN SITU GELLING SYSTEM IN PERIODONTAL DISEASE

Periodontitis is an inflammatory condition that leads to destruction of periodontum, resorption of the alveolar bone and frequently tooth loss. It is one of the most prevalent oral diseases in humans and generally affects humans above 35 years of age.

Periodontal diseases include chronic periodontitis, aggressive periodontitis and systemic disease periodontitis. The clinical sign of periodontitis are changes in morphology of gingival tissue, bleeding upon probing as well as periodontal pocket formation. The etiology of periodontal diseases is gm (-) ve bacteria, mainly anaerobes like *Porphyromonas gingivalis, Tanerella forsynthesis, Trepenoma denticola, etc.* Periodontal disease is considered to be one of the possible risk factor in other systemic diseases such as cardiovascular disease including coronary heart disease, stroke and preterm low birth weight infants.

Currently, most commonly used procedure for the treatment of severe periodontitis is the use of mechanical disruption of the bacterial flora by a procedure called scaling and root planning. However studies have shown that this mechanical disrupment is insufficient in altering the

makeup of the flora. As periodontal disease is associated with bacteria, an adjunctive anti microbial therapy appears to be appropriate. But systemic route of antibiotic may not be ideal because of the concern over the development of bacterial resistance that may be induced over a prolonged period of time, and also the rise in number of undesirable side effects such as nausea, vomiting, diarrhea, abdominal pain, pseudo membranous enterocollitis etc.

Hence local delivery of in situ gelling system to periodontal pockets has the benefit of putting more drugs at target site while minimizing exposure of the total body to the drug .This in situ gelling system helps in maintaining effective levels of drug in gingival cervicular fluid (GCF) to produce desirable clinical effects. With respect to solid devices, the injectable gels posses a higher biocompatibility and bioadhesivity by allowing adhesion to mucosa in dental pocket. Finally, they can be eliminated through normal catabolic pathways, decreasing risk of irritation or allergic host reaction at the application site.

In situ gel for controlled drug delivery system of periodontal pocket has received greater interest and appears to hold some promise in periodontal therapy. They are designed to release drug slowly with more prolonged drug availability and sustained drug action. Controlled release systems offer an advantage of decrease in frequency of administration, thereby improving patient compliance. The dose of the drug can also be decreased and hence, the toxicity when compared to conventional therapy. In controlled drug delivery, the drug is released over an extended period of time by zero order kinetics and hence constant plasma drug concentration can be achieved.

IN SITU GEL

In-situ gel refers to polymer solution which can be administered as liquid & undergoes a phase transition to semisolid gel upon exposure to physiological environment[16]. The gelation can be triggered by temperature, pH change, ionic change & also UV induced gelation, Solvent exchange induced gelation[17-19].

Change in temperature

Change in temperature sustained drug delivery can be achieved by use of polymer that changes from sol- gel at temperature of the body. Temperature dependent system includes pluronics F1278-10 & tetronic. The poloxamers F127 are polyols which thermal gelling properties where solution viscosity increases when temperature is rised to critical temperature.

Change in pH

Change in pH triggered system shows sol – gel transformation when pH is raised by gingival cervicular fluid to pH 7.4. PH triggered system cellulose acetate Hydrogen phthalate latex (pH 5.0 to 7.2 -7.4 from a gel with lacrimal fluid), carbopol pH 4.0 – 7.4 sol – gel transformation.

Cellulose acetate phthalate is polymer with potentially useful properties for a sustained drug delivery to eye since latex is free flowing solution at pH 4.4 which undergoes coagulations. When pH raised by tear fluid of pH 7.4. pH triggered in situ gelling system are low viscosity polymeric dispersion in water which undergoes spontaneous coagulations & gelation after instillation in conjunctiva curl –de-sac[20].

Change in electrolyte composition

Change in electrolyte composition ion activated system shows sol- gel transformation in presence of the mono or divalent cations (Na+, Ca++ etc) typically found in tear fluids. Ion activated system include Gelrite & Alginate. 12-14 Gellan gum is an anionic extracellular polysaccharide secreted by pseudomonas elodea. Gellan gum formulated in aqueous solution forms clear gel in the presence of mono or divalent cations. These system shows sol-gel transformation in presence of ions.

APPLICABILITY OF IN SITU POLYMERIC DRUG DELIVERY SYSTEM

Oral drug delivery system

The pH-sensitive hydrogels have a potential use in site-specific delivery of drugs to specific regions of the GI tract. Hydrogels made of varying proportions of PAA derivatives and crosslinked PEG allowed preparing silicone microspheres, which released prednisolone in the gastric medium or showed gastro protective property [21]. Cross-linked dextran hydrogels with a faster swelling under high pH conditions, likewise other polysaccharides such as amidaded pectins, guar gum and insulin were investigated in order to develop a potential colon-specific drug delivery system[22]. The formulations of gellan and sodium alginate both containing complexed calcium ions that undergo gelation by releasing of these ions in the acidic environment of the stomach. Oral delivery of paracetamol was studied. For the oral in situ gel delivery system pectin, xyloglucan & gellan gum natural polymers are used. Pectin formulation for sustained delivery of paracetamol has been reported [23]. Advantages of pectin is water soluble so, no need to add organic solvent.

Ocular drug delivery system

In ocular delivery system natural polymers like gallen gum, alginic acid & xyloglucan are most commonly used. For local ophthalmic delivery system various compounds like antimicrobial agent, anti-inflammatory agent & autonomic drugs are used to relieve intra ocular tension in glaucoma. Conventional delivery system often results in poor availability & therapeutic response because high tear fluid turns over & dynamics which cause rapid elimination of the drug from

the eye so, the overcome the bioavailability problem ophthalmic in-situ gel were developed [24]. To improve the bioavailability viscosity enhancers such as Hydroxy Propyl Methyl Cellulose, Carboxy Methyl Cellulose, Carbomers, Poly Vinyl alcohol used to increase the viscosity of formulation in order to prolong the precorneal residence time & improve the bioavailability, ease to manufacture [25]. Penetration enhancer such as preservatives, chelating agent, surfactants are used to enhance corneal drug penetration [26].

Nasal drug delivery system

In nasal in-situ gel system gallan gum & xanthan gum are used as in-situ gel forming polymers momethasone furoate was evaluated for its efficacy for the treatment of allergic rhinitis [27]. Animal study were conducted using allergic rhinitis model & effect of in-situ gel on antigen induced nasal symptoms in sensitizes rats was observed. In-situ gel was found to inhibit the increase in nasal symptoms are compared to marketed preparation nosonex (Momethasone furoate suspension 0.05%).

Rectal drug delivery system

The rectal route may be used to deliver many types of drugs that are formulated as liquid, semisolid (ointments, creams and foams) and solid dosage forms (suppositories). Conventional suppositories often cause discomfort during insertion. In addition, suppositories are unable to be sufficiently retained at a specific position in the rectum, sometimes they can migrate up-wards to the colon that makes them possible for drug to undergo the first-pass effect [28]. Novel in situ gelling liquid suppositories with gelation temperature at 30–36°C. Poloxamer 407 and/ or poloxamer 188 were used to confer the temperature-sensitive gelation property. In-situ gel possesses a potential application for rectal & vaginal route. Miyazaki et al. investigated the use of xyloglucan based thermo reversible gel for rectal drug delivery of Indomethacin. Administration of Indomethacin loaded xyloglucan based system to rabbit indicated broad drug absorption & a longer drug residence time as compared to that resulting after administration of commercial suppository. For better therapeutic efficacy & patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating Clotrimazole-β-cyclodextrin complex formulated for treatment of vaginitis[29].

Vaginal drug delivery system

The vagina, in addition to being an important organ of reproductive tract, serves as a potential route for drug administration. Formulations based on a thermo-plastic graft copolymer that undergo in situ gelation have been developed to provide the prolonged release of active ingredients such as nonoxynol- 9, progestins, estrogens, peptides and proteins[30]. A mucoadhesive

thermo-sensitive gel (combination of poloxamers and polycar-bophil), which exhibited, increased and prolonged antifungal activity of clotrimazole in comparison with conventional PEG-based formulation[31].

Injectable drug delivery system

One of the most obvious ways to provide sustained- release medication is to place the drug in delivery system and inject or implant the system into the body tissue. Thermo reversible gels mainly prepared from poloxamer are predominantly used[32]. The suitability of poloxamer gel alone or with the addition of hydroxypropylmethylcellulose (HPMC), sodium carboxymethylcellulose (CMC) or dextran was studied for epidural administration of drugs in vitro[33]. The compact gel depot acted as the rate limiting step and significantly prolonged the dual permeation of drugs in comparison with control solutions. Pluronic F127 gels, which contained either insulin or insulin-PLGA nanoparticles with conclusion, that these formulations could be useful for the preparation of a controlled delivery system[34]. Likewise, poloxamer gels were tested for intramuscular and subcutaneous administration of human growth hormone or with the aim to develop a long acting single dose injection of lidocaine[35, 36]. A new class of injectable controlled release depots of protein which consisted of blends of Pluronics with poly (D, L-lactide)/1- methyl-2- pyrrolidone solutions. Some other thermo sensitive hydrogels may also be used for parenteral administration. ReGel ® (triblock copolymer PLGAPEG- PLGA) was used as a drug delivery carrier for the continuous release of human insulin. Steady amounts of insulin secretion from the Re- Gel ® formulations up to day 15 of the subcutaneous injections were achieved. The synthesis of a biodegradable poly (ethylene oxide) and poly (L-lactic acid) hydrogel, which exists in a form of sol at an elevated temperature (around 45°C) and forms a gel after subcutaneous injection and subsequent rapid cooling to body temperature[37]. In-situ forming Injectable drug delivery system, cross linking of hydrazide modified by aleuronic acid with aldehyde modified version of cellulose derivatives such as carboxy methyl cellulose, methyl cellulose, hydroxy propyl methyl cellulose are used. These in-situ forming gel were used for preventing postoperative peritoneal adhesion thus avoiding pelvic pain, bowel obstruction & infertility. For a better therapeutic efficacy & patient compliance, mucoadhesive, thermo sensitive, prolonged release vaginal gel incorporating Clotrimazole-β- cyclodextrin complex was formulated for treatment of virginities[38].

Dermal and transdermal drug delivery

Thermally reversible gel of Pluronic F127 was evaluated as vehicle for the percutaneous administration of Indomethacin. In-vivo studies suggest that 20% w/w aqueous gel may be of

practical use as a base for topical administration of the drug. Poloxamer 407 gel was found suitable for transdermal delivery of insulin[39]. The combination of chemical enhancers and iontophoresis resulted in synergistic enhancement of insulin permeation.

EVALUATION AND CHARACTERIZATION OF IN SITU GELLING SYSTEM

In-situ gel evaluated & characterized by the following parameters-

Clarity

The clarity of formulated solution is determined by visual inspection under black & white Background [40-42].

Texture analysis

The consistency, firmness &cohesiveness of in situ gel are assessed by using texture profile analyzer which mainly indicated gel strength & easiness in administration in vivo higher value of adhesiveness of gel are needed to maintain an intimate contact with mucus surface[43].

pH of gel

pH can be determined formulation is taken in beaker & 1ml NaOH added drop wise with continuous stirring. pH is checked by using pH meter[44-46].

Rheological studies

The viscosity measured by using Brookfield viscometer, cone & plate viscometer. In-situ gel formulation is placed in sample tube. Formulation should have viscosity 5-1000 mPas , before gelling & after ion gel activation by periodontal pocket will have viscosity of from about 50-50,000 mPas[47-49].

Swelling studies [50-53]

Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature. The cell contains artificial gingival cervicular fluid[54]. Swelling medium equilibrating at 370c one milliliter of formulated solution is placed in dialysis bag & put into the swelling medium. At specific time interval the bag is removed from the medium & weight is recorded. The swelling of the polymer gel as a function of time is determined by using the following relationship[55-58].

$$\% St = (Wt - W0)\ 100/W0$$

Where,

St = Swelling at time‘t’.

W0=Initial weight of gelling solution.

Wt=Final weight of gel.

Stastical analysis

Analysis of variance (ANOVA) is used the testing the difference between calculated parameters using SPSS statistical package. Statistical difference yielding $P \leq 0.05$ is considered[59]. Duncan multiple comparison is applied when necessary to identify which of the individual formulations are significantly different[60].

High performance liquid chromatography

The HPLC system is used in reversed phase mode. Analysis is performed on a Nova pack C18 packed column (150 mm length X 3.9 mm i.d) [61].

Fourier transformer infra red

The possibility of drug excipient interaction is investigated by FTIR studies. The FTIR graph of pure drug & combination of drug with excipient are recorded by using KBR pellets[62, 63].

Thermal analysis

Thermo gravimetric analysis can be conducted for in situ forming polymeric system to quantitative the percentage of water in hydrogel. Different scanning calorimetry is used to observed, if there are many changes in thermo grams as compared with pure ingredients used thus indicating the interaction[64].

In vitro drug release studies

In vitro release study of in situ gel solution is carried out by using Franz diffusion cell. The formulation is placed in donor compartment & freshly prepared simulated gingival cervicular fluid in receptor compartment. Between receptor & donor compartment dialysis membrane is placed (0.22 μm pore size). The whole assembly is placed on thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37 ^{0}C ± 0.5 ^{0}C. 1ml sample is withdrawn at predetermined time interval of 1hr for 6 hrs the sample volume of fresh medium is replaced. The withdrawn sample is diluted to 10 ml in volumetric flask with respective solvent & analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers peppas & Fickinian diffusion mechanism for their kinetics.

Antimicrobial activity

Antimicrobial efficacy studies are carried out to ascertain the biological activity of sol-gel-system against microorganisms. This is determined in agar diffusion medium employing ‘Cup Plate Techniques’. The microbial growth of bacteria is measured by conc. Of antibiotic &

compared with that produced by known conc. Of standard preparation of antibiotic & carried out the microbial assay serial dilution method is employed.

Sterility testing

Sterility testing is carried out as per the IP 1996. The formulation is incubating for not less than 14 days at 300-350c in the fluid thioglycolate medium to find the growth of bacteria & at 200-250 c in Soya bean casein digest medium to find the growth of fungi in formulation.

Accelerated stability studies

Formulation is replaced in amber colored vials & sealed with aluminum foil for the short term accelerated stability study at 40°C± 20°C & 75 ±5% RH as per International Conference of Harmonization (ICH) State Guidelines. Sample is analyzed at every month for clarity, pH, gelling capacity, drug content, rheological evaluation & in vitro dissolution[65].

CONCLUSION

In conclusion, there are various formulations available for the treatment of Periodontitis. Despite the reported clinical successes, currently available controlled release formulations suffer from several disadvantages including: (i) the requirement for mechanical binding of the drug delivery system to a tooth surface to prevent removal of the system from the periodontal pocket as a result of the positive flow of GCF from the pocket into the oral cavity. (ii) The requirement for removal of tooth bound, non-biodegradable drug delivery systems at the termination of treatment, (iii) poor retention of oil-based delivery systems within the aqueous environment of the periodontal pocket. (iv)Potential deleterious effects of plasticizers leached from solid polymeric drug delivery systems on the periodontal tissues. To overcome these disadvantages development of in situ gelling system for treatment of Periodontitis. They are designed to release drug slowly with more prolonged drug availability and sustained drug action, decrease in frequency of administration, improving patient compliance. The dose of the drug can also be decreased and hence, the toxicity when compared to conventional therapy.

REFERENCES

1. Epidemiology of Periodontal Diseases. J Periodontol 2005; 76:1406-1419.
2. Lovegrove JM. Dental plaque revisited: bacteria associated with periodontal disease. J NZ Soc Periodontol 2004; 87:7-21.
3. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal disease. Periodontology 2000; 1994:78-111.

4. Socransky SS, Haffajee AD, et al. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998; 25:134-144.
5. Epidemiology of Periodontal Diseases. Position Paper, Academy of Periodontology, 2005. J Periodontol 2005; 76: 1406-1419.
6. Page RC, Offenbacher S. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. Periodontol 1997; 14:216-248.
7. Tsilingardis G, Yucel-Lindberg T, Modeer T. Enhanced levels of prostaglandin E2, leukotrine B4, and matrix metalloproteinase-9 in gingival crevicular fluid from patients with Down syndrome. Acta Odontol Scan 2003; 61(3):154-158.
8. Koreeda N, Iwano Y. Periodic exacerbation of gingival inflammation during the menstrual cycle. J Oral Sci 2005; 47(3):159-164.
9. American Academy of Periodontology Research, Science and Therapy Committee Position Paper: Epidemiology of periodontal diseases. J Periodontol 2005; 76:1406-1419.
10. Palmer RM, Matthews JP, Wilson RF. Non-surgical periodontal treatment with and without adjunctive metronidazole in smokers and non-smokers. J Clin Periodontol 1999; 26(3):158-163.
11. Slots J, Rams TE. Antibiotics in periodontal therapy: advantages and disadvantages. J Clin Periodontol 1990; 17: 479–493.
12. Vandekerckhove BNA. The use of tetracycline-containing controlled release fibres in the treatment of refractory periodontitis. J Periodontol 1997; 68: 353–361.
13. Pitcher GR. Access to Subgingival plaque by disclosing agents using mouth rinsing and direct irrigation. J Clin Periodontol 1980; 7:300–308.
14. Gates KA. A new bioerodible polymer insert for a controlled release of metronidazole. Pharm Res 1994; 11:1605–1609.
15. Greenstein G. Local drug delivery in the treatment of periodontal diseases: assessing the clinical significance of the results. J Periodontol 2006; 77: 565–578.
16. Bochot A, Fattal E, Gulik A, Couarraze G, Couvreur P. Liposome dispersed within a thermosensitive gel; a new dosage form for ocular delivery of oligonucleotide. Pharm Res 1998; 15:1364-1369.
17. Millar SC, Donovan MD. Effect of Poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. Int J Pharma 1982; 12:147-152.
18. Gurny R, Boye T, Ibrahim H. Ocular therapy with nanoparticulate system for controlled drug delivery. J Controlled Release 1985; 2:353-361.

19. Moorhouse R, Colegrove GT, Sandford PA, Baird JK, Kang KS. A new gel forming polysaccharide, solution properties of polysaccharide. ACS symposium series. Washington- DC 1981; 111-124.
20. Desai SD, Blanchard J. Encyclopedia of Pharmaceutical Technology (Swarbrick, J.and Boylan, J.C., eds). New York: Marcel Dekker; 1995: 43–75.
21. Carelli V, Coltelli S, Di Colo G, Nannipieri E, Serafini MF. Silicone microspheres for pH controlled gastrointestinal drug delivery. Int J Pharm 1999; 179:73-83.
22. Kubo W, Miyazaki S, Attwood D. Oral sustained delivery of paracetamol from in situ gelling gellan and sodium alginate formulations. Int J Pharm 2003; 258:55-64.
23. Schoenwala RD, Smlen VF. Drug absorption analysis from Pharmacological data: Transcorneal biphasic availability of tropicamide. J Pharm Sci 1971; 60:1039-1045.
24. Durrani AM, Davies NM, Thomas M, Kellaway IW. Pilocarpine bioavailability from a mucoadhesive liposomal ophthalmic drug deli. Sys. Int J Pharma 1992; 88: 409.
25. Gariepy ER, Leroux JC. In situ-forming hydrogels— reviews of temperature-sensitive systems. Eur J Pharma and Biopharma 2004; 58: 409–426.
26. Lin HR, Sung KC. Carbopol/pluronic phase change solutions for ophthalmic drug delivery. J Controlled Release 2000; 69: 379.
27. Carelli V, Coltelli S, Di Colo G, Nannipieri E, Serafini MF. Silicone microspheres for PH controlled gastrointestinal drug delivery. Int J Pharm 1999; 179:73-83.
28. Kubo W, Miyazaki S, Attwood D. Oral sustained delivery of paracetamol from in situ gelling gellan and sodium alginate formulations. Int J Pharm 2003; 258:55-64.
29. Schoenwala RD, Smlen VF. Drug absorption analysis from Pharmacological data: Transcorneal biophasic availability of tropicamide. J Pharm Sci 1971; 60:1039-1045.
30. Durrani AM, Davies NM, Thomas M, Kellaway IW. Pilocarpine bioavailability from a mucoadhesive liposomal ophthalmic drug deli. Sys. Int J Pharma 88; 1992:409.
31. Chang JY, Oh YK, Kong HS, Kim EU, Jang DD, Nam KT. Prolonged antifungal effects of clotrimazole-containing Mucoadhesive thermo sensitive gels on vaginitis. J Control Release 2002; 82: 39-50.
32. Ricci EJ, Bentley MV, Farah M, Bretas RES, Marchetti JM. Rheological characterization of Poloxamer 407 lidocaine hydrochloride gels. Eur J Pharm Sci 2007; 17:161-167.
33. Choi HG, Oh YK, Kim CK. In situ gelling and mucoadhesive liquid suppository containing acetaminophen: enhanced bioavailability. Int J Pharm 1998; 165:23-32.
34. Liaw J, Robinson J, Rin mitra AK. Ophthalmic drug delivery system.1993; 369-381.

35. Vermani K, Garg S. The scope and potential of vaginal drug delivery. Pharm Sci & Technol Today 2000; 3:359-364.
36. Chang JY, Oh YK, Kong HS, Kim EU, Jang DD, Nam KT. Prolonged antifungal effects of clotrimazole-containing Mucoadhesive thermo sensitive gels on vaginitis. J Control Release 2000; 82:39-50.
37. Ricci EJ, Bentley MV, Farah M, Bretas RES, Marchetti JM. Rheological characterization of Poloxamer 407 lidocaine hydrochloride gels. Eur J Pharm Sci 2007; 17:161-167.
38. Paavola AY, liruusi J, Rosenberg P. Controlled release mater permeability of lidocaine and ibuprofen from Injectable poloxamer-based gels. J Control Release 1998; 52:169-178.
39. Barichello JM, Morishita M, Takayama K, Nagai T. Absorption of insulin from Pluronic F-127 gels following subcutaneous administration in rats. Int J Pharm 1999; 184:189-198.
40. Katakam M, Ravis WR, Banga AK. Controlled release of human growth hormone in rats following parenteral administration of poloxamer gels. J Control Release 1997; 49:21-26.
41. DesNoyer JR, McHugh AJ. The effect of Pluronic on the protein release kinetics of an Injectable drug delivery system. J Control Release 2003; 86:15-24.
42. Kim YJ, Choi S, Kohl JJ, Lee M, Kim SW. Controlled release of insulin from Injectable biodegradable triblock copolymer. Pharm Res 2001; 18:548-550.
43. Jeong B, Bae YH, Lee DS, Kim SW. Biodegradable block copolymers as injectable drug delivery systems. Nature 1997; 388:860-862.
44. Coa S, Ren X, Zhang Q, Chen E, Xu F, Chen J. In situ gel based on gellan gum as a new carrier for nasal administration of Momethasone furoate. Int J pharm 2009; 365:109-15.
45. Miyazaki S, Tobiyama T, Takada M, Attwood D. Percutaneous absorption of indomethacin from pluronic F127 gels in rats. J Pharm Pharmacol 1995; 47:455-457.
46. Pillai O, Panchagnula R. Transdermal delivery of insulin from poloxamer gel: ex vivo and in vivo skin permeation studies in rat using iontophoresis and chemical enhancers. J Control Release 2003; 89:127-14.
47. Bilensoy E, Rouf MA, Imran V, Murat S, Hincal AA. Mucoadhesive thermo sensitive prolonged release vaginal gel for Clotrimazole: β- Cyclodextrin complex. AAPS Pharm Sci Tech 2006; 7:38.

48. Ito T, Yeo Y, Highley CB, Bellas B, Benitez CA, Kohane DS. The prevention of peritoneal adhesion by in situ cross linking Hydrogel of Hyaluronic acid & cellulose derivatives biomaterial. 2007; 28:975-83.
49. Nirmal HB, Bakliwal SR, Pawar SP. In situ gel: New trends in controlled & sustained drug delivery system. Pharm Tech Research 2010; 2:1398-1408.
50. Sautou Miranda V, Labret F, Grand-Boyer A, Gellis C, Chopineau J. Impact of deep frizzing on the stability of 25 mg/ml vancomycin ophthalmic solution. Int J Pharm 2002; 243:205-207.
51. Gupta H, Jain S, Mathur R, Mishra P, Mishra AK. Sustained ocular drug delivery from a temperature & pH triggered novel in situ gel system. Drug delivery 2007; 14:507-515.
52. Doijad RC, Manvi FV, Malleswara Rao VSN, Prajakta, Alsae. Sustained ophthalmic delivery of gatifloxacin from in situ gelling system. Indian J Pharm Sci 2006; 68:814-818.
53. Lin HR, Sung KC. Carbopol/ Pluronic phase change solution for ophthalmic drug delivery. J control Rel 2000; 69:379-388.
54. Bhowmik M, Das S, Chattopadhyay D, Lakshmi K, Ghosh. Study of thermo sensitive in situ gel for ocular delivery. Sci Pharm 2011; 79: 351-58.
55. Rathore KS. In situ gelling ophthalmic drug delivery system: An overview. Int J Pharm Sci 2010; 4:30-34.
56. Tho I, Kjoniksen AL, Nystrom B. Characterization of association & gelation of pectin in methanol-water mixer. Biomacromolecules 2003; 4:1623-1629.
57. Amal El, Kamel, Heba Al, Dosari & fahad Al. Environmentally responsive ophthalmic gel formulation of carteolol Hydrochloride. Drug Delivery 2006; 13:55-59.
58. Sasaki H, Igarachi Y, Nagano T, Nishida K, Nkamura J. Different effects of absorption promoter on corneal & conjuctival penetration of ophthalmic beta blockers. Pharm Res 1995; 12:1146-50.
59. Kashap N, Viswanad B, Sharma G, Bhardwaj V, Ramarao P, Kumar MNV. Design & evaluation of biodegradable, bio-sensitive in situ gelling system for pulsatile delivery of insulin biomaterials.2007; 28:2051-60.
60. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B, Dharmesh MM. A pH triggered in situ forming opthalmic drug delivery system for tropicamide. Drug Deliv Technol 2007; 5:44-49.

61. Nassem A, Charoo, Kanchan Kohhli, Asgar. A preparation of in situ forming ophthalmic gels of Ciprofloxacin Hydrochloride for treatment of Bacterial Conjunctivitis: In Vitro & In Vivo studies. Pharm Sci 2003; 2:407-417.
62. Government of India. Ministry of Health & family welfare. Indian Pharmacopoeia vol.1. The controller of publication, Delhi; 1996: 100-124.
63. Shivanand Swamy P, Fatima Sanjeri Dasankoppa, Sreeniwas SA, Hasan Pasha N, Nanjun daswamy NG. Formulation & evaluation of a Novel in situ Gum based ophthalmic drug delivery system of Linezolid. Sci Pharm 2008; 6:515-532.
64. Sindhu Abraham, Sharon Furtoda, Bharath S, Basava BV, Deveswaran R, Madhavan N. Sustain ophthalmic delivery of ofloxacin from an ion activated in situ gelling system. Pak J Pharm Sci 2009; 2:172-179.
65. Jain SP, Shah SP, Rajadhyaksha N, Singh PS, Purnima A. In situ ophthalmic gel for Ciprofloxacin Hydrochloride for once a day sustained delivery drug development. Ind Pharm 2008; 34:445-452.
66. Goodson JM. Periodontal therapy by local delivery of tetracycline. J. Clin. Periodontol 1979; 6: 83–92.
67. Coventry J, Newman HN. Experimental use of a slow release device employing chlorhexidine gluconate in areas of acute periodontal inflammation. J Clin Periodontol 1982; 9: 129–133.
68. Tonetti M. Zero-order delivery with periodontal placement of tetracycline-loaded ethylene vinyl acetate fibres. J Periodontol Res 1990; 25: 243–249.
69. Addy M, Langeroudi M. Comparison of the immediate effects on the sub-gingival microflora of acrylic strips containing 40% chlorhexidine, metronidazole or tetracycline. J Clin Periodontol 1984; 11: 379–386.
70. Noguchi T. New method for local drug delivery using resorbable base material in periodontal therapy. Bull Tokyo Med Dent Univ 1984; 31: 145–153.
71. Higashi K. Local ofloxacin delivery using a controlled release insert (PT- 01) in the human periodontal pocket. J Periodontol Res 1990; 25: 1–5.
72. Deasy PB. Use of strips containing tetracycline hydrochloride or metronidazole for the treatment of advanced periodontal disease. J Pharm Pharmacol 1989; 41: 694–699.
73. Maze GI. Response to intracrevicular controlled delivery of 25% tetracycline from poly (lactide:glycolide) film strips in SPT patients. J Clin Periodontol 1995; 22: 860–867.

74. Friedman M Golomb G. New sustained release dosage form of chlorhexidine for dental use. J Periodont Res 1982; 17: 323–328.
75. Golomb G. Sustained release device containing metronidazole for periodontal use. J Dent Res 1984; 63. 1149–1153
76. Steinberg D. A new degradable controlled release device for treatment of periodontal disease: in vitro release study. J Periodontal 1990; 61: 393–398.
77. Goffin G. Efficacy of sustained local delivery of chlorhexidine Periochip as an adjuvant to scaling and root planning in the treatment of chronic periodontal disease. Int Dent Rev 1998; 18: 1–18.
78. Ozmeric N. Chitosan film enriched with an antioxidant agent taurine in fenestration defects. J Biomed Mater Res 2005; 1:500–503.
79. Perugini P. Periodontal delivery of ipriflavone: new chitosan/PLGA film delivery system for a lipophilic drug. Int J Pharm 2003; 252: 1–9.
80. El-Kamel AH. Micrometrical metronidazole benzoate film as a local mucoadhesive delivery system for treatment of periodontal diseases. AAPS Pharm Sci Tech 2007; 8: E75.
81. Wang LC. Study on poly (vinyl alcohol)/carboxy methyl-chitosan blend film as local drug delivery system. J Mater Sci Mater Med 2007; 18: 1125–1133.
82. Agarwal RK. Development and characterization of tetracycline–poly (lactide/glycolide) films for treatment of periodontitis. J Control Release 1993; 23: 137–146.
83. Vasavada RC, Junnarkar GH. Release of metronidazole from poly (ortho ester) matrices. Proc Int Symp Control Release Bioact Mater 1997; 24: 499–500.
84. Higashi K. Local drug delivery systems for the treatment of periodontal disease. J Pharmacobiodyn 1991; 14: 72–81.
85. Kyun KD. Development of minocycline containing polycaprolactone film as a local drug delivery. Taehan Chikkwa Uisa Hyophoe Chi 1990; 28: 279–290.
86. Akncbay H. Application of chitosan gel in the treatment of chronic periodontitis. J Biomed Mater Res B Appl Biomater 2007; 80: 290–296.
87. Jones DS. Development and mechanical characterization of bioadhesive semi-solid. Polymeric systems containing tetracycline for the treatment of periodontal diseases. Pharm Res 1996; 13: 1734–1738.

88. Jones DS. Mucoadhesive. syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: in vitro release kinetics, syringe ability, mechanical and mucoadhesive properties. J Control Release 1997; 49: 71–79.
89. Bruschi ML. Semisolid systems containing propolis for the treatment of periodontal disease: in vitro release kinetics. Syringe ability, rheological, textural, and mucoadhesive properties. J Pharm Sci 2007; 96: 2074–2089.
90. Polson AM. Two multi-center trials assessing the clinical efficacy of 5% sanguinarine in a biodegradable drug delivery system. J Clin Periodontol 1996; 23: 782– 788.
91. Noyan U. A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult Periodontitis patients. J Clin Periodontol 1997; 24: 158–165.
92. Maze GI. Gingival fluid tetracycline release from bioerodible gels. J Clin Periodontol 1996; 23: 133–136.
93. Baker RW. A controlled release drug delivery system for periodontal pocket. Proc Int Symp Control Release Bioact Mater 1988; 140: 238a–238b.
94. Esposito P. Biodegradable microparticles for sustained delivery of tetracycline to the periodontal pocket: formulatory and drug release studies. J Microencapsul 1997; 14: 175–187.
95. Mundargi RC. Development and evaluation of novel biodegradable microspheres based on poly (D.L-lactide-co-glycolide) and poly (epsiloncaprolactone) for controlled delivery of doxycycline in the treatment of human periodontal pocket: in vitro and in vivo studies. J Control Release 2007; 119: 59–68.
96. Bako J. Synthesis of nanoparticles for dental drug delivery systems. Fogorv Sz 2007; 100: 109–113.
97. Moulari B. Potentiation of the bactericidal activity of Harungana madagascariensis Lam. ex Poir. (Hypericaceae) leaf extract against oral bacteria using poly (D.L-lactide-co-glycolide) nanoparticles: in vitro study. Acta Odontol Scand 2006; 64: 153–158.
98. Dung TH. Chitosan-TPP nanoparticle as a release system of antisense oligonucleotide in the oral environment. J Nanosci Nanotechnol 2007; 7: 3695–3699.
99. Pinon SE. Preparation and characterization of triclosan nanoparticles for periodontal treatment. Int J Pharm 2005; 294: 217–232.
100. Jones MN, Kaszuba M. Polyhydroxy-mediated interactions between and bacterial biofilms. Biochim Biophys Acta 1994; 1193: 48–54.

101. Jones MN. The interaction of phospholipid liposomes with bacteria and their use in the delivery of bactericides. J Drug Target 1997; 5: 25–34.

102. Tallury P. Poly (ethylene-co-vinyl acetate) copolymer matrix for delivery of chlorhexidine and acyclovir drugs for use in the oral environment: effect of drug combination, copolymer composition and coating on the drug release rate. Dent Mater 2007; 23: 404–409.