CHAPTER-2

Literature Review

CHAPTER 2

Literature Review

2.1. The Anatomy and Physiology of periodontium

The basic structure of tooth is demarcated into three parts; Crown, neck and root as depicted in Fig 2.1. Anatomically, crown is the part of tooth covered with enamel above the cementoenamel junction (CEJ). CEJ is the anatomical border where crown of tooth and cementum covering the roots meet. Majority part of crown is composed of dentin containing roots and pulp chamber in the center. A tooth could have single or multiple roots for instance canines and most premolars usually have one root.

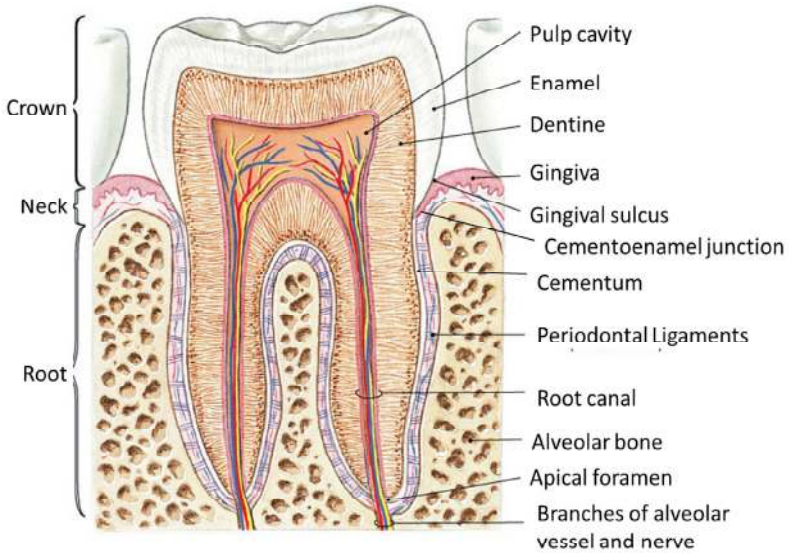

Fig. 2.1: The anatomical structure of tooth (obtained from intranet.tdmu.edu.ua).

The periodontium is a supporting tissue for the teeth made up of cementum, periodontal ligament, alveolar mucosa, gingiva, and alveolar bone. These periodontal tissues are have different structures but they act together to support and fix tooth in the alveolar bones of jaw (Caton and Ryan, 2011; Palumbo, 2011).

A healthy gingiva is light pink in colour but may vary depending on geographical origin of the races. The gingival sulcus is the invagination around a tooth holded by free gingival margin (Palumbo, 2011). At the gingival margin, the junctional epithelium attaches the gingiva to the tooth surface *via.* hemidesmosomes and a basement lamina. This epithelial attachment is the junction where bacterial biofilm could form and causes a destructive host derived immune-inflammatory response in the subjacent attachment apparatus including the alveolar bone, gingiva, and periodontal ligament (Caton and Ryan, 2011). These host-derived immune-inflammatory responses account for generation of periodontal diseases.

2.2. Periodontal diseases

Gingivitis and periodontitis constitute two most prevalent periodontal diseases. The inflammation of gingival tissue is known as gingivitis *viz.* "gingiv" = gingival tissue + "itis" = inflammation. The further spread of infection and inflammation into deeper tissues associated with bone loss is known as periodontitis *viz.* "peri" = around, "odont" = tooth, "itis" = inflammation (Armitage, 1999; Schwach-Abdellaoui *et al.,* 2000). Gingivitis unambiguously develops in subjects with healthy gingiva who refrain from maintenance of tooth hygiene and thereby allow microorganisms to colonize into the supragingival part of the tooth as plaque. These plaques have been regarded as biofilms and are responsible for initiating host defense mechanism such as inflammation.

Progression of host defense action against infectious microbes, liberates inflammatory cell infiltrates in the marginal gingival which brings destruction of alveolar bone, ligaments leading to pocket formation and concomitant loss of tooth bringing down speech, mastication, confidence and esthetics of a person (Greenstein, 2005; Mishra and Yadav, 2015). Loss of teeth is not only the problem with periodontitis but its severity is more related to systemic condition; this aspect is discussed in later section *viz.* oral-systemic connections. Periodontal pockets are characteristic symptom of periodontitis and depth of pocket decides the severity of disease such as mild (< 3 mm), moderate (3 to 4 mm), or severe (≥ 5 mm) (Armitage,

1999). Periodontal pockets are formed due to degeneration of supporting collagen of the periodontium, loss of attachments, supportive connective tissue and bone. These pockets are the favorable dwelling place for anaerobic microbes, which perpetuate host defense mechanism leading to deepening of pockets (Caton and Ryan, 2011). Fig. 2.2 illustrates the progression of periodontal diseases from healthy gingival to advanced periodontitis.

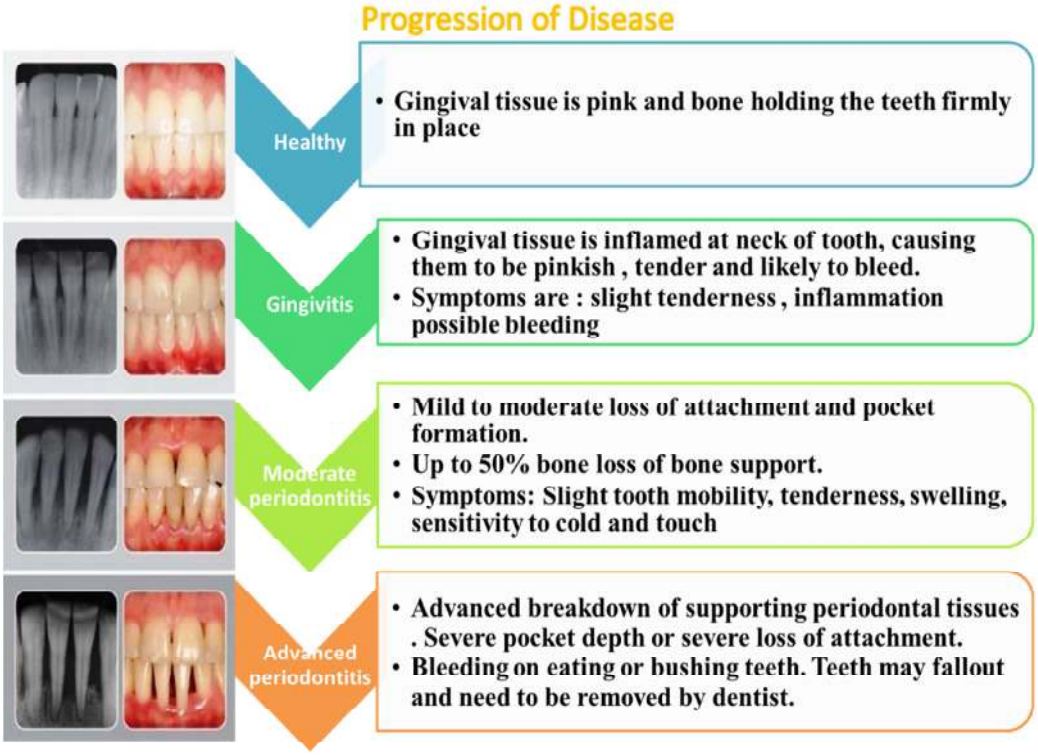

Figure 2.2: The symptoms associated with progression of periodontal disease from healthy gingiva to advanced periodontitis (modified from Alliance Dental Center)(Bermudez and Grau, 2011; Center, 2017).

Warning signs of periodontal diseases includes swollen gums, bright red or purplish gums, gums feel tender when touched and recede from teeth making it appear longer, new spaces are formed between teeth, pus formation between teeth and gums, bad breath, and bad taste in mouth.

The detailed classification of periodontal diseases based on progression of disease are given in Table 2.1.

Table 2.1: Classification of Periodontitis (Armitage, 1999)

I. Gingival Diseases	
II. Chronic Periodontitis	A. Localized B. Generalized
III. Aggressive Periodontitis	A. Localized B. Generalized
IV. Periodontitis as a Manifestation of Systemic Diseases	
A. Associated with hematological disorders	*1.Acquired neutropenia* *2. Leukemias* *3. Other*
B. Associated with genetic disorders *1.Familial and cyclic neutropenia* *2.Down syndrome* *3.Leukocyte adhesion deficiency syndromes* *4. Papillon-Lefèvre syndrome* *5. Chediak-Higashi syndrome* *6. Histiocytosis syndromes* *7. Glycogen storage disease*	*8.Infantile genetic agranulocytosis* *9.Cohen syndrome* *10.Ehlers-Danlos syndrome (Types IV and VIII)* *11.Hypophosphatasia* *12.Other*
C. Not otherwise specified (NOS)	
V. Necrotizing Periodontal Diseases	A. Necrotizing ulcerative gingivitis (NUG) B. Necrotizing ulcerative periodontitis (NUP)
VI. Abscesses of the Periodontium	A. Gingival abscess B. Periodontal abscess C. Pericoronal abscess
VII. Periodontitis Associated With Endodontic Lesions	
A. Combined periodontic-endodontic lesions	
VIII. Developmental or Acquired Deformities and Conditions	
A. Localized tooth-related factors that modify or predispose to plaque-induced gingival diseases/periodontitis	
B. Mucogingival deformities and conditions around teeth	
C. Mucogingival deformities and conditions on edentulous ridges	
D. Occlusal trauma	

Chronic and aggressive periodontitis are most common forms of disease (Armitage, 1999) (Table 2.1). Chronic periodontitis is also known as adult periodontitis due to high prevalence in adults but it can occur at any age. It is the most frequently occurring form of periodontitis and is characterized by inflammation within the supporting tissues of the teeth, pocket formation, and recession of the gingiva progressing to attachment and bone loss. If left untreated over time, may progress to damage and the breakdown of smaller or larger parts of the periodontal attachment apparatus (Greenstein, 2005). Aggressive periodontitis is also known as early-onset periodontitis due to rapid progression of disease and its destructive nature, occurs in otherwise clinically healthy patients. Common trait of disease includes rapid bone destruction, attachment loss and familial aggregation. Aggressive periodontitis can be localized or generalized. Localized aggressive periodontitis (LAgP) patients suffers from interproximal attachment loss including at least two permanent first molars and incisors, with attachment loss on no more than two teeth other than first molars and incisors. Generalized aggressive periodontitis (GAgP) patients have generalized interproximal attachment loss in at least three teeth that are not first molars and incisors (Oh *et al.,* 2002).

2.3. Etiological factors

Most commonly the etiology of disease is associated with presence of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Dialister pneumosintes, Bacteroides forsythus* and *Treponema denticola* (Oh *et al.,* 2002). Other disease causing anaerobic bateria includes *Porphyromonas (Bacteroides) gingivalis, Bacteroides forsythus, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum, Eubacteria nodatum, Selenomonas noxia, Porphyromonas (Bacteroides) gracilis, PROS Spirochete (Treponema vincentii), Peptostreptococcus micros Eubacterium sp., Selenomonas sp., Streptococcus intermedius, and some micro-aerophillic bacterias Actinobacillus actinomycetemcomitans, Wolinella (Campylobacter) recta, Eikenella corrodens* (Loesche, 1999).

Invasion of pathogenic bacteria alters the local environment of pockets around tooth. Also, bacterial products and biofilms with different bacterial species get established. Overall there is shift in microbial population from "an endogen poly-microbial opportunistic flora" to "gram-negative proteolytic and predominantly anaerobic" microorganisms in the gingival tissue (Oh *et al.,* 2002). The bacterial biofilms or plaques contain more than $1*10^{11}$ bacteria/cm^3 with about 400 species. Due to alteration in microbial components, host defense mechanism gets activated leading to damage to connective tissues of dentine. Lipo-polysaccharide (LPS) present on the outer membrane of bacteria evokes the production of inflammatory mediators such as IL-β, tumor necrosis factor (TNFα), IL-6 and cytokines *via.* activation of p38 mitogen activated protein kinase pathways (Kirkwood and Rossa Jr, 2009). Not all bacteria release noxious products responsible for evoking host inflammatory response (Havemose-Poulsen and Holmstrup, 1997).

There are two plaque theories proposed for microbial contribution in the disease, *nonspecific plaque hypothesis* and *specific plaque hypothesis*. The *nonspecific plaque hypothesis* proposes contribution of entire microorganism present in a plaque deposited on tooth surfaces and gingival crevice, for the development of periodontal disease. On the other hand, *specific plaque hypothesis*, proposes that only particular microbial species of plaque are pathogenic. In spite of presence of more than hundred microbial species only twenty species have been found in higher concentrations during periodontitis (Southard and Godowski, 1998). Research are undergoing for identification of most causative components of biofilms or plaques. These biofilms are also responsible for failure of therapy due to development of resistance or tolerance for antibiotics (Eick *et al.,* 2004; Larsen, 2002; Southard and Godowski, 1998). *Enterococcus faecalis* had been reportedly found responsible for failure of endodontic treatments (Verdugo *et al.,* 2016).

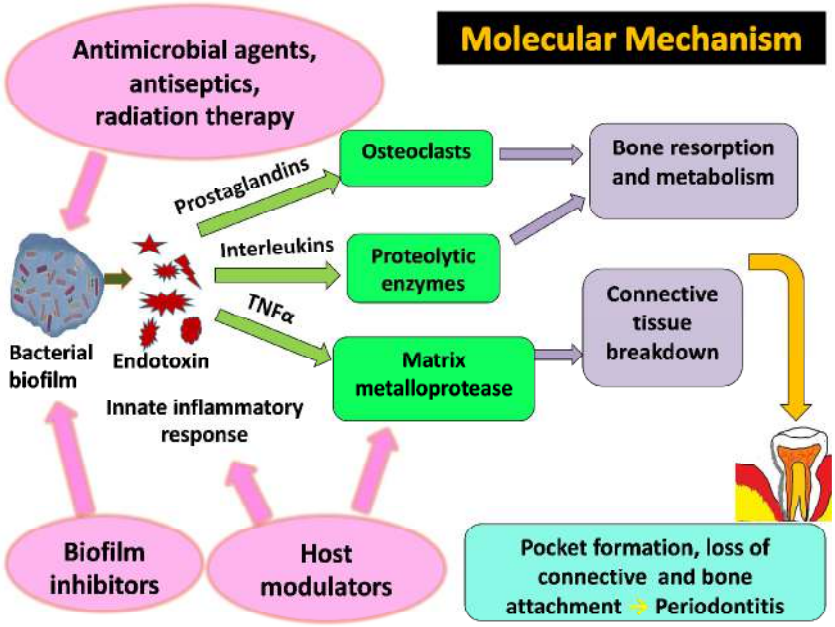

Figure 2.3: Illustration of pathogenesis and progression of periodontitis and various treatment strategies, SRP- Scaling and Root Planning (Yadav *et al.,* 2015).

Thus, it has been observed that bacteria are necessary to drive inflammatory response for occurrence of periodontitis, but they alone cannot cause disease. Presence of a susceptible host is another parameter as periodontal breakdown (bone loss, attachment loss) is predominantly caused by host-derived destructive enzymes including osteoclasts, proteolytic enzymes and MMPs, and inflammatory mediators such as cytokinins, prostaglandin E2 (PE_2), and interleukins (IL-1 and IL-6) secreted during inflammatory cascade. Most susceptible host includes old age people (> 45 years) who have weakened immune system (Fig. 2.3). Besides, in children neutrophil dysfunction may lead to development of periodontitis causing loss of primary and permanent teeth (Eskan *et al.,* 2012; Pihlstrom *et al.,* 2005).

As a serious consequence of this, loss of physiological and psychological functions may occur. Paradoxically, host immune response which is for the protection against microbes, here is responsible for the breakdown of the soft and hard periodontal tissues (Caton and Ryan, 2011; Ryan, 2002).

2.4. Oral-systemic connections

A common phrase says 'A healthy mouth is a gateway to a healthy body'. Thus a good oral health contributes towards good overall health.

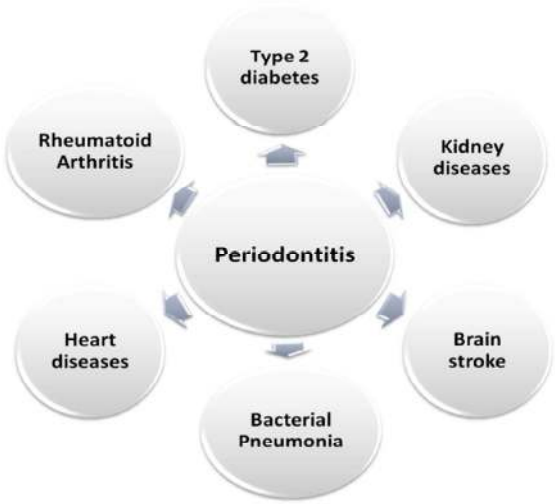

Figure 2.4: Representation of diseases with oral-systemic connections with periodontitis.

Periodontitis is not only associated to irreversible bone and tooth loss but it introduces complications in various systemic diseases including cardiovascular diseases, stroke, atherosclerosis, overt nephropathy, end-stage renal disease, diabetes, adverse pregnancy outcomes, obesity, Alzheimer's disease (Fig. 2.4) (Cotti *et al.,* 2011; Craig *et al.,* 2002; Gupta, 2010; Katz *et al.,* 2001; Pischon *et al.,* 2007).

Periodontitis and systemic diseases are interconnected *via* circulation of cytokines and other inflammatory mediators in blood. The untreated periodontal diseases spread infection to other tissues and systemic circulation. Periodontal patients often appear asymptomatic, however they have been reported to have a significantly increased risk for the development of cardiovascular diseases including atherosclerosis, myocardial infarction, stroke and atherosclerosis (Cotti *et al.,* 2011; Katz *et al.,* 2001).

As per the reports of World biomedical literature during 1930 to 1996, infective endocarditis has been found to be associated with periodontal infection. The

incidence of infective endocarditis was about 0.70 to 6.8 per 100,000 people. Of all cases, 8% cases were related with periodontal disease without any dental procedure and the risk of infective periodontitis after dental procedure was about 1 per 3,000 to 5,000 procedures (Li *et al.,* 2000).

2.4.1. Pathways Linking Oral Infection to Secondary Non-oral Disease

Metastatic spread, metastatic injury, and metastatic inflammation are the three basic pathways linking oral infections to secondary systemic diseases. *Metastatic spread* of infection may occur from oral cavity to systemic circulation due to transient bacteremia (caused by presence of bacteria in blood), *metastatic injury* occurs from the effects of circulating oral microbial toxins which may enter other organs, and *metastatic inflammation* caused by immunological injury provoked by oral microorganisms.

2.4.2. Role of inflammatory mediators

Periodontium acts as a reservoir for inflammatory mediators. The most common circulating cytokines include: C-reactive protein (CRP), IL-1, IL-6, TNF-α and PE2 were found in high concentration in periodontium (Seymour *et al.,* 2007). The spillover concentrations of mediators make them to enter blood circulation to perpetuate systemic effects. For instance, IL-1β augments coagulation and thrombosis and retards fibrinolysis, whereas IL-1, TNF-α, and thromboxane can cause platelet aggregation and adhesion, formation of lipid laden foam cells, and deposition of cholesterol (Li *et al.,* 2000).

The hypothesis of systemic inflammation model states that there is increased circulation of cyctokines and other inflammatory mediators emanating from diseased periodontium, which in turn could damage the vascular endothelium and result in atherosclerosis (Seymour *et al.,* 2007). Studies indicated high levels of CRP were present in patients with cardiovascular diseases and advanced periodontitis (Seymour *et al.,* 2007). They further account for preterm labor and low birth weight of infants (Li *et al.,* 2000).

2.5. Treatment strategy

Current treatment strategies follows controlling the disease at different stages as illustrated in Fig. 2.5. Treatments can be done by scaling and root planning (SRP), use of antibiotics, antiseptics, radiation therapy, biofilms inhibitors and host modulators (Yadav *et al.,* 2015). Broadly, periodontal treatments can be done by SRP, systemic and localized drug delivery systems.

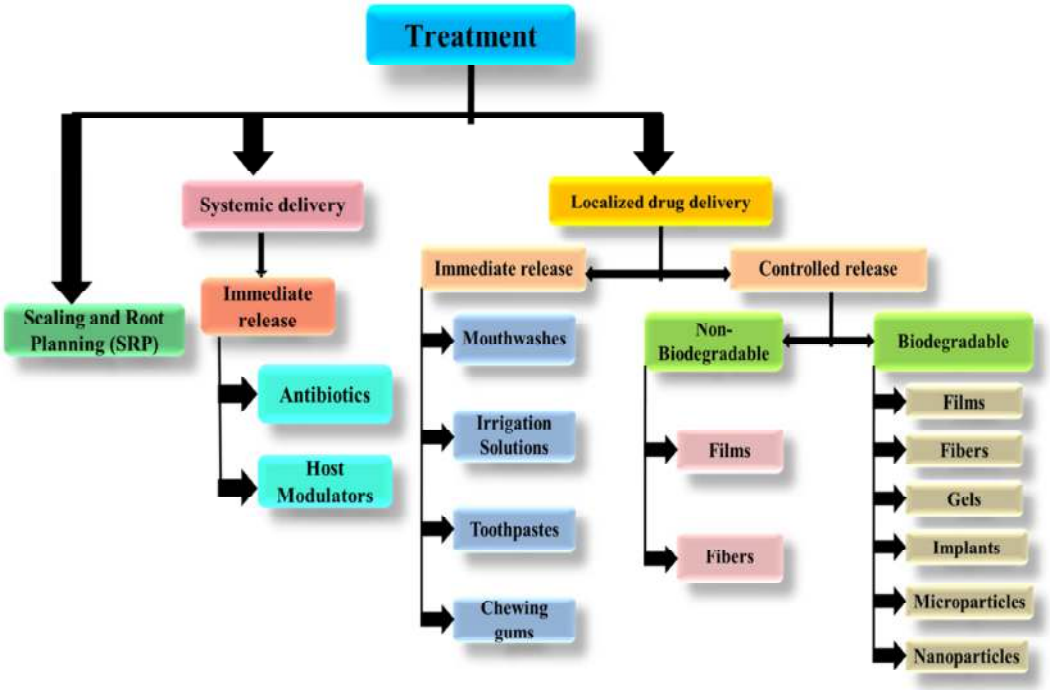

Figure 2.5: Current treatment strategies for the treatment of periodontitis (modified from Yadav *et al.,* 2015).

2.5.1. Scaling and Root Planing (SRP)

First line strategy for periodontal disease treatment involves conventional mechanical therapy *i.e.* scaling and root planing (SRP), which can improve the overall gingival health and halts the progression of disease (Fig. 2.5). In scaling, removal of tartar or calculus above and gum line is done. Root planing involves elimination of rough spots or plaque containing colonies of microbes. Overall, SRP helps in removal of contamination, toxins bacteria, plaque and calculus from tooth surface. SRP cleans gums, promotes reattachment of gum tissue so that it becomes pink and firm.

However, SRP produces little discomfort to the patients and does not get rid of all the microbes which further cause persistence and reoccurrence of infection with attachment loss. Moreover, SRP requires frequent patient visit to dentist for the maintenance of therapy. Therefore, to improve outcomes of SRP, antimicrobial therapy could be given as an adjunct by systemic and localized routes (Southard and Godowski, 1998).

2.5.2. Systemic delivery of antimicrobials

The systemic usage of antibiotics deals with a number of issues *viz.* requirement of higher dose and long term administration, increased risk of antibiotic resistance, adverse drug reactions (nausea, diarrhea, pseudomembranous colitis) and potential liver and kidney damage (Table 2.2) (Southard and Godowski, 1998; Walker, 1996). Consequently, systemic therapy has poor patient compliance. Also, systemic administration unnecessarily exposes whole body towards drug while only very little amount of drug reaches the desired sub-gingival microflora of the periodontal pocket and GCF (Goodson, 1994; Yadav *et al.,* 2015).

Many a times drug may not reach periodontal pockets in sufficient concentration because of first pass metabolism and excretion of drug systemically. These challenges associated with oral delivery can be addressed by administration of drugs directly to the intended site of action *i.e.* periodontal pockets with significantly lesser dose. Since the periodontal infections are restricted to periodontal pockets, intrapocket administration of drugs will prove to be more beneficial than the traditional systemic therapy (Schwach-Abdellaoui *et al.,* 2000; Southard and Godowski, 1998).

2.5.3. Localized intrapocket delivery of antimicrobial agents.

With the incline in knowledge about the etiology and pathogenesis of the disease, many types of dosage forms have been developed (Nair and Anoop, 2012; Yadav *et al.,* 2015). Hierarchy of advances in periodontal drug delivery systems starting from systemic delivery to localized intrapocket delivery are illustrated in Fig.

2.6. The pocket acts as a natural reservoir and provides easy access for the insertion of a medicinal device. Drug release and distribution throughout the pocket is provided by GCF, which acts as the leaching medium where drug concentration can be maintained for prolonged period. All these factors make the intrapocket drug delivery an ideal choice (Nair and Anoop, 2012).

Table 2.2: Comparison of localized intrapocket delivery and systemic delivery (Yadav *et al.,* 2015)

Localized drug delivery	Systemic drug delivery
High concentration of drug achieved in the pocket	Less concentration of drug reaches pocket
Less chances of resistance development	Development of resistance
Avoids exposure of drug to non-oral sites	Whole body is exposed to the drug
Relatively lower dose is required	A higher dose is required
Minimal side effects	More adverse effects
Avoids inactivation of drugs	Inactivation of drugs due to first pass metabolism and excretion
Fast action	Slow action
Provides controlled release	Immediate release
More patient compliance due to noninvasive and painless	Patient compliance is relied on

Essentially localized delivery of antimicrobial agents could be done by two mechanisms namely immediate release (irrigation of liquids) and controlled release systems. Immediate release localized delivery includes irrigation and mouth rinsing. Slow and controlled release systems includes films, chip, strips, gels, ointments, *in-situ* gels, microparticles and nanoparticles (Table 2.3 and 2.4).

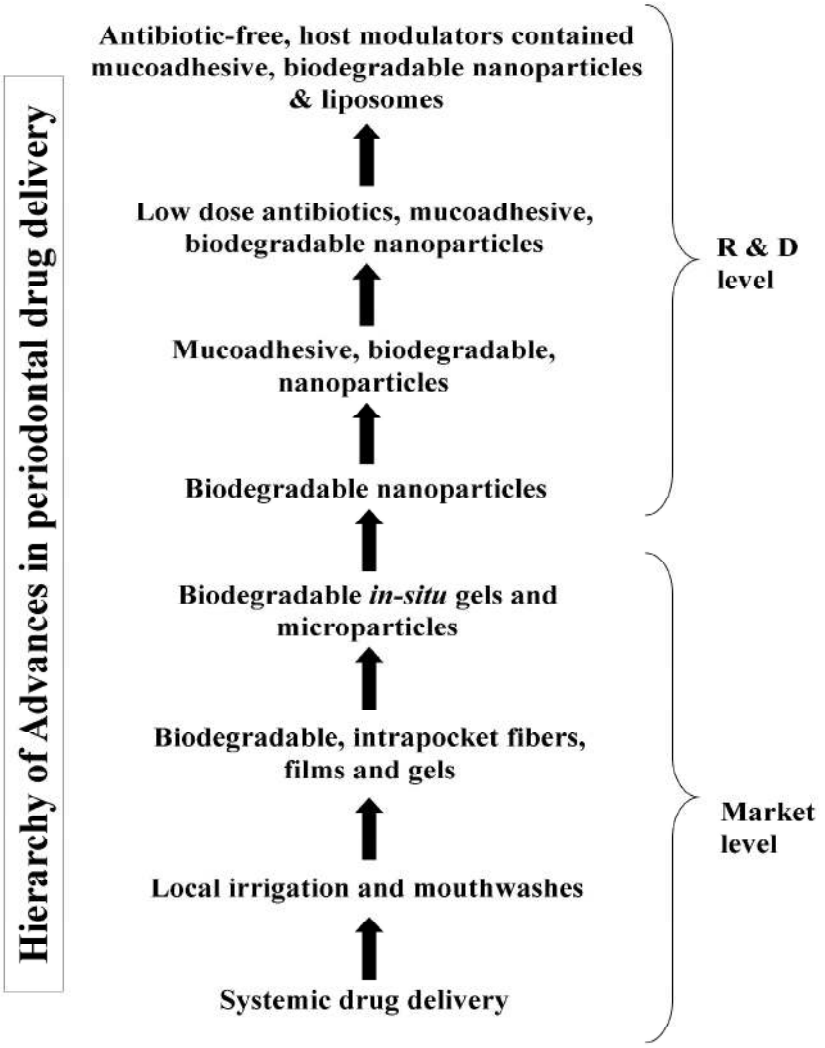

Figure 2.6: Hierarchy of advances in periodontal drug delivery (Yadav *et al.,* 2015).

Table 2.3: Marketed intrapocket drug delivery systems

Brand	Company	Drug/Delivery	Polymer	Biodegradable	Approval
Arestin®	OraPharma, US	Minocycline Hydrochloride Microspheres	Poly (glycolide-co-dl-lactide)	Biodegradable (14-21 days)	USFDA
Actisite®	Schiff and Company	Tetracycline hydrochloride Fiber	Ethylene Vinyl Acetate	Non-biodegradable	USFDA
Atridox®	Atrix Laboratories	Doxycycline Gel	Poly(dl-lactide) and N methyl pyridine	Bioabsorbable (21 days)	USFDA
Periochip®	Perio Products Ltd., Israel	Chlorhexidine gluconate Chip	Gelatin crosslinked with glutaraldehyde	Biodegradable (7 to 10 days)	USFDA
Dentomycin®	Wyeth, United Kingdom	Minocycline HCl Ointment	Hydroxyethyl-cellulose, aminoalkyl-methacrylate, triacetine and glycerine	Biodegradable	European Union but non-FDA cleared
Elyzol 25	Dumex-Alpharma	Metronidazole benzoate Gel	Glyceryl mono-oleate and sesame oil	Biodegradable	Non-FDA
Chlo-Site	Ghimas Company, Italy	Chlorhexidine Gel	Xanthan gel	Biodegradable	Non-FDA

Table 2.4: Benefits and limitations of commonly used localized drug delivery systems

Drug delivery systems	Benefits	Limitations
Irrigation or immediate release (Paquette, 2009)	Ease of administration using simple syringes	Do not reach deeper pockets, Short term effect
Gels/Ointments (Esposito *et al.,* 1996)	Ease of administration using simple syringes and deep pocket penetration	Lack of prolonged release and get washed away by high GCF turnover
Film/Chip (Ahuja *et al.,* 2006)	Provide controlled release	Less flexible for deep pocket penetration, get folded during insertion
Strips (Moran *et al.,* 1990)	Flexible	Difficulty in insertion using forceps
In-situ gels (Do *et al.,* 2014)	Easily syringeable and deep pocket penetration, solidify as per pocket size and shape	Requires expertise for administration
Microparticles (Álvarez *et al.,* 2011)	Provides controlled and prolonged release	Lack of ease of administration and uniform dosing
Nanoparticles	Provides controlled and prolonged release	Expensive, safety and toxicity issues, still not reached market

2.5.3.1. Advantages and disadvantages of intrapocket formulations are as follows

(Joshi *et al.,* 2016)

Advantages

- Direct access to diseased pocket
- Improved patient compliance
- Avoidance of gastrointestinal-related issues of oral drug delivery
- Bypasses first-pass metabolism
- Enhanced therapeutic efficacy of the drug
- Reduced treatment cost
- Reliable drug delivery route in very sick patients who are unable to swallow
- Safer and more convenient route of drug administration
- Longer duration of action

- Noninvasive, painless and simple application

Disadvantages

- Local irritants cannot be administered
- Dose is limited because of relatively small area.
- The enzymes like peptidase and esterase may cause presystemic metabolism.
- Peptide administration is not practicable due to peptidases.
- This route understood the needs for high-potency drugs.
- Manufacturing cost of the patches or devices is a matter for consideration.

2.5.3.2. Desirable characteristics of localized drug delivery systems required to be inserted into periodontal pockets are:

- ***Biocompatible*:** Should be non-toxic and biocompatible
- ***Biodegradable*:** It should be biodegradable in nature so that patient does not require a second visit to the dentist to remove the systems. This further improves patient compliance.
- ***Mucoadhesive*:** For good retention in the periodontal pocket for prolonged time
- ***Ease of Application*:** Should be easily inserted in to the pocket with less pain to the patient.
- ***Sustained and prolonged release*:** Should produce sustained/prolonged release of drugs so that constant concentration (above MIC) of antimicrobial is maintained into the GCF for longer duration.
- ***Broad antimicrobial activity:*** Inhibit or kill all the causative microorganisms.
- ***Targeted action*:** It should reach the diseased site in adequate concentration.

2.5.3.3. Literature review on development of localized drug delivery systems

About more than three decades ago, Deasy et al. (1989) formulated strips/films containing tetracycline hydrochloride or metronidazole in

polyhydroxybutyric acid (biodegradable polymer matrix) which showed sustained release in simulated gingival fluid pH 6·6 at 37 °C. The strips inserted into pockets provided greatest response reduction of plaque index, gingival index and pocket depth in patients during clinical studies as compared to untreated control group (Deasy *et al.,* 1989). Later on, Higashi et al. (1990) suggested that controlled-release insert (PT-01) of ofloxacin as suitable pharmaceutical preparation for periodontal chemotherapy (Higashi *et al.,* 1990). Roskos et al. (1995) formulated tetracycline loaded poly(ortho esters) viscous, semisolid materials by the condensation of 1,2,6 -hexanetriol and an alkyl orthoacetate. The materials were injected using a blunt needle into pockets and released antibiotic for 24 h (Roskos *et al.,* 1995). Şenel et al. (2000) fabricated chitosan based local delivery gels of chlorhexidine for application to the oral cavity. These gels showed release for 3 h (Şenel *et al.,* 2000).

Schwach-Abdellaoui et al. (2002), developed bioerodible semisolid gel containing tetracycline-free base based on auto-catalyzed poly (ortho esters) containing lactoyl lactyl dimers in the polymer backbone which modulated release of tetracycline over two weeks when injected or placed as a solid device directly into the periodontal pocket (Schwach-Abdellaoui *et al.,* 2002). Later, İkinci et al. (2002) observed that antimicrobial activity of chitosan films or gels containing chlorhexidine gluconate showed a higher activity when compared to that of chlorhexidine gluconate alone, against a periodontal pathogen, *Porphyromonas gingivalis* (Ikinci *et al.,* 2002). This study indicated chitosan films or gels seem to be promising delivery systems for local therapy of periodontal diseases due to enhanced bioadhesive property and antimicrobial activity.

Biodegradable intrapocket dental films, were prepared by Ahuja *et al.,* (2006) which could be easily placed into the periodontal pocket, and be capable of delivering therapeutic concentrations of amoxycillin and metronidazole for prolonged period of time with a much lower dose, hence obviating untoward side effects (Ahuja *et al.,* 2006). Gad et al. (2008) studied biodegradable polymers [poly (lactide) (PLA) and poly (lactide-co-glycolide) (PLGA)], *in-situ* implants containing doxycycline hydrochloride and/or secnidazole that could be used in the treatment of periodontitis

by direct periodontal intrapocket administration. Results revealed that the pharmaceutical formulation based on 25% PLGA containing secnidazole and doxycycline hydrochloride has promising activity in treating periodontitis in comparison with Atridox® (Gad *et al.,* 2008). However, the release was controlled only for 24 h by use PLGA type polymer.

Garripelli et al. (2010) utilized a class of thermosensitive biodegradable multiblock copolymers with acid-labile acetal linkages, synthesized from Pluronic triblock copolymers (Pluronic® P85 and P104) and di-(ethylene glycol) divinyl ether (Garripelli *et al.,* 2010). These novel polymers hold great potential as a thermosensitive controlled drug delivery system owing to their interesting phase transition behavior and biocompatibility and find their applicability into periodontal drug delivery.

Bermudez and Grau, (2011) explored the potential of combining two different poloxamers (P407 and P188) with κ-carrageenan and sodium chloride for designing of thermosensitive injectable depot systems for drug release. These systems offers a promising alternative to development of injectable depot type controlled drug release platforms (Bermudez and Grau, 2011).

Selection of suitable drug delivery system for prolonged intrapocket retention and maintenance of effective drug concentration in GCF is the greatest challenge today. There are many generics like metronidazole, levofloxacin, tetracycline, doxycyline and minocycline and products such as Periostat, Periochip®, Atridox®, Arestin®, Actisite®, Dentomycin®, and Elyzol®, are available in the market for intrapocket application (Table 2.3). All existing products are recommended to be used as an adjuvant to SRP. Although a number of drug delivery systems are researched and available in the market with their own pros and cons, the effective treatment means are still lacking (Table 2.4). There is need to develop devices which can surpass or produce equivalent effect to mechanical intervention.

Development of novel compounds and delivery systems having more efficacy than existing ones could circumvent the issues regarding intrapocket delivery.

Moreover, biodegradability, biocompatibility, bioadhesivity and inertness are the critical characteristics to be possessed by developed intrapocket delivery systems. The achievement of these characteristics is a function of selection of suitable polymers. Further, ease of drug delivery and good penetration into the infected tissue is also desirable approach for periodontal drug delivery.

2.6. Formulation specific review

2.6.1. Multiparticulate based delivery systems

In general, multiparticulate based drug delivery systems are constituted by granules, pellets, minitablets, microparticles, and nanoparticles depending on the size and applicability. A controlled release multiparticulate system consists of multiple mini drug depots wherein the drug is either dispersed in a matrix or encapsulated in a reservoir system. Most importantly it can be divided into desired dosage units without any effect on the dimension and properties of individual particles as compared to films or strips. They are more flexible and offer more therapeutic benefits than single unit dosage forms depending on their surface area (Shukla *et al.,* 2011). Micro- and nano- forms of drug delivery are ideal for intrapocket delivery due to their small size. The basic criterion of selection of microspheres over nanoparticles is disclosed in the next section.

2.6.2. Microspheres

Microspheres are applied in drug delivery since decades and are considered as one of the novel dosage forms due to benefits offered by them. They are free-flowing powders and provide sustained and controlled drug release at target site (Jayaprakash *et al.,* 2009)). They are defined as solid spherical polymeric structures with size falling below 1 mm (1-1000 µm) containing drug dispersed throughout the polymeric matrix or reservoir. (Jayaprakash *et al.,* 2009). Both non-biodegradable and biodegradable polymers from natural and synthetic origin can be used to formulate microspheres (Goyal *et al.,* 2013). They can be directly injected into the pocket or can be loaded into gels, films and dental paste (Jain *et al.,* 2008).

Microspheres put forth a number of advantages like shielding of unstable drug before and after administration, controlled drug release, improved patient compliance, sustained therapeutic effect, enhanced bioavailability and decreased frequency and intensity of adverse effects (Garg *et al.,* 2013). Unlike nanoparticles, microspheres are non-toxic, more stable and easy to prepare and handle without need of sophisticated instruments (Yadav *et al.,* 2013). They provide controlled release of entrapped drug with high encapsulation efficiencies and are useful alternatives to nanoparticles for targeting drugs to the monocyte-macrophage system as compared to liposomal formulations (Jha *et al.,* 2011; Pandey *et al.,* 2015; Smith, 2005).

2.6.2.1. Formulation methods for microspheres

Formulation of microspheres should full-fill the following parameters:(1) Able to load drugs at high concentrations. (2) should be stable with a clinically acceptable shelf life. (3) Small controllable particle size and dispersible in injectable solutions. (4) provide prolonged and controlled release of drug. (5) Biocompatibility and biodegradable.

Single emulsion technique – This method is applicable to natural (chitosan) and water soluble polymers. The natural polymers are dissolved in aqueous solvent and then dispersed in oil phase *i.e.* non-aqueous medium (Sinha *et al.,* 2004). Followed by this, two crosslinking methods are followed methods (1) Cross-linking by heat: by adding the dispersion into heated oil (unsuitable for the thermolabile drugs). (2) Chemical cross-linking agents: by using chemicals *i.e.* formaldehyde, tripolyphosphate, glutaraldehyde etc. the safety of microspheres depends on the type of crosslinker used.

Double emulsion technique - It deals with formation of multiple emulsions *i.e.* w/o/w by pouring the primary w/o emulsion into an aqueous solution of polyvinyl alcohol under stirring. The primary emulsion was prepared by pouring aqueous drug-polymer mixture to the organic phase like liquid paraffin. It is best suited to water-soluble drugs, peptides, proteins and the vaccines. Natural as well as the synthetic polymer can use for this method.

Interfacial polymerization - In this techniue, various monomers form a film of polymer that encapsulate the dispersed phase contaning drug, present at the interface between the two immiscible liquid phases. Here, two types of monomers are employed; one is dissolved in dispersed phase while other is dissolved in the continuous phase (aqueous in nature) and both are emulsified. Depending on the differrential solubility of polymers two conditions arise; if the polymer is soluble in droplet, monolithic type of carrier is formed. If the polymer is insoluble in droplet, capsular type formed (Jayaprakash *et al.,* 2009).

Spray drying and spray congealing – Both methods depend on removal of solvent or cooling of drug-polymer solution. Spray drying is based on evaporation of solvent, while spray congealing is based on inversion of liquid to a solid. Spray drying is the mostly used in industrial process. Thus, spray drying is applicable where the product is required to comply with precise quality standards for particle size, shape and distribution, residual moisture content, and bulk density (Broadhead *et al.,* 1992; Mishra *et al.,* 2017).

Solvent evaporation method - In this method, first drug is dissolved (in acetone) and dispersed in chitosan solution. The drug and polymer mixture is then emulsified into liquid paraffin and agitated. For the formation of the emulsion between polymer solution and an immiscible continuous phase in aqueous (o/w) as well as non-aqueous phase (w/o). Magnesium stearate could be added for preventing agglomeration (Sinha *et al.,* 2004).

. ***Phase separation coacervation technique*** - It is simplest method involves simple separation of micromolecular solution into two immiscible liquid phase. the basic principle involves the coacervation or formation of polymer rich phase due to decreased solubility of polymer in organic phase. This is an ideal method for encapsulation of water soluble drugs *i.e.* peptides, proteins *etc.* to form reservoir type of system (Nihant *et al.,* 1995).

Solvent extraction – This method is based on removal of the organic phase by extraction of the organic solvent. Isopropanol is commonly used water-miscible

solvent. This method requires less time for hardening of microspheres. Further, the plus point of this method is that the drug could be directly added to polymer-solvent mixture. The rate of removal of solvent by extraction method depends phase ratio and temperature and solubility of the polymer (Freitas *et al.,* 2005).

Microspheres had been applied in drug delivery for the treatment of various diseases and could be advantageous for periodontal drug delivery where prolonged treatment is desirable. The development of a noteworthy technique for micro-encapsulating minocycline hydrochloride in a bioresorbable, bioadhesive gel for intrapocket delivery resulted in the introduction of Arestin® (OraPharma, US) in 2001. The system's pharmacokinetics results in gradual hydrolyzation of the microspheres with sustained release over 14 days (Bader, 2010). It is FDA approved product supplied in a sterilizable syringe with disposable tips.

Novel biodegradable microspheres (90 to 200 µm) based on poly(D,L-lactide-co-glycolide) and poly(ε-caprolactone) had been developed by w/o/w technique for controlled delivery of DX for the treatment of diseased pocket. *In-vitro* release indicated a burst effect initially, which was followed by controlled release up to 11 days (Mundargi *et al.,* 2007). Further, Patel *et al.,* (2008) demonstrated microparticles (10-25 µm) encapsulated with 86% of DX can be used for treatment of periodontitis. It was produced by spray drying of poly(lactide-co-glycolide) w/o/w multiple emulsions. *In-vitro* drug release was above the minimum inhibitory concentration for *Staphylococcus aureus* growth and continued up to three days (Patel *et al.,* 2008).

Monolithic biodegradable systems containing microspheres made from PLGA and Zein, incorporated with tetracycline were prepared by de Sousa and coworkers (2012). Prolonged release of tetracycline and significant antimicrobial activity was obtained against *S. aureus* for over 30 days (de Sousa *et al.,* 2012). In 2013, Pichayakorn and Boonme, formulated chitosan microspheres loaded with metronidazole by emulsion cross-linking process. They further incorporated optimized microspheres into mucoadhesive hydrogels and films and compared their *in-vitro* release. The studies convinced that microspheres incorporated hydrogels

provided prolonged and more preferable pattern of drug release than microsphere loaded films (Pichayakorn and Boonme, 2013).

Some recent drug-loaded microparticles/microspheres have been researched so far as listed in Table 2.5. The limitations of developed microspheres for intrapocket delivery in the management of periodontitis till date includes;

1. Use of single drug
2. Use of costlier synthetic polymers (PLGA, Poly-e-caprolactone *etc*)
3. Use of toxic crosslinkers
4. Less prolonged delivery with some exceptions
5. Incorporated with traditional drug metronidazole and tetracyclines
6. Directly injecting microspheres (without immersing in gel) into pockets creates friction and decreases ease of injectability.

Table 2.5: Literature survey of microspheres based periodontal drug delivery

Topic	Reference
Doxycycline hyclate-loaded bleached shellac *in-situ* forming microparticle for intraperiodontal pocket local delivery	(Phaechamud *et al.,* 2016)
Microparticles containing propolis and metronidazole: *in-vitro* characterization, release study and antimicrobial activity against periodontal pathogens	(de Souza Ferreira *et al.,* 2014)
Controlled-release and antibacterial studies of doxycycline-loaded poly (ε-caprolactone) microspheres	(Raval *et al.,* 2014)
Evaluation of cross-linked chitosan microparticles containing metronidazole for periodontitis treatment	(Pichayakorn and Boonme, 2013)
Efficacy of locally-delivered doxycycline microspheres in chronic localized periodontitis and on Porphyromonas gingivalis	(Rao *et al.,* 2012)
Effect of zein on biodegradable inserts for the delivery of tetracycline within periodontal pockets	(de Sousa *et al.,* 2012)
Formulation, antimicrobial and toxicity evaluation of bioceramic based ofloxacin loaded biodegradable microspheres for periodontal infection	(Jamal *et al.,* 2012)
The effectiveness of scaling and root planing with adjunctive time-release minocycline using an open and closed approach for the treatment of periodontitis	(Zingale *et al.,* 2011)
Preparation and characterization of chitosan microspheres for doxycycline delivery	(Shanmuganathan *et al.,* 2008)
Collagen-coated polycaprolactone microparticles as a controlled drug delivery system	(Aishwarya *et al.,* 2008)
Precursor system of liquid crystalline phase containing propolis microparticles for the treatment of periodontal disease: development and characterization	(Bruschi *et al.,* 2008)
Micromatricial metronidazole benzoate film as a local mucoadhesive delivery system for treatment of periodontal diseases	(El-Kamel *et al.,* 2007)
Development and evaluation of novel biodegradable microspheres based on poly (d, l-lactide-co-glycolide) and poly (ε-caprolactone) for controlled delivery of doxycycline in the treatment of human periodontal pocket: *in-vitro* and *in-vivo* studies	(Mundargi *et al.,* 2007)
Optimisation and characterization of bioadhesive controlled release tetracycline microspheres	(Govender *et al.,* 2005)
Comparative analysis of tetracycline-containing dental gels: Poloxamer- and monoglyceride-based formulations	(Esposito *et al.,* 1996)

2.6.3. *In-situ* forming implants

In-situ forming drug delivery systems (IFDDS) or *in-situ* gels are liquid formulations initially that transforms into semisolid gel or implants at physiological conditions after administration. They undergo phase transition or sol/gel conversion when entering biological fluid or cavity in response to biological stimuli like pH and temperature. The most promising feature of these injectable gels is that they can be introduced at the site of action at the minimal invasive way and get solidified at the site after injection. Thus, they are beneficial over preformed or solid implants which require surgery for placement. Other than this, they fully fill the cavity or defects, and sets as per the size and shape of the cavity.

Thermosensitive IFDDS are aqueous solutions before administration that solidify or form a gel upon phase transition at physiological body temperature (37 ºC). In a similar manner pH sensitive IFDDS shows a pH-dependent behavior. They form depot at the site of injection, therefore drug delivery for prolonged periods can be achieved typically ranging from days to several months by polymeric manipulation of such systems.

By definition gels or hydrogels are polymeric networks with a three-dimensional configuration capable of imbibing high amounts of water or biological fluids (Peppas and Khare, 1993). In particular, they are composed of water-insoluble polymer in a water-miscible, biocompatible solvent. They get solidified based on solvent exchange mechanism at physiological conditions (Fig. 2.7). Upon contact with body fluids, water permeates inside the formulations and due to non-solubility of polymers at physiological conditions (pH and temperature), it precipitates to form solid polymeric implant (Dumortier *et al.,* 2006).

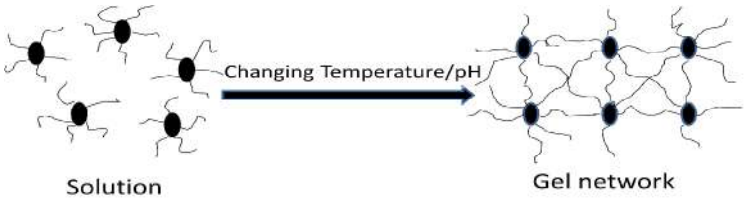

Fig 2.7: Mechanism of Sol-gel transformation

The mechanism of sol-gel conversion is generally governed by the ratio of hydrophilic to lipophilic groups of the polymer. A common characteristic feature of thermosensitive polymers is the presence of the hydrophobic groups, such as methyl, ethyl and propyl groups. These polymers possess have lower critical solution temperature (LCST). LCST is the temperature above which the polymeric monophasic system becomes hydrophobic and insoluble (gel phase), leading to phase separation, whereas below the LCST the polymers are soluble (sol phase). For polymers having LCST, a small increase in temperature results in negative free energy of the system (ΔG) leading to a higher entropy term (ΔS) with respect to increase in the enthalpy term (ΔH) in the thermodynamic relation as follows (Cabana *et al.,* 1997; James *et al.,* 2014);

$$\Delta G = \Delta HT\Delta S$$

Alternatively, on increasing temperature some amphiphilic polymers (poloxamers) self-assemble in solution by forming micelle packing leading to gelation because of polymer-polymer interactions. An ideal *in-situ* system would be a solution that is a free-flowing, injectable liquid at ambient temperature and gels at body temperature with minimal syneresis. Moreover, loaded with drugs or cells should be achieved by simple mixing. When administered parenterally, these systems should exhibit a pH close to neutrality and should be bioresorbable (Cabana *et al.,* 1997; James *et al.,* 2014).

Various polymers like PLGA, cholesterol, shellac, collagen, chitosan, polyacrylic acid, carbomer are significantly used for the preparation of gel dosage form. This drug delivery system can be made *in-situ* in nature by mixing with specific polymers *viz*. pluronic® F127 (Table 2.6) (Jain *et al.,* 2008).

Table 2.6: Different polymers used in the formulation of thermosensitive *in-situ* gel system

Polymers	Reference
Poloxamers (P407 and P188) with κ-carrageenan and sodium chloride	Bermudez and Grau, (2011)
Hydroxypropylmethylcellulose (HPMC), metolose (MTL), and poloxamer 407	Baloglu et al. (2010)
Poly (e-caprolactone)-poly (ethylene glycol)-poly (e-caprolactone) (PCEC)	Gong et al. (2009)
Chitosan and Pluronic F127	Gupta et al. (2009)
Poly (lactide) (PLA) and PLGA)	Gad et al. (2008)
Glycerol-phosphate disodium salt to a chitosan	Chenite et al. (2000)

In 1998, Atrix Laboratories developed Atridox, a thixotropic intrapocket gel containing 10% DX, used as an adjunct to SRP. The term "thixotropic" refers to the product's conversion from a gel to a hard, plastic state when it comes in contact with moisture in the pocket. This allows the antibiotic-containing material to remain in place for 7 to 21 days. Chitosan hydrogels were also able to deliver ipriflavone, a lipophilic drug that promotes bone density, into the periodontal pockets. For this purpose, mono and multilayer composite systems consisting of chitosan and PLGA were designed and were shown to prolong drug release *in-vitro* (Perugini *et al.,* 2003).

A study was conducted by Gupta et al. (2008) on a physiologically activated *in-situ* gel of chitosan and pluronics for local periodontal application of Prilocaine hydrochloride exhibited ease of administration and prolonged duration of action. The formulations were liquid at pH of 6 and 25 °C and got converted to gel at body pH of 7.4 and 37 °C (Gupta *et al.,* 2008) (Table 2.7).

Table 2.7. Literature survey of *in-situ* gelling systems in the management of periodontitis.

Title	Reference
Cholesterol *in-situ* forming gel loaded with doxycycline hyclate for intra-periodontal pocket delivery	(Phaechamud and Setthajindalert, 2017)
Formulation, *in-vitro* characterization and clinical evaluation of curcumin *in-situ* gel for treatment of periodontitis	(Nasra *et al.,* 2017)
Clinical evaluation of thermoresponsive and mucoadhesive Chitosan *in-situ* gel containing Levofloxacin and Metronidazole in the treatment of periodontal pockets–A split-mouth, clinical study	(Bansal *et al.,* 2016)
Pluronic and chitosan-based *In-situ* gel system for periodontal application	(Gupta *et al.,* 2014)
Formulation development of physiological environment responsive periodontal drug delivery system for local delivery of metronidazole benzoate	(Dabhi and Sheth, 2013)
Composition and characterization of *in-situ* usable light cured dental drug delivery hydrogel system	(Bakó *et al.,* 2013)
Dual controlled release, *in-situ* gelling periodontal sol of metronidazole benzoate and serratiopeptidase: statistical optimization and mechanistic evaluation	(Kumari and Pathak, 2012)
Systemic ornidazole as an adjunct to non-surgical periodontal therapy in the treatment of chronic periodontitis: a randomized, double-masked, placebo-controlled clinical trial	(Pradeep *et al.,* 2012)
Efficacy and safety of adjunctive local moxifloxacin delivery in the treatment of periodontitis	(Flemmig *et al.,* 2011)
Thermosensitive poloxamer-based injectables as controlled drug release platforms for veterinary use: Development and *in-vitro* evaluation	(Bermudez and Grau, 2011)
A novel injectable chlorhexidine thermosensitive hydrogel for periodontal application: preparation, antibacterial activity and toxicity evaluation	(Ji *et al.,* 2010b)
Design and formulation of mebeverine HCl semisolid formulations for intraorally administration	(Baloğlu *et al.,* 2010)
pH-Induced in situ gel for periodontal anesthesia	(Gupta *et al.,* 2008)
Formulation and evaluation of PLA and PLGA *in-situ* implants containing secnidazole and/or doxycycline for treatment of periodontitis	(Gad *et al.,* 2008)

Semisolid systems containing propolis for the treatment of periodontal disease: *in-vitro* release kinetics, syringeability, rheological, textural, and mucoadhesive properties	(Bruschi *et al.,* 2007)

2.7. Design of Experiments (DoE)

Quality by Design (QbD) deals with the design of experiments (DoE) which uses statistical, systematic, scientific approach for identifying critical quality attributes (CQA) associated with any process by reducing a number of experimental runs. Fabrication of mucoadhesive, controlled and prolonged release microspheres is a complex process and are influenced by many factors. The complexity of process increases the number of experimental runs. It can be simplified by identifying the most important influencing factors by using QbD approach. QbD involves factorial designs or screening methods which identify important and critical factors and responses in formulation process (Fisher *et al.,* 1926). This statistical approach decreases the experimental load by providing maximum information out of the minimum number of experiments (Boussès *et al.,* 2015; Ghasemian *et al.,* 2013).

Plackett-Burman factorial design (PBFD) is the best-suited screening design for estimating the correlations between factors and responses by classifying significant and non-significant factors affecting any process (Vardhan *et al.,* 2017). A PBFD is highly efficient, economical screening design applied in several fields to study the impact of processing factors on physicochemical properties of formulations (Khan and Jiabi, 1998; Kim, 1995). PBFD is the best-suited most economical and commonly used screening design over others like Box-Behnken experimental design (BBED) and central composite design which generated 92 and 53 runs respectively for 6 factors. In PBFD, full factorial designs are fractionalized to create runs multiple of 4 (12 runs for 6 factors), rather than the power of 2. It is also known as Hadamard matrix designs (Tye, 2004). In PBFD, the experimental runs can be 8 (up to7 factors), 12 (up to 11 factors) and so on. The maximum runs that can be studied are 4n-1, where n = ¼, 1, 2, 3 for a 4n number of experimental runs (Analytical Methods Committee, 2013).

After screening designs, final optimization using BBED is novel approach utilized by various scientists. Likewise PBFD, BBED requires fewer runs as compared to full factorial central composite design. Additionally, BBED avoids extremes by allowing to work around extreme factor combinations, provides good lack of fit detection, internal estimate of error and estimation of transformations (Ferreira *et al.,* 2007).

2.7.1. Screening, Validation and optimization parameters

Pareto plots- Pareto chart was named after Vilfredo Pareto, gives the magnitude and importance of an effect and screens significant variables from non-significant variables.

Contour and surface plots- contour plots are the two-dimensional (2D) view of the surface formed by points having similar responses, connected to form contour lines of constant responses. Parallel lines indicate no interaction. Surface plots are the three-dimensional (3D) view of contour plots. They both are based on regression model and are important for establishing the desirable response values and operating conditions. Surface plots display clear concept of response surface than contour plots.

Polynomial equations: Determines the type of relation between variables and responses; linear, quadratic, cubic and so on. The positive coefficient and negative coefficient of variables suggest direct and inverse effect respectively.

*The standard error of regression (S)***:** S-value is measured in the similar units of responses and represents the standard deviation of observed values from the fitted values. For best model description a lower S-value indicates results are closer to fitted line.

Regression coefficient (R^2): R^2 provides the percentage of variation in the results. Predicted R^2 determines the predictive ability of model for future responses.Adjusted R^2 determines the fitting of the model with the observed results when adjusted for some predictors in the model. The closeness of R^2 and predicted R^2

values indicate that the model can predict future response precisely. The higher the R^2 value and closer to 100%, the better the model fits data.

Fisher test value (F-value): The obtained F-value gives the significance of regression model.

Sum of squares (SS): Gives the estimated effect of factors and measures the variation from the mean. It is calculated by summation of the squares of the differences from the mean value.

Mean square (MS) = Degrees of freedom X SS

Percentage Bias (%B): used for validation of predicted responses. The value lies between ±15%.

QbD is approved by International Conference on Harmonization (ICH Q8) guidelines with the basic objective of introduction of quality into the product during its manufacturing by applying knowledge and risk management (Savic *et al.,* 2012).

The utility of QbD includes;

- Determination influential variables (factors)
- Determination where to set influential factors to optimize response
- Determination where to set influential factors to minimize response variability
- Determination where to set influential factors to minimize the effect of the uncontrollable factors
- Improves process yield
- Reduces variability
- Reduces development time
- Reduces overall costs of experiments

2.8. Drug Specific review

2.8.1. Ornidazole (OZ)

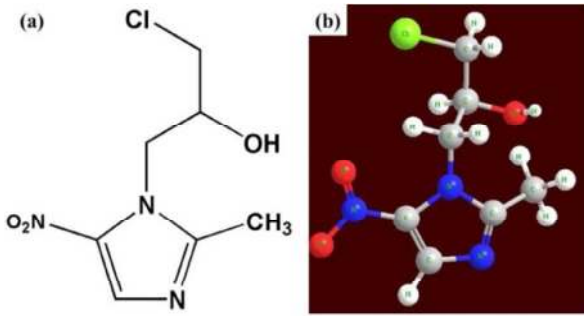

Fig 2.8. 2D and 3D structures of ornidazole (Yadav and Mishra, 2016)

Nitroimidazole drugs have been used for over 20 years, not only as major antimicrobial drugs but also as sensitizers of hypoxic tumors in conjunction with radiotherapy, thus possessing a wider spectrum of useful clinical activity than any other antibiotics. OZ is a 5-nitroimidazole derivative and is used in the treatment of susceptible protozoal infections and also in anaerobic bacterial infections. It has been used for amoebic liver abscesses, duodenal ulcers, giardiasis, intestinal lambliasis and vaginitis (Hizarciolu *et al.,* 2004).

2.8.1.1. Physicochemical Properties

Molecular Formula: $C_7H_{10}ClN_3O$

Molecular Weight: 219.62 g/mol

IUPAC name: 1-chloro-3-(2-methyl-5-nitroimidazole-1-yl)propan-2-ol

pKa: 2.4±0.1

Appearance: white crystalline powder

Density: 1.53 g/cm^3

Boiling Point: 443.2 °C at 760 mmHg

Melting Point: 85-90 °C

Refractive index: 1.617

Solubility: 4330 mg/L at 25 °C in water. It is soluble in water, ether, ethanol and chloroform

Stability: Stable at normal temperatures and pressures.

2.8.1.2. Mechanism of action

Unionized metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduce metronidazole to its active form. OZ covalently binds primarily to DNA, RNA or intracellular proteins, produces DNA strand breakage, inhibited repair and ultimately disrupted transcription and cell death. Bactericidal activity appears to be dependent on the formation of a redox intermediate metabolite in the bacterium (Breccia, 2012; Edwards, 1993; Lamp *et al.,* 1999).

2.8.1.3. Therapeutic use

OZ is given by mouth in tablets after food, or intravenously. When given intravenously, solutions of OZ should be diluted to 5 mg or less per ml and 100 or 200 ml infused over 15 to 30 minutes. It has also been given by vaginal pessary.

2.8.1.4. Adverse effects and contraindications

Nitroimidazoles and also OZ are considered safe drugs as only minor side effects have been observed. The most frequent side effects are an unpleasant taste, nausea, vomiting, abdominal discomfort and diarrhea. Serious side effects such as seizures and peripheral neuropathy are very rarely encountered in conventional doses (Rossignol *et al.,* 1984).

2.8.1.5. Role of OZ in the management of periodontitis

There are only few reports available for use of OZ in localized periodontal drug delivery (Table 2.8). However, many reports are available on nitromidazole prototype drug metronidazole. Systemic use of OZ as an adjunct to Scaling and root planing provided significantly better clinical results in treating moderate to advanced chronic periodontitis (Pradeep *et al.,* 2012). The short-term clinical trial reported although both OZ and metronidazole are effective, clinically and microbiologically, OZ was slightly better than metronidazole due to its higher plasma half-life (14.4 h) as compared to metronidazole (7.3 h). The study concluded that both the drugs are equally effective but recommend clinicians to prefer OZ over metronidazole as

adjunct in the treatment of generalized chronic periodontitis because of its better patient compliance (Jaswal *et al.,* 2008).

Table 2.8. Literature survey of the studies done on ornidazole.

Title	Reference
Systemic ornidazole as an adjunct to non-surgical periodontal therapy in the treatment of chronic periodontitis: a randomized, double-masked, placebo-controlled clinical trial	(Pradeep *et al.,* 2012)
Thermal characterization of antimicrobial drug ornidazole and its compatibility with a solid pharmaceutical product	(Soares *et al.,* 2011)
Formulation and evaluation of colon targeted tablets of Ornidazole for the treatment of amoebiasis	(Patel *et al.,* 2011)
Characterization and ornidazole release *in-vitro* of a novel composite film prepared with chitosan/poly (vinyl alcohol)/alginate	(Pei *et al.,* 2008)
Short-term clinical and microbiological effects of systemic ornidazole vs. Metronidazole in the treatment of generalized chronic periodontitis patients	(Jaswal *et al.,* 2008)
Treatment of rheumatoid arthritis with ornidazole: a randomized, double-blind, placebo-controlled study	(Ogrendik, 2006)
The clinical and microbiological effects of systemic ornidazole in sites with and without subgingival debridement in early-onset periodontitis patients	(Kamma *et al.,* 2000)

2.8.2. Doxycycline hyclate (DX)

DX (6-Deoxy-5-hydroxytetracycline) is a semi-synthetic tetracycline. It was first invented and clinically developed in 1967 by Pfizer Inc. Currently, it is one of the most commonly used broad-spectrum antibiotics and is relatively inexpensive (Holmes and Charles, 2009).

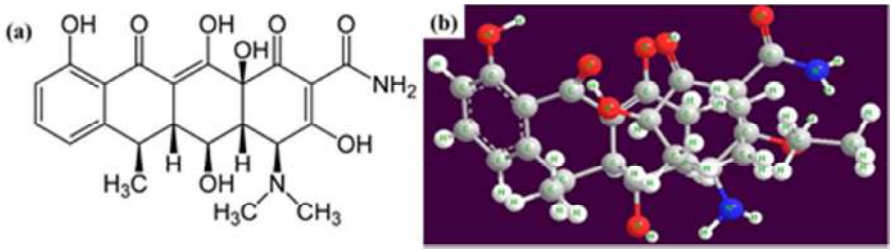

Fig 2.9. 2D and 3D structures of Doxycycline (Yadav and Mishra, 2016)

2.8.2.1. Physicochemical Properties

Molecular Formula: $(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$

Molecular Weight: 1025.89 g/mol

IUPAC name: (4S,4aR,5S,5aR,6R,12aR)-4-(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide;ethanol;hydrate; hydrochloride

Synonyms: Doxycycline Hyclate/Monohydrate; Doxycycline monohydrate

CAS number: 24390-14-5

pKa: 3.5, 7.7, 9.5

Appearance: Yellow crystalline powder

Boiling point: 685.2 °C at 760 mmHg

Melting point: 201 °C

Solubility: It is soluble in water and methanol giving a clear, yellow-green solution. DX dissolves in dilute mineral acids, solutions of alkali hydroxides and carbonates. It is sparingly soluble in ethanol and insoluble in chloroform or ether.

Stability: Stable at 2-8 °C. Solutions of sodium chloride or glucose should be used within 48 hours of preparation and protected from direct sunlight.

2.8.2.2. Mechanism of action

DX is basically a bacteriostatic antibiotic which may become bactericidal at high concentrations. DX enters the bacterial cell by passive diffusion as well as active carrier-mediated transport. It then reversibly binds to the 30S ribosomal subunit of susceptible organisms and prevents the binding of aminoacyl transfer RNA. This

inhibits protein synthesis and bacterial cell growth. It has been also reported that DX inhibits MMP mainly by disrupting the active zinc domains (Nordström *et al.,* 1998).

2.8.2.3. Therapeutic uses

FDA has approved the use of DX in the treatment of respiratory tract infections, typhus fever, lymphogranuloma, trachoma, endocervical or rectal infections, relapsing fever, plague, cholera, brucellosis, anthrax, gonorrhea and syphilis. Based on MMP inhibitor activity, DX has been used for chronic wounds, periodontal disease (Golub *et al.,* 2001), rheumatoid arthritis ((Nordström *et al.,* 1998), corneal erosion and skeletal muscle reperfusion injuries (Roach et al. 2002).

2.8.2.4. Adverse effects and contradiction

DX should be used with caution due to the risk of dose-dependent side effects, such as oesophageal ulceration, photosensitivity, anemia, teratogenicity, hepatotoxicity, pseudomembranous colitis and gastro-oesophageal reflux disease on chronic use.

2.8.2.5. Role of DX in the management of periodontitis

DX or sub-antimicrobial dose DX (SDD) is only MMP enzyme inhibitor approved for clinical use in the US, Canada and Europe for the treatment of periodontitis (Giovagnoli *et al.,* 2010). In early studies, Golub *et al.,* (1990) reported that semisynthetic compound DX is more effective than the parent compound tetracycline in reducing excessive collagenase activity in the GCF of chronic periodontitis patients. Because DX was found more effective inhibitor of collagenase due to its safety profile, pharmacokinetic properties, and ready systemic absorption during clinical trials it is preferred over minocycline or tetracycline (Golub *et al.,* 1985; Golub *et al.,* 1995). In an effort to eliminate the side effects of long-term tetracycline therapy, especially the emergence of tetracycline-resistant organisms, SDD is preferred (Golub *et al.,* 2001). Subsequent studies using SDD therapy adjunctive to routine SRP indicated continued reductions in the excessive levels of collagenase in the GCF after one month of treatment (Caton and Ryan, 2011). The

results of title search for applicability of DX in periodontal diseases has been elaborated in Table 2.9.

Table 2.9: Recent literature survey of doxycycline for the management of periodontitis

Title	Reference
Cholesterol *in-situ* forming gel loaded with doxycycline hyclate for intra-periodontal pocket delivery	(Phaechamud and Setthajindalert, 2017)
Doxycycline hyclate-loaded bleached shellac *in-situ* forming microparticle for intraperiodontal pocket local delivery	(Phaechamud *et al.,* 2016)
Characterization of the release profile of doxycycline by PLGA microspheres adjunct to non-surgical periodontal therapy	(Moura *et al.,* 2015)
Controlled-release and antibacterial studies of doxycycline-loaded poly (ε-caprolactone) microspheres	(Raval *et al.,* 2014)
Efficacy of locally-delivered doxycycline microspheres in chronic localized periodontitis and on *Porphyromonas gingivalis*	(Rao *et al.,* 2012)
Clinical studies on the management of periodontal diseases utilizing subantimicrobial dose doxycycline (SDD)	(Caton and Ryan, 2011)
Formulation and release behavior of doxycycline–alginate hydrogel microparticles embedded into Pluronic F127 thermogels as a potential new vehicle for doxycycline intradermal sustained delivery	(Giovagnoli *et al.,* 2010)
Formulation and evaluation of PLA and PLGA *in-situ* implants containing secnidazole and/or doxycycline for treatment of periodontitis	(Gad *et al.,* 2008)
Development and evaluation of novel biodegradable microspheres based on poly (d, l-lactide-co-glycolide) and poly (ε-caprolactone) for controlled delivery of doxycycline in the treatment of human periodontal pocket: *in-vitro* and *in-vivo* studies	(Mundargi *et al.,* 2007)
Susceptibility of *Porphyromonas gingivalis* in biofilms to amoxicillin, doxycycline and metronidazole	(Larsen, 2002)

Adjunctive treatment with subantimicrobial doses of doxycycline: effects on gingival fluid collagenase activity and attachment loss in adult periodontitis	(Golub *et al.,* 2001)

2.9. Polymer specific review

2.9.1. Chitosan (CS)

CS is the second most abundant natural polymer after cellulose; obtained by alkaline N-deacetylation of chitin, which is the primary structural component of the outer skeletons of many marine creatures such as crustaceans, crab, shrimp shells and many other species such as insects and fungi chitin (Kas, 1997; Suheyla, 1997).

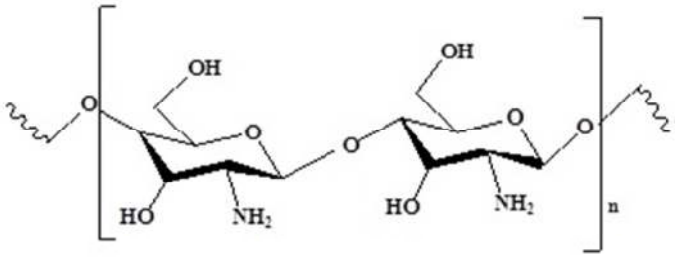

Fig 2.10. Structure of chitosan

2.9.1.1. Physicochemical properties

Chemical Name: Poly-b-(1,4)-2-Amino-2-deoxy-D-glucose

Molecular formula: $[C_{16}H_{18}O_8N_2]n$

Molecular weight: medium molecular weight

CAS Registry Number: 9012-76-4

Synonyms:2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; chitosani hydrochloride; deacetylated chitin; deacetylchitin; b-1,4-poly-D-glucosamine; poly-D-glucosamine; poly-(1,4-b-D-glucopyranosamine).

Description: CS occurs as odourless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and CS may look 'cottonlike'.

pH: 4.0 to 6.0 (1% w/v aqueous solution

Density: 1.35 to1.40 g/cm^3

Glass transition temperature: 203 °C

Solubility: Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. CS dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulfuric acids).

Functional Category: Coating agent; disintegrant; film-forming agent; mucoadhesive; tablet binder; viscosity increasing agent; cosmetic.

Stability and Storage: CS powder is a stable material at room temperature, although it is hygroscopic after drying. CS should be stored in a tightly closed container in a cool, dry place. The European pharmacopoeia specifies that CS should be stored at a temperature of 2-8 ºC.

Incompatibility: CS is incompatible with strong oxidizing agents.

Safety: Nontoxic and non irritant material, biocompatible with healthy and infected skin, biodegradable. CS is generally regarded as a non-toxic and non-irritant material. It is biodegradable, biocompatible with both healthy and infected skin.

Regulatory Status: CS is registered as a food supplement and approved by FDA.

2.9.1.2. *Drug delivery aspects*

CS is a non-toxic, biocompatible, biodegradable, and mucoadhesive natural polysaccharide polymer obtained by N-deacetylation of chitin and contains repeating units of 2-amino and 2-acetamido-2-deoxy-β-D-glucopyranose (Ma *et al.,* 2014; Songsurang *et al.,* 2011). On dissolution in the acidic medium it becomes positively charged attributable to the presence of $-NH_3^+$ ions and reverts back to neutral form in alkaline medium. The positively charged $-NH_3^+$ ions could interact with negatively charged molecules to form neutral complexes capable of controlling drug release. This characteristic feature of CS has made its widespread applicability in controlled release carriers Table 2.10.

Table 2.10: Literature survey of studies done on chitosan-based drug delivery systems.

Title	Reference
Fabrication of chitosan microspheres using vanillin/TPP dual crosslinkers for protein antigens encapsulation	(Walke *et al.,* 2015)
Preparation and characterization of vanillin cross-linked chitosan microspheres of pterostilbene	(Zhang *et al.,* 2014)
Immobilized lipase on magnetic chitosan microspheres for transesterification of soybean oil	(Xie and Wang, 2012)
Evaluation of mucoadhesive hydrogels loaded with diclofenac sodium–chitosan microspheres for rectal administration	(El-Leithy *et al.,* 2010)
Glutaraldehyde cross-linked chitosan microspheres for controlled delivery of Zidovudine	(Nayak *et al.,* 2009)
Preparation and characterization of chitosan microspheres for doxycycline delivery	(Shanmuganathan *et al.,* 2008)
Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin	(Anal *et al.,* 2006)

2.9.2. Sodium Alginate (SA)

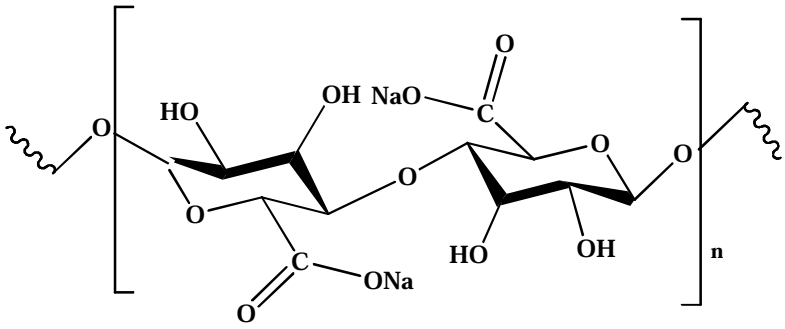

Fig 2.11. Structure of sodium alginate

2.9.2.1. *Physicochemical properties*

Chemical Name: Sodium alginate

Molecular formula: $(C_6H_8O_6)_n$

Molecular Weight: 1170.93 g/mol

CAS Registry Number: 9005-38-3

Synonyms: Alginato sodico; algin; alginic acid, sodium salt; E401; Kelcosol; Keltone; natrii alginas; Protanal; sodium polymannuronate.

Description: SA occurs as an odorless and tasteless, white to pale yellowish-brown colored powder.

pH: 7.2 (1% w/v aqueous solution)

Density: 2.11 g/cm^3

Solubility: Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3. Slowly soluble in water, forming a viscous colloidal solution

Functional Category: Stabilizing agent; suspending agent; tablet and capsule disintegrant; tablet binder; viscosity increasing agent.

Stability and storage: Aqueous solutions of SA are most stable at pH 4-10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of SA exposed to differing temperatures had a viscosity 60-80% of its original value after storage for 2 years. Solutions should not be stored in metal containers. SA is a hygroscopic material and should be stored in an airtight container in a cool, dry place.

Incompatibility: SA is incompatible with acridine derivatives, crystal violet, phenylmercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%.

Safety: It is generally regarded as a nontoxic and non-irritant material, although excessive oral consumption may be harmful. The WHO has not specified an acceptable daily intake for alginic acid and alginate salts as the levels used in food do not represent a hazard to health.

Regulatory Status: GRAS listed and accepted in Europe as a food additive. Included in the FDA Inactive Ingredients Database, Canadian List of Acceptable Non-medicinal Ingredients and in non-parenteral medicine licensed in the UK.

2.9.2.2. *Drug delivery aspects*

SAs are a natural anionic polysaccharide polymers, formed of alternating units of 1-4 linked α-L-guluronic (G) and β-D-mannuronic (M) acid residues (Zeeb *et al.,* 2015). It is the sodium salt of alginic acid extracted from the cell walls of brown algae. They are favored in biomedical and drug delivery area due to its biodegradable, biocompatible and mucoadhesive characteristics. In addition to its use in drug delivery, it is also identified as food product due to its non-toxic nature at large concentrations (Hoad *et al.,* 2009). The success of SA in drug delivery is established on its competence of interactions with cationic divalent ions such as calcium (Ca). Ca has strong affinity for carboxylic acid moieties of G and M blocks of SA. These cationic-anionic interactions forms "egg-box like structure" whereby carboxylate units of SA molecules (box) are coordinated by divalent cations (eggs) (Li *et al.,* 2011; Zeeb *et al.,* 2015). Ca-SA networks had shown entrapment of large range of products as listed in Table 2.11.

Table 2.11: Literature survey of studies done on role of sodium alginate in drug delivery systems.

Title	Reference
Design, optimization and characterizations of chitosan fortified calcium alginate microspheres for the controlled delivery of dual drugs	(Yadav *et al.,* 2017)
Preparation methods and applications behind alginate-based particles	(Lopes *et al.,* 2017)
Preparation and characterization of sodium alginate/chitosan microparticles containing esculin	(Tsirigotis-Maniecka *et al.,* 2016)
Retention and release of oil-in-water emulsions from filled hydrogel beads composed of calcium alginate: impact of emulsifier type and pH	(Zeeb *et al.,* 2015)
Retention and release of oil-in-water emulsions from filled hydrogel beads composed of calcium alginate: impact of emulsifier type and pH	(Martinez *et al.,* 2013)
Quality by design approach for developing chitosan-Ca-alginate microspheres for colon delivery of celecoxib-hydroxypropyl-β-cyclodextrin-PVP complex	(Mennini *et al.,* 2012)
Control of lipase digestibility of emulsified lipids by encapsulation within calcium alginate beads	(Li *et al.,* 2011)
Preparation of alginate coated chitosan microparticles for vaccine delivery	(Li *et al.,* 2008)
Emulsion and macromolecules templated alginate based polymer microspheres	(Zhang *et al.,* 2006)

2.9.3. Pluronics® (P127 and P68)

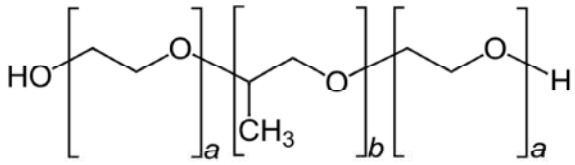

Fig 2.12. General structure of pluronics. Pluronic F127: a= 200.45, b=65.17 and Pluronic F68: a= 152.73, b=80.97

2.9.3.1. Physicochemical properties

Chemical Name: a-Hydro-o-hydroxypoly(oxyethylene)poly(oxypropylene) poly-(oxyethylene) block copolymer

Empirical Formula: $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$.

CAS Registry Number: 9003-11-6

Synonyms: Poloxamer (non-proprietary name), Lutrol, Synperonics, and Kolliphor.

Functional Category: Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

Description: Poloxamers generally occur as white, waxy, free-flowing prilled granules, or as cast solids. They are practically odorless and tasteless. At room temperature, poloxamer 124 occurs as a colorless liquid.

Chemistry: Pluronics are difunctional block copolymer surfactant terminating in primary hydroxyl groups. They are non-ionic surfactant that is 100% active and relatively nontoxic. They are class of 'aba' triblock copolymers commercially available as Pluronic® (non-proprietary name "poloxamers") offers a pool of more than 50 amphiphilic, water-soluble and polymorphic materials (a = hydrophilic block poly(ethylene oxide) (PEO) and b = hydrophobic block poly(propylene oxide) (PPO) (Table 2.12). The original manufacturer BASF introduced a specific nomenclature consisting of a letter indicating the morphism of each copolymer (liquid (L), paste (P), and flake (F)) and providing 1/300 of the molar mass of the PPO block per unimer. The last digit shows one-tenth of the molar mass percentage of PEO content per unimer (Pitto-Barry and Barry, 2014).

Therapeutic use: Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

Stability and Storage Conditions: Aqueous solutions are stable in the presence of acids, alkalis, and metal ions. However, aqueous solutions support mold growth. The bulk material should be stored in a well-closed container in a cool, dry place.

Incompatibilities: Depending on the relative concentrations, poloxamers are incompatible with phenols and parabens.

Safety: Poloxamers are used in a variety of oral, parenteral, and topical pharmaceutical formulations, and are GRAS. Poloxamers are not metabolized in the body.

Regulatory Status: Included in the FDA Inactive Ingredients Database (IV injections; inhalations, ophthalmic preparations; oral powders, solutions, suspensions, and syrups; topical preparations). Included in non-parenteral medicines licensed in the UK and Canadian List of Acceptable Non-medicinal Ingredients.

Table 2.12. Difference between physical properties of Pluronic F127 and Pluronic F68.

Physical properties	Pluronic F127 (P127)	Pluronic F68 (P68)
Average molecular weight (Da)	12600	8400
Specific gravity, 77/25 °C	1.05	1.06
Viscosity, cps at 77 °C	3100	1000
Melting point	56 °C	52 °C
Surface tension (0.1% aqueous) at 25 °C	41 dynes/cm	50 dynes/cm
HLB value	18 – 23	29
Solubility in water at 25 °C	> 10%	> 10%
Critical micellar concentration (CMC)	$2.8X10^{-6}$	$2.8X10^{-6}$
pH (2.5% aqueous)	6.0-7.0	6.0-7.5

2.9.3.2. *Drug delivery aspects*

Over the past two decades, this copolymer has been investigated extensively for various applications Table 2.13. Early studies evaluated P127 thermosensitive solutions for the treatment of burns, topical administration of anticancer agents, and sustained delivery of drugs after extravascular parenteral injection. P68 does not have thermosensitive behaviour; however it is used together with P127 for adjusting transition temperature of thermosensitive gel. Pluronics® or poloxamers are used in injectable formulations as they can be sterilised by autoclaving (Dimitrova *et al.,* 2000). After parenteral injection, poloxamer gels can prolong drug release compared to solutions, but the delivery period rarely exceeds a few days. This characteristic makes poloxamer gels interesting for short-term therapies like pain management,

infection treatment, and fertility control (Bermudez and Grau, 2011; Bilensoy *et al.,* 2006; Shen *et al.,* 2012; Yuan *et al.,* 2012). P68 has also been used as an emulsifying agent for fluorocarbons used as artificial blood substitutes, and in the preparation of solid-dispersion systems (Rowe *et al.,* 2009).

Table 2.13. Literature survey of applications of pluronics or poloxamers in drug delivery systems.

Topic	Reference
Formulation, *in-vitro* characterization and clinical evaluation of curcumin *in-situ* gel for treatment of periodontitis	(Nasra *et al.,* 2017)
Poloxamer-based thermoresponsive ketorolac tromethamine *in-situ* gel preparations: Design, characterisation, toxicity and transcorneal permeation studies	(Fathalla *et al.,* 2017)
Tromethamine loaded nanodispersion incorporated into thermosensitive *in-situ* gel for prolonged ocular delivery	(Morsi *et al.,* 2016)
Poloxamer-based *in-situ* hydrogels for controlled delivery of hydrophilic macromolecules after intramuscular injection in rats	(Zhang *et al.,* 2015)
Thermoresponsive ophthalmic poloxamer/tween/carbopol *in-situ* gels of a poorly water-soluble drug fluconazole: preparation and *in-vitro–in-vivo* evaluation	(Lihong *et al.,* 2014)
Enhancement in bioavailability of ketorolac tromethamine via intranasal *in-situ* hydrogel based on poloxamer 407 and carrageenan	(Li *et al.,* 2014)
Thermosensitive and mucoadhesive *in-situ* gel based on poloxamer as new carrier for rectal administration of nimesulide	(Yuan *et al.,* 2012)
Thermosensitive poloxamer-based injectables as controlled drug release platforms for veterinary use: Development and *in-vitro* evaluation	(Bermudez and Grau, 2011)
A poloxamer/chitosan *in-situ* forming gel with prolonged retention time for ocular delivery	(Gratieri *et al.,* 2010)
Development of a poloxamer analogs/bioadhesive polymers-based *in-situ* gelling ophthalmic delivery system for tiopronin	(Jiang *et al.,* 2009)

In-situ gel forming systems of poloxamer 407 and hydroxypropyl cellulose or hydroxypropyl methyl cellulose mixtures for controlled delivery of vancomycin	(Talasaz *et al.,* 2008)
Optimization and physicochemical characterization of thermosensitive poloxamer gel containing puerarin for ophthalmic use	(Qi *et al.,* 2006)
Sustained release of lidocaine from Poloxamer 407 gels	(Ricci *et al.,* 2005)
rhEGF/HP-β-CD complex in poloxamer gel for ophthalmic delivery	(Kim *et al.,* 2002)
Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of poloxamer gels containing diclofenac sodium	(Yong *et al.,* 2001)

2.9.4. Sodium Tripolyphosphate (TPP)

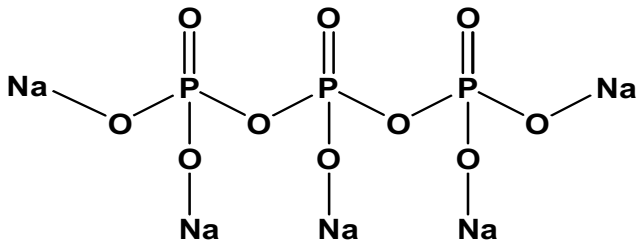

Fig 2.13. Structure of sodium tripolyphosphate

2.9.4.1. Physicochemical properties

Chemical name: pentasodium;[oxido(phosphonatooxy)phosphoryl] phosphate

Formula: $Na_5O_{10}P_3$

Molecular Weight: 367.86 g/mol

CAS Number: 7758-29-4

Synonyms: triphosphoric acid, pentasodium salt, triphosphoric acid, sodium salt triphosphoric acid, sodium tripolyphosphate anhydrous, tetrasodium tripolyphosphate

Description: white crystalline powder

Melting point: 622 °C

Solubility: Freely soluble in water 20 g/100 ml (20 ºC) and insoluble in ethanol

Density: 2.52 g/cm³

pH: Between 9.1 and 10.2 (1% solution)

Category: Food Additives, sesquestrant, texturiser, thickner, stabilizers and oxidants.

Stability and storage: Storage temperature: no restrictions.

Incompatibility: Incompatible with strong oxidizing agents, strong acids, hygroscopic.

Safety: It has met Safer Choice Criteria for its functional ingredient class and best-in-class chemical and among the safest available for a particular function.

FDA Requirements: TPP has been approved as a GRAS reagent by US FDA when used in accordance with good manufacturing practice (Huang and Lapitsky, 2011)

2.9.4.2. *Drug delivery aspects*

TPP is a non-toxic, ionic multivalent anion (carrying five negative charges) capable of reacting with positively charged amino groups of chitosan (Ji *et al.,* 2010a; Shu and Zhu, 2001). Extent of interaction is dependent on pH of solution. The literature reported on TPP are listed in Table 2.14.

Table 2.14. Literature survey of research done on tripolyphosphate based drug delivery systems.

Topic	Reference
Guided bone regeneration with tripolyphosphate cross-linked asymmetric chitosan membrane	(Ma *et al.,* 2014)
Rheological properties of chitosan–tripolyphosphate complexes: From suspensions to microgels	(Li and Huang, 2012)
Insight on the formation of chitosan nanoparticles through ionotropic gelation with tripolyphosphate	(Koukaras *et al.,* 2012)
Effect of crosslinked condition on characteristics of chitosan/tripolyphosphate/genipin beads and their application in the selective adsorption of phytic acid from soybean whey	(Yang *et al.,* 2011)
Electrospray fabrication of doxorubicin-chitosan-tripolyphosphate nanoparticles for delivery of doxorubicin	(Songsurang *et al.,* 2011)
Development of chitosan–tripolyphosphate fibers through pH dependent ionotropic gelation	(Pati *et al.,* 2011)

Monovalent salt enhances colloidal stability during the formation of chitosan/tripolyphosphate microgels	(Huang and Lapitsky, 2011)
A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery	(Shu and Zhu, 2000)

2.9.5. Glutaradehyde (GLU)

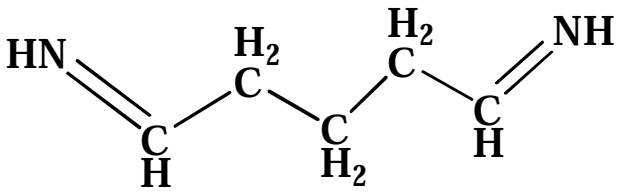

Fig 2.14. Structure of glutaraldehyde

2.9.5.1. Physicochemical Properties

Chemical name: 1,5-Pentanedial

Molecular Formula: $C_5H_8O_2$

Molecular Weight: 100.117 g/mol

CAS registry number: 111-30-8

Synonyms: Cidex, Diswart, Gludesin, Glutaral, Glutaraldehyde, Glutardialdehyde, Glutarol, Korsolex, Novaruca, Sekumatic, Sonacide, Sporicidin,5-Pentanedial; Glutaral;mGlutaric dialdehyde;

Physical description: glutaraldehyde solution is colorless liquid with pungent odor.

Boiling point: 187-189 °C (decomposes)

Freezing point: 14 °C

Solubility: Miscible with water, Soluble in ethanol, benzene, ether, Miscible with ethanol

Density: 1.10

pH: Mildly acidic (50% solution)

Stability: Stable under recommended storage conditions. Stable in light, oxidizes in air, polymerizes in heat

Storage: Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. The recommended storage temperature is -20 °C under inert gas.

Incompatibilities: Incompatible with strong bases, strong oxidizing agents, and strong acids. Alkaline solutions of gluaraldehyde react with alcohol, ketones, amines, hydrazines, and proteins.

Health Hazard: Contact with liquid causes severe irritation and corrosive to eyes, skin and respiratory tract. A harmful contamination of the air will be reached rather slowly on evaporation at 20 °C. It is classified as a non-carcinogen by the European Union or the US Environmental Protection Agency. Its use as a biocide in water treatment, hydraulic fracturing fluids and oil-field applications, may direct release to the environment (Takigawa and Endo, 2006). The persons working with GLU are advised to wear safety goggles or eye protection in combination with breathing protection.

FDA Requirements: Microcapsules may be formulated for encapsulating discrete particles of flavoring substances that are generally recognized as safe.

Therapeutic use: Disinfectant, sanitizer, crosslinking agent, tanning agent for leather, paper and textile industries to improve wet strength and dimensional stability of fibers.

2.9.5.2. *Drug delivery aspects*

The reactive aldehyde groups present in GLU tend to form covalent bonds with the $-NH_2$ groups of chitosan. The covalent bond networks are exploited in drug delivery since many decades. Some of the recent researches are listed in Table 2.15.

Table 2.15: Literature survey of applications of glutaraldehyde in drug delivery systems.

Title	Reference
Development and Evaluation of Biodegradable Chitosan Films of Metronidazole and Levofloxacin for the Management of Periodontitis	(Khan *et al.,* 2015)
Studies on Glutaraldehyde Crosslinked Chitosan Hydrogel Properties for Drug Delivery Systems	(Mirzaei B *et al.,* 2013)
Glutaraldehyde–chitosan and poly (vinyl alcohol) blends, and fluorescence of their nano-silica composite films	(Hu *et al.,* 2013)
Formulation and characterization of glutaraldehyde cross-linked chitosan biodegradable microspheres loaded with famotidine	(Ramachandran *et al.,* 2011)
Glutaraldehyde cross-linked chitosan microspheres for controlled delivery of Zidovudine	(Nayak *et al.,* 2009)
Glutaraldehyde cross-linked chitosan microspheres for controlled release of centchroman	(Gupta and Jabrail, 2007)

2.9.6. Vanillin (VAN)

VAN is chemically 4-hydroxy-3-methoxybenzadehyde, obtained by extraction from bean or pod of climbing orchid *Vanilla planifolia* (Walton *et al.,* 2003).

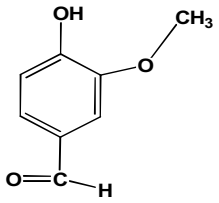

Figure 2.15. Structure of vanillin

2.9.6.1. Physicochemical Properties

Chemical name : 4-hydroxy-3-methoxybenzaldehyde

Molecular Formula: $C_8H_8O_3$

Molecular Weight: 152.14 g/mol

Synonyms:Vanillic aldehyde, Vanillaldehyde, 4-hydroxy-m-anisaldehyde, methylprotocatechuic aldehyde, 4-Hydroxy-m-anisaldehyde; p-hydroxy-m-methoxybenzaldehyde; 3-methoxy-4-hydroxybenzaldehyde.

Description: White or cream, crystalline needles or powder with characteristic vanilla odor and sweet taste.

CAS Registry Number: 121-33-5

Functional Category: Flavoring agent

Use category: Food/foodstuff additives

Melting point: 80-81 °C

Solubility: 25ºC- 10 g/l

pH Value: 4.3 (5% in water at 25 ºC)

Acidity/alkalinity : Aqueous solutions are acid to litmus.

Boiling point: 284-285 ºC (with decomposition)

Density (bulk): 0.6 g/cm^3

Flash point: 153 ºC (closed cup)

Melting point: 81-83 ºC

Specific gravity: 1.056 (liquid)

Incompatibilities: Incompatible with acetone, forming a brightly colored compound. A compound practically insoluble in ethanol is formed with glycerin.

Stability and Storage Conditions: VAN oxidizes slowly in moist air and is affected by light. The bulk material should be stored in a well-closed container, protected from light, in a cool, dry place.

Safety: There have been few reports of adverse reactions to VAN, although it has been speculated that cross-sensitization with other structurally similar molecules, such as benzoic acid, may occur. Adverse reactions that have been reported include contact dermatitis and bronchospasm caused by hypersensitivity. According to WHO allocated estimated acceptable daily intake for VAN, it is considered safe for consumption.

Regulatory approval Status: VAN was given GRAS (Generally Recognized As Safe) status by FEMA (Flavor and Extract Manufacturers' Association) in 1965 and is approved by the FDA for food use (GRAS). The Joint FAO/WHO Expert Comittee on Food Additives (1967) has published a monograph and specifications for VAN, giving an unconditional ADI (Acceptable Daily Intake) of 0-10 mg/kg (Opdyke, 1977).

Therapeutic use: VAN is used as pharmaceutic aid (flavour), as flavouring agent in confectionary, beverages, foods, as a odour agent in perfumery and as reagent in analytical chemistry (Bogdan *et al.,* 2009; Burri *et al.,* 1989; Stroescu *et al.,* 2013). Besides, VAN is also useful in the synthesis of drugs such as Aldomet, L-dopa and Trimethoprim. It is also used in perfumes and to mask unpleasant odors or tastes of bitter drugs (Bogdan *et al.,* 2009).

2.9.6.2. *Drug delivery aspect*

The structure of VAN has reactive phenolic –OH group. The aldehyde groups tend to form Schiff base with the $–NH_2$ groups of chitosan and while phenolic -OH group forms H-bonds with CS. These crosslink bonds or networks are responsible for controlled drug release from CS-VAN crosslinks (Cheng et al. 2010; Peng et al. 2010). Very few researches and reports are available on CS-VAN crosslinked formulations for controlled drug deliveries are discussed in Table 2.16.

Table 2.16. Literature survey of applications of vanillin in drug delivery systems.

Topic	Reference
Development of drug-loaded chitosan-vanillin nanoparticles and its cytotoxicity against HT-29 cells	(Li *et al.,* 2016)
Preparation and characterization of vanillin-crosslinked chitosan therapeutic bioactive microcarriers	(Zou *et al.,* 2015)
Fabrication of chitosan microspheres using vanillin/TPP dual crosslinkers for protein antigens encapsulation	(Walke *et al.,* 2015)
Preparation and Characterization of Binary Blend Films Containing Chitosan and Vanillin	(Ravindra *et al.,* 2015)
Preparation and characterization of vanillin cross-linked chitosan microspheres of pterostilbene	(Zhang *et al.,* 2014)
Formulation of vanillin cross-linked chitosan nanoparticles and its characterization	(Wang *et al.,* 2011)
Vanillin cross-linked chitosan microspheres for controlled release of resveratrol	(Peng *et al.,* 2010)