DEVELOPMENT AND EVALUATION OF NANOGEL SYSTEM LOADED WITH TRICLOSAN AND FLURBIPROFEN FOR TREATMENT OF PERIODONTITIS

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DEVELOPMENT AND EVALUATION OF NANOGEL SYSTEM LOADED WITH TRICLOSAN AND FLURBIPROFEN FOR TREATMENT OF PERIODONTITIS

by

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DEDICATION

I dedicate this thesis to my beloved parents, wife, children (Maimunah, Adnan and Saimah), brothers, and sisters for their endless support with love, encouragement, and prayers. They firmly stood by me during happy and difficult times, and keep encouraging me to move on. This unquantifiable support helped me tremendously to conquer all the confronted challenges in my PhD journey. Thank you for all your sacrifices which enabled me to achieve my goals.

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Long live Usmanu Danfodiyo University Sokoto!

Long live Universiti Sains Malaysia!

Long live Federal Republic of Nigeria!

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degrees centigrade
AB	Alveolar bone
ANOVA	Analysis of variance
APIs	Active pharmaceutical ingredients
ATR	Attenuated total reflection
CDER	Center for Drug Evaluation and Research
$CHPNH_2$	Cholesterol-bearing pullulan modified with amino-groups
COX	Non-selective cyclooxygenase
CS	Chitosan
DMAHDM	Dimethylaminohexadecyl methacrylate
DOE	Design of experiments
DSC	Differential Scanning Calorimetry
DTGS	Deuterated triglycine sulfate
e.g.	exempli gratia
En	Enamel
ENR	Enoyl-acyl carrier protein reductase enzyme
EP	Experimental periodontitis
FDA	Food and Drug Administration
FLB	Flurbiprofen
FTIR	Fourier transform infrared spectroscopy
h	Hour
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
i.e.	That is; in other words

ICH	International Conference on Harmonization
IL	Interleukin
IST	Isothermal stress testing
KBr	Potassium bromide
KP	Kolliphor® P188
LOD	Limit of Detection
LOQ	Limit of Quantification
M	Molar
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute
mL	Milliliter
mm	Millimeter
MPC	2-methacryloyloxyethyl phosphorylcholine
N	Number of theoretical plates
NG	Nanogels
NIC	Non-induced control
nm	Nanometer
NOHSA	National Oral Health Survey of Adults
NPs	Nanoparticles
NSAIDs	Non-steroidal anti-inflammatory drugs
NT	Non-treated
PCL	Poly-ε-caprolactone
PDI	Polydispersity index
PEG-PLA	Poly(ethylene glycol)–poly(lactic acid)

PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PVAL	Poly(vinyl alcohol)
RE	Relative Error
RH	Relative Humidity
rpm	Rotation per minute
RSD	Relative Standard Deviation
SD	Standard deviation
SEM	Scanning Electron Microscopy
TCS	Triclosan
TCS-loaded NPs	Triclosan loaded nanoparticles
TEM	Transmission Electron Microscopy
TNF-a	Tumor Necrosis Factor-alpha
UK	United Kingdom
USP	United States Pharmacopoeia
UV	Ultraviolet
UV-VIS	Ultraviolet-Visible
v/v	Volume by Volume
w/v	Weight by Volume
XRPD	X-ray powder diffraction
ΔH_{fus}	Heat of fusion
λ_{max}	Maximum absorption
$\mu g\ mL^{-1}$	Microgram per milliliter

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PEMBANGUNAN DAN PENILAIAN SISTEM NANOGEL YANG DIMUATKAN DENGAN TRICLOSAN DAN FLURBIPROFEN UNTUK RAWATAN PERIODONTITIS

ABSTRAK

Periodontitis sangat berleluasa dan merupakan salah satu penyakit pergigian utama yang memberi kesan kepada berjuta manusia di seluruh dunia. Bakteria gram negatif anaerob dan toksinnya telah dikenalpasti sebagai penyebab utama penyakit ini. Banyak sistem penghantaran drug setempat telah dicadangkan, tetapi fokus utama kebanyakannya ialah membunuh mikrob penyebab dan bukannya penyasaran keradangan yang memainkan peranan penting dalam etiologi penyakit. Lebih-lebih lagi, terdapat kesukaran untuk mengakses poket periodontal dan mencapai masa kediaman yang mencukupi oleh sistem-sistem sedia ada, oleh itu menjadikan sistem-sistem sedia ada hanya sebahagiannya berjaya. Oleh itu, matlamat kajian ini adalah untuk membangunkan dan menilai suatu nanogel (NG) bioserasi yang baru yang dimuatkan bersama dengan triclosan (TCS) dan flurbiprofen (FLB) untuk rawatan periodontitis intra-poket yang berkesan. TCS dan FLB merupakan sebatian berhablur dan tidak larut air. Sifat ini menghadkan penghantarannya dalam bentuk tulen ke dalam poket periodontal, maka wajar diformulasikan dalam sistem nanopartikulat. Kaedah HPLC, kajian keserasian, dan nanopartikel (NP) dan NG telah dibangunkan. Formulasi-formulasi telah dicirikan dengan ekstensif dengan menggunakan mikroskop elektron pengimbasan (SEM), mikroskop elektron transmisi (TEM), saiz partikel, indeks polidispersiti (PDI), keupayaan zeta, kalorimetri pengimbasan pembezaan (DSC), difraksi serbuk sinar-X (XRPD), spektroskopi inframerah fourier transform (FTIR), reologi, pembengkakan, pelepasan *in vitro*, kestabilan dan kajian *in vivo*.

Kaedah HPLC yang dibangunkan berjaya memencilkan dan mengkuantifikasikan TCS dan FLB pada masa retensi (t_R) TCS dan FLB pada 12.5 min dan 10.1 min masing-masing. Hasil kajian keserasian mencadangkan ketiadaan ketakserasian farmaseutikal antara semua komponen dipilih. Penggunaan perisian Design-Expert® dalam pembangunan NP membantu dalam mengenal pasti atribut kualiti kritikal untuk formulasi NP yang dimuatkan TCS. NP yang dimuatkan TCS yang teroptimum telah diikatsilang kepada hidrogel chitosan (CS) yang dimuatkan FLB yang menghasilkan NG yang dimuatkan dengan TCS dan FLB. Kandungan drug TCS dan FLB dalam NG ialah 93.67 ± 3.51 dan 96.33 ± 2.08% masing-masing. NG yang dimuatkan TCS dan FLB mempunyai saiz partikel dan PDI 313.62 ± 66.60 nm dan < 0.3, masing-masing. SEM dan TEM menunjukkan bahawa kebanyakan NG berbentuk sfera. Secara fizikal, DSC dan XRPD menunjukkan perubahan dalam bentuk pepejal drug-drug di dalam NG. Tambahan lagi, FTIR menunjukkan interaksi drug-polimer yang minima dalam formulasi NG yang dibangunkan. Daya bioadhesif NG yang dibangunkan didapati sebanyak 137.22 ± 5.05 g. Kajian pelepasan drug *in vitro* dari sistem NG menunjukkan pelepasan panjang dan cepat untuk TCS dan FLB, masing-masing. Hal ini disebabkan enkapsulasi TCS dalam NP poly-ε-caprolactone (PCL), dan FLB dalam hidrogel CS yang digunakan sebagai pembawa NP yang boleh dihakis dalam keadaan berasid air liur simulasi pada 37 °C. Hasil kajian antimikrob mengesahkan aktiviti antimikrob NG terhadap kedua-dua bakteria gram-negatif dan gram-positif. Kajian *in vivo* dalam tikus periodontitis yang disebabkan oleh ligatur menunjukkan peningkatan pertumbuhan tulang alveolar yang luar biasa dan kesan anti-radang yang ditingkatkan dalam NG yang dibangunkan. Oleh itu, NG yang dimuatkan TCS dan FLB yang telah dibangunkan didapati berpotensi untuk rawatan periodontitis dengan berkesan.

DEVELOPMENT AND EVALUATION OF NANOGEL SYSTEM LOADED WITH TRICLOSAN AND FLURBIPROFEN FOR TREATMENT OF PERIODONTITIS

ABSTRACT

Periodontitis is a highly prevalent major dental disease that affects millions of people around the globe. Anaerobic gram-negative bacteria and its toxins have been strongly implicated as a prime cause of the disease. A great number of local drug delivery systems have been proposed, but they were mainly made to focus on killing the causative microbes rather than also targeting the inflammation which also plays a significant role in the aetiology of the disease. Moreover, there is difficulty in accessing the periodontal pocket and attaining adequate residence time by these systems, hence being only partially successful. Therefore, the aim of this study was to formulate and evaluate a novel biocompatible nanogel (NG) co-loaded with triclosan (TCS) and flurbiprofen (FLB) for effective intra-pocket treatment of periodontitis. TCS and FLB are highly crystalline compounds and practically insoluble in water. These properties restrict delivery of their pure forms into the periodontal pocket, hence warranted their formulation into a nanoparticulate system. HPLC method development, compatibility study, nanoparticles (NPs) and NG development were performed. The formulations were extensively characterised by scanning electron microscopy (SEM), transmission electron microscopy (TEM), particle size, polydispersity index (PDI), zeta potential, differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), fourier transform infrared spectroscopy (FTIR), rheology, swelling, erosion, bioadhesion, *in vitro* release, antimicrobial, stability and *in vivo* studies. The developed HPLC method was able to successfully separate and quantify TCS and FLB at retention times (t_R) of 12.5 and 10.1 min, respectively. The

findings from the compatibility study suggested lack of pharmaceutical incompatibility between all the selected components. Design-Expert® software which was used in the design and optimisation of NPs has helped in identifying critical quality attributes that led to the development of optimised TCS-loaded NPs. The optimised TCS-loaded NPs were physically crosslinked with FLB-loaded chitosan (CS) hydrogel which resulted in TCS and FLB-loaded NG. Drug content of the loaded TCS and FLB in the NG was found to be 93.67 ± 3.51 and 96.33 ± 2.08 %, respectively. The TCS and FLB-loaded NG exhibited particle size and PDI of 313.62 ± 66.60 nm and < 0.3, respectively. SEM and TEM displayed the NG mostly in spherical shape. Physically, DSC and XRPD revealed a change in the solid state of the drugs in the NG. Moreover, FTIR indicates minimal drug-polymer interaction in the developed NG formulation. Bioadhesive force of the developed NG was found to be 137.22 ± 5.05 g. *In vitro* drug release study from the NG system displayed sustained and rapid release of TCS and FLB, respectively. This was mainly due to the encapsulation of TCS in the poly-ε-caprolactone (PCL) NPs, and FLB in the CS hydrogel which was used as the NPs' carrier that could be eroded in an acidic condition of simulated saliva at 37 °C. The result of the antimicrobial study confirmed the antimicrobial activities of the NG against both gram-negative and gram-positive bacteria. *In vivo* study on the ligature-induced periodontitis rats revealed remarkable alveolar bone regeneration and anti-inflammatory effects of the developed NG. Thus, the formulated TCS and FLB-loaded NG was found to be a potentially useful candidate for the effective treatment of periodontitis.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Background of Study

1.1.1 Periodontal Disease

The term "periodontal disease", also known as gum disease, refers to the common inflammatory pathological conditions that affects the structures of periodontium such as gums (gingiva), periodontal ligament, alveolar bone and cementum (Aminu and Toh, 2017; Jain et al., 2008; Page and Kornman, 1997; Schwach-Abdellaoui et al., 2000). The disease is a major dental illness that affects millions of people around the globe. A study published in Lancet in 2005 revealed that up to 90% of the world population might be affected by periodontal disease (Pihlstrom et al., 2005). Similarly, a recent study that was published in 2017 indicated that the disease might be the most common disease of human beings and its global burden has increased by more than 50% from the year 1990 to 2010 (Tonetti et al., 2017). In Malaysia, the 2010 report of the National Oral Health Survey of Adults revealed that about 94% of all Malaysian dentate adults have some kind of periodontal diseases (Abdulaziz, 2014). The main categories of periodontal disease are gingivitis and periodontitis.

1.1.1(a) Gingivitis

At the early stage of periodontal disease, a condition called gingivitis will manifest, where the bacterial plaque and tartar will build up and trigger a reaction that causes gums to become swollen and reddish especially at the margins, and may bleed during brushing, mostly as a result of poor oral hygiene (Cochran, 2008; Mizrahi and Domb, 2008; Tanner, 2015). Gingivitis is the mildest form of periodontal disease, and it is very common and readily reversible by simple oral hygiene. The symptoms of

gingivitis are less pronounced in many cases, and it may even go unnoticed, so, many individuals neglect to treat it. Left untreated, gingivitis may gradually transform into irreversible periodontitis (an advanced form of periodontal disease) (Kim and Amar, 2006). Studies have shown that gingivitis always appears to precede periodontitis (Albandar and Rams, 2002; Mizrahi and Domb, 2008).

1.1.1(b) Periodontitis

Periodontitis is a prevalent dental inflammatory disorder that affects the soft tissue, as well as bone that surrounds and supports the tooth. The word "periodontitis" originates from the Greek terms, i.e., *peri-*, *-odont* and *-itis* referring to "around", "tooth" and "inflammation", respectively. Therefore, its literal meaning is "inflammation around the tooth" (European Federation of Periodontology, 2018; MedicineNet.com, 2018; Nordqvist, 2018). Anaerobic gram-negative bacteria and its toxins have been strongly implicated for causing the disease (Aminu and Toh, 2017; Jain et al., 2008; Page and Kornman, 1997). Some of the identified members of microbiota that were associated with periodontitis are *Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Campylobacter rectus, Aggregatibacter (Actinobacillus) actinomycetemcomitans, Eubacterium nodatum, Tannerella forsythia, Prevotella spp., Peptostreptococcus micros, Streptococcus intermedius, and Fusobacterium nucleatum* (Lakshmyya Kesavalu et al., 2007; Tanner, 2015). Additionally, host-mediated responses have also been found to be involved in the pathogenesis of the disease (Cai et al., 2008; Page and Kornman, 1997). It is well established that the inflammatory and immune responses play a critical role in the overall aetiology of periodontitis, through several host-related intrinsic or induced factors which causes the body to turn on itself resulting in serious injury. Pro-inflammatory mediators and cytokine networks such as

cyclooxygenase enzymes (which lead to development of prostaglandins, especially prostaglandin E2), interleukin (IL)-1, leukaemia inhibitory factor, tumour necrosis factor-alpha (TNF-a) and oncostatin M have been found to play essential roles in this process (Cai et al., 2008; Cochran, 2008; Graves and Cochran, 2003; Lerner, 2006). The accumulation of microbiota triggers the release of these mediators, which in turn stimulate the breakdown of connective tissue and destruction of periodontium. Figure 1.1 shows a comparison between a healthy tooth and periodontitis.

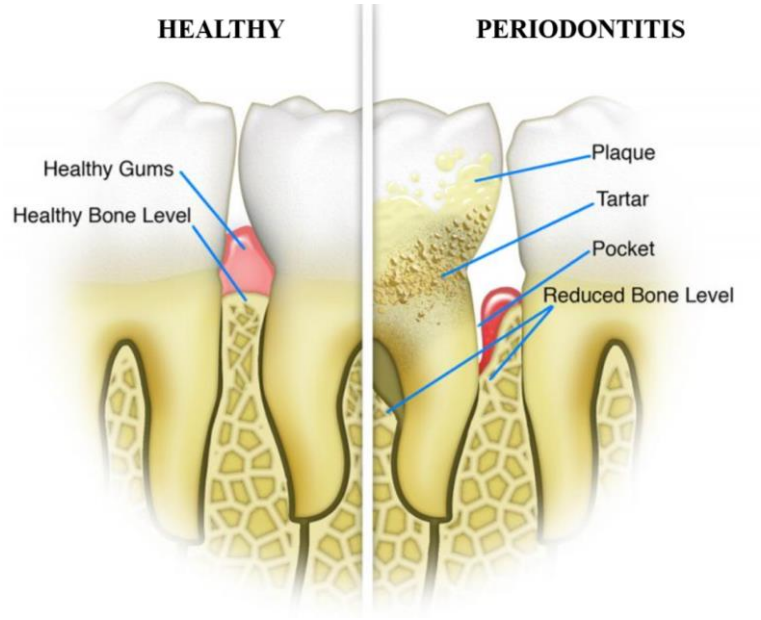

Figure 1.1: A comparison between healthy and diseased tooth. Periodontal pocket has developed on the diseased tooth. The figure was adopted from (Zuerlein, 2018)

During the progression of periodontitis, the epithelial and subepithelial connective tissue of the gingiva detaches from the tooth surface leading to an unusual depth of the gingival sulcus termed as ‘periodontal pocket’ (Vyas et al., 2000). This pocket provides an ideal medium for the proliferation of gram-negative anaerobic pathogenic bacteria. Thus, periodontitis is characterised by inflammation of the

structures adjacent to the teeth that extends deep into the tissues and causes loss of supporting connective tissue and alveolar bone (Pihlstrom et al., 2005). Without proper treatment, the bacteria's tartar and calculus will greatly build up on the teeth surfaces and in the periodontal pockets, and the pockets may perforate up to the periodontal ligament, resulting in loosening and loss of teeth as a consequence (Darveau, 2010; Jain et al., 2008).

There are several other risk factors besides poor oral hygiene that may directly or indirectly contribute to the development of periodontitis. Some of these important risk factors are: certain systemic diseases; smoking or chewing tobacco; older age; genetics; hormonal changes (in pregnancy or menopause) (Mayo Foundation for Medical Education and Research, 2018); malnutrition (including deficiency in vitamin C) (Nishida et al., 2000); excessive alcohol consumption (Pitiphat et al., 2003); stress (Genco et al., 1999); and medications such as steroids, anti-epileptic and chemotherapeutic drugs (Ireland Dental Health Foundation, 2018; Petersen and Ogawa, 2012; U.S. National Institute of Dental and Craniofacial Research, 2013).

1.1.2 The Potential Relationship between Periodontitis and Systemic Health

Many authors have reported studies that associated periodontitis to various systemic diseases (Ameet M. et al., 2013; Cullinan et al., 2009; Garcia et al., 2001). A review article summarised evidence linking periodontal diseases with cardiovascular disorders such as atherosclerosis, ischemic heart disease, coronary heart disease and myocardial infarction. Epidemiologic studies demonstrated these potential links through periodontal infections (Dhadse et al., 2010). Researchers have identified some common pathogens associated with periodontal infection in cardiovascular areas such as atherosclerotic, carotid, coronary and aortic plaques (Cairo et al., 2004; Iwai et al.,

2005). Several other studies have also demonstrated positive correlations between these two diseases.

Periodontitis has also been found to be a manifestation of other systemic diseases, especially in immuno-compromised patients such as those with human immunodeficiency virus (HIV) and those suffering from general infection (Petersen and Ogawa, 2012). Therefore, patients diagnosed with periodontitis are likely to be at higher risk of developing certain systemic diseases because the lesions arising from the disease have been recognised as reservoirs for the continuous systemic spread of anaerobic gram-negative bacteria, its antigens and some pro-inflammatory mediators like cytokines. Significant associations between periodontitis and diabetes mellitus, osteoporosis, preterm low birth weight and some other diseases have also been discovered (American Academy of Periodontology, 2018; Cullinan et al., 2009; Kim and Amar, 2006). All these findings strongly suggest that oral health may be an important indicator of systemic health.

Therefore, these studies imply the importance of effective interventional periodontal care that may improve not only oral health but also the overall systemic health. Hence, researchers must continue to explore novel treatment strategies for enhancing the therapeutic outcomes of periodontitis.

1.2 Statement of the Problem

Delivering a therapeutic agent to the targeted site has been a major hurdle in the treatment of periodontitis. The drawback of most conventional periodontal drug delivery systems after administration is the poor penetration of drug into the junctional epithelium (Aminu et al., 2017, 2013). Administering systemic medication such as antibiotics may serve as a vital supplement to scaling and root planning in the treatment

of periodontitis. For example, tetracycline, metronidazole, and amoxicillin either alone or in their combination have been employed to target a broad range of bacteria in the treatment of the disease (Addy and Langeroudi, 1984; Chaturvedi et al., 2012; Yadav et al., 2015). However, systemic antibiotic therapy is mostly unspecific and may lead to the inhibition of beneficial microflora in the gastro-intestinal tract (Bourrain et al., 2013; Martínez, 2017). Furthermore, frequent use of these drugs may lead to the development of resistance. Therefore, systemic antibiotics are now seldom used for the treatment of periodontitis because of its drawbacks such as: gastrointestinal intolerance, inadequate antibiotic concentration at the site of periodontal pocket, rapid decline of the plasma antibiotic concentration to subtherapeutic levels, requiring frequent dosing, lack of patient compliance, and hypersensitivity (Jain et al., 2008; Kataria et al., 2014).

Similarly, conventional local drug delivery systems of periodontitis are characterised by limited effectiveness, poor biodistribution, and lack of selectivity (Wilczewska et al., 2012). A great number of local drug delivery devices for this disease have been proposed (Table 1.1), but unfortunately, the difficulty in accessing the periodontal pocket has rendered these approaches only partially successful (Kashi et al., 2012). These apparent disadvantages have evoked an interest in the development of more effective intra-pocket drug delivery systems for the treatment of periodontitis. Since the disease is primarily confined in periodontal pockets, effective localised intra-pocket drug delivery systems may be far more beneficial than systemic medications.

Table 1.1: Some investigated localised drug delivery systems for the treatment of periodontal disease

Type of delivery system	Drug(s)/Active agent(s) used	Polymer(s) used	References
Microparticles	Metronidazole	Cross-linked CS	(Pichayakorn and Boonme, 2013)
	Ofloxacin	PLGA	(Jamal et al., 2012)
	Doxycycline	PLGA + PCL	(Shanmuganathan et al., 2008)
	Tetracycline	PLGA	(Esposito et al., 1997)
	Chlorhexidine	PLGA	(Yue et al., 2004)
Films	Ciprofloxacin & Diclofenac	CS	(Ahmed et al., 2009)
	Chlorhexidine	Ethyl cellulose	(Hirschfeld et al., 1984)
	Metronidazole	CS & PCL	(El-Kamel et al., 2007)
	Minocycline	PCL	(Kyun et al., 1990)
Chips	Chlorhexidine	Cross-linked hydrolysed gelatin & glycerin	(Mızrak et al., 2006)
	Chlorhexidine	PLGA	(Yue et al., 2004)
Strips	Chlorhexidine	Acrylic polymer	(Addy and Langeroudi, 1984)
	Metronidazole	Hydroxypropyl cellulose	(Wade et al., 1992)
	Doxycycline	Hydroxypropylmethylcellulose and methylcellulose	(Taner et al., 1994)
Gels	Tetracycline HCl & Metronidazole benzoate	CS	(Popa et al., 2013)
	Metronidazole	CS	(Akncbay et al., 2007)
Fibers	Tetracycline	PCL & cellulose acetate propionate	(Raheja et al., 2013)
Fibers	Chlorhexidine	Cellulose acetate	(Coventry and Newman, 1982)
Wafers	Benzylpenicillin & Tetracycline	PLGA & ethyl cellulose	(Bromberg et al., 2001)
Dendrimers	Triclosan	Polyamidoamine	(Gardiner et al., 2008)

Type of delivery system	Drug(s)/Active agent(s) used	Polymer(s) used	References
Implants	Secnidazole and/or Doxycycline	PLA and PLGA	(Gad et al., 2008)
Micelles	Triclosan	Micelles of poloxamine T1107	(Chiappetta et al., 2008)

Poly (lactic-co-glycolic acid) (PLGA); poly lactic Acid (PLA); poly-ε-caprolactone (PCL); chitosan (CS)

1.3 Nanoparticles in Periodontitis Therapy

Some important solutions to the problems discussed in Section 1.2 above came through the recent advancement and innovations in nanotechnology. Nanotechnology was described to comprise particles or structures that fall within the size range of 1 to 100 nm in diameter (US National Nanotechnology Initiative, 2017). However, from the pharmaceutical viewpoint, all particles or structures that are less than 1 µm in diameter are recognised as nanoparticles (NPs) (Williams III and Vaughn, 2007).

NPs are complex mixtures which involved interactions of two or more different type of components. In most cases, the exact composition of the NPs' structure, both surface and internal, is intimately related to their intended applications and commonly comprises multilayers and/or multiphase packed with APIs to provide the desired functions (Christian et al., 2008; Naito et al., 2018). These layers can be of two or three types, i.e. (1) a surface which can be functionalised, (2) a shell which may be intentionally added and it's often chemically different from the core of the NP, and (3) a core which is essentially the inner part of the NP and commonly associated with its fundamental properties that results to most of its applications (Christian et al., 2008; Khan et al., 2017).

The applications of NPs in modern medicine has a profound positive influence on pharmaceutical and biomedicines by contributing to better paths of healthcare delivery, ranging from disease prevention to ultimate treatment (Sahu et al., 2017).

There is growing interest in nanoparticulate systems which is attributable to the search for alternatives to some of the conventional drug delivery systems that lack desirable effectiveness. In recent years, formulation scientists and researchers have been deeply involved in investigating novel delivery systems which would enhance the pharmacological action of drugs at the same time decreasing its toxic side effects (Ding et al., 2013; Thassu et al., 2007). One outcome is the use of NPs that can provide site-specific or targeted drug delivery combined with optimal drug release profiles. Studies have shown that NPs has excellent potentials as drug carriers for effective delivery of therapeutic agents into the periodontal pockets (Piñón-Segundo et al., 2005). The advantages of NPs which include small size, site specificity, high dispersibility in aqueous medium, better stability during storage as compared to liposomes, controlled release rate, feasibility of incorporating both hydrophilic and hydrophobic substances, high carrier capacity, and improved dissolution and bioavailability, make them suitable candidates for delivering drugs into the periodontal pockets in the treatment of periodontitis. In addition, these systems may reduce the frequency of drug administration which in turn may improve patient compliance. It also has the potential to provide a uniform release of active agent over an extended period thus resulting in a high benefit to low-risk ratio as compared to conventional dosage forms (Pramod et al., 2014; Puri and Puri, 2013).

Researchers have studied that NPs can transport active pharmaceutical ingredients (APIs) across the junctional epithelium and can also be a potential intra-pocket carrier system for the delivery of APIs to the periodontal pocket. Piñón-Segundo et al. (2005) developed and characterised triclosan (TCS)-loaded NPs as a novel delivery system for the treatment of periodontitis, using emulsification-diffusion method. In their study, polylactic (PLA), poly(lactic-co-glycolic acid) (PLGA) and

cellulose acetate phthalate were used as carrier polymers, and poly(vinyl alcohol) (PVAL) as a stabiliser to prepare the NPs. Their results showed the NPs were less than 500 nm, with entrapment efficiency (EE) above 63.8% for all batches. An *in-vivo* study in dogs with experimental periodontitis (EP) suggested that TCS-loaded NPs were able to permeate across the junctional epithelium and deliver the loaded TCS, which in turn lead to a decrease in gingival inflammation (Piñón-Segundo et al., 2005).

In a similar development, TCS-loaded poly-ε-caprolactone (PCL) NPs for the treatment of periodontal infections was synthesised by Aminu et al. (2013). Box–Behnken statistical model was used for the design of experiments and optimisation, and solvent displacement method for the NPs development. The NPs were characterised and evaluated as per their morphology, particle size and distribution, polydispersity index, zeta potential, EE and drug loading, differential scanning calorimetry (DSC), cell viability assay, *in vitro* and accelerated stability studies. The results revealed that the synthesised NPs are less than 230 nm. The NPs demonstrated nearly 100% cell viability in L929 cell lines, which indicated the absence of cytotoxicity and hence confirmed its safety. The optimised NPs were mainly spherical and exhibited biphasic *in vitro* release pattern. The study concluded that TCS-loaded PCL NPs could serve as a novel colloidal drug delivery system for the treatment of periodontal infections (Aminu et al., 2013).

Osorio and associates designed bioactive and cytocompatible NPs loaded with calcium and zinc for the treatment of periodontal disease. The PolymP-*n* active NPs was fabricated with 2-hydroxyethyl methacrylate as the backbone monomer, ethylene glycol dimethacrylate as a cross-linker, and methacrylic acid as a functional monomer using polymerisation precipitation method. They used scanning electron microscopy (SEM) that were attached to an energy dispersive analysis system to evaluate the

biomimetic precipitation on polymeric particles after immersion in a simulated body fluid for seven days. They also conducted other evaluation studies such as X-ray diffraction and cell viability analysis. Results confirmed that calcium and phosphate had precipitated on the surfaces of NPs loaded with calcium. Cell viability assays indicated that calcium and zinc-loaded NPs have a dose-dependent but very minor cytotoxic effect. The authors concluded that the observed less toxic calcium-loaded NPs and its ability in promoting precipitation of calcium phosphate deposits might offer new strategies for the treatment of periodontal disease (Osorio et al., 2016).

Kashi and associates prepared minocycline-loaded PLGA NPs for periodontal infection treatment, with the aim to improve low EE of hydrophilic drugs. They employed different techniques for the preparations of polymeric NPs which include nanoprecipitation, single and double solvent evaporation, and ion pairing. The resulting NPs were characterised with respect to particle size and size distribution, morphology, drug loading and EE, thermal properties, and antibacterial activity. They observed that the prepared NPs were spherical with a particle size in the range of 85–424 nm. The best EE, i.e. 30% of the study was obtained with solid/oil/water ion pairing method, hence the method was selected. The minocycline-loaded NPs exhibited a minimum bactericidal concentration and minimum inhibitory concentration (MIC) which was at least two times lesser than that of the free drug, while its *in vitro* antibacterial activity was higher than the free drug (Kashi et al., 2012).

In another similar investigation, a team of researchers prepared and characterised minocycline-loaded poly(ethylene glycol)–poly(lactic acid) (PEG-PLA) NPs for enhancing localised treatment of chronic periodontitis in beagle dogs. They used emulsion/solvent evaporation technique to prepare the NPs. The minocycline-loaded NPs were subjected to *in vitro* release and *in vivo* pharmacokinetics studies of

minocycline in phosphate-buffered saline (PBS; 0.01 M, pH 7.4) and gingival crevicular fluid of beagle dogs with induced periodontitis, respectively. Results showed that the minocycline-loaded NPs were mainly spherical, with an average particle size of 100 nm in diameter, and demonstrated a sustained release characteristic of minocycline during *in vitro* release study. The researchers also observed that the prepared NPs have better drug retention in dogs' gingival crevicular fluid than Periocline®, and hence, suggested that minocycline-loaded NPs may be a potential drug delivery candidate for the treatment of periodontitis (Yao et al., 2014).

1.4 Nanogels in Periodontitis Therapy

Incorporation of polymeric NPs into strands of another polymer network such as a hydrogel may lead to the formation of an interestingly novel class of dosage form called nanoparticles-hydrogel composite or nanogel (NG). As indicated in Figure 1.2, NG is a nanoscale multicomponent network that consists of NP structures entwined into hydrogel (a three-dimensional, cross-linked hydrophilic polymer network). NG has attracted growing interest in recent years as one of the most promising nanoparticulate drug delivery systems. This may be due to its unique potential of yielding the beneficial characteristics of NPs (as mentioned in Section 1.3) and that of hydrogel system (such as hydrophilicity, softness, rubbery, low interfacial tension with aqueous medium or biological fluid, and extremely high water content), in a unit delivery system (Pérez et al., 2014; Yao et al., 2016). In addition, the size and unique surface properties of NG enable them to be involved in more chemical reactions and multivalent bioconjugation. NG can also be loaded with a myriad of drugs and biomolecules by entrapment or encapsulation due to its structural features. Hence, it can be highly useful for delivery of drugs and other bioactive molecules (Bencherif et

al., 2009). This drug loading is achieved through self-assembly mechanisms involving Van der Waals, electrostatic, and/or hydrophobic interactions between molecules of the drug and that of the polymer (Yao et al., 2016).

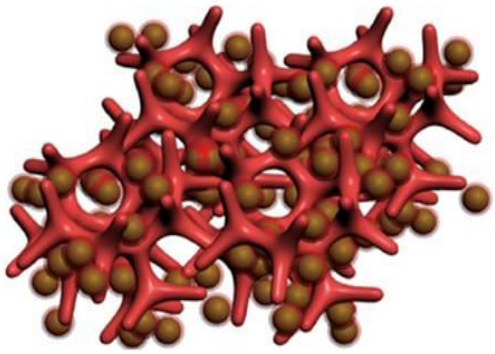

Figure 1.2: Schematic illustration in 3D of nanoparticles-hydrogels composite (nanogel), showing how nanoparticles are entwined in a hydrophilic polymer network (Wu et al., 2013)

1.4.1 Advantages of Nanogel as Drug Delivery Systems

NG can exhibit several advantages when used as drug delivery systems. These advantages are:

a) NG is often biocompatible, biodegradable and bioadhesive (if polymers with such features are used in its composition) because of their physical properties' similarity with living tissue
b) Through the NG, multiple drugs can be loaded and delivered to the targeted site with or without chemical cross-linkage. Non-chemical cross-linkage loading has the benefit of maintaining the original drug activity
c) NG system can be suitably fabricated for various routes of administration such as to the oral cavity which include the periodontal area, parenteral, intra-ocular and nasal cavity
d) NG is easy to produce, handle, and administer

e) NG can swell up to more than 30 times of its initial volume, a property embodied in hydrogels, hence providing more surface area for accommodation of APIs
f) NG can enhance solubility as well as pharmacokinetics of the conjugated drugs
g) NG can be designed to have controlled and sustained release characteristics
h) NG provides an opportunity for designing both passive and active targeting because of its surface nature and molecular size
i) NG has high stability

Thus, NG represent a promising carrier system for effective delivery of drugs and other biologically active agents (Aminu and Toh, 2017; Moya-Ortega et al., 2012; Pérez et al., 2014; Rejinold et al., 2012; Sahu et al., 2017; Yao et al., 2016).

Studies have shown evidence where NG techniques were employed to develop drug delivery systems in which two drugs were loaded in a unit delivery system. Researchers from Massachusetts Institute of Technology, USA have carried out a study where two drugs were loaded in a mesh-like network of NG. They developed PEG-PLA NPs which were suited to carry hydrophobic small-molecules of drug. On the other hand, they made a gel with a cellulose polymer suited to carry hydrophilic drug and then mixed the two components which resulted in NG (Trafton, 2015). Thus, this is important progress in formulation science especially for simultaneous delivery of multiple drug substances for the treatment of certain diseases.

Bako and coworkers (2008) developed nanocomposite biocompatible hydrogels (NG) which could be used as a model system for *in situ* local drug delivery devices for treatment of periodontal infections. This NG was composed of NPs, gel matrix and chlorhexidine as an antibacterial drug. The researchers initially prepared NPs by free radical initiated copolymerisation of monomers which comprises 2-hydroxyethyl methacrylate and polyethylene glycol dimethacrylate. They used the

same monomers to prepare crosslinked gel matrices. Finally, they developed NG by mixing NPs, gel matrices and the drug in an aqueous solution by photopolymerisation crosslinkage. This integrated gel system showed distinct advantages compared to simple hydrogels as a drug delivery system. Based on this study, they concluded that *in situ* polymerisations of hydrogels offers flexibility for local placement of drugs in the treatment of periodontal disease (Bako et al., 2008).

In another study, NG with thermoresponsive properties was prepared and characterised. Inverse emulsion polymerisation technique was used for its fabrication via cross-linkage of acrylate derivatives of poly(ethylene glycol) and poly(ethylene glycol)-bl-poly(propylene glycol)-bl poly (ethylene glycol) copolymers, also known as Pluronics®. These synthesised hydrogel-NPs were of a size range of 100 to 500 nm and were found to be stable (Missirlis, 2005).

Fukui et al. (2007) investigated whether cholesterol-bearing pullulan modified with amino-groups ($CHPNH_2$) NG could be used as a carrier to introduce quantum dots into the periodontal ligament cells that were obtained from a primary culture. The $CHPNH_2$ NG was found to form complexes with quantum dots resulting in monodisperse hybrid NPs which were able to be delivered into live cells by endocytosis in significant quantities. Therefore, the $CHPNH_2$ NG was found to be useful as a carrier to introduce quantum dots into periodontal ligament cells in cell culture, and it demonstrated feasibility for further characterisation of periodontal ligament cells as well as investigation of regenerative processes (Fukui et al., 2007). This feature further demonstrates the capacity of the NG system in periodontal therapy.

In another investigation, doxycycline nano-liposome slow-release NG was evaluated for therapeutic effects on an established rat model of periodontitis. The biocompatibility of the system was examined by oral perfusion of the sample gel for

prolonged observation. The results revealed that doxycycline NG exhibit excellent biocompatibility from weight measure and tissue section evaluation. Furthermore, the NG system demonstrates the ability to improve periodontitis condition on rats with periodontitis defects (Jin et al., 2010).

Just of recent, Madi and co-researchers prepared and evaluated nanostructured doxycycline gel and then compared its anti-inflammatory effectiveness against conventional doxycycline gel when used locally as an adjunct to scaling and root planning in the treatment of chronic periodontitis. They developed the nanostructured doxycycline gel by spray-drying technique, using CS as a matrix polymer and polyvinyl alcohol as a stabiliser. This NG, as well as the conventional doxycycline gel, were tested on patients suffering from chronic periodontitis that possessed a deep periodontal pocket. Results showed that all the tested treatment plans significantly reduced the level of inflammatory mediators of the investigated patients. However, only doxycycline-loaded NG significantly decreased probing depth when compared with the conventional doxycycline gel and placebo CS gel during the study period. The researchers concluded that nanostructured doxycycline gel could impart anti-inflammatory effect when used as an adjunct to scaling and root planning, by enhancing clinical parameters as well as inflammatory markers (Madi et al., 2018).

1.5 Rationale of the Present Study

The oral administration of antibiotics as a therapeutic remedy for the treatment of periodontitis is no longer a preferred choice due to the reasons mentioned in Section 1.2. Local or targeted drug delivery approaches are the best strategy currently being adopted for the effective management of periodontal disease. This is because they are more favourable as compared to systemic approach, and also because of its site-

specific delivery, reduction of dosing frequency and gastrointestinal side effects, low dose requirement, and bypass of first-pass metabolism effect (Joshi et al., 2016). Additionally, local drug delivery approach can provide a safe and effective medium of treatment.

Several researchers have reported nanoparticulate drug delivery approaches in which a single drug (mostly antibacterial) were loaded in polymer-based system and used for the treatment of periodontitis (Abou Neel et al., 2015; Joshi et al., 2016; Kong et al., 2006; Nguyen and Hiorth, 2015). There is little research done on delivery approaches where multiple drugs are loaded and delivered into the periodontal pockets. The reported approaches suffer from adhesion problem at the periodontal pocket (site of action), as they are rapidly flushed away by saliva or during ingestion of food substances. Moreover, they mainly focused on neutralising the causative bacteria rather than also targeting the pro-inflammation factors. It is well established that inflammation is one of the major pathological outcomes of periodontitis (Cai et al., 2008; Cochran, 2008; Graves and Cochran, 2003; Lerner, 2006; Pihlstrom et al., 2005), hence countering it should be one of the primary goals for an ideal treatment strategy. Besides causing periodontal tissue destruction, inflammation can also inflict severe pain and cause discomfort to the patient. Therefore, by blocking inflammatory pathways through the use of non-steroidal anti-inflammatory drugs (NSAIDs), periodontitis should be better treated. This is because NSAIDs can significantly decrease the periodontal tissue breakdown, promote healing and regeneration of alveolar bone (Vyas et al., 2000).

As of the time of planning the present study, there was no reported investigated study where a nanoparticulate drug delivery system loaded with antimicrobial and anti-inflammatory agents for the enhancement of therapeutic outcomes of periodontitis.

Therefore, to fill in this gap, as well as to address the identified shortcomings mentioned above and in Section 1.2, development of a bioadhesive polymeric NG drug delivery system that can be effectively delivered and retained for an extended period in the periodontal pockets was envisaged. The NG would be developed from NPs and hydrogel. Hence, it may possess the essential properties of both systems. This developed NG would be loaded with dual therapeutic agents, i.e., antimicrobial and anti-inflammatory drugs, in order to simultaneously halt the causative pathogenic microbes and the triggered inflammation and pain. This study can be considered as a significant contribution in the field of formulation science and dentistry, since up to now there are no reported experimental studies which investigated the potential benefits of using a NG strategy with antimicrobial and anti-inflammatory effects, as an alternative therapeutic remedy for the treatment of periodontitis.

Therefore, NG was selected as a drug delivery system due to its ability to penetrate the gingival sulcus and periodontal pocket, and be retained for a prolonged period thereby providing and maintaining an effective drug release rate. The pocket allows easy access to the medication delivery tool and dislodgment of its content. The environmental conditions of the periodontal pocket, i.e. temperature and acidic pH would be used as trigger factors for *in situ* gelation of the NG, swelling, erosion and release of its cargo. This may provide controlled drugs release from the bioadhesive polymer matrix, improve the treatment's effectiveness, reduce the treatment cost, and improve patient's compliance.

In this work, triclosan (TCS) and flurbiprofen (FLB) were selected as the drugs for the intended pharmacological actions, while poly-ε-caprolactone (PCL) and chitosan (CS) were the selected polymeric carriers. Kolliphor® P188 (KP) was the

selected stabiliser during NPs development. The details of these selected components are discussed in the following sections.

1.5.1 Triclosan

TCS (Figure 1.3) is a non-ionic broad-spectrum antimicrobial agent that has a recognised efficacy against several plaque-forming bacteria and has been used extensively in various products for more than four decades (Piñón-Segundo et al., 2005; Rosling et al., 1997a).

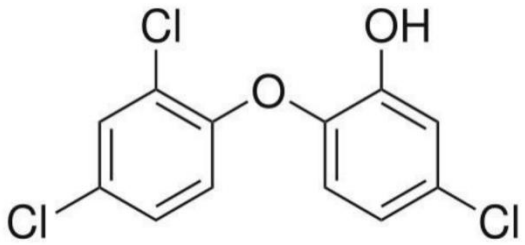

Figure 1.3: Structure of triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol)

Studies showed that the broad spectrum antimicrobial effect of TCS covered a wide range of gram-positive and gram-negative bacteria found in the oral cavity (Ciancio, 2007; Davies, 2007). The antimicrobial profile of TCS has been reported in the literature (Australian National Industrial Chemical Notification and Assessment Scheme, 2009; Bhargava and Leonard, 1996; U.S. Food & Drug Administration, 2008). TCS has been included in several oral health care products such as dentifrices and oral rinses due to its efficacy in preventing and reducing bacterial plaque (Jones et al., 2000). A review study on the efficacy of TCS in dentifrices revealed that it acts as an effective anti-plaque and anti-gingivitis agent in dentifrices, and hence, it is an essential drug candidate for the treatment of periodontitis (Gunsolley, 2006).

Researchers had demonstrated that TCS reduced the already established supragingival plaque and resolved gingival lesions. It also has some *in-vitro* antiviral and antifungal activity (Bhargava and Leonard, 1996; Jones et al., 2000). A study which involved 60 adult human subjects with recurrent periodontitis was conducted to determine whether the use of a dentifrice containing TCS can influence clinical symptoms of the disease (Rosling et al., 1997b). The subjects were instructed to use the dentifrice containing 0.3% TCS for a specified period. Results demonstrated that this dentifrice with TCS content reduced bone loss and frequency of deep of periodontal pockets (Rosling et al., 1997b). Similar results were obtained when NPs loaded with 9% of TCS was used to treat beagle dogs with induced periodontal disease (Piñón-Segundo et al., 2005).

With solubility of ≤ 0.01 g L^{-1} at 20 °C, TCS is practically insoluble in the water (Aminu et al., 2013; Australian National Industrial Chemical Notification and Assessment Scheme, 2009; Bhargava and Leonard, 1996; Scientific Committee on Consumer Products of European Commission, 2009; U.S. Food & Drug Administration, 2008; Vyas et al., 2000). The drug is also highly crystalline in nature (Celebioglu et al., 2014; Scientific Committee on Consumer Products of European Commission, 2009; Sweetman, 2009). These properties restrict delivery of pure TCS into the periodontal pocket, hence warranted its formulation into a suitable dosage form such as nanoparticulate system in order to facilitate its delivery and release at the site of action. Several researchers have employed TCS for various antimicrobial effects. However, due to its low water solubility and extreme crystallinity, these researchers have to prepare NPs formulation of the drug to counter these challenges and to enable its delivery to the targeted site of action as well as to improve its bioavailability (Davachi and Kaffashi, 2015; Davoodi et al., 2016; Domínguez-Delgado et al., 2011; Piñón-Segundo et al., 2005; Wais et al., 2018). Therefore, in the

present study, TCS will be loaded into polymeric NPs to change it solid state from highly crystalline to amorphous, as well as to facilitate its transportation to the periodontal pocket for enhancement of its therapeutic effect.

1.5.1(a) Mechanism of Action of Triclosan

TCS acts by blocking the active site of the enoyl-acyl carrier protein reductase enzyme (ENR), an essential enzyme in fatty acid synthesis in bacteria. By blocking the active site, TCS inhibits the enzyme and therefore prevents the bacteria from carrying out the fatty acid synthesis, which is necessary for building cell membranes and for reproduction (Levy et al., 1999; McMurry et al., 1998). TCS is a very potent inhibitor of ENR enzyme, and even a small amount can exert powerful antimicrobial action. Humans do not have this ENR enzyme and hence are not affected. TCS has long been determined to be fairly harmless to humans, thus in classical toxicological terms, it is relatively non-toxic to them and other mammals (Glaser, 2004).

1.5.2 Flurbiprofen

FLB (Figure 1.4) belongs to NSAIDs class of drugs that has been well investigated during the last two decades, as modulators of the inflammatory response of the host having periodontitis (Heasman et al., 1993; Ribeiro et al., 2012). Studies have revealed evidence which indicated that supplementing periodontitis treatment with NSAIDs can have a positive effect on the outcome of the therapy (Hersh et al., 1991; Jeffcoat et al., 1995; Ribeiro et al., 2012).

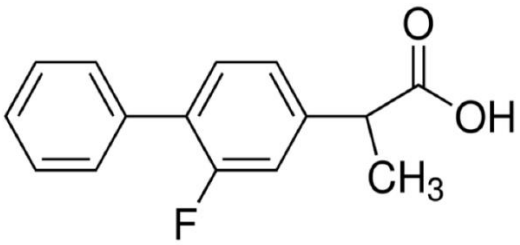

Figure 1.4: Structure of flurbiprofen ((±)-2-(2-fluoro-4-biphenylyl) propionic acid)

FLB, a non-selective cyclooxygenase (COX) enzyme inhibitor (Agrawal et al., 2016), has been used for the management of periodontitis in both animals and humans (Jeffcoat et al., 1988; Williams et al., 1987), and has been found to be efficacious when used as a topical rinse or systemic medication (Heasman et al., 1993; Jeffcoat et al., 1995). FLB blocks the COX enzymes which cause inflammation and pain (Heasman et al., 1993). COX enzymes are inflammatory enzymes triggered by cytokines – critical immune factors in periodontal disease. The drug is used not only for relieving pain in periodontitis but also for slowing the disease process (Jeffcoat et al., 1988). Several studies have found FLB to significantly decrease gingival inflammation and slow the progression of periodontitis (Howell and Williams, 1993). Similarly, many studies have reported the ability of FLB to aid in healing and reduce alveolar bone loss in patients with periodontitis, for both surgical and nonsurgical treatment, and these successes were evident with both short-term and long-term use of the medication (Dicke, 2011; Jeffcoat et al., 1988). However, long-term systemic use of COX enzyme inhibitors such as FLB can result in gastrointestinal disturbances (Heasman et al., 1993; Ribeiro et al., 2012). Because of this reason, topical application of the drug is logically favourable in circumventing the gastrointestinal adverse effect of FLB. Fortunately, studies have shown that FLB is well absorbed through the gingival tissues (Salvi and Lang, 2005). Additionally, an investigation conducted to evaluate the effectiveness of topically applied FLB had yielded favourable outcomes by promoting

bone gain following nonsurgical therapy of periodontitis (Dicke, 2011; Heasman et al., 1993). Therefore, in the present study, FLB will not be incorporated in NPs but rather directly into the hydrogel system since it is well absorbed on the gingiva. Moreover, this may lead to its immediate release after application of the NG formulation in order to manage the gingival inflammation and reduce pain arising from the application site. The ability to use FLB for local effect reduces concerns of adverse effects and toxicity that may arise from long-term ingestion of the drug. Thus, the incorporation of FLB in topical formulations for periodontitis treatment may improve their effectiveness.

1.5.3 Combination of Two Drugs for the Treatment of Periodontal Disease

There are few reported studies which developed drug delivery systems that contained two drugs. For example, Popa and co-workers developed chitosan (CS) gel containing two antibacterial drugs (tetracycline hydrochloride and metronidazole benzoate) for local intra-pocket drug delivery for the treatment of periodontal disease. Based on kinetic release and rheological studies' data, the researchers concluded that 3% *w/w* concentration of CS could offer a base for an optimum modulation in drug dose, and its gels are efficient in the local treatment of periodontal disease (Popa et al., 2013). A suitable formulation of TCS and FLB combination would be highly desirable for periodontal therapy, owing to the therapeutic efficacy of the drugs as discussed in Section 1.5.1 and 1.5.2 above.

1.5.4 Poly-ε-caprolactone Polymer

PCL (Figure 1.5) is a family member of aliphatic polyesters which is highly hydrophobic and semi-crystalline in nature, and has been found to be biodegradable, biocompatible, having *in vitro* stability, relatively low cost and having good drug

permeability properties (Azimi et al., 2014; Ranjha et al., 2009; Zamani et al., 2010). These essential features have led to its applications in drug delivery systems and biomedicals such as prosthetics, bandages, and sutures (Azimi et al., 2014; Jayakumar and Tamura, 2008).

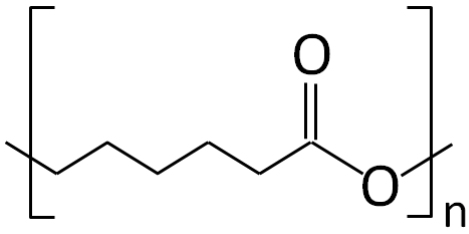

Figure 1.5: Structure of poly-ε-caprolactone ((1,7)-polyoxepan-2-one)

PCL is structurally an achiral polymer, a feature that restricts chances of modulation of its properties via the structural configuration of polymer chains, and therefore, it is highly resistant to chemical hydrolysis (Chawla and Amiji, 2002). The polymer has been approved for use in human beings by the United States Food and Drug Administration (FDA) (Azimi et al., 2014).

Thus, PCL was selected as the polymer to prepare the NPs for the present study because of its numerous advantages that include biocompatibility, biodegradability, non-toxicity, high permeability to small drug molecules, high encapsulation efficiency, and does not generate an acidic environment during degradation as compared to polylactides and glycolides. The polymer undergoes degradation in a slow manner, a property desirable for prolong delivery systems (Koleske, 1978; Murthy, 1997; Ranjha et al., 2009; V.R. Sinha et al., 2004), and many varieties of APIs have been encapsulated or entrapped in the polymer for controlled drug release as well as targeted drug delivery (V.R. Sinha et al., 2004).