GHENT UNIVERSITY

FACULTY OF PHARMACEUTICAL SCIENCES

Department of Pharmaceutics

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QUEEN'S UNIVERSITY BELFAST

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FORMULATION AND CHARACTERIZATION OF LIQUID CRYSTAL PLATFORMS FOR THE TREATMENT OF PERIODONTAL DISEASE

Kaat DE CLERCQ

Master of Drug Development

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SAMENVATTING

Het doel van deze studie is het ontwikkelen en karakteriseren van liquid crystals ter behandeling van periodontitis. Er wordt gebruikt gemaakt van een ternair systeem bestaande uit twee niet-ionische surfactanten, Tetronic® (T) 904/704 en Procetyl® (PC), in combinatie met water (W). Een fasediagram wordt opgesteld met behulp van gepolariseerd licht microscopie. Twee formulatielijnen worden geselecteerd met als vloeibare isotrope precursoren 10%T/25%W/65%PC en 30.40%T/21.60%W/48%PC. Rheologische testen, i.e. flow, oscillatie en creep rheologie, worden uitgevoerd om de structurele eigenschappen van de formulatielijnen te analyseren. De tweede formulatielijn met Tetronic® 904 als surfactant, is de voorkeurskeuze. 1% tetracycline HCl wordt toegevoegd aan de systemen. De precursor is vloeistofachtig met een lage viscositeit wat het eenvoudig maakt om te injecteren in de periodontische beurs. 20% verdunning met water naar de hexagonale fase induceert een transitie naar een vastere toestand met G'>G", een viscositeit van 183 ± 25 Pa.s en een retentietijd van 479 ± 110 s. Verdere verdunning tot 60%, i.e. kubieke fase, verbetert de rheologische eigenschappen. De rheologische resultaten (viscositeit van 721 ± 59 Pa.s, G'>G" en een retentietijd van 1 464 ± 184 s) duiden aan dat de kubieke fase de beste karakteristieken vertoont voor de behandeling van periodontitis. De vrijstelling van tetracycline HCl gedurende 6 uur is gecontroleerd en vertoont nulde-orde kinetiek. Ongeveer 34% van de initiële hoeveelheid tetracycline HCl wordt vrijgegeven. Het mechanisme van geneesmiddelvrijstelling is diffusie-gecontroleerd. Een mucosale irritatietest wordt uitgeoefend op naaktslakken om de verenigbaarheid met mucosa na te gaan. De T904 precursor van de eerste formulatielijn wordt getest en irritatie is duidelijk zichtbaar, aangezien er een geel mucus wordt geproduceerd. 48% gewichtsverlies, 27% mucusproductie en lactaatdehydrogenase vrijstelling hoger dan die van de positieve controle, toont de hoge graad van irritatie aan veroorzaakt door de formulatie.

SUMMARY

The aim of this study is to develop and characterize liquid crystal platforms for the treatment of periodontal disease. A ternary system that consists of two non-ionic surfactants, Tetronic® (T) 904/704 and Procetyl® (PC), in combination with water (W) is used. A phase diagram is constructed using polarized light microscopy. Two formulation lines are selected, the liquid isotropic precursors are 10%T/25%W/65%PC and 30.40%T/21.60%W/48%PC. Rheological tests, i.e. flow, oscillation and creep rheology, are conducted to analyse the structural properties of the formulation lines. The second formulation line containing Tetronic® 904 is the choice of preference. 1% tetracycline HCl is added to the systems. The precursor is liquid-like with a low viscosity which offers ease of syringeability into the periodontal pocket. 20% dilution with water to the hexagonal phase induces a transition to the solid-like state with G'>G", a viscosity of 183 ± 25 Pa.s and a retention time of 479 ± 110 s. Further dilution to 60%, i.e. the cubic phase, improves the rheological properties. The rheological results (a viscosity of 721 ± 59 Pa.s, G'>G" and a retention time of 1 464 ± 184 s) indicate that the cubic phase displays the best characteristics for the treatment of periodontal disease. The release of tetracycline HCl during 6 h is controlled and displays zero-order kinetics. Around 34% of the initial amount of tetracycline HCl is released. The mechanism of drug release is diffusion-controlled. A slug mucosal irritation test is performed to test the compatibility with mucosa. The T904 precursor of the first formulation line is tested and irritation is clearly noticed, as a yellow mucus is produced. Weight loss of 48%, mucus production of 27% and lactate dehydrogenase release higher than the positive control demonstrate the high irritation caused by the formulation.

CONTENTS

List of abbreviations

BKC: benzalkoniumchloride

EO: ethylene oxide

HPLC: high performance liquid chromatography

LVR: linear viscoelastic region

LDH: lactate dehydrogenase

PC: Procetyl

PEO: poly(ethylene oxide)

PLM: polarized light microscopy

PPG-5-CETEH-20: Polyoxypropylene (5) poloxyethylene (20) cethylether

PO: propylene oxide

RSRT: relative stable retention time

T: Tetronic

TC: Tetracycline HCl

W: water

1 INTRODUCTION

1.1. PERIODONTITIS

The goal of this study is to find a drug delivery platform to treat periodontal disease. Periodontitis is an inflammatory condition which affects the supporting structures of the teeth (the periodontal ligament and the alveolar bone). As a consequence, morphological changes of the gingival tissue, gingival haemorrhage and the formation of a periodontal pocket between the gingiva (gum) and the tooth have been noticed (Figure 1.1). *(Jones, Woolfson et al. 2000; Bruschi, de Freitas et al. 2008; Feher, Urban et al. 2008; Jones, Bruschi et al. 2009)*

Periodontal disease is the collective noun for conditions including gingivitis and periodontitis. Gingivitis can be seen as the inflammation of the gingiva, whereas periodontitis encloses inflammation into deeper tissues. Untreated periodontal disease can induce increased tooth mobility and finally tooth loss. (*Bruschi, de Freitas et al. 2008)*

The treatment of periodontal disease is mainly focused on the mechanical cleaning of the tooth surface to remove bacterial plaque and calculus. At the present, specific bacteria are thought to play a key role in the disease development. The periodontal pocket provides supreme conditions for the colonization and multiplication of pathogenic anaerobic bacteria. *(Jones, Woolfson et al. 2000; Bruschi, de Freitas et al. 2008; Feher, Urban et al. 2008)*

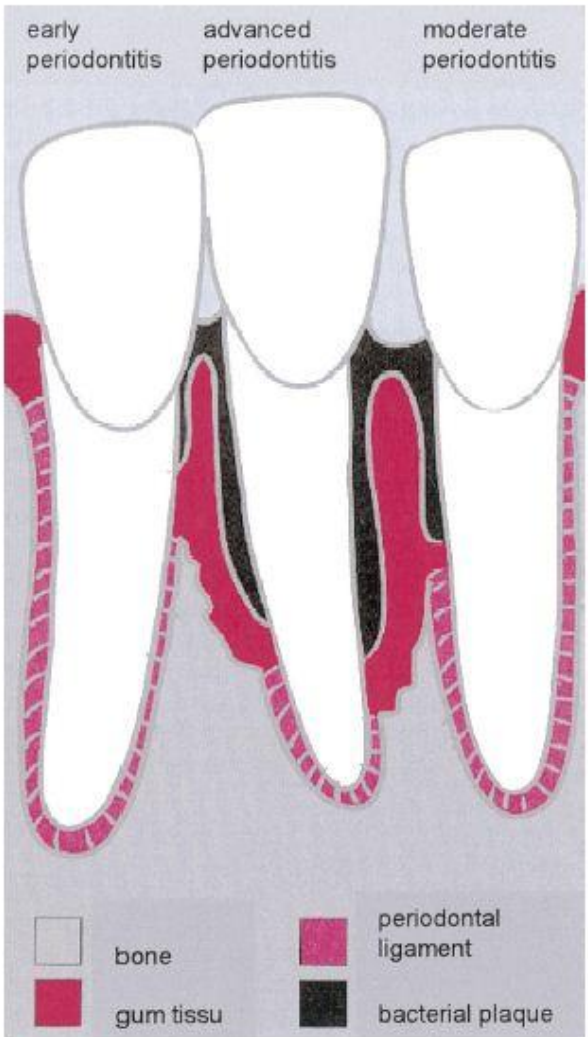


Figure 1.1: Bacteria in subgingival plaque have caused a periodontal pocket to develop, inflaming surrounding tissue and causing loss of alveolar bone

(Schwach-Abdellaoui, Vivien-Castioni et al. 2000)

The bacteria are principally responsible for the beginning of the inflammation and for the destruction of tissues. Certain bacteria produce a large quantity of biologically active substances, which affect directly the surrounding tissues, leading to destruction. As a consequence, antimicrobial drugs have been used as an addition to the traditional mechanical treatment, mostly in treating early-onset and refractory cases. *(Jones, Woolfson et al. 2000; Bruschi, de Freitas et al. 2008; Feher, Urban et al. 2008)*

The interest in the sustained delivery of therapeutic molecules within the pocket has increased because administering of systemic antibiotics has the potential risk of side effects. Side effects include hypersensitivity reactions, gastrointestinal intolerance and the development of bacterial resistance. Furthermore, poor results are reported because the active ingredient could not attain an adequate concentration at the site of action and because of the inability of the active ingredient to be sustained locally for a sufficient period of time. Antiseptic mouthwashes seem to be unable to penetrate into the periodontal pocket. A sustained release formulation would ensure an effective high concentration of the antimicrobial agent within the pocket. *(Jones, Woolfson et al. 2000; Schwach-Abdellaoui, Vivien-Castioni et al. 2000; Feher, Urban et al. 2008)*

Topical drug delivery is most frequently performed for the treatment of localised disorders like periodontitis. It has the advantage of being able to deliver the therapeutic agents immediately to the target tissue. On the other hand, an important problem of topical formulations is the poor retention at the site of application. This is caused by limited interaction of the formulation with the host epithelium and by mechanical removal due to protective self-cleansing mechanisms by the action of saliva, mucosal turnover and applied stresses during normal physiological functions as chewing and swallowing. Consequently, a key design parameter will be the ability of the drug implant to be sustained at the application site*. (Andrews and Jones 2006; Jones, Bruschi et al. 2009)*

These problems can be defeated by the use of antimicrobial-containing formulations that can be effortlessly introduced into the periodontal pocket and whilst in this environment, interact with mucin-coated epithelial and tooth surfaces by specific interfacial forces in a process called mucoadhesion. Mucoadhesion is classified under bioadhesion. Bioadhesion can be described as "*the binding of a natural or synthetic polymer to a*

biological substrate". If the substrate is a mucous layer, the term mucoadhesion is used. *Carvalho, Barbi et al. 2010)*

Mucoadhesive drug delivery systems are retained within the pocket for an extended period, thereby ameliorating drug bioavailability whereas lower drug concentrations can be used for the treatment. Furthermore, these systems offer controlled drug delivery for the period of retention. The mechanism of mucoadhesion of liquid crystal phases can be assigned to the rheological properties of the system, which are similar to those of *in situ* gelling vehicles. The liquid crystal matrix can be very strong and viscous. This supports mucosal retention and thus hindering of instant removal of the formulation. *(Jones, Woolfson et al. 2000; Andrews and Jones 2006; Carvalho, Barbi et al. 2010)*

Furthermore, the success of the clinical performance of a formulation is dependent on the mechanical properties of the formulation. The ideal characteristics of a formulation include the ease of delivery into the periodontal pocket, controlled release of the drug molecule into the gingival crevicular fluid and retention within the pocket for the desired time. The formulation also has to be biodegradable, non-toxic and non-irritant. (*Jones, Woolfson et al. 2000)*

In addition, a major disadvantage of implantable semi-solid drug delivery systems for topical application to the oral cavity is the destruction of the rheological properties of the formulation *in vivo* following dilution with the associated body fluid, which finally directs to rapid leakage and hence poor retention of the formulation and inappropriate control of drug release. Thus, since rheological properties of gel systems are of significant importance to their clinical success, there has been an increased interest in the formulation of rheologically structured gels for pharmaceutical application. Drug delivery implant formulations are subjected to a broad range of shearing stresses, which may be destructive and/or non-destructive. Additionally, besides the high stresses associated with mastication, formulations applied into the oral cavity will be exposed to non-destructive oscillatory stresses (>0.5 Hz). Therefore, the characterization and thus selection of semi-solids as topical implantable systems must engage complete rheological evaluation, including both transient and destructive testing methods. *(Andrews and Jones 2006)*

Different drug delivery devices have been designed in order to treat periodontal disease such as fibers, strips and compacts, films and injectable systems. Two types of injectable delivery systems are considered in the treatment of periodontitis, namely biodegradable microparticles and gels. (*Schwach-Abdellaoui, Vivien-Castioni et al. 2000)*

Goodson, Holborow et al. (1983) developed ethylene vinyl acetate fibers providing a sustained release matrix of tetracycline HCl *in vivo* for a period of 9 days. Nevertheless, since the fibers are non-resorbable, they must be removed after 10 days. *(Maheshwari, Miglani et al. 2006)*

Killoy (1998) developed Periochip®, a biodegradable gelatin matrix containing chlorhexidine gluconate as active drug substance.

Jones, Woolfson et al. *(*2000*)* have found that a 3% hydroxyethylcellulose, 3% polyvinylpyrrolidine, 1% polycarbophil and 5% tetracycline HCl semi-solid formulation provides a promising drug delivery system that shows a compromise between optimal tetracycline release and mucoadhesive and rheological properties. One week following administration of the formulation, a significant improvement in periodontal health was detected by reduced numbers of subgingival microbial pathogens.

Jones, Bruschi et al. *(*2009*)* characterized rheological, mechanical and mucoadhesive properties of thermoresponsive, bioadhesive binary mixtures composed of poloxamer 407 and carbopol 974P platforms for implantable drug delivery systems for use in the oral cavity. These systems offer rheological structuring and mucoadhesion at body temperature, thus resolving aforecited challenges. Formulations composed of 15% poloxamer 407 and 20% carbopol 974P, with high elasticity and mucoadhesive characteristics, render potentially useful platforms for controlled topical drug delivery within the oral cavity.

Féher et al. *(*2008*)* formulated preconcentrates of liquid crystal of oil and surfactants having a low viscosity. Once injected into the periodontal pocket the formulation transforms into a highly viscous liquid crystalline phase by absorption of the saliva in the periodontal pocket. The active ingredient is metronidazole-benzoate. The carrier is a 4:1 mixture of a non-ionic surfactant Cremophor EL or Cremophor RH40 and an oil phase (Miglyol 810).

1.2. LIQUID CRYSTALS

The interest in liquid crystal platforms as formulations for pharmaceutical application is increasing. Liquid crystals have properties related to both liquids and crystals. Molecules in a crystal are highly ordered, while molecules in a liquid are free to diffuse in a random way. Thus, molecules in liquid crystal phases diffuse like the molecules of a liquid but they contain some degree of order. *(Collings and Hird 1997)* A generally used synonym for liquid crystal is the mesophase, indicating the unique structure intermediate between that of a true liquid and a solid crystal phase. *(Rochow and Tucker 1994; Makai, Csanyi et al. 2003; Guo, Wang et al. 2010)*

In general liquid crystals systems can be classified in two categories, i.e. thermotropic and lyotropic mesophases. Thermotropic liquid crystal phases are formed by a change of temperature, whereas lyotropic phases when mixed with a solvent. *(Rochow and Tucker 1994; Dierking 2006)*

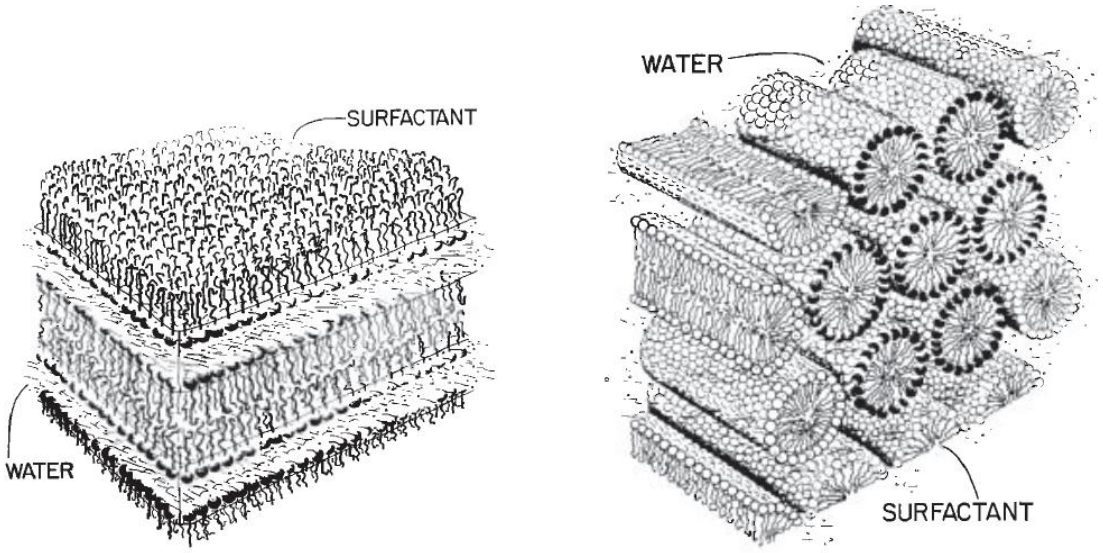
Lyotropic liquid crystals usually consist of amphiphilic substances like surfactants and solvents. An amphiphilic molecule is characterized by the possession of both a non-polar, hydrophobic tail and a polar, hydrophilic head on the same molecule which allows this molecule to form ordered structures in polar and non-polar solvents. At low concentration the basic unit of an amphiphile is the micelle, a cluster of molecules with their polar groups oriented in the water. This phase is indicated as liquid isotropic phase, isotropic meaning that the structure shows identical properties in all directions. *(Attwood and Florence 1983; Rochow and Tucker 1994; Collings and Hird 1997; Siddig, Radiman et al. 2004; Guo, Wang et al. 2010)*

At higher concentrations, more ordered structures are formed such as hexagonal, lamellar and cubic phases. These structures are formed since there is insufficient water to fill the spaces between the spherical or elongated micelles. They are formed with low energy input or by means of spontaneous structural organization. The production of liquid crystals is therefore relatively simple and energy-saving. They are thermodynamically stable and can be stored for long periods of time without phase separation. Depending on the concentration of the solvent and the polarity of the solvated mesogen, these systems can undergo phase transitions and structure modifications. Thus, their consistency and

rheological properties can be changed systematically as required. *(Attwood and Florence 1983; Makai, Csanyi et al. 2003)*

Studies have shown that these systems can be used as matrices for sustained release of drug molecules with a variety of physicochemical properties. Liquid crystal systems can form semi-solid phases with exclusive structural properties in which drugs can be incorporated. *(Fong, Hanley et al. 2009; Guo, Wang et al. 2010)*

Lyotropic liquid crystal systems formed from aqueous surfactants can absorb water from the environment inducing spontaneously phase-transitions, forming lamellar, hexagonal and cubic phases, depending on the water content of the environment (Figure 1.2). A lamellar phase can be considered as alternate double flat layers of surfactant molecule tail-to-tail with the polar groups facing the intervening layers of water molecules. Noticeable is that this phase is relative fluid despite the high surfactant concentration. The structure responsible for the fluidity of the lamellar phase is indicated as smectic. The layers can glide easily over one another, but at the same time their crystal-like composition, i.e. the arrangement of the layers, is preserved. *(Rosevear 1968; Attwood and Florence 1983; Rochow and Tucker 1994; Carvalho, Barbi et al. 2010)*



(a) lamellar phase (b) hexagonal phase

Figure 1.2: (a) structure of the lamellar liquid crystal phase and (b) structure of the hexagonal liquid crystal phase *(F. B. Rosevear 1968)*

Hexagonal and cubic mesophases are particularly of high interest in the drug delivery field due to their exceptional potential as drug vehicles. Drugs can be incorporated in these gel-like phases. Additionally they have non-toxic, biodegradable and bioadhesive characteristics which make them supplementary interesting for the drug delivery function. *(Guo, Wang et al. 2010)*

The structure of a hexagonal mesophase can be seen as an array of hexagonally close-packed water layers sheltered by a surfactant monolayer. The long-range order is two-dimensional. Two types of hexagonal mesophase can be encountered: normal and reverse mesophase, respectively in aqueous and anhydrous organic solvents. The hexagonal phase is much stiffer than the lamellar phase. *(Rosevear 1968; Attwood and Florence 1983; Fraser, Separovic et al. 2009; Guo, Wang et al. 2010)*

The structure of cubic mesophases is exceptional (Figure 1.3). The structure consists of continuous curved bilayers and a pair of interpenetrating, separate and non-intersecting aqueous channels. The cubic phase has a large interfacial area of 400 m^2/g. The unique microstructure of a cubic phase offers advantageous properties for controlled drug release. The close packing of the micelles accounts for the marked flow-resistance of the phase, which is stiffer than the mesophase. *(Fraser, Separovic et al. 2009; Guo, Wang et al. 2010) (Rosevear 1968)*

The cubic phase is formed spontaneously in excess water. Furthermore, the structure provides a slow release matrix for drugs of varying polarity and size because of its dual polar-apolar nature. Additionally, release of the drug from within the cubic phase is controlled to a degree because of its unique microstructure.

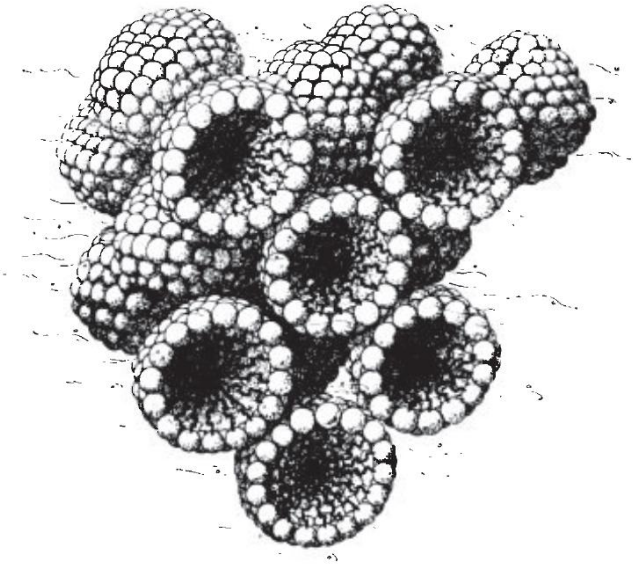


Figure 1.3:Structure of the cubic mesophase *(Rosevear 1968)*

Subsequently, the cubic phase displays bioadhesive properties. Thus, the structure is appropriate for gastrointestinal, pulmonar, nasal, oral, buccal, rectal, and vaginal drug delivery. As a final point, the cubic mesophase is stable *in vitro*. *(Clogston and Caffrey 2005; Rizwan, Hanley et al. 2009)*

Instead of a binary water-surfactant system, this study examines a ternary liquid crystal system. A ternary system usually consists of water, oil and surfactant. Carvalho, Barbi et al. (*2010*) describe a ternary system that consists of the non-ionic PEO surfactant polyoxypropylene (5) polyoxyethylene (20) cetyl alcohol (PPG-5-CETETH-20), water and oleic acid. Since it has previously been reported that high concentrations of oleic acid can cause irritation, non-ionic surfactants poly(ethylene oxide)-poly(propylene oxide) block copolymers Tetronic® 904 and 704 are used in this study instead of oleic acid. *(Henschler, Greim et al. 2002).* Non-ionic surfactants are mostly less toxic than ionic surfactants and have the advantage over ionic surfactants that they are compatible with all other types of surfactants and that their properties are generally not affected by pH. The use of two non-toxic, non-ionic surfactants has an additional advantage of increasing the solubility of the drug via the solubilisation of hydrophobic drug substances. *(Attwood and Florence 1983)*

1.3 LIQUID CRYSTALS AND DRUG DELIVERY

Liquid crystals have a wide range of applications. Liquid crystals loaded with an active drug component can be administered orally, to increase the oral bioavailability of poorly water-soluble drugs. To exhibit sustained release of oral drugs *in vivo*, the formulations must remain stable in the gastrointestinal fluids to provide a persistent matrix from which drugs can be slowly released. Thus, the formulations need to resist the digestive process to a certain extent, this can be technically challenging. Furthermore, because of the bioadhesive properties the retention time of the formulations in the gastrointestinal tract prolongs, providing more time for drug absorption. *(Guo, Wang et al. 2010)* Nguyen, Hanley et al. (2011) proved the ability of liquid crystalline particles to sustain the absorption of a poorly water soluble drug, cinnarizine, after oral administration. The oral bioavailability was significantly enhanced compared to a suspension or emulsion. They used phytantriol to form liquid crystals, which establish non-digestible liquid crystal particles as a novel sustained drug delivery system.

Liquid crystals are also used for topical drug delivery. Aytekin, Gursoy et al. (2012) developed stable and suitable lamellar liquid crystal formulations for topical drug delivery, that consists of liquid paraffin as the oil phase, a mixture of two non-ionic surfactants (Brij 721P and Brij 72) and azelaic acid as active drug substance. Liquid crystals show advantages above classical topical platforms. They attain controlled release for both hydrophilic and hydrophobic drugs, increase solubility of drugs and provide long-term hydration of the skin. Adjustment of parameters as temperature, pH of release medium and the composition of substances in the formulation, will fine-tune drug transport across the skin by liquid crystals.

Furthermore, liquid crystals show their potential as good candidates for mucosal drug delivery. For example, *in situ* formed cubic phases have bioadhesive properties because of the high viscosity. Nielsen, Schubert et al. (1998) described cubic liquid crystal phases of glycerol monooleate and monolinoleate as bioadhesive mucosal drug delivery systems. The bioadhesive nature of cubic liquid crystals allows them to target drugs to oral and vaginal cavity and to different parts in the gastrointestinal tract. *(Shah, Sadhale et al. 2001)*

1.4 RHEOLOGY AND ITS APPLICATION FOR THIS STUDY

1.4.1 Introduction

Rheology can be defined as the science of deformation of materials including flow. Deformation is the movement of parts of a material body relatively to one another. Just a small number of materials are in the form of perfect viscous liquid obeying Newton's law of viscosity or in the form of perfect solids obeying Hooke's laws of elasticity. Hooke's law states that, for an elastic solid, the strain is directly related to the applied stress. This proportionality constant is called the Young's modulus. *(Blair 1969; Jones 1999; Malkin, Malkin et al. 2006)*

$$\varepsilon = \frac{\sigma_E}{E} \tag{1.1}$$

where ε is deformation, σ_E is tensile stress and E is elastic or Young's modulus.

Newton's law expresses that, for simple liquids, the applied stress is proportional to the rate of strain. The proportionality constant is called the viscosity.

$$\gamma = \frac{\sigma}{\eta} \tag{1.2}$$

where η is viscosity, σ is shear stress and γ is rate of shear formation *(Blair 1969; Jones 1999; Malkin, Malkin et al. 2006)*

In reality, pharmaceutical preparations are complex rheological materials which often have both fluid and solid properties. Thus, the basic laws of Newton and Hooke are not applicable as such. This type of behaviour is called viscoelasticity when the behaviour is essentially solid-like and elasticoviscosity when the behaviour is principally liquid-like. *(Blair 1969; Barry 1971; Swarbrick and Boylan 1995)*.

To understand the ideas of viscoelasticity better, some typical behaviours of viscoelastic materials are explained by simple mechanical models. The simplest model to describe the viscous behaviour of material is a dashpot, a piston moving inside a cylinder filled with liquid. It is assumed that the speed of a movement, V = dX/dt, of the piston is relational to the applied force, F:

$$F = \eta \cdot V \tag{1.3}$$

where X is displacement, t is time and η is the coefficient of proportionality.

This equation is analogous to the basic equation for Newtonian liquid and one assumes that the speed is an analogue of the rate of deformation, the force F is an analogue of a stress, σ and the coefficient η is an analogue of viscosity. *(Malkin, Malkin et al. 2006)*

The simplest mechanical model to term an elastic body is a spring. The displacement X is proportional to the applied force, F:

$$X = \frac{F}{G} \tag{1.4}$$

In order to compare this equation with the standard formulation of Hooke's law, X can be treated as a relative deformation, F as an analogue of stress and G as an analogue of the elastic modulus. *(Malkin, Malkin et al. 2006)*

Both equations are quite trivial and do not add anything new to the initial concepts of viscous (Newtonian) liquid and elastic (Hookean) material. However, the following mechanical models, Kelvin-Voigt and Maxwell model, combine the spring and dashpot elements. Viscoelasticity can be described using a combination of the Maxwell and Kevin-

Voigt models. The Maxwell model (Figure 1.5) consists of a spring in series with a Newtonian dashpot while the Kevin-Voigt model includes a spring in parallel connection with a Newtonian dashpot (Figure 1.4). The spring represents the elastic response and a dashpot the viscous response. Combination of both models gives a basic system to describe viscoelastic behaviour of semi-solid materials. *(Malkin, Malkin et al. 2006)*

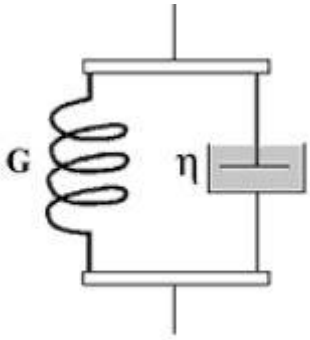


Figure 1.4: A spring and dashpot joined in parallel, a model of the Kelvin-Voigt viscoelastic liquid
(Malkin, Malkin et al. 2006)

Figure 1.5: A spring and a dashpot element joined in series, a model of the Maxwellian viscoelastic liquid
(Malkin, Malkin et al. 2006)

1.4.2 Flow rheological analysis

Flow rheology is a destructive rheological test method because stress or strain values are applied which exceed the linear viscoelastic region (LVR). Despite the fact that this test is destructive, it is of high importance to understand how a formulation will respond during high shear rates, in high stress environments. *(Yu, Malcolm et al. 2011)*

Using continuous shear experiments, one of the most general rheological parameters of gel structures, the viscosity, can be examined. Viscosity can be determined from the slope of a flow rheogram, i.e. a shear rate versus shear stress plot following the application of either a unidirectional controlled stress or strain whilst recording the resultant strain or stress, respectively. Generally, determination of flow behaviour is executed by enclosure of the formulation between two surfaces that rotate relative to one another about a common axis. *(Yu, Malcolm et al. 2011)*

Gel-like formulations generally have a viscosity dependent on the applied degree of the stress or the rate of shear. If the flow rheogram is a linear plot, the behaviour of the formulation can be described as a simple liquid, defined as Newtonian. Non-Newtonian materials, on the other hand, display a change in viscosity in function of shear stress/shear rate. There are three types of non-Newtonian flow behaviour: plastic, pseudoplastic and dilatant. *(Yu, Malcolm et al. 2011)*

1.4.3 Oscillation rheological analysis

Oscillatory analysis is a dynamic mechanical technique. A sinusoidal strain (or stress) is applied to a sample whilst the stress (or strain) response is recorded. This test gives information about the response of materials at very short times. The relationship between viscous flow and elasticity at small stress values, during passive leakage between epithelial surfaces, can be examined using oscillation analysis. *(Jones 1999; Yu, Malcolm et al. 2011)*

Typically, measurements of oscillatory rheology are conducted within the linear viscoelastic region (LVR). In the LVR, as the stress applied to a sample is increased, the relationship between the stress and strain remains a constant, although strain varies with time. The response on application of a small strain to the material might not be instantaneous, it is dependent on the elastic or viscous properties of the material. A highly elastic formulation shows a near linear relation between the stress and strain, the applied energy is used for the instantaneous recovery from deformation. The opposite is noticed for a highly liquid formulation. The relationship between the stress and strain is linear but the strain is behind the stress by 90°. Formulations demonstrating this behaviour display perfect spreading properties. The stress-strain relationship of a viscoelastic material will be somewhere between these two extremes (0-90°). *(Jones 1999; Yu, Malcolm et al. 2011)*

The main rheological parameters obtained from an oscillatory rheogram are the storage modulus (G’), the loss modulus (G”) and the loss tangent (tan δ). The storage modulus is a measure of energy stored and recovered per cycle of oscillation. It represents the solid-like component of a viscoelastic material. When a sample is chiefly elastic or very structured, the storage modulus is high. The loss modulus is a measure of the energy dissipation per cycle. It represents the liquid-like component. The loss tangent is the ratio of loss modulus:storage

modulus. Values of tan δ <1 are suggestive of an elastic gel structure. *(Malkin, Malkin et al. 2006; Yu, Malcolm et al. 2011)*

Three operation modes are generally used in oscillatory analysis, i.e. frequency, time and temperature sweep methods. Frequency sweeps are the most commonly used and useful since the variety of structural information that can be obtained. Frequency sweep experiments measure stress at a range of frequencies executed at a constant strain and temperature. The information derived from this rheological analysis provides an understanding of the structural characteristics of the formulation. The shape of the rheogram is connected to the structure of the material. Oscillatory analysis is a useful tool to study the effect of formulation composition on elastic structure. *(Yu, Malcolm et al. 2011)*

1.4.4 Creep rheology analysis

Creep tests record strain or compliance response as a function of time after the administration of a sudden, quickly imposed, unidirectional stress. At the end of a defined time period, the stress is removed whilst measurement of the strain is continued. The time-dependent strain or compliance (strain per unit stress) response to this application of a constant stress is termed creep rheology and the response when the stress is removed supplies the recovery curve. By the use of creep analysis, the balance of viscous-elastic character can be verified within the viscoelastic gel. Additionally the technique can reveal how this balance shifts as a function of time under the application of small stress values. *(Barry 1971; Yu, Malcolm et al. 2011)*

Viscoelasticity of semi-solid materials can be described using a combination of the Maxwell and Kevin-Voigt models. Based on Boltzmann superposition principle, creep behaviour should be stated by infinite Kelvin models in series with a Maxwell model. In this study five Kelvin models (0-50s, 50-100s, 100-400s, 400-800s, 800-1200s) are used to measure a creep curve.

A typical creep curve can be seen in Figure 1.6. The curve consists of three regions. The first part of the curve represents the elastic region. Application a small stress results in an instantaneous elastic response. *(Yu, Malcolm et al. 2011)*

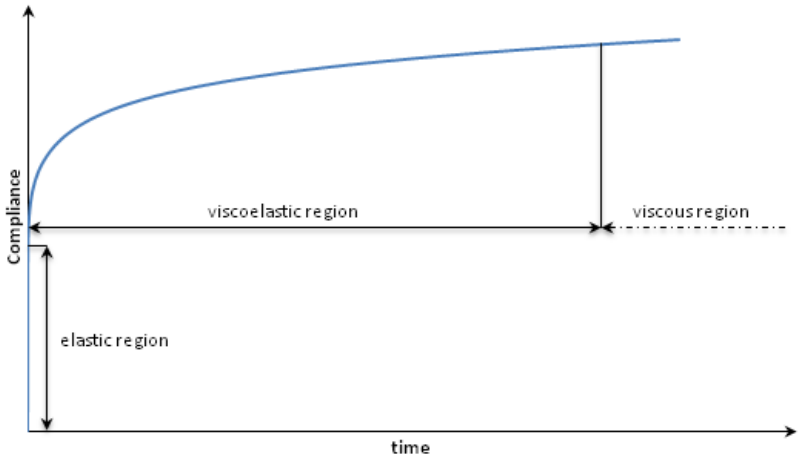


Figure 1.6. : a typical creep curve that consists of three regions

The applied stress is directly proportional to the resultant strain in very short time. It indicates the elongation of the molecular backbone of a viscoelastic material. A loaded spring represents elasticity. The instantaneous response is due to the extension of the residual spring of the Maxwell unit. *(Yu, Malcolm et al. 2011)*

The second region is the viscoelastic region, described by Kelvin models. It represents the retarded elastic response of the individual Kelvin-Voigt unit. Relative movement between molecules occurs to produce viscous flow while molecular chains continue being stretched to produce elastic deformation. *(Yu, Malcolm et al. 2011)*

The third region of the curve is called the viscous region, due to extension of the Maxwell dashpot. It starts from the end of the viscoelastic region to infinity time. A linear relationship between strain and time consists. *(Yu, Malcolm et al. 2011)*

For this study, creep experiments are used to determine the relative stable retention time (RSRT). The relative stable retention time is defined as the period from elastic stretching occurred on molecular chains till its limitation is reached. Thus, creep analysis provides a useful tool to examine whether the formulation is retained at the site of application and gives an idea of the time of retention.

2 OBJECTIVES

The aim of this study is to develop a liquid crystal platform for the treatment of periodontal disease. An ideal formulation needs to be easily implanted into the required site of administration yet possess high elasticity to control drug diffusion and enhance retention at the site of application, i.e. periodontal pocket, for the duration of drug delivery.

A liquid crystal platform is designed for oral application to treat periodontal disease using tetracycline HCl as active drug component. The liquid crystal platform is a ternary system composed of two non-ionic surfactants, Tetronic® 904/704 and Procetyl AWS®, and water. A liquid isotropic precursor is used to be injected into the periodontal pocket. Dilution with saliva from the environment will generate the hexagonal and cubic liquid crystal phase, providing a sustained drug release depot *in situ*.

Using polarized light microscopy, a ternary phase diagram is constructed. It is of high importance to analyse the phase behaviour of liquid crystal systems once exposed to artificial saliva since it has been noticed that when the aqueous phase increases, the phase behaviour of the systems is changing.

Rheological tests are carried out to evaluate the characteristics of the liquid crystal formulations. Flow, oscillation and creep rheology are conducted to determine the appropriateness of the designed formulations in terms of structural behaviour. Flow rheology data yield the initial viscosity, a fundamental property of the mesophase. Oscillation rheological tests analyse the viscoelastic nature of the formulation. Creep rheology is used to determine whether the formulation is retained at the site of application.

Drug release tests are conducted to analyse if the platform provides a matrix for controlled drug release.

Subsequently, a slug mucosal irritation test is carried out to evaluate the compatibility of the formulations with mucosa.

3 MATERIALS

3.1 SURFACTANTS

3.1.1 Procetyl AWS®

Polyoxypropylene (5) polyoxyethylene (20) cethylether (PPG-5-CETEH-20), commercially available as Procetyl AWS® was kindly donated by Croda *(Goole, UK).* It's a non-ionic polyethylene oxide (PEO) surfactant. The chemical name is alkoxylated cetyl alcohol. *(www.croda.com)* (*Carvalho, Barbi et al. 2010)*

PEO-based polymers, like PPG-5-CETETH-20, are frequently used in mucoadhesive drug delivery. Mucoadhesive polymers are usually hydrophilic systems with many polar side-groups. They attach and bind to biological substrates essentially through interpenetration of polymer chains, thereafter by secondary non-covalent bonding between polymer and substrate, mostly by formation of hydrogen bonds, as the polymers contain hydrophilic groups like poly(ethylene oxide) (PEO). PEO-based polymers can join together to form a variety of liquid crystal structures. (*Carvalho, Barbi et al. 2010)*

3.1.2 Tetronics®

Poloxamines or Tetronics® are tetrafunctional poly(ethylene oxide)-poly(propylene oxide) (PEO-PPO-PEO) block copolymers. The copolymers are four branched and each branch has the structure $(EO)_x$-$(PO)_y$. A PO unit connects the four branches to an ethylene diamine. They are available in a wide range of molecular weights and EO/PO ratios *(Habas, Pavie et al. 2004; Chiappetta and Sosnik 2007)*. The general molecular structure of a polaxamine is illustrated in Figure 3.1. Tetronics® can be used for several functions such as anti-foaming agents, wetting agents, dispersants, thickeners, and emulsifiers. *(www.basf.com)* In this study Tetronic® 704 and Tetronic® 904 are used.

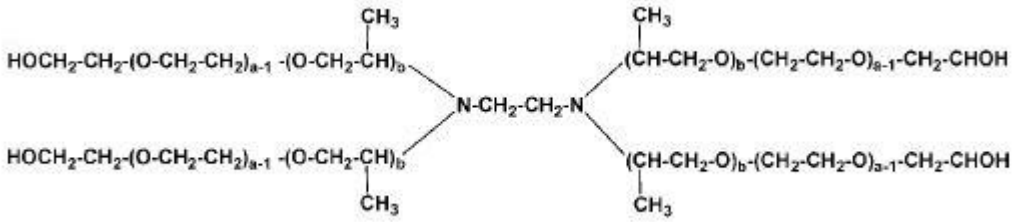


Figure 3.1 : general molecular structure of a poloxamine *(Gonzalez-Lopez, Alvarez-Lorenzo et al. 2008)*

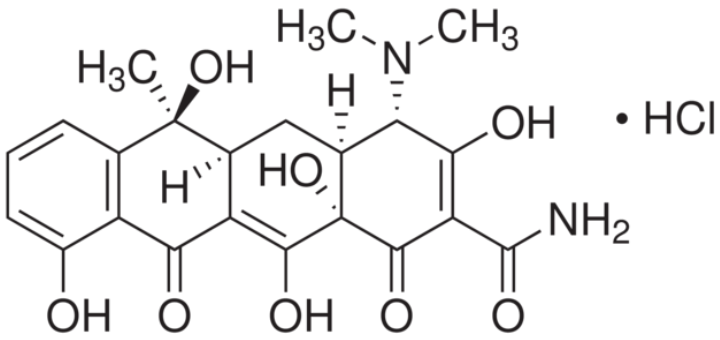
Poloxamines will undergo a phase transition from liquid to mucoadhesive gel at body temperature. This permits *in situ* gelation at the site of application. Aqueous solutions of poloxamine can be implanted as low viscosity liquids and they form a solid implant upon heating (37 °C). It has been reported that they provide ease of administration and prolonged drug delivery. Furthermore, these materials are non-irritating when applied topically or subcutaneously. *(Chiappetta and Sosnik 2007; Carvalho, Barbi et al. 2010; Jones, Muldoon et al. 2010)*

On the other hand, aqueous poloxamines show relatively low retention at the site of implantation due to the absence of specific molecular interactions between host epithelium and dosage form. Secondly, the system relies on a thermal transition to enhance viscoelastic structure of the implant. These deficiencies can be overcome with the development of bioadhesive formulations in which rheological structuring occurs following exposure to the biological fluids present at the site of application. Since poloxamines do not exhibit bioadhesive properties, such systems require the inclusion of polymers that will provide this essential property, for example the PEO-surfactant PPG-5-CETEH-20. *(Chiappetta and Sosnik 2007; Carvalho, Barbi et al. 2010; Jones, Muldoon et al. 2010)*

Tetronic® 904 *(BASF Corporation, New Jersey, USA)* is a non-ionic surfactant. It is a slightly milky, cloudy coloured liquid, with an odour of polyol. It has a viscosity of 8 200 cPs. The molecular weight is 6 700 Da with an average of 60.9 EO units and 69.3 PO units. Tetronic 704 *(BASF, New Jersey, USA)* is a liquid with an amber like colour and mild odour of polyol. The viscosity of T704 is 1 390 cPs. The molecular weight is 5 500 Da with an average of 50.0 EO units and 56.9 PO units. *(www.basf.com) (Prokop, Kozlov et al. 2002)*

3.2 DRUG

Tetracycline hydrochloride *(Sigma-Aldrich, Steinheim, Germany)* is an antibiotic active against Gram-negative and Gram-positive bacteria. It is also active against β-lactamase producing strains, which frequently grow in ≥6 mm deep periodontal pockets. Tetracycline hydrochloride inhibits the elongation step in bacterial protein synthesis by preventing binding of aminoacyl-tRNA to the 30S subunit. Furthermore, the drug inhibits tissue



collagenase activity. Collagenase is a protein that destroys connective tissue and bone. *(van Winkelhoff, Winkel et al. 1997; Schwach-Abdellaoui, Vivien-Castioni et al. 2000)*

Figure 3.2: Chemical structure of tetracycline HCl

It is a yellow, crystalline powder. The solubility of tetracycline HCl in water is 10 mg/mL, thus the drug is good water soluble. The chemical structure, $C_{22}H_{24}N_2O_8 \cdot HCl$, is shown in Figure 3.2. The drug has a molecular weight of 480.90 g/mol. *(European Pharmacopoeia 7.0; www.sigmaaldrich.com)*

3.3 ARTIFICIAL SALIVA

Artificial saliva is prepared using the following substances. Potassium phosphate dibasic is purchased from Fisher Scientific *(Leicestershire, UK).* Potassium phosphate monobasic, potassium chloride and calcium chloride dehydrate are purchased from BDH *(Poole, UK)*. Sorbitol and sodium chloride are supplied by Sigma Aldrich *(Steinheim, Germany)*. Magnesium chloride hexahydrate is purchased from DH Scientific *(Huddersfield, UK).* Finally, methylparaben is provided by Aldrich Chemical *(Gillingham, England)*.

The water used for all the samples and experiments is purified water (resistivity > 18.2 MΩcm; *Elga Purelab Maxima, Co. Kildare, Ireland*)

4 METHODS

4.1 LIQUID CRYSTAL PREPARATION

Formulations are prepared by weighing Tetronic® 904 or 704, purified water and Procetyl® into a glass vial or plastic container. The materials are then thoroughly mixed using a spatula.

4.2 PHASE DIAGRAM DETERMINATION

The conditions over which each type of mesophase exists are explored via a phase diagram, which represents the phase equilibria within ternary systems. The phase diagram makes it possible to verify the different types of systems formed from the mixture of components. Subsequently, the most appropriate range for the development of a drug delivery system can be chosen. *(Carvalho, Rocha e Silva et al. 2012)*

Samples of different compositions of Tetronic® (T) 904/704, water (W), Procetyl® (PC) are transferred in glass tubes (2g in total), homogenized and stored overnight in the fridge in order to determine the boundaries of different phases.

Table 4.1: Composition of liquid crystal samples containing Tetronic 904, water and Procetyl, prepared to determine the boundaries of the different phases in order to compose a phase diagram

Sample number	Composition (%, w/w)		
	Tetronic 904	Water	Procetyl
1	10	30	60
2	28	30	42
3	37.5	30	32.5
4	40	30	30
5	33	40	27
6	40	35	25
7	42	37	21
8	29	41.5	29.5
9	10	45	45

10	3	50	47
11	10	80	10
12	4	80	16
13	10	74	16
14	16	78	6
15	16	73	11
16	43	47	10
17	43	53	4
18	39	54	7
19	50	20	30
20	50	23	27
21	43	24	33

Table 4.2: Composition of liquid crystal samples containting Tetronic 704, water and Procetyl, prepared to determine the boundaries of the different phases in order to compose a phase diagram

Sample number	Composition (%, w/w)		
	Tetronic 704	Water	Procetyl
22	7	50	43
23	14	40	46
24	21	45	34
25	20	50	30
26	28	46	26
27	44	40	16
28	50	37	13
29	50	20	30
30	45	21	34
31	40	27	33
32	64	14	22
33	80	13	7
34	72	14	14

Polarized light microscopy (PLM) (Olympus BX50F4, *Microscope Service and Sales, Surrey, England*) at room temperature is used to determine the phase boundaries. Photomicrographs are taken at a magnification of 40x or 100x using a Pixelink® Megapixel Firewire Camera. PLM can determine whether a material has an isotropic or anisotropic behaviour. It is designed to observe specimens that are visible principally due to their optically anisotropic character. The properties of anisotropic structures are dependent of direction, while isotropy, means that the material has identical properties in all directions. To achieve this task, the microscope is equipped with both a polarizer and an analyzer (a second polarizer).
(Rochow and Tucker 1994) (www.olympusmicro.com/primer/techniques/polarized/polarizedhome.html)

PLM is suitable for detection of lyotropic liquid crystals (except cubic mesophases) because liquid crystals demonstrate birefringence. Each liquid crystal shows typical black and white textures under PLM. The lamellar and hexagonal phases are classified as anisotropic structures. Evidence of the anisotropic character can be found in the double refraction or birefringence. *(Muller-Goymann 2004)*

The interaction of plane-polarized light with birefringent (also called doubly-refracting) material yields two individual wave components that are each polarized in mutually perpendicular planes. The velocities of the wave components are different and vary with the propagation direction through the specimen. After exiting the material, the light components are out of phase, but are subsequently recombined with constructive and destructive interference when passing through the analyzer. The sample appears bright when viewed between two light-polarizers arranged in the so-called crossed position. Isotropic materials appear dark between crossed polarizers. *(www.olympusmicro.com/primer/techniques/polarized/polarizedhome.html)*

Both cubic phase and liquid isotropic phase are isotropic systems, presented as a dark view under PLM. However distinction can be made because of their difference in viscosity. Liquid isotropic phases are fluid, whereas cubic phases display high viscosity. The hexagonal and lamellar lyotropic liquid crystalline phases are clear but anisotropic gels. Both hexagonal and lamellar lyotropic liquid crystals show characteristic structures under the microscope.

Malta crosses are observed in the lamellar liquid crystal phase. Hexagonal mesophases can be recognized by their typical fan texture. *(Muller-Goymann 2004; Siddig, Radiman et al. 2004; Carvalho, Barbi et al. 2010; Carvalho, Rocha e Silva et al. 2012)*. Based on the structures examined using PLM a phase diagram is constructed using SigmaPlot Software®.

4.3 RHEOLOGICAL CHARACTERIZATION

Two different formulation lines of the phase diagram are prepared and compared in order to obtain the ideal drug delivery formulation (Table 4.3).

Table 4.3: Composition of liquid crystal formulations that are characterized using rheological analysis.

Sample	Composition (%, w/w)		
	Tetronic® 904/704	**Water**	**Procetyl®**
First formulation line			
Precursor 1	10.00	25.00	65.00
20% dilution	8.33	37.50	54.17
60% dilution	6.25	53.125	40.625
Second formulation line			
Precursor 2	30.40	21.60	48.00
20% dilution	25.00	35.00	40.00
60% dilution	19.00	51.00	30.00

The drug tetracycline HCl is added to each drug delivery systems (drug concentrations: 1% and 5%) by suspending the drug powder immediately into the systems. Identical rheological tests are executed to examine whether the drug affects the rheological properties of the system.

4.3.1 Flow rheological analysis

Flow rheology is conducted in order to attain the initial viscosity of the formulations. Flow rheometry is performed at 37 ± 0.1°C using an AR 2000 rheometer (T.A. Instruments, Surrey, England) operating in continuous flow mode using a 4 cm solvent trap steel plate and a gap of 1000 µm. The system is equilibrated during three minutes. Flow rheology is conducted using a continuous ramp test in which the shearing rate is increased gradually

from a minimum (0 s^{-1}) up to a predetermined maximum (50 s^{-1}) within 10 minutes and subsequently returned to the starting shear rate under the same conditions. The data from the flow rheometry are modelled using a power-law model, using Rheology Advantage Data Analysis (T.A. Instrument):

$$\sigma = k \cdot \gamma^n \quad (3.1)$$

where σ is the shearing stress, γ is the rate of shear, k is the consistency and n is the pseudoplastic index.

The precursors are liquid formulations, flow rheometry is conducted at 37 ± 0.1 °C using an AR 2000 rheometer operating in continuous flow mode using standard-size double concentric cylinders (rotor outer radius of 21.96 mm and inner radius of 20.38 mm, stator inner radius of 20.00 mm, height cylinder of 59.50 mm). A gap of 500 µm is used. Flow rheology is conducted using a continuous ramp test in which the shear rate is gradually increased from 0 s^{-1} to 50 s^{-1} within 10 minutes and subsequently returned to the starting shear rate under the same conditions.

4.3.2 Oscillatory rheological analysis

The oscillatory analysis is performed at 37°C with a AR2000 rheometer in oscillatory mode, using a 4 cm solvent trap steel plate, separated by a gap of 1000 µm. Samples are carefully applied to the lower plate, ensuring that formulation shearing is minimized and allowed to equilibrate for 3 minutes before analysis. Sweep oscillatory analysis is executed after determination of the linear viscoelastic region at 37°C, where stress is directly proportional to strain and the storage modulus remained constant. 30 Pa is selected as stress amplitude, which is found to be in the LVR. Frequency sweep analysis is carried out over the frequency range of 0.1-10 Hz, following application of a constant stress of 30 Pa.

4.3.3 Creep rheological analysis

The creep analysis of samples is performed at 37°C with a AR2000 rheometer in creep mode, using a 4 cm solvent trap steel plate, separated by a gap of 1000 µm. Samples are carefully applied to the lower plate, ensuring that formulation shearing is minimized and allowed to equilibrate for 10 minutes before analysis. Creep analysis is conducted for 20

minutes using a constant shear stress of 30 Pa. To analyze the weight sum retardation time of each formulation, five Kelvin models (0-50s, 50-100s, 400-800s, 800-1200s) are introduced to measure creep curves, based on the mathematical model developed by *Yu Tao.* The relative stable retention time (RSRT) is seven times of the weight sum retardation time. RSRT is defined as the period from elastic stretching occurred on molecular chains till its limitation is reached.

4.4 *IN VITRO* DRUG RELEASE

The *in vitro* drug release test is based on dissolution test for transdermal patches. (Ph. Eur. 7.0, 2.9.4) *In vitro* release of tetracycline HCl from the formulations is executed using a Cellu Sep® regenerated cellulose tubular membrane *(Orange Scientific, Braine-l'Alleud, Belgium)*. The release medium is 50.00 mL artificial saliva, prepared as follows: 0.82% potassium phosphate dibasic, 0.036% potassium phosphate monobasic, 4.27% sorbitol solution 70%, 0.063% potassium chloride, 0.087% sodium chloride, 0.013% magnesium chloride hexahydrate, 0.007% calcium chloride dehydrate, 0.180% methylparaben and 94.524% water. *(Bruschi, de Freitas et al. 2008)* 0.50 mL of the precursors of the formulation lines, the liquid isotropic phases, is injected using a 1.00 mL syringe into the membrane. The membrane is closed between two plastic carriers and emerged into 50.00 mL artificial saliva. The temperature is maintained at 37°C using an Unitron incubator. The beakers are covered using Parafilm during the test to minimize evaporation. At predetermined intervals (0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 4h, 5h, 6h), 1 mL sample of the release medium is withdrawn from the zone midway between the surface of the dissolution medium and the membrane. The sample is filtered through a Millex®GS filter Unit 0.22 µm *(Millipore, Carrigtwohill, Co. Cork, Ireland)*. Each drug release experiment is conducted three times.

Drug concentration is determined using a high performance liquid chromatography (HPLC) system: a Waters binary HPLC pump 1525, a Waters Plus Autosampler 717, a Waters In-line Degasser AF and a Waters Dual ʎ Absorbance Detector 2487 (*Waters, Hertfordshire, UK)*. The mobile phase consists of 40% methanol and 60% water + 0.2% trifluoracid. The flow rate is 1 mL/min, injection volume is 10 µL and a running time of 15 minutes is used. A Thermo Scientific 5 µm Hypersil® BDS C18 column *(Hertfordshire, UK)* with dimensions of 250 x 4.6 mm is used. The temperature is set at 37°C. A wavelength of 254 nm (Ph.Eur. 7.0)

is used for detection of tetracycline HCl. Chromatograms are interpreted using Waters Breeze® software.

4.5 SLUG MUCOSAL IRRITATION TEST

The slug mucosal irritation test is used to compare the mucosal toxicity of the liquid crystal formulations with two negative controls. Mucus production is an indicator of epithelial cell damage. Another marker used in this assay is lactate dehydrogenase (LDH). LDH is release from the foot of a slug in response to cell damage. The testing protocol is adapted from a method described by Forbes, Lowry et al. (2011). Keel slugs *(Tandonia budapestensis)* weighing between 4-6 g were collected locally (Belfast, Northern Ireland). The slugs are housed individually in ventilated plastic boxes, which contained lettuce and cucumber for 4 days before the start of the experiment. The precursor (5 mL) is spread onto the base of a standard plastic Petri dish (90 mm diameter). A slug is placed on top of the material, left in contact for 30 minutes and then transferred to a fresh Petri dish containing 5 mL PBS (pH 7.4) for a further 60 minutes. The slug is subsequently transferred to another Petri dish containing 5 mL PBS for a further 60 minutes. Both volumes of PBS solution are collected and analysed for LDH concentrations. LDH levels are expressed as a percentage of the total LDH released in response to the positive control irritant (1% benzalkoniumchloride in PBS). The cumulative weight of produced mucus is determined by weighing each Petri dish before and after the slug is added. Each formulation is assessed over three consecutive days. At the end of each daily experiment, the slugs are returned to their plastic boxes until the next contact period. LDH levels are determined using an *in vitro* toxicology assay kit (Sigma Aldrich®) and a Bio-Tek Powerwave XS UV multi-well plate reader *(Bedfordshire, UK)*. *(Forbes, Lowry et al. 2011)*

5 RESULTS AND DISCUSSION

5.1 LIQUID CRYSTAL PLATFORM FOR ORAL APPLICATION: TETRONIC® 904, WATER AND PROCETYL AWS®

5.1.1 Phase diagram

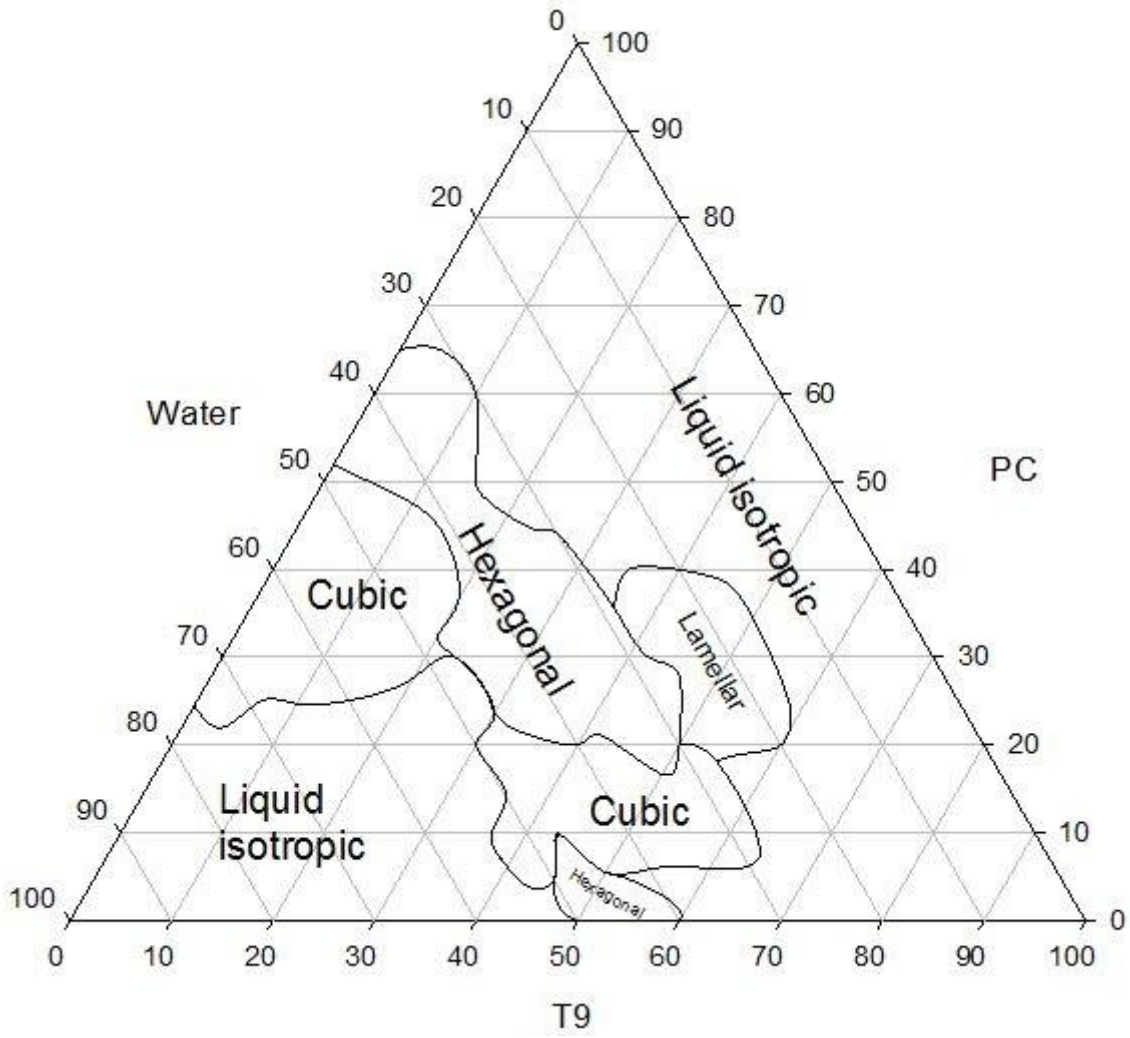


Figure 5.1: Phase diagram (at 25°C) of ternary system containing Tetronic®904, water and Procetyl®, constructed based on visual observations via PLM

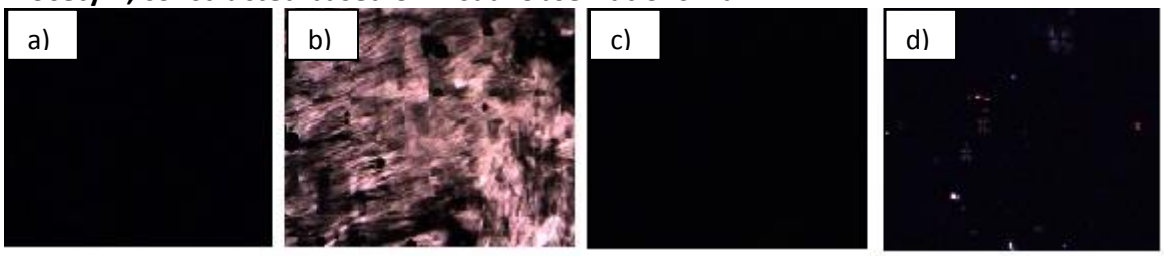


Figure 5.2: Polarized light microscopy pictures (magnification 40x) of ternary liquid crystal systems containing Tetronic® 904 (T9), water (W) and Procetyl® (PC). (a) 10% T9/80%W/10% PC (liquid isotropic), (b) 10%T9/30%W/60% PC (hexagonal, fan structure), (c) 10%T9/45%W/45%PC (cubic phase, isotropic), (d) 50% T9/20%W/30% PC (lamellar phase, Malta crosses).

The phase diagram of Tetronic® 904/water/Procetyl® system at 25°C is illustrated in Figure 5.1. Figure 5.2 demonstrates the polarized light images used to determine the phase diagram based on the observed structures. Derived of the phase diagram, it can be seen that liquid isotropic phases exist at low concentrations of surfactants and high water concentrations or at low concentrations of water and high surfactant concentrations. The cubic phase can be seen at low concentration of T9 and intermediate concentrations of water and PC or at low concentration of PC and intermediate concentrate of T9 and water. At a concentration around 40% water a hexagonal phase area establishes. The phase diagram can be used to prepare formulations with the desirable liquid crystal phase. The aim is to choose a liquid isotropic precursor which can undergo phase transition to hexagonal and cubic phase by absorbing saliva once injected into the periodontal pocket, thereby forming a sustained release depot *in vivo*.

The following formulation line is chosen as liquid crystal platform for further rheological characterization and drug release tests:

Table 5.1: Composition of precursor 1 and diluted samples (i.e. first formulation line)

Sample	Phase behaviour	Composition (%, w/w)		
		Tetronic® 904	Water	Procetyl®
Precursor 1	liquid isotropic	10.00	25.00	65.00
20% dilution	Hexagonal	8.33	37.50	54.17
60% dilution	Cubic	6.25	53.125	40.625

5.1.2 Rheological characterization of the blank formulations

The results of the rheological analysis of the formulations are displayed in Table 5.3 and 5.4. Oscillation and creep results of the first formulation line show that the hexagonal phase looses the stable and rigid hexagonal structure at 37°C. The liquid-like state is observed visually and confirmed by oscillation rheology since G”>G’. Furthermore, the formulation is not retained at the site of application according to the creep results. As a result, another line of the phase diagram (Table 5.2) is evaluated (i.e. second formulation line).

Table 5.2: Composition of precursor 2 and diluted samples (i.e second formulation line)

Sample	Phase behaviour	Composition (%, w/w)		
		Tetronic® 904	Water	Procetyl®
Precursor 2	liquid isotropic	30.40	21.60	48.00
20% dilution	Hexagonal	25.00	35.00	40.00
60% dilution	Cubic	19.00	51.00	30.00

Table 5.3: Rheological properties (n=3, average ± standard deviation) of liquid crystals containing Tetronic®904 (T9), water (W) and Procetyl® (PC).

Sample	Flow rheology	Creep rheology
	Initial viscosity (Pa.s)	RSRT (s)
First formulation line		
Precursor 1	0.90 ± 0.03	-
20% dilution	69 ± 21	0.42 ± 0.39
60% dilution	1 599 ± 283	3 044 ± 693
Second formulation line		
Precursor 2	1.34 ± 0.07	-
20% dilution	196 ± 12	1 085 ± 79
60% dilution	398 ± 26	2 029 ± 205

Table 5.4: Oscillation rheology (n = 3, average ± standard deviation) of liquid crystals containing Tetronic® 904 (T9), water (W) and Procetyl® (PC)

Sample	Oscillatory frequency (Hz)	Viscoelastic properties (at 37°C)	
		G' (Pa)	G'' (Pa)
First formulation line			
20% dilution	0.1	1.63 ± 0.13	8.34 ± 0.29
	10	112 ± 1.84	220 ±1.4
60% dilution	0.1	55 653 ± 1053	9 062 ± 330
	10	76 687 ± 2082	3 947 ± 324
Second formulation line			
20% dilution	0.1	3 486 ± 793	4 059 ± 643

	10	35 370 ± 2065	12 530 ± 283
60% dilution	0.1	12 660 ± 1601	2 881 ± 222
	10	19 877 ± 1853	1 640 ± 104

Flow results of the liquid isotropic precursor of the first line demonstrate that initial viscosity is very low, 0.90 Pa.s, confirming the liquid isotropic phase. Because of the low viscosity, the formulation can be used as a precursor for the more structured and rigid hexagonal and cubic phase. Highly viscous formulations like the hexagonal and cubic phase are clumsy to handle and difficult to inject, thus a liquid isotropic precursor is required. The precursor can be easily injected into the periodontal pocket. Once in the pocket, saliva from the environment will be gradually absorbed inducing phase transition to the hexagonal and cubic phase. Consequently, a sustained release depot is formed *in situ*.

Furthermore, flow rheology indicates that the liquid isotropic formulation shows pseudoplastic behaviour, since the viscosity is decreased when strain is increased. While injecting, strain is applied on the formulation, which offers ease of syringeability.

20% dilution of the precursor with water to the 8.33% T9, 37.50%W and 54.17% PC hexagonal formulation does not present promising rheological characteristics. Oscillation analysis indicates the liquid-like state of the semi-solid with results of G" higher than G' for both low and higher oscillatory stresses. Furthermore, creep test shows a retardation time around 0 seconds. Thus, the formulation is not retained at the site of application and immediately starts to flow. These results demonstrate that the formulation is not appropriate as a matrix for the sustained release of a drug molecule. The formulation melts at 37°C with a loss of the rigid structure. To explain this phenomenon, it is useful to construct a new phase diagram at 37°C instead of room temperature to determine changes of phase boundaries. Additionally, differential scanning calorimetry could be conducted in order to detect the melting point of the hexagonal phase.

Upon further dilution of the formulation with water (60% dilution), the cubic phase renders more advantageous rheological properties. A high viscosity of 1 599 ± 283 Pa.s is observed from flow rheology. Oscillation rheology confirms that the formulation is a solid-like semi-solid since G' is significantly higher than G" for both low and higher oscillatory

stresses. The rigid solid-like state is thus maintained independent of applied stresses. Creep rheology indicates that the formulation is retained in the periodontal pocket for 3 044 ± 693 s. Summarized, the cubic phase can be used as a matrix for sustained drug release which is retained at site of application. Although the 20% dilution formulation does not display good rheological properties, the first line formulation can be used as a liquid crystal platform. Since the amount of saliva is dependent on each individual patient, it is for example possible, that the amount of saliva of the patient is sufficient to dilute the precursor 40% with the opportunity to induce a phase transition to a stable and rigid liquid crystal that can be retained at the site of application.

Nevertheless, the ideal situation would be to have stable formulations for both 20% and 60% dilutions. It is desirable that once the precursor is injected, saliva dilutes the formulation in order to form a sustained release depot *in situ*. Since the amount of saliva is dependent on each patient, it is favourable to have a wide phase range where stable formulations can be formed at different dilution rate.

Similar to the first formulation line, the precursor of the second formulation line is a liquid isotropic phase with a low initial viscosity of 1.34 Pa.s and a pseudoplastic behaviour. Again, it can be stated that the liquid isotropic phase can be used as a suitable precursor for the stiff hexagonal and cubic phase and can be injected into the periodontal pocket by a syringe.

The hexagonal formulation (i.e. 20% dilution), displays advantageous rheological characteristics. A viscosity of 196 ± 12 Pa.s indicates the elastic nature of the mesophase. A retention time of 1 085 ± 79 s points out that the formulation will be retained at the site of application for a prolonged period of time. Oscillation rheology confirms these results since G’ is remarkable higher than G”.

Further absorption of saliva from the environment (60% dilution) will induce a phase transition to the cubic liquid crystal phase. A high viscosity of 398 ± 26 Pa.s is noticed. The cubic phase is a very rigid structure which can be derived from the oscillation rheological results, G’ is remarkable higher than G” at both low and higher oscillatory stresses. The formulation will be retained since creep results present a retardation time of 2 029 ± 205 s. Thus, the second line is a good candidate for a sustained release liquid crystal matrix. The

second line is the choice of preference since 20% dilution of the precursor with saliva induces phase transition to a solid-like and sustained release matrix. Further dilution to the cubic phase even increases this ability to retain the drug at the site of application.

5.1.3 Rheological characterization of liquid crystals containing tetracycline HCl

First, 5% tetracycline HCl (TC) was added to the formulations. The first formulation line showed similar rheological characteristics as the blank formulations. However, addition of 5% tetracycline HCl to the formulations of the second line significantly affected the structure of the samples (data not shown). Examination of the samples indicates that the drug reduces the rheological properties of the semi-solid formulations. Instead of the observed elastic semi- solid hexagonal and cubic phases in the blank samples, addition of tetracycline HCl introduces a phase transition to a liquid isotropic system. The formulations cannot be used as candidates for sustained drug delivery platform. The same formulations are prepared but with a lower concentration of tetracycline HCl (1%) and stored overnight. Rheological results of the 1% tetracycline HCl formulations are displayed in Table 5.5 and 5.6.

Table 5.5: Rheological properties (n=3, average ± standard deviation) of liquid crystals containing Tetronic®904 (T9), water (W), Procetyl® (PC) and 1% tetracycline HCl.

Sample	Flow rheology	Creep rheology
	Initial viscosity (Pa.s)	RSRT (s)
First formulation line		
Precursor 1	1.28 ± 0.26	-
20% dilution	1.04 ± 0.20	-([a])
60% dilution	1 006 ± 179	4 466 ± 629
Second formulation line		
Precursor 2	1.54 ± 0.05	-
20% dilution	183 ± 25	479 ± 110
60% dilution	721 ± 59	1 464 ± 184

([a]) As the formulation immediately melts at 37°C, rheological tests could not be executed.

Table 5.6: Oscillation rheology (n=3, average ± standard deviation) of liquid crystals containing Tetronic®904 (T9), water (W), Procetyl® (PC) and 1% tetracycline HC

Sample	Oscillatory frequency (Hz)	Viscoelastic properties at 37°C	
		G' (Pa)	G" (Pa)
First formulation line			
20% dilution	0.1	4.93 ± 1.34	20 ± 3
	10	310 ± 11	439 ± 17
60% dilution	0.1	41 813 ± 1 040	5 746 ± 127
	10	57 237 ± 1 085	2 833 ± 127
Second formulation line			
20% dilution	0.1	4 573 ± 643	3 486 ± 738
	10	46 993 ± 1884	18 357 ± 838
60% dilution	0.1	39 407 ± 999	7 114 ± 84
	10	58 817 ± 1 113	5 429 ± 41

The rheological properties of the 1% tetracycline HCl formulations of the first formulation line are similar to the blank formulation. The precursor is liquid-like with a low viscosity which enables injection in the periodontal pocket. The 20% dilution, hexagonal phase, looses the rigid and stable structure at 37°C as seen by an initial viscosity around 1 Pa.s and the liquid-like state (G”>G’). Furthermore, the formulation is not retained in the periodontal pocket because of its liquid-like state. Microscopic analysis reveals that tetracycline HCl has an influence on the liquid crystal structure since the hexagonal structure cannot be detected strongly, a dark field is present (see 5.1.4). The 60% dilution, cubic phase, offers a very rigid structure with a viscosity of 1 008 ± 179 Pa.s, a storage modulus significantly higher than the loss modulus and a retention time of 4 466 ± 629 s. Despite the advantageous characteristics of the cubic phase, the first formulation line is not the first choice to treat periodontal disease. The hexagonal phase melts at 37°C, thereby losing its rheologically structured properties. It is favourable that the formulation line displays stable formulations that are retained in the periodontal pocket for both 20% and 60% dilution.

Since the phase transitions of the precursor are dependent on the amount of saliva of each patient, it is critical to obtain stable formulations at different dilution ranges.

The precursor of the second formulation line is liquid isotropic with a viscosity of 1.04 ± 0.20 Pa.s. Dilution with water induces phase transition to solid-like semi-solids. The 20% dilution has an initial viscosity of 183 ± 25 Pa.s. Oscillation tests confirm the solid nature of the mesophase since G'>G". Creep test indicates good sustained release properties with a retention time of 479 ± 110 s. The 60% dilution offers a good sustained release matrix with a viscosity of 721 ± 59 Pa.s, solid-like phase (G'>G") and retention at the site of application (retention time of 1 464 ± 184 s). Thus, the second line formulation provides a good liquid crystal platform for the sustained release of tetracycline HCl in order to treat periodontal disease efficiently.

Noticeable is that oscillation (as illustrated in Figure 5.3) and flow results of the 1% tetracycline HCl cubic phase of the second formulation line are significantly higher than their blank formulations. Tetracycline HCl might reduce the hexagonal phase present in the blank formulation and thus enhance rheological properties.

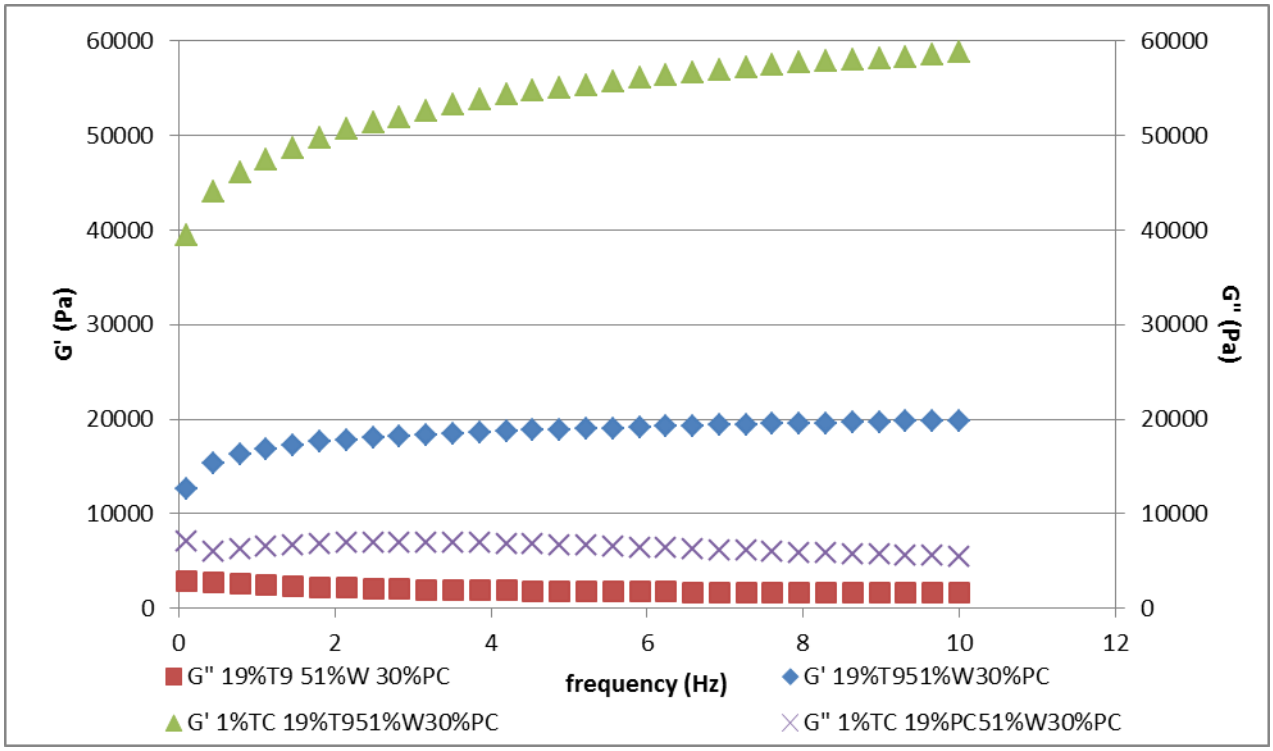


Figure 5.3: Oscillation rheology (n=3) of 60% dilution sample of the second formulation line in comparison with 1% tetracycline HCl samples.

5.1.4 Microscopic analysis

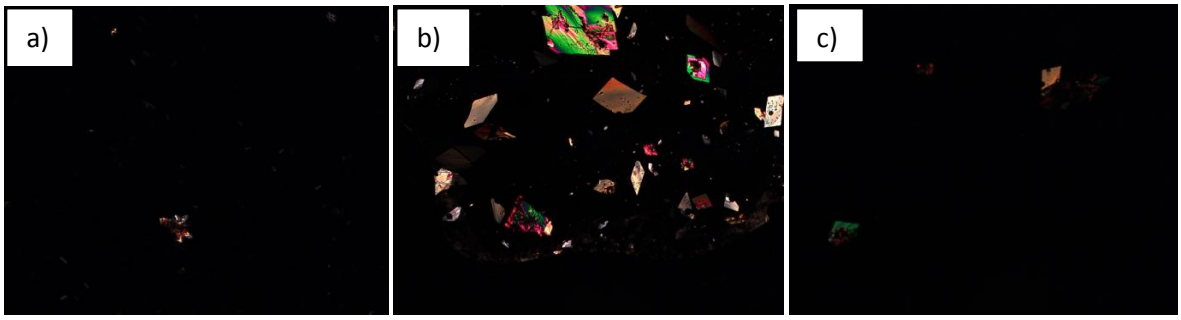


Figure 5.4: Polarized light microscopy images (magnification 40x) of ternary systems containing Tetracycline HCl (TC),Tetronic®904 (T9), water (W) and Procetyl® (PC). (a) 1%TC/ 10%T9/25%W/65%PC (liquid isotropic phase), (b) 1%TC/8.33%T9/37.5%W/54.17%PC (20% dilution, dark field), (c) 1%TC/6.25%T9/53.125%W/48%PC (60% dilution, cubic phase)

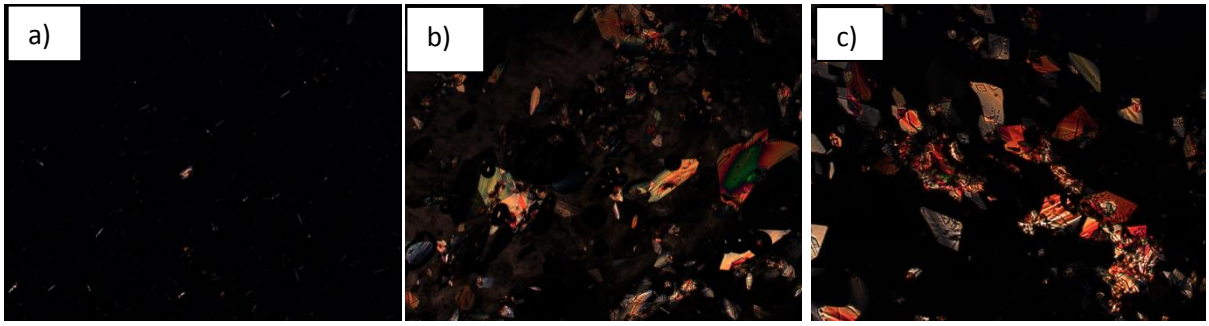


Figure 5.5: Polarized light microscopy images (magnification 40x) of ternary systems containing Tetracycline HCL (TC),Tetronic®904 (T9), water (W) and Procetyl® (PC). (a) 1%TC/30.40%T9/21.60%W/48%PC (liquid isotropic phase, (b) 1%TC/25%T9/35%W/40%PC (20% dilution, fan structure), (c) 1%TC/19%T9/51%W/30%PC (60% dilution, dark field)

The influence of tetracycline HCl on the liquid crystal structures is examined using PLM. Tetracycline HCl itself is observed as crystals under PLM. It can be seen that tetracycline HCl does not influence the liquid isotropic and cubic phase. Nevertheless, it is noticed that the 20% dilution of the precursor of the first formulation line, does not display the typical fan structure of a hexagonal phase. The 20% dilution formulation is a rigid and isotropic gel, thus the addition of tetracycline HCl induces a phase transition to the cubic phase. The cubic phase is dominating the hexagonal phase. This change in structure will be the reason for the loss of structure noticed during rheological characterization. The 20% dilution of first formulation line remains a hexagonal phase, as the typical structure can be seen using PLM.

5.1.5 *In vitro* drug release

The drug release profiles of the tetracycline HCl formulations can be seen in Figure 5.6. The drug release curve of the 1% tetracycline HCl formulation corresponds to a zero-order release model in both formulations. Zero-order kinetics can be expressed by following equation:

$$Q_t = Q_0 + K_0 t \quad (5.1)$$

where Q_0 is the initial amount of drug, Q_t the cumulative amount of drug release at time t, K_0 zero order release constant and t is time in h.

The release of tetracycline HCl is controlled and displays zero-order kinetics since $R^2 > 0.9$.

The amount of tetracycline HCl released in six hours is about 34%. The formulations show controlled drug release. Typically for liquid crystal systems is the slow release of the active agent. Since tetracycline HCl is a hydrophilic drug, the release from the matrix is controlled by diffusion through the aqueous channels of the liquid crystal systems. *(Boyd, Whittaker et al. 2006; Costa-Balogh, Sparr et al. 2010)*

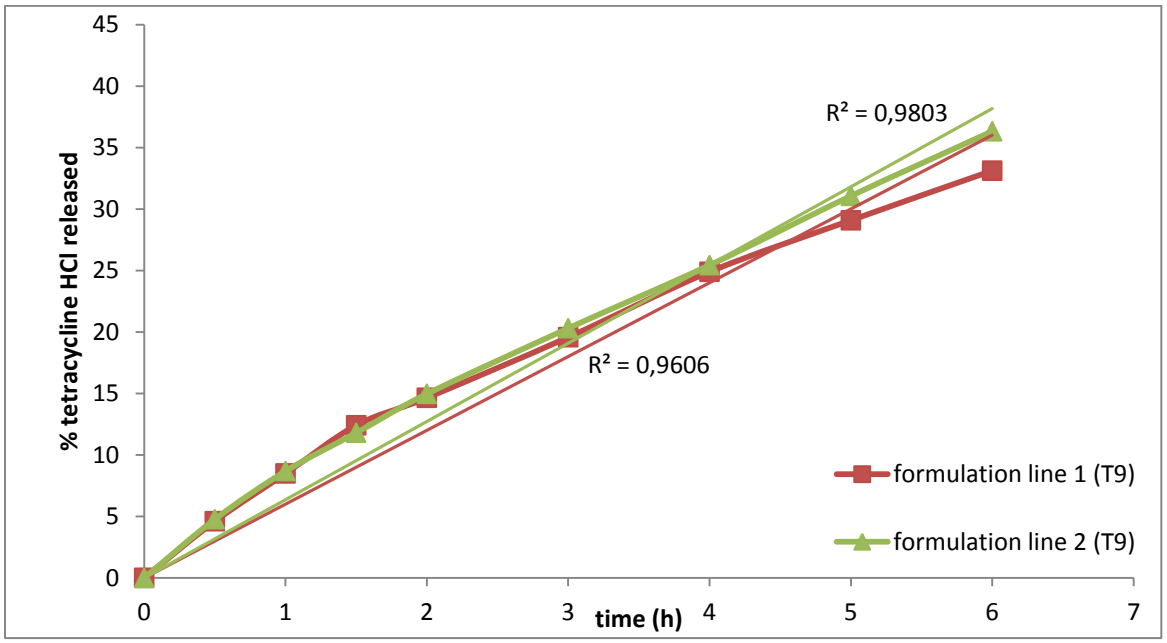


Figure 5.6. : Drug release profile (n=3) of liquid crystals liquid crystals containing Tetronic®904 (T9), water (W), Procetyl® (PC) and 1% tetracycline HCl.

Subsequently, drug release of 1% tetracycline HCl, suspended in artificial saliva, is conducted (Figure 5.7). The amount of tetracycline HCl released during the drug release experiment is significantly higher, and the drug release rate is not controlled.

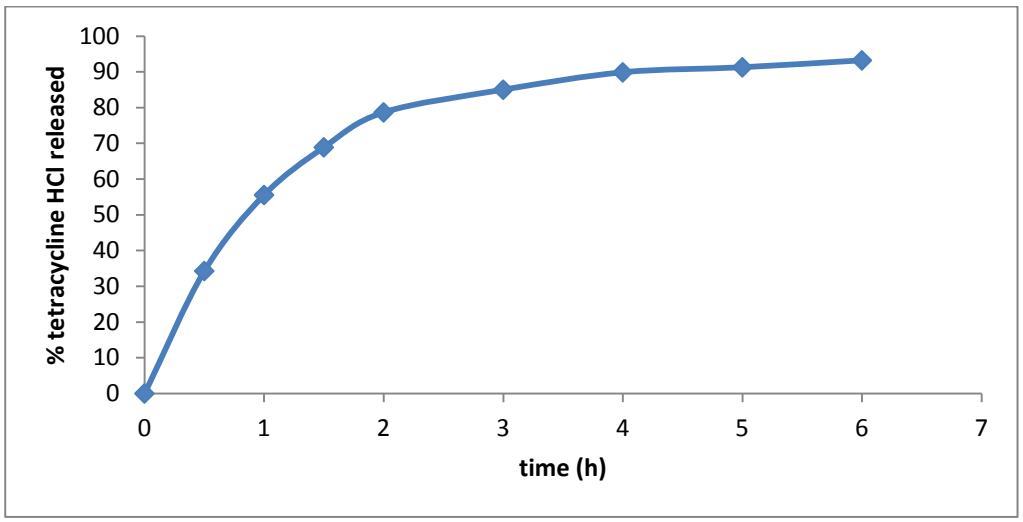


Figure 5.7: Drug release profile (n=3) of pure tetracycline HCl.

Furthermore, tetracycline HCl release is plotted against the square root of time (Figure 5.8), because it has previously been reported that release of drugs from mesophase matrices is controlled by diffusion through the matrix.*(Boyd, Whittaker et al. 2006; Costa-Balogh, Sparr et al. 2010)* Drug release can be described by the Higuchi diffusion equation given by:

$$Q = (D_m C_d (2A - C_d)t)^{1/2} \quad (5.2)$$

where Q is the mass of drug released at time t which is proportional to the apparent diffusion coefficient of the drug in the matrix D_m, the initial amount of drug in the matrix A, and the solubility of the drug in the matrix C_d. The slope of this plot is proportional to the apparent diffusion coefficient for the drug in the matrix. *(Boyd, Whittaker et al. 2006)*

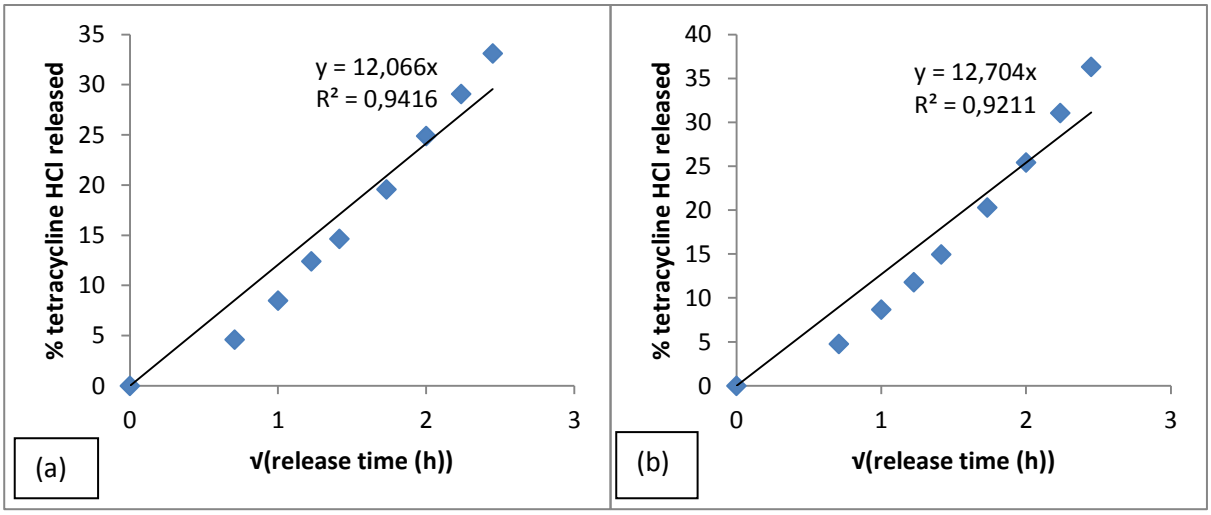


Figure 5.8: Release of tetracycline HCl (vs. square root of time) from the precursor formulations. (a) Precursor 1, (b) Precursor 2

The plots are linear (R^2 > 0.9) which shows that the release of tetracycline HCl is determined by diffusion through the matrix. The slope of the curves is proportional to the apparent diffusion coefficient of the drug in the matrix and presented in Table 5.7. The velocity of drug release is equal for both precursors. In summary, it can be said that release of tetracycline HCl from the formulations shows very similar profiles.

Table 5.7: Slope of the drug release profiles from liquid isotropic phases

Sample	Slope of % released vs. released time$^{1/2}$
Precursor 1	12.07
Precursor 2	12.70

5.1.6 Slug mucosal irritation test

The precursor of the first formulation line is spread onto the base of a petri dish. When the slug is brought into contact with the formulation, it is immediately noticeable that the colour of the produced mucus is not clear but displays a yellow colour. The mucus production after 30 minutes of contact is illustrated in Figure 5.9 (a). Mucus production is an indicator of epithelial cell damage.

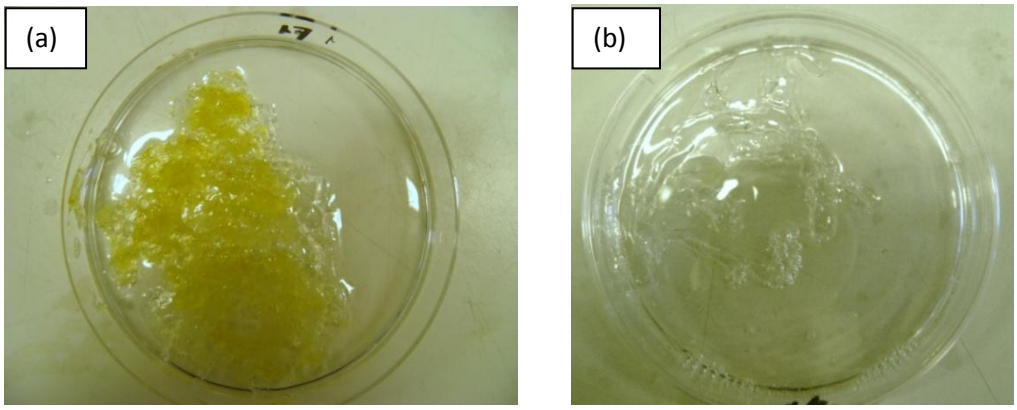


Figure 5.9. : (a) Mucus production after 30 minutes of contact with the precursor of first formulation line, (b) mucus production after 30 minutes of contact with 75% Tween 80 and 25% water.

The slug mucus production and weight loss in response to each formulation are illustrated in Table 5.8. Slugs exposed to the negative controls (i.e. blank and PBS) produced low levels of clear mucus (range 7-12%). The mucus production after exposure to the precursor of the first formulation line is remarkable higher, 26.8% and the weight loss is around 48.3%. These results clearly indicate that the formulation causes mucosal irritation. Further research is necessary to investigate whether Procetyl® or Tetronic® is responsible for the irritation. One of the surfactants can be replaced by Tween 80. When an irritation test is conducted using 25% water and 75% Tween 80 as precursor formulation, mucus production is 8.67% (comparable to the negative control) and a clear mucus is noticed (Figure 5.9 (b)).

Table 5.8 : Mucus production and weight loss over three consecutive days (relative to total body mass) for slugs exposed to control solutions and test formulations.

Test substance	**Day 1**		**Day 2**		**Day 3**	
	Mucus (%)	Weight loss (%)	Mucus (%)	Weight loss (%)	Mucus (%)	Weight loss (%)
1% BZC in PBS	42.6 ± 14.9	43.1 ± 3.2	-	-	-	-
Blank control	7.5 ± 2.2	21.2 ± 3.8	11.5±3.7	23.4 ±4.8	7.9 ±1.2	7.5 ± 2.2
PBS control	11.4 ± 1.1	13.9 ± 11.3	8.8 ±0.3	11.6 ±5.5	6.8 ±0.1	6.4 ± 5.7
Precursor formulation line 1	26.8 ± 9.5	48.3 ± 3.6	-	-	-	-
75% Tween 80 25% water	8.7	15.5	-	-	-	-

These results are confirmed by the LDH assay. The percentage of LDH released by the slugs relative to the positive control (i.e. 1% benzalkoniumchloride (BKC) solution) is displayed in Figure 5.10. LDH release of the slugs after contact with the precursor of the first formulation line is even significantly higher than the positive control irritant. In comparison, LDH release of slugs after contact with the 25% water/75% Tween 80 formulation is similar to that of the negative controls. Before commercialising of a liquid crystal platform containing high concentrations of surfactant, careful examination of irritant properties is necessary. Other formulations are not tested since the precursor of the first formulation line demonstrates severe irritation of the mucosa.

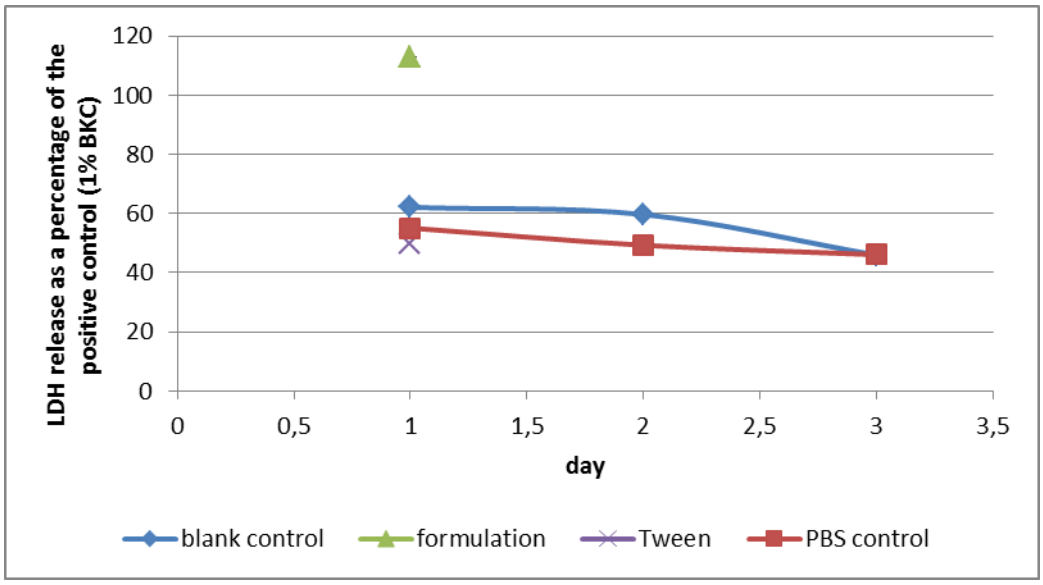


Figure 5.10: Percentage LDH released from slugs relative to the positive control (i.e. 1% aqueous BKC solution) following 3-day exposure to test formulations and control solutions (n=3)

5.2 LIQUID CRYSTAL PLATFORM FOR ORAL APPLICATION: TETRONIC® 704, WATER AND PROCETYL AWS®

5.2.1 Phase diagram

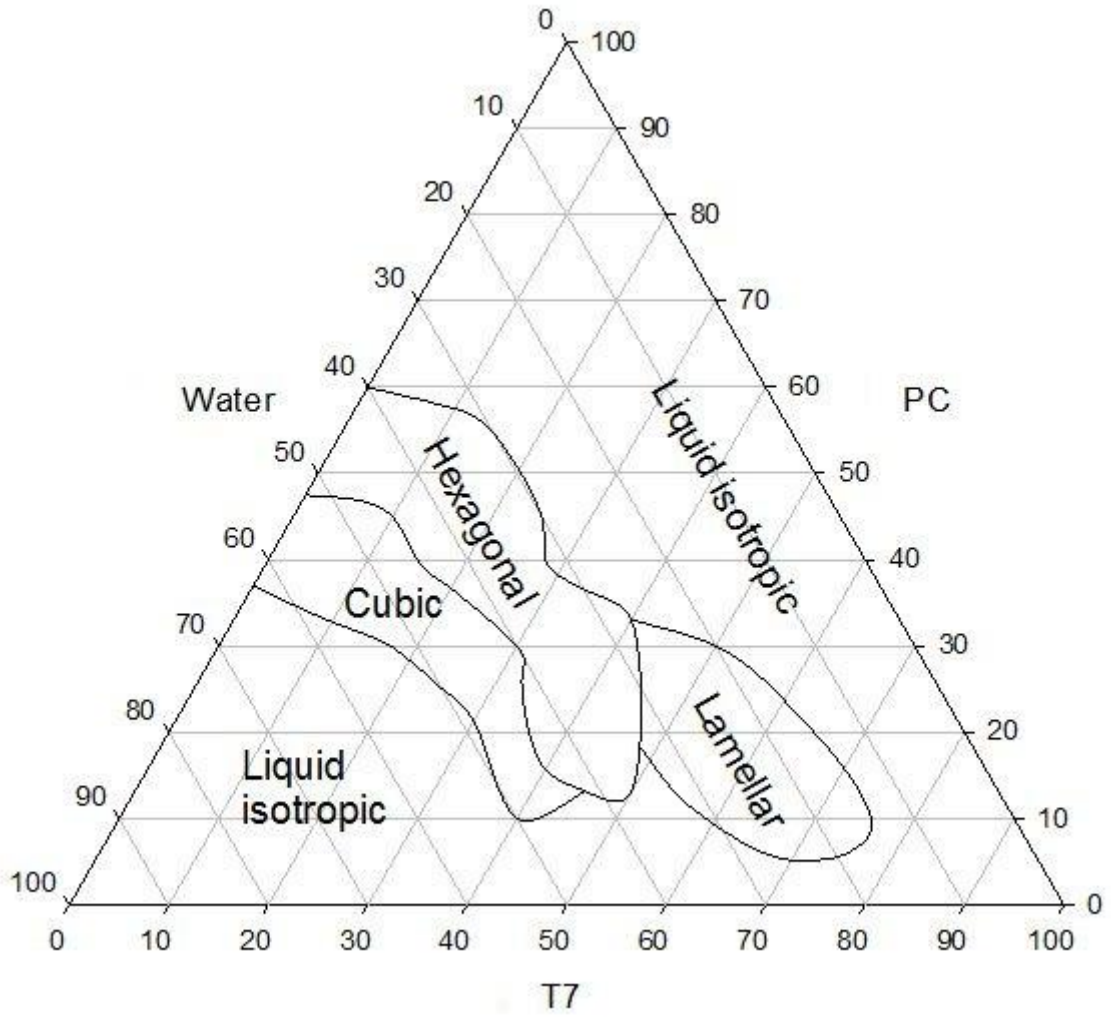


Figure 5.11: Phase diagram (at 25°C) of ternary system containing Tetronic® 704, water and Procetyl®, constructed based on visual observations via PLM

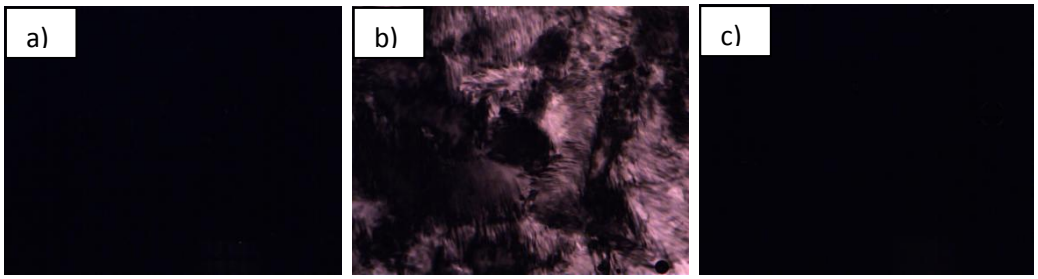


Figure 5.12: **Polarized light microscopy pictures (magnification 40x) of ternary liquid crystal systems containing Tetronic® 704 (T7), water (W) and Procetyl® (PC). (a) 45%T7/21%W/ 34%PC (liquid isotropic), (b) 14%T7/40%W/46%PC (hexagonal, fan structure), (c) 7% T7/50%W/43% PC (cubic phase, isotropic)**

The phase diagram of the ternary system Tetronic® 704, water and Procetyl® can be seen in Figure 5.11. Hexagonal phase can be found in the area 0-50% T7, 40-50% W, 15-60% PC. Cubic phase appears in the area 0-40% T7, 52-62% water and 10-47% PC. Lamellar phase is observed in the range 45-75% T7, 15-35% water and 7-30% PC. Other compositions result in a liquid isotropic phase. Interest goes out to the cubic and hexagonal phase as potential sustained release drug platforms.

5.2.2 Rheological characterization

Two formulation lines are prepared using Tetronic® 704 instead of Tetronic® 904 to compare which one of the two surfactants is the most appropriate for the application. Results of the blank formulations are displayed in Table 5.9 and 5.10, while results for the drug formulations are displayed in Table 5.11 and 5.12. Formulations of the first line show similar rheological properties as the formulations with T9 as surfactant. The hexagonal formulation melts at body temperature (37 °C) while the cubic phase shows very favourable properties to achieve prolonged drug delivery. The retention time of the T7 formulations both for blank and drug formulations is slightly higher than those of the T9 formulations, 4 765 ± 1 157 s for the blank T7 formulation and 5 998 ± 475 s for the drug T7 formulation versus 3 044 ± 693 s for the blank T9 formulation and 4 466 ± 629 s for the drug T9 formulation. Although Tetronic® 704 is more liquid-like and less viscous than Tetronic® 904, the rheological properties of the 60% dilution of the first formulation line are enhanced.

Table 5.9: Rheological properties (n=3, average ± standard deviation) of liquid crystals containing Tetronic®704 (T7), water (W) and Procetyl® (PC).

Sample	Flow rheology	Creep rheology
	Initial viscosity (Pa.s)	RSRT (s)
First formulation line		
Precursor 1	0.86 ± 0.027	-
20% dilution	1.75 ± 0.024	-([a])
60% dilution	1 377 ± 165	4 765 ± 1 157
Second formulation line		
Precursor 2	1.11 ± 0.03	-
20% dilution	220 ± 64	192 ± 67

60% dilution	2.19 ± 1.38	-

[(a)] As the formulation immediately melts at 37°C, rheological tests could not be executed.

Table 5.10: Oscillation rheology (n=3, average ± standard deviation) of liquid crystals containing Tetronic® 704 (T7), water (W) and Procetyl® (PC).

Sample	Oscillatory frequency (Hz)	Viscoelastic properties at 37°C	
		G' (Pa)	G'' (Pa)
First formulation line			
20% dilution	0.1 10	0.018 ± 0.00059 130 ± 0.50	0.84 ± 0.007 79 ± 0.24
60% dilution	0.1 10	3 482 ± 738 46 933 ±1884	4 573 ± 643 18 357 ± 838
Second formulation line			
20% dilution	0.1 10	27 967 ± 211 48 073 ± 973	7 026 ± 111 8 220 ± 147
60% dilution	0.1 10	0.013 ± 0.00035 129 ± 0,23	0.75 ± 0.0035 74.44 ± 0,37

It can be noticed for the second formulation line that both blank and tetracycline HCl formulations with Tetronic® 704 as surfactant demonstrate less favourable properties in comparison with the Tetronic® 904 formulation. Flow, oscillation and creep results are significantly lower when compared to the T9 formulation. The T7 formulations are not retained at the site of application and the 60% dilution seems to be liquid-like instead of a rigid viscous structure. This result can be explained by examination of the phase diagram of T7-W-PC. The 60% dilution formulation is located in the cubic phase area but close to the border of liquid isotropic phase. Thus, the 60% dilution probably is a mixture of cubic and liquid isotropic phase which clarifies the low rheological characteristics. On the other hand, the 20% dilution of the second line does not display advantageous properties since the formulation is not retained at the site of application (RSRT of 8.39 ± 1.61 s). Compared to the first formulation line, the second formulation line contains a higher percentage of Tetronic®,

25.00% versus 8.33%. Since T7 is a liquid with a lower viscosity than T9, T7 might affect the rheological properties negatively.

Table 5.11: Rheological properties (n=3, average ± standard deviation) of liquid crystals containing Tetronic®704 (T7), water (W), Procetyl® and 1% tetracycline HCl (TC).

Sample	Flow rheology	Creep rheology
	Initial viscosity (Pa.s)	RSRT (s)
First formulation line		
Precursor 1	0.83 ± 0.019	-
20% dilution	1.13 ± 0.21	-([a])
60% dilution	3 695 ± 763	5 998 ± 475
Second formulation line		
Precursor 2	1.035 ± 0.015	-
20% dilution	111 ± 29	8.39 ± 1.61
60% dilution	2.54 ± 0.69	0.014 ± 0.0011

([a]) As the formulation immediately melts at 37°C, rheological tests could not be executed.

Table 5.12: Oscillation rheology (n=3, average ± standard deviation) of liquid crystals containing Tetronic®704 (T7), water (W), Procetyl® (PC) and 1% tetracycline HCl.

Sample	Oscillatory frequency (Hz)	Viscoelastic properties at 37°C	
		G' (Pa)	G'' (Pa)
First formulation line			
20% dilution	0.1 10	0.0010 ± 0.00022 13 ± 0.067	0.63 ± 0.015 65 ± 1.37
60% dilution	0.1 10	83 240 ± 3267 $1{,}26 \cdot 10^5$ ± 153	18 467 ± 3 597 9 601 ± 1 185
Second formulation line			
20% dilution	0.1 10	1 128 ± 353 21 403 ± 1993	1 564 ± 301 12 287 ± 301
60% dilution	0.1 10	0.0023 ± 0.0017 18 ± 0.42	0.76 ± 0.0019 77 ± 1.68

5.2.3 Microscopic analysis

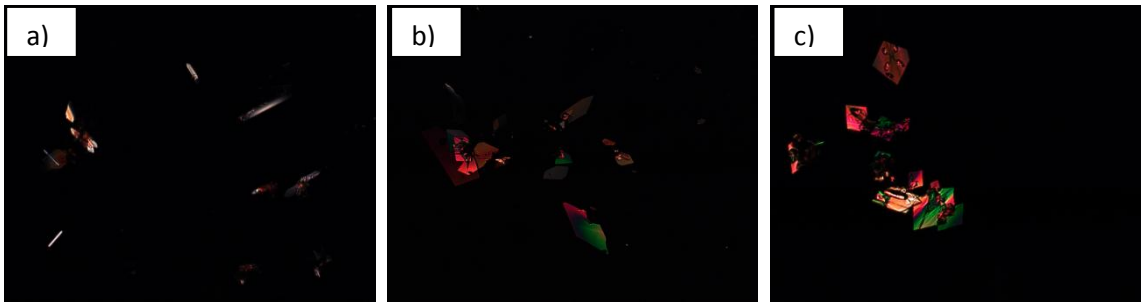


Figure 5.12: Polarized light microscopy images (magnification 40x) of ternary systems containing Tetronic® 704 (T7), water (W), Procetyl® (PC) and 1% Tetracycline HCl (TC). (a) 1%TC/10%T7/25%W/65%PC (liquid isotropic phase), (b) 1%TC/8.33%T7/37.5%W/54.17% PC (20% dilution, dark field), (c) 1%TC/6.25%T7/53.125%W/48%PC (60% dilution, cubic phase)

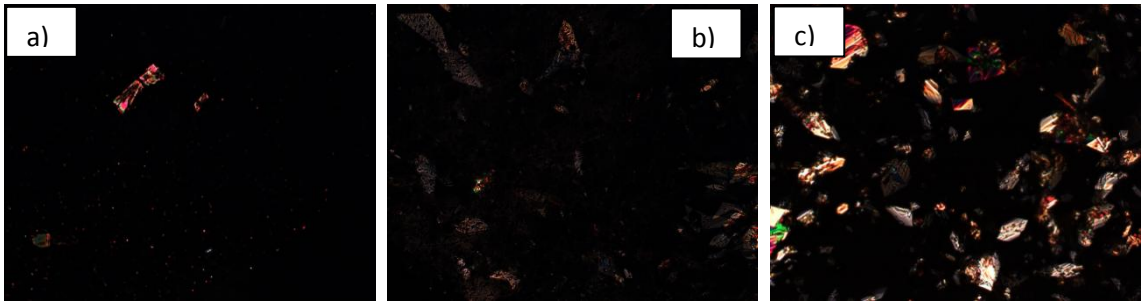


Figure 5.13: Polarized light microscopy images (magnification 40x) of ternary systems containing Tetronic® 704 (T7), water (W), Procetyl® (PC) and 1% Tetracycline HCl (TC). (a) 1%TC/30.40%T7/21.60%W/48%PC (liquid isotropic phase, (b) 1%TC/25%T7/35%W/40%PC (20% dilution, fan structure), (c) 1%TC/19%T7/51%W/30%PC (60% dilution, dark field)

Microscopic analysis to investigate the potential influence of tetracycline HCl on the liquid crystal structure gives similar results as the T9 samples (Figure 5.12 and 5.13). The liquid isotropic and cubic phases remain stable, visualised as a dark field under PLM and by visual investigation of the viscosity of the samples. The 20% dilution of the first formulation line (Figure 5.12 (b)) has lost its hexagonal structure since a dark field dominates. In comparison, the 20% dilution of the second formulation line (Figure 5.13 (b)) still displays the hexagonal structure as the typical fan structure is seen using PLM.

5.2.4 *In vitro* drug release

The drug release profile of the T7 formulation of the first line is displayed in Figure 5.14 (a). Drug release of the T7 formulation of the second line is not conducted since creep rheology indicates that there is no retention at the site of application. The drug release profile is similar to those obtained with T9 formulations. The curve displays almost a straight line, indicating a zero order kinetic and controlled release.

During six hours of drug release experiment, 34% of tetracycline HCl is released from the formulation. Tetracycline HCl release versus the square root of time is plotted in Figure 5.14 (b). The release of the hydrophilic tetracycline HCl is diffusion controlled ($R^2 > 0.9$). The slope of the curve is proportional to the apparent diffusion coefficient and has a value of 13.90.

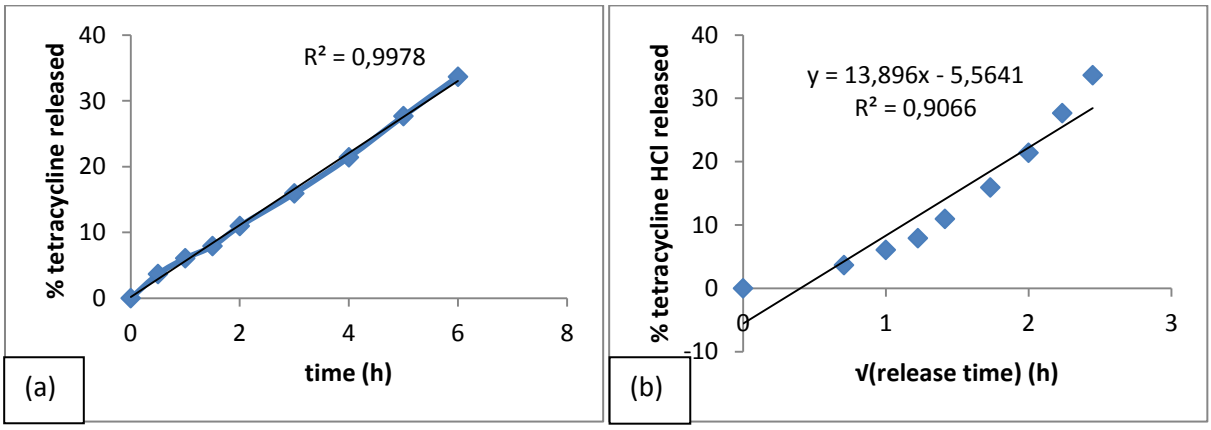


Figure 5.14: Release (n=3, at 37°C) of tetracycline HCl from the precursor of the first formulation line containing Tetronic® 704: (a) a function of time at 37°C (n = 3), (b) as a function of square root of time

6 CONCLUSION

This study describes the formulation and characterization of liquid crystal platforms for the treatment of periodontitis. Periodontitis is an inflammatory condition that affects the supporting structures of the teeth, inducing mobility of the teeth and finally teeth loss. Since bacteria play an important role in the development of periodontal disease, the antibacterial agent tetracycline HCl is used as drug substance in the formulation.

The formulation of clinical successful preparations for the treatment of periodontitis is technical challenging. Firstly, the ease of administration is important. The formulation has to be injected into the periodontal pocket. Furthermore, it is desired that the formulation is retained at the site of application and offers a controlled drug release. Since it will be subjected to a wide range of stresses, associated with mastication and physiological conditions as chewing and swallowing, the importance of rheologically structured gel-like formulations is not to be underestimated.

Liquid crystals display characteristics in accordance to these technical requirements. A liquid isotropic precursor can be injected into the periodontal pocket. Once in contact with the artificial saliva of the environment, phase transitions are induced to the hexagonal and cubic phases which offer exceptional drug delivery properties.

A ternary system of two surfactants Tetronic® 904/704 and Procetyl® in combination with water is used. In order to determine where the phase transitions take place, a phase diagram is constructed using PLM. Two precursors are selected: 10%T/25%W/65%PC and 30.40%T/21.60%W/48%PC. To analyse the structural appropriateness, rheological tests are performed on the precursors, the hexagonal phase (i.e. 20% dilution) and the cubic phase (i.e. 60% dilution). First, blank formulations are analysed, subsequently 1% tetracycline HCl is added to the formulations. The best candidate for the treatment of periodontal disease is the second formulation line. The starting point is a liquid-like formulation with a low initial viscosity of 1.54 ± 0.057 Pa.s. 20% dilution with water yields the solid-like hexagonal phase, with an initial viscosity of 183 ± 25 Pa.s and G'>G" at low and higher oscillatory stresses. Creep rheology indicates that the formulation is retained at the site of application for 479 ±

110 s. Further dilution of 60% of the formulation to the cubic phase, offers a high initial viscosity of 721 ± 59 Pa.s. The cubic phase is a very rigid structure, as seen from oscillation results with G' significantly higher than G" at both low and high oscillatory stresses. The formulation has a retention time of 1 464 ± 184 Pa.s. Thus, the precursor yields liquid crystalline platforms capable to be sustained in the periodontal pocket, in a wide range of amount of saliva.

Subsequently, the precursor formulations (except for the T7 precursor of the second line since the rheological results are not promising) are subjected to drug release experiments during 6h. The drug release profiles show zero-order kinetics and a controlled release of tetracycline HCl. The amount of tetracycline HCl released is in the range of 34%. The mechanism of release is diffusion controlled.

A slug mucosal irritation test is conducted. The precursor of the T9 first formulation line is spread onto the base of a petri dish and for 30 minutes the slug is in contact with the formulation. The formulation causes irritation of the mucus layer. A yellow mucus is produced. LDH release is higher than the positive control, and a weight loss of 48% and mucus production of 27% are noticed. Before commercialising a liquid crystal platform it is of high importance to verify the compatibility with mucosa due to the high concentrations of the surfactants present in liquid crystals.

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