DEVELOPMENT AND ASSESSMENT OF MINOCYCLINE SUSTAINED RELEASE CAPSULE FORMULATIONS

By

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ABSTRACT

The use of minocycline for the treatment of a broad range of systemic infections and for severe acne has been associated with vestibular side effects. The severity of side effects may lead to poor adherence to therapy by patients. The use of sustained release formulations of minocycline that display slow dissolution of minocycline following administration may be beneficial in reducing the incidence and severity of side effects. Therefore, sustained release capsule dosage forms containing 100 mg minocycline (base) were manufactured and assessed for use as sustained release oral dosage forms of minocycline.

Minocycline sustained release capsules were manufactured based on matrix technologies using hydroxypropylmethyl cellulose (HPMC) and Compritol® as release retarding polymers. The rate and extent of minocycline release from the capsules was evaluated using USP Apparatus 1 and samples were analysed using a validated High Performance Liquid Chromatographic (HPLC) method with ultraviolet (UV) detection.

Differences in the rate and extent of minocycline release from formulations manufactured using HPMC or Compritol® were influenced by the concentration of polymer used in the formulations. The rate and extent of minocycline release was faster and greater when low concentrations of polymer were used in formulations.

The effect of different excipients on the release pattern(s) of minocycline and particularly their potential to optimise minocycline release from experimental formulations was investigated. The use of diluents such as lactose and microcrystalline cellulose (MCC) revealed that lactose facilitated minocycline release when HPMC was used as the polymer matrix. In contrast, the use of lactose as diluent resulted in slower release of minocycline from Compritol® based formulations.

The addition of sodium starch glycolate to HPMC based formulations resulted in slower release of minocycline than when no sodium starch glycolate was used. Compritol® based formulations were observed to release minocycline faster following addition of sodium starch glycolate and Poloxamer 188 to experimental formulations.

In vitro dissolution profiles were compared to a target or reference profile using the difference and similarity factors, f_1 and f_2, and a one way analysis of variance (ANOVA). In addition, the mechanism of minocycline release was elucidated following fitting of dissolution data to the Korsmeyer-Peppas, Higuchi and Zero order models.

Minocycline release kinetics were best described by the Korsmeyer-Peppas model and the values of the release exponent, n, revealed that drug release was a result of the combined effects of minocycline diffusion through matrices and erosion of the matrices. These in vitro dissolution profiles were better fit to the Higuchi model than to the Zero order model.

Two formulations that displayed a fit to the Zero order model were identified for further studies as potential dosage forms for sustained release minocycline.

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STUDY OBJECTIVES

Minocycline, a second generation tetracycline antibiotic is prescribed for a broad range of systemic infections [1]. In addition, minocycline is used for the management and treatment of severe acne that is unresponsive to treatment with other tetracycline antibiotics [1]. Chronic use of minocycline is associated with vestibular side effects, the severity of which may cause patients to default on treatment [2-4]. The incidence and severity of side effects has been shown to be greater when dissolution of minocycline following administration is rapid [5]. The vestibular side effects associated with rapid dissolution of the API may be reduced by the administration of a sustained release formulation, thereby potentially promoting adherence to therapy by the patient.

The objectives of this study were:

1. To develop and validate a stability-indicating, simple, sensitive and selective High Performance Liquid Chromatographic (HPLC) method with the necessary accuracy and precision for the quantitation of minocycline in aqueous solutions and in pharmaceutical dosage forms.
2. To investigate the use of hydrophilic and lipophilic polymers for the development of minocycline (100 mg base) sustained release dosage forms that released at least 80% according to a zero order kinetic model for 12 hours.
3. To assess and evaluate the rate and extent of minocycline release from the formulations using an appropriate dissolution method.
4. To study the dissolution kinetics and release mechanisms of minocycline from manufactured capsule dosage forms.

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CHAPTER ONE

1 MINOCYCLINE HYDROCHLORIDE

1.1 INTRODUCTION

Minocycline is a second generation semi-synthetic tetracycline antibiotic [6,7]. Although not the first to be discovered, tetracycline is the generic structure for nomenclature purposes in the entire tetracycline family [8]. Tetracycline, originally known as terramycin, was named due to the presence of the linearly arranged naphthacene ring system that forms the basic chemical and structural makeup of tetracycline antibiotics depicted in Figure 1.1 [6]. Structurally, minocycline possesses the perhydronaphthacene skeleton characteristic of all tetracyclines but lacks the 6-methyl and 6-hydroxyl substituents and possesses a 7-dimethyl substituent as indicted in Figure 1.1 [8]. As a consequence of this difference in chemical structure, minocycline has a broader spectrum of activity and better tissue penetration characteristics when compared to other tetracycline molecules and which is more than likely due to the fact that the lipophilicity of minocycline in the physiological pH range is much more pronounced than with other tetracycline analogues [3].

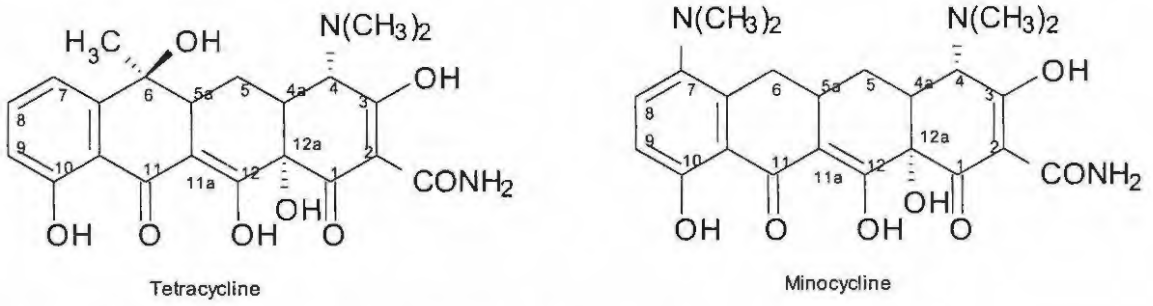


Figure 1.1. Chemical structure of tetracycline and minocycline

Following the discovery and isolation of chlortetracycline, the first tetracycline, from cultures of *Streptomyces aureofaciens* by scientists at Lederle in the late 1940s, and subsequent development of the first generation tetracyclines, researchers undertook studies with blocked mutant strains of *Streptomyces* which led to the discovery of demeclocycline in 1956 [6]. Although not chemically useful due to its potential to precipitate phototoxic episodes, demeclocyline became an important starting material for the manipulation of the tetracycline molecule using semi-synthetic techniques resulting in modification at the C-6 and C-7 positions leading to the discovery of minocycline by chemists at Cyanamid [6].

Minocycline has similar indications to those of other tetracycline antibiotics, which include the treatment of rickettsial, chlamydial and vibrio infections, brucellosis, chronic bronchitis, acne and mycoplasma and spirochaetal infections [9]. Minocycline is administered via the oral or parenteral routes and is usually used as the hydrochloride salt.

1.2 PHYSICO-CHEMICAL PROPERTIES

1.2.1 Description

Minocycline hydrochloride is chemically known as 4, 7-bis (dimethlyamino) 1, 4-4a, 5, 5a, 6, 11, 12a-octahydro-3, 10, 12, -12a-tetrahydroxy-1, 11-dioxo-2-napthacenecarboxamide monohydrochloride and by the trivial name 7-dimethylamino-6-demethyl-deoxytetracycline hydrochloride [10]. The chemical structure of minocycline hydrochloride is depicted in Figure 1.2.

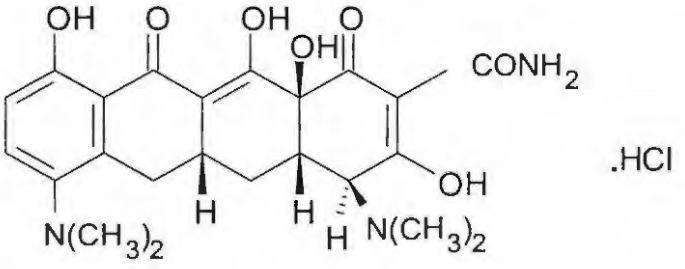


Figure 1.2. Minocycline hydrochloride [10]

Minocycline hydrochloride occurs as a yellow crystalline powder that is slightly hygroscopic [11]. One milligram (1 mg) of minocycline hydrochloride contains not less than 890 µg and not more than 950 µg of minocycline calculated on an anhydrous basis [12].

1.2.2 Solubility

Minocycline hydrochloride is sparingly soluble in water, slightly soluble in alcohol and soluble in solutions of alkali hydroxides and carbonates [11]. It is practically insoluble in chloroform and ether [13]. A list of the solubilities of minocycline hydrochloride dihydrate in a variety of solvents is shown in Table 1.1 [10].

Table 1.1. Solubility of minocycline hydrochloride dihydrate in various solvents at 25°C

Solvent	Solubility mg/ml	Solubility % w/v
Hexane	0.004	0.0004
Benzene	0.02	0.002
Chloroform	0.13	0.013
Ethyl acetate	0.3	0.03
Methyl ethyl ketone	0.4	0.04
1-Octanol	0.5	0.05
Acetone	0.6	0.06
Dioxane	0.7	0.07
1-Butanol	4.4	0.44
2-Propanol	7	0.7
Methanol	14	1.4
Water	16	1.6
Absolute Ethanol	42	4.2

1.2.3 Dissociation constant (pKa)

The apparent dissociation constants of tetracycline antibiotics are quite similar within the class [14]. The three functional groups associated with each of the reported dissociation constants of tetracycline antibiotics and the respective dissociation constants of tetracycline, the parent compound for tetracycline antibiotics are depicted in Figure 1.3 [15].

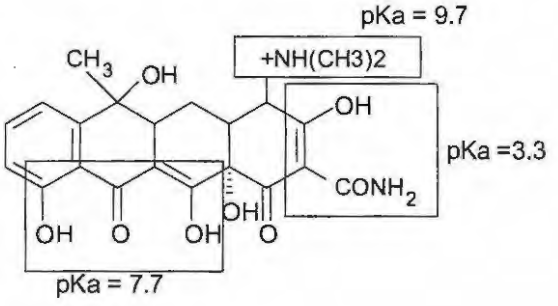


Figure 1.3. Dissociation constants of tetracycline

Minocycline has a second dimethylamino group located at position C-7 of the tetracycline backbone resulting in minocycline having four ionisable groups as depicted in Table 1.2 [16] and an isoelectric point of 6.4 [17].

Table 1.2. Dissociation constants of minocycline

Ionisable groups	Dissociation constants
Amine groups	5 and 9.5
Hydroxyl groups	2.8 and 7.8

1.2.4 Partition coefficient

The partition coefficient of a compound is a measure of the extent to which that compound is distributed between water and a water-immiscible liquid phase as determined by their relative concentrations (weight per unit volume) in each of the phases [18]. The partition coefficient is frequently determined in binary systems of octanol and water and is expressed as a coefficient (*P*) or as its logarithim, *log P* [18]. Partition coefficients have been extensively used to infer a correlation of the relative lipophilic characteristics of a molecule with its biological properties within a set of congeners [19]. Partition coefficient measurements are important for predicting membrane transfer properties, protein binding, receptor affinity and pharmacological activity of molecules [18]. In drug design and formulation, partition coefficient measurements may be used to predict dissolution and partitioning rate of drugs and for establishing structure-activity parameters that are involved in the hydrophobic bonding ability of a drug [19].

Colaizzi and Klink [15] studied the pH-partitioning behaviour of tetracyclines in octanol/ buffer system. The results of these studies indicated that the zwitterionic form of tetracycline molecules are the most lipophilic form of the molecule. Furthermore, the zwitterionic form was found to be present in the highest concentration in solutions of between pH 4 and pH 7. The partition coefficients of minocycline observed in these studies are summarised in Table 1.3.

Table 1.3. Apparent Partition coefficients (Octanol/ Buffer) of minocycline hydrochloride

pH	2.1	3.9	5.6	6.6	8.5
Partition coefficient	0	0.051	1.11	1.48	0.36

1.2.5 pH of solution

A 1% (w/v) solution of minocycline hydrochloride in water has a pH of between 3.5 and 4.5 [20].

1.2.6 Melting range

Differential thermal analysis studies have shown that minocycline hydrochloride exhibits one melting and/or decomposition endotherm at 217°C [10].

1.2.7 Infra-red absorption spectrum

Infrared spectroscopy is a relatively simple, fast and reliable method for assigning a substance to a particular class of compounds [21]. Many functional groups in organic compounds exhibit molecular vibrations which correspond to absorption bands in defined regions of the infrared spectrum [21]. The infrared spectrum of minocycline hydrochloride (Lederle House Standard No. 7516B-172) in the range of 4000-700 cm^{-1} was determined by the KBr method using a Perkin-Elmer 21 Instrument [10]. The infrared spectrum of minocycline and the relevant band assignments are shown below in Figure 1.4 and Table 1.4, respectively.

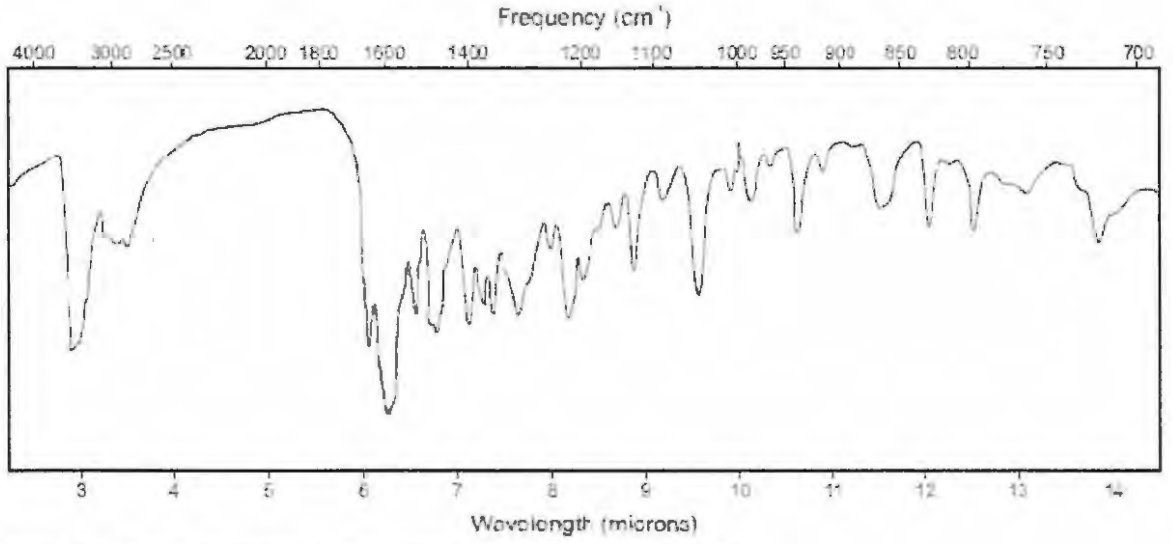


Figure 1.4. Infrared spectrum of Minocycline $HCl.2H_20$ in KBr Pellet

Table 1.4. Characteristic band assignment of Minocycline $HCl.2H_20$ infrared spectrum

Wavelength (μ)	Assignment
2.9	NH_2 stretching of 2-carboxamido and 12-hydroxy
>2.9-5	Hydrogen bonded phenolic and enolic hydroxyl groups and hydrogen atom on protonated dimethylamino group
6.07	Carbonyl of 2-carboxamido group
6.25	Conjugated hydrogen bonded ketones and conjugated double bond systems
7.7	Hydrogen bonded phenolic and enolic hydroxyl groups and 2-carboxamindo
8.2	Relatively unbonded phenolic hydroxy groups

1.2.8 Ultra-violet absorption spectrum

The use of ultraviolet/visible (UV/ Vis) spectroscopy is valuable for qualitative and quantitative analysis, photometric titration, determination of equilibrium and dissociation constants in addition to trace analysis

of compounds [21]. Furthermore the use of UV detection in High Performance Liquid Chromatography (HPLC) is well known [21]. In the absence of physico-chemical factors such as for example solubility of the compound, the UV absorbance of a substance is proportional to the path length through which the radiation passes and the concentration of the substance in solution [22].

The ultraviolet absorption spectra of minocycline hydrochloride in solution in methanol, mobile phase (methanol: acetonitrile:0.08 M phosphate buffer pH 7.0, 13:17:70v/v/v) and water at a concentration of 20 µg/ml are shown in Figures 1.5, 1.6 and 1.7, respectively. The spectra were generated using a double beam Model GBC 916 UV VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Melbourne, Victoria, Australia). The resultant spectra reveal a peak at 253.6nm (methanol), and two peaks at 244.8 nm and 273.6 nm (mobile phase). The spectrum obtained from water revealed three peaks at wavelengths of 221.6 nm, 248.8 nm and 273.6 nm.

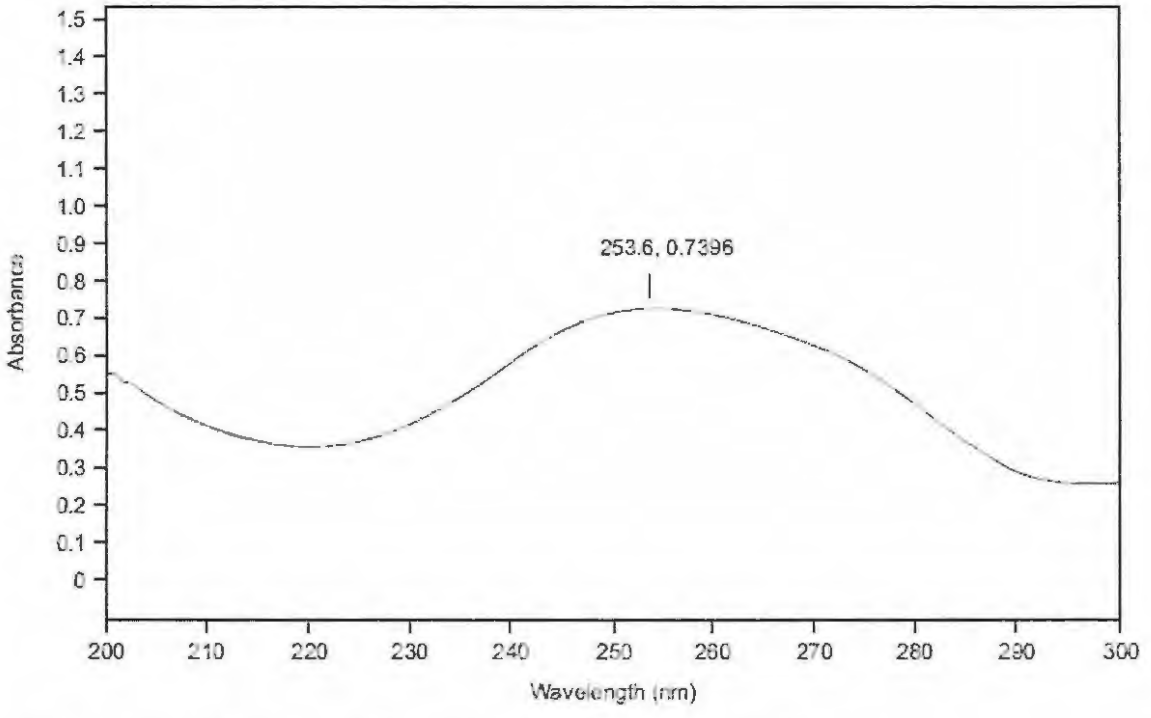


Figure 1.5. The ultraviolet spectrum of minocycline hydrochloride in methanol

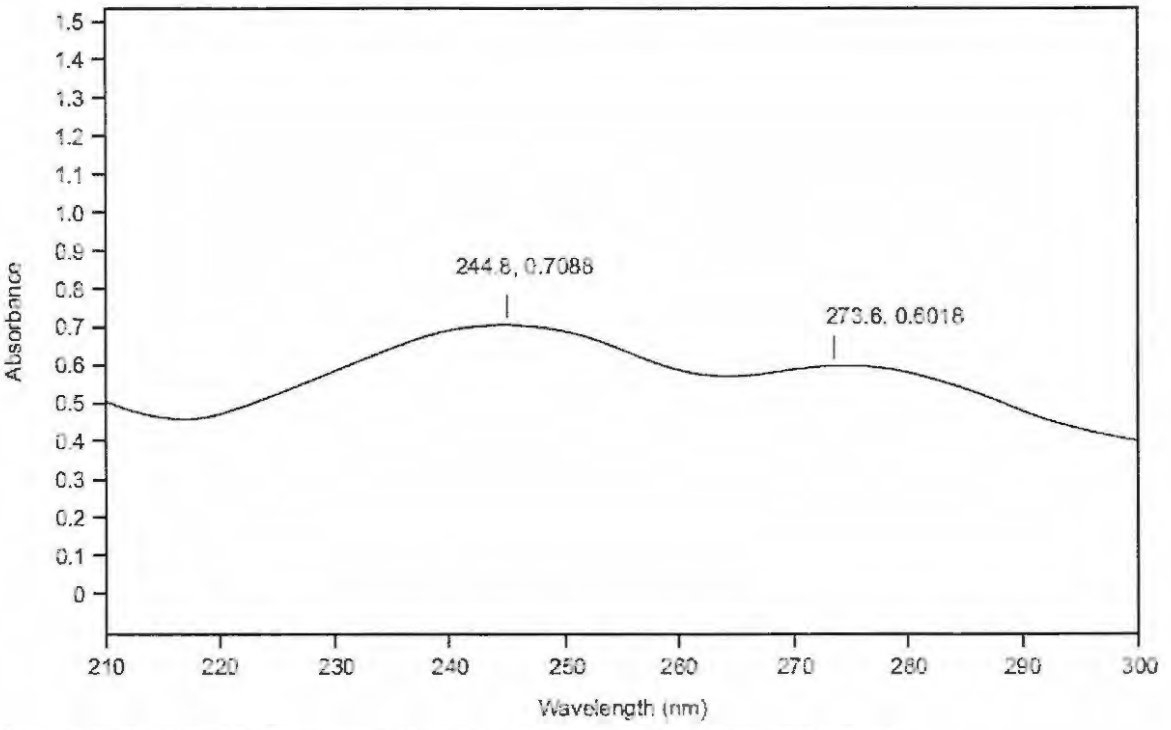


Figure 1.6. The ultraviolet spectrum of minocycline hydrochloride in mobile phase

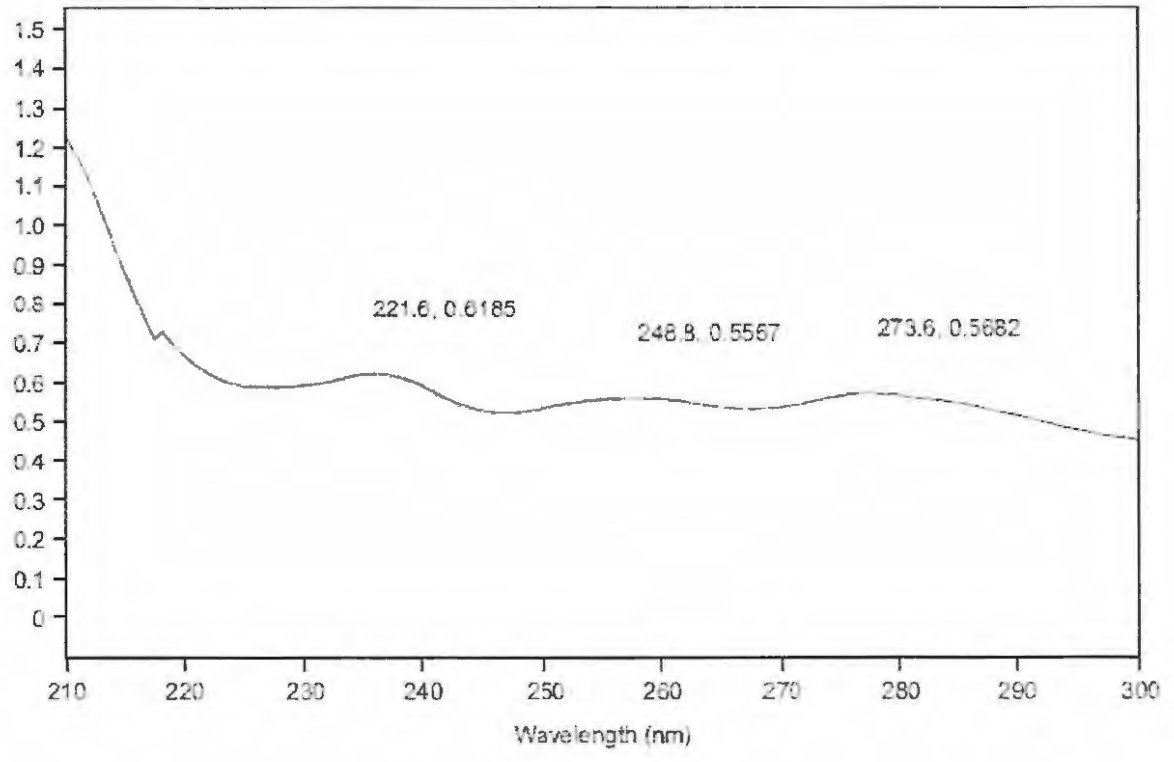


Figure 1.7. The ultraviolet spectrum of minocycline hydrochloride in water

1.2.9 Nuclear magnetic resonance spectrum

Nuclei such as protons possess a permanent magnetic moment which when placed in an external magnetic field takes on well-defined orientations which correspond to distinct energy levels determined with respect to direction of the applied magnetic field [22]. A proton magnetic resonance spectrum is used to characterise the behaviour of protons with respect to their nuclear and electronic environment within a molecule [22]. A chemical (δ) shift, expressed in parts per million (ppm), results when there is difference between a given sample and reference standard [22]. As a consequence of the magnetic field emanating from an adjacent nucleus, signals are frequently split into groups of related peaks that are referred to as doublets, triplets or multiplets [22].

The proton magnetic resonance spectrum of minocycline hydrochloride in hexadeuterodimethylsulfoxide with tetramethyl silane as the internal standard with the corresponding proton assignments observed are depicted in Figure1.8 and Table1.5, respectively [10].

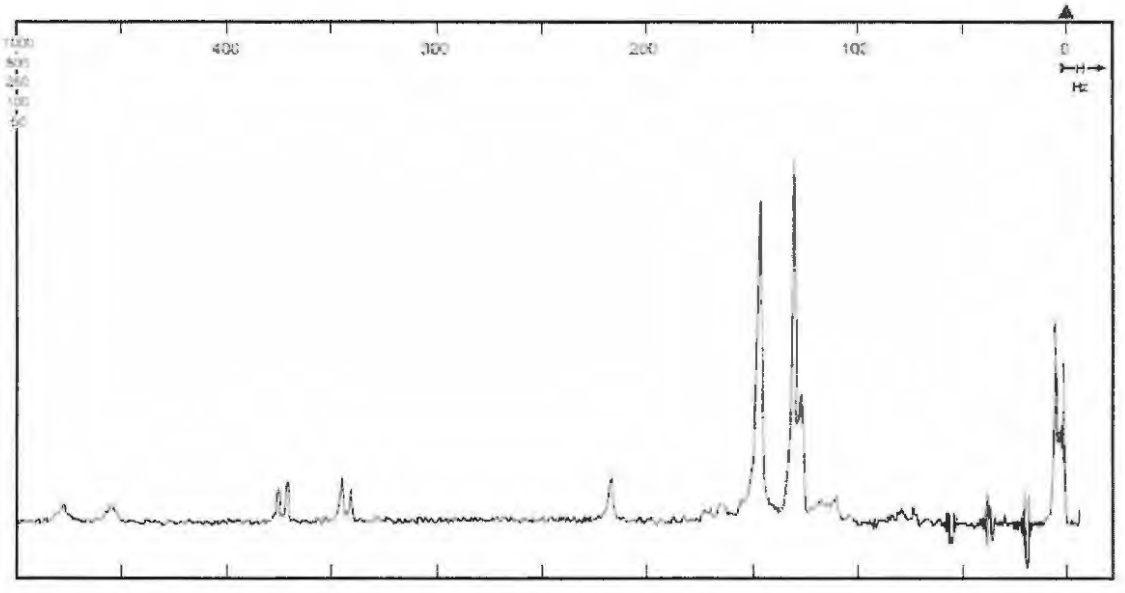


Figure 1.8. Proton magnetic resonance spectrum of minocycline HCl.2H_20

Table 1.5. Proton magnetic resonance spectral assignments of minocycline Hydrochloride

Proton site	Chemical shifts (ppm)	Multiplicity
$N(CH_3)_2$ (at C_4)	2.60	singlet
$N(CH_3)_2$ (at C_7)	2.94	singlet
C_4	4.34	singlet
C_8	7.41	doublet
C_9	6.83	doublet
$CO(NH_2)$	9.05; 9.53	2 broad singlets
C_{10}-OH	11.30	

1.3 SYNTHESIS

1.3.1 Synthetic procedure/pathway

Minocycline was originally synthetically produced by chemical modification of demeclocycline [6]. Due to an increased potential for phototoxicity, demeclocycline has restricted clinical use but was considered an important starting material for the manufacture of semi-synthetic derivatives of tetracycline [6].

The synthesis of minocycline from demeclocycline (I) is comprised of four main steps and is illustrated in Figure 1.9. Initially following catalytic reduction, the acid labile 6-hydroxy group is removed to form 6α-deoxy-6-demethyl tetracycline, also known as sancycline (II) [6]. The acid stable sancycline is reacted with nitration reagents under strong acidic conditions to allow aromatic substitution at positions C-7 and C-9 on the D-ring yielding the 7-nitro (III) and 9-nitro derivatives of sancycline [6]. Hydrogenation of the nitro groups yields the 7-amino (IV) and 9-amino compounds, after which reductive alkylation of the C-7 amino derivative using formaldehyde in the presence of catalysts yields 7-dimethylamino-6-demethyl-6-deoxytetracycline, minocycline (V) [6].

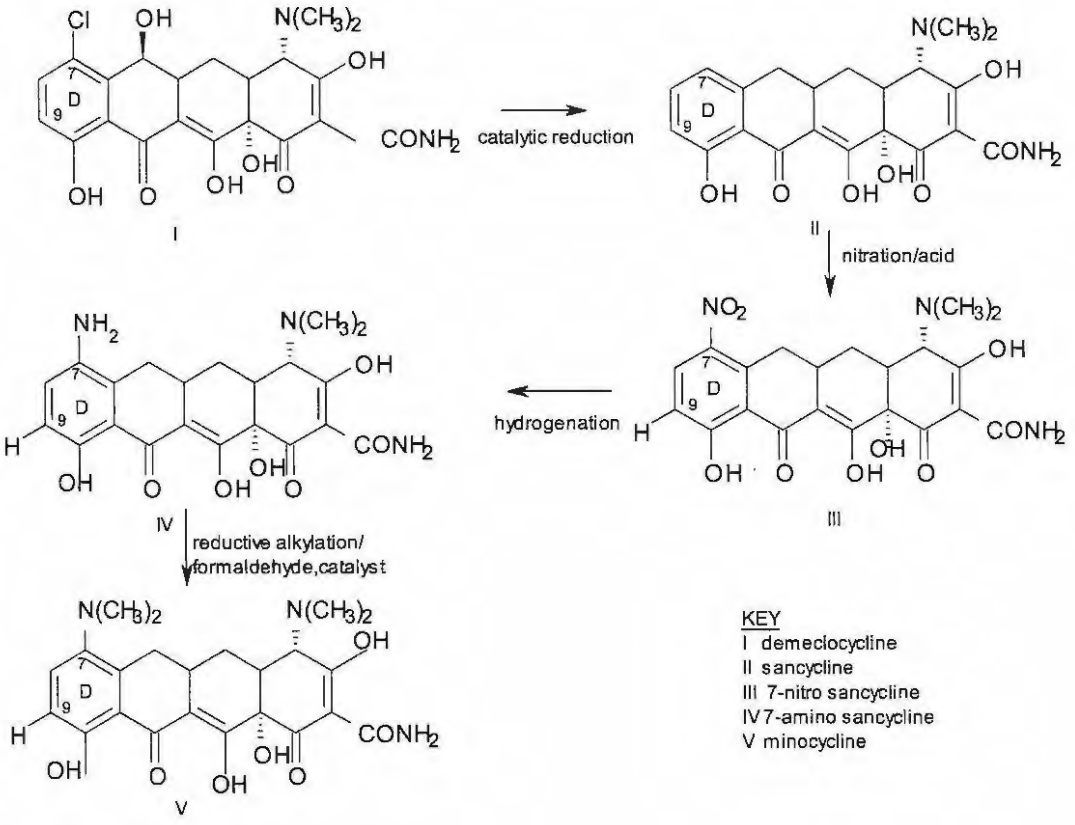
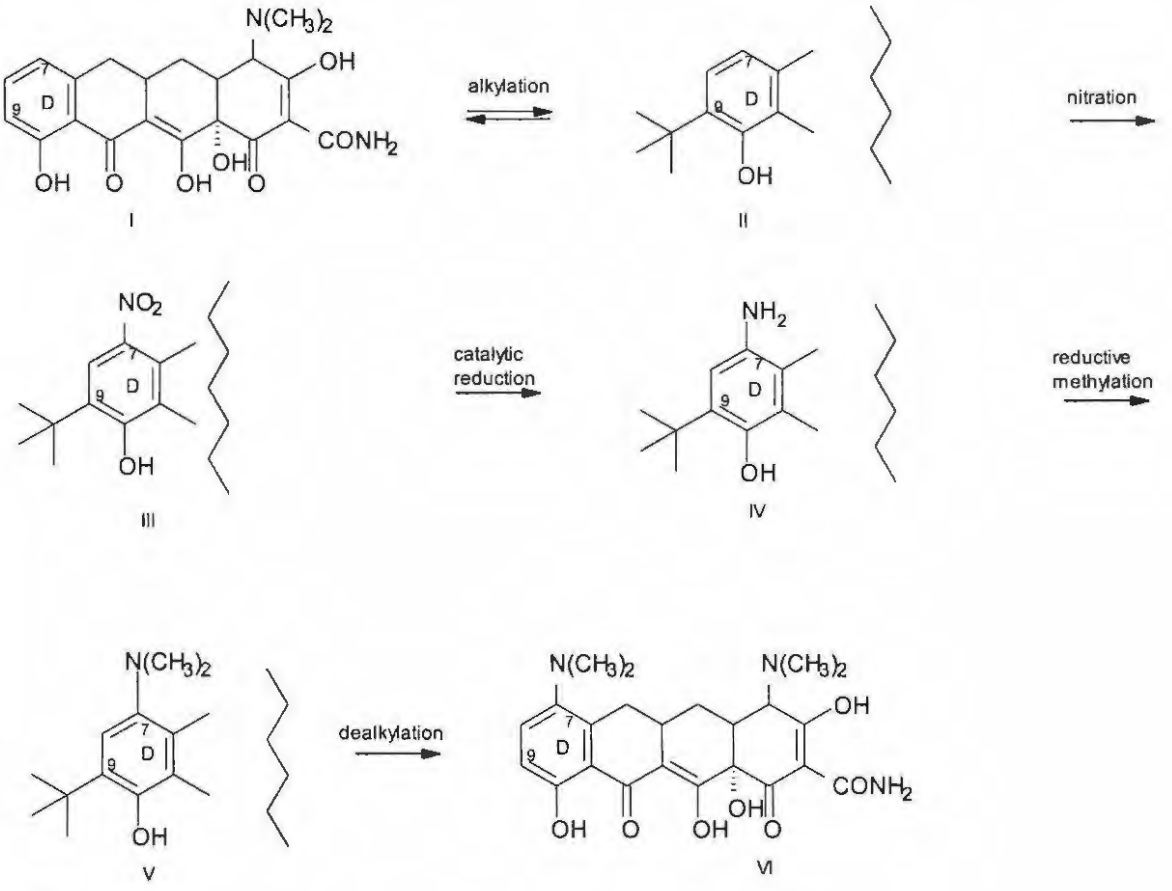


Figure 1.9. Original synthesis of minocycline

It is clear that the synthesis of minocycline using the procedure depicted in Figure 1.9 is likely to yield both the 7-nitro and 9-nitro tetracycline derivatives, thereby posing challenges. Consequently it is necessary to separate the 7-substituted and 9-substituted derivatives to provide an appropriate reactant for the synthesis of minocycline. In order for electrophilic substitution to occur in such a way to exelusively form the 7-nitro derivative, position 9 must be sterically protected in some way [23]. Bernardi et al. [23] reported that 9-alkyl derivatives of 6-demethyl-6-deoxytetracycline, which could revert back to the non-substituted 6-demethyly-6-deoxytetracycline, could be formed by reacting 6-demethyl-6-deoxytetracycline with alkylating agents in the presence of a strong acid. By reacting the resulting 9-alkyl derivative obtained with anhydrous hydrofluoric acid, methanesulfonic acid or boiling trifluoric acid, the non-substituted 6-demethly-6-deoxytetracycline could be regenerated. Bernardi et al. used the above concept to synthesise minocycline as summarized in Figure 1.10.



KEY
I 6-demethyl-6-deoxytetracycline
II 9-tert-butyl-6-demethyl-6-deoxytetracycline
III 9-tert-butyl-7-nitro-6-demethyl-6-deoxytetracycline
IV 7-amino-9-tert-butyl-6-demethyl-6-deoxytetracycline
V 9-tert-butyl-7-dimethylamino-6-demethy-6-deoxytetracycline
VI 7-dimethylamino-6-demethyl-6-deoxytetracycline/minocycline

Figure 1.10. Stereo-specific synthesis of minocycline

Additional methods for synthesizing minocycline involve the reductive dimethylamination of 7-nitro-6-demethy-6-deoxytetracycline, 7-[1,2-bis(carbobenzyloxy) hydrazino]-6-demethyl-6-deoxytetracycline or 7-(4-sulfophenylazo) -6-demethyl-6-deoxytetracycline with formaldehyde [24]. Using any of the aforementioned methods, minocycline is isolated from the reaction liquid containing formaldehyde by using either dilution or extraction. Both methods involve the use of large volumes of organic solvents and often leave residual formaldehyde, which has the potential to form unwanted by-products, particularly in aqueous media [24]. Hasegawa et al. [24] have described a process for synthesising high purity minocycline and with a higher yield and less residual formaldehyde after reductive methylation of 7-amino-6-methyl-6-deoxytetracycline. This process uses a reaction liquid containing minocycline and

formaldehyde that is treated with hydroxylamine or urea and subsequently subjected to an adsorption process using non-ionic adsorptive resins onto which minocycline is preferentially adsorbed.

1.3.2 Structure activity relationship

All tetracycline antibiotics share the perhydronaphthacene skeleton as depicted in Figure 1.11 [8]. Tetracycline is denoted the parent compound for nomenclature purposes [8]. 6-demethyl-6-deoxy-4-dedimethylaminotetracycline is the simplest known tetracycline that displays some anti bacterial activity, although only against Gram-positive bacteria in vitro [8]. A basic functional group at the C-4 position is essential to ensure in vivo activity of tetracylines, thus making 6-demethyl-6-deoxytetracycline the simplest tetracycline compound to display broad-spectrum activity both in vitro and in vivo [8]. In order to ensure broad-spectrum activity, tetracycline antibiotics must contain the two UV-absorbing chromophoric groups i.e. the A-ring and BCD-ring systems. Modification (breaking, aromatization, extension, blockage) of these components of the structure results in the formation of compounds that lack activity or compounds that display weak antibacterial activity and through a mode of action different to that of other tetracycline derivatives [8].

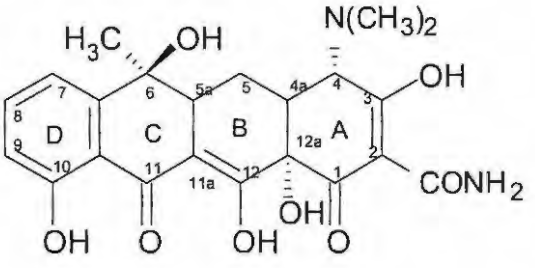


Figure 1.11. Stereochemistry of tetracyclines (tetracycline)

Neither the 6-methyl nor the 6-hydroxy substituent is essential for antibacterial activity [8] and minocycline lacks both these substituents. The presence of the 6-hydroxy substituent renders tetracyclines acid labile and modification of the molecule at the C-6 position may yield 6-deoxytetracycline derivatives that are acid stable and which have facilitated substitution at the C-7 and/or C-9 positions [8]. Substitution with electron withdrawing groups at the C-7 position has also been shown to enhance in vitro activity [8]. Minocycline possesses a 7-dimethylamino substituent which is protonated and therefore electron withdrawing near the surface of bacteria thereby enhancing the in vitro activity of the compound [8].

With the increase of bacterial resistance to tetracycline antibiotics, so the development of newer semi-synthetic tetracyclines that exhibit a broader spectrum of activity has commenced [25]. Sum et al. [25] synthesised and studied 9-substituted minocycline derivatives for possible enhanced microbial activity. Substitution with a tert-butyl-glycylamido side chain at the C-9 position of minocyline, has yielded tigecycline the first antibiotic of the glycylcycline class [26].

1.4 STABILITY

1.4.1 Temperature

Minocycline is stable in the dry-powder state for a three to four year period when stored at 25 °C [10]. Solutions of minocycline, as solutions of other tetracyclines, are not stable enough to permit the preparation of pre-constituted aqueous solutions as feasible practical dosage forms [10]. In a study conducted to determine the stability of minocycline hydrochloride and rifampicin in intravenous solutions, minocycline was found to be stable in both 5% dextrose and 0.9% sodium chloride with its initial concentration declining by 8% at 24°C and by 2% at 4°C over a period of seven days [27].

1.4.2 pH

The stability of minocycline in solution is affected by pH, with solutions stored at pH 4.2 and 5.2 maintaining at least 90% of their initial potency following a week of storage at 25 °C. The stability of minocycline in solution of different pH is depicted in Figure 1.12 [10].

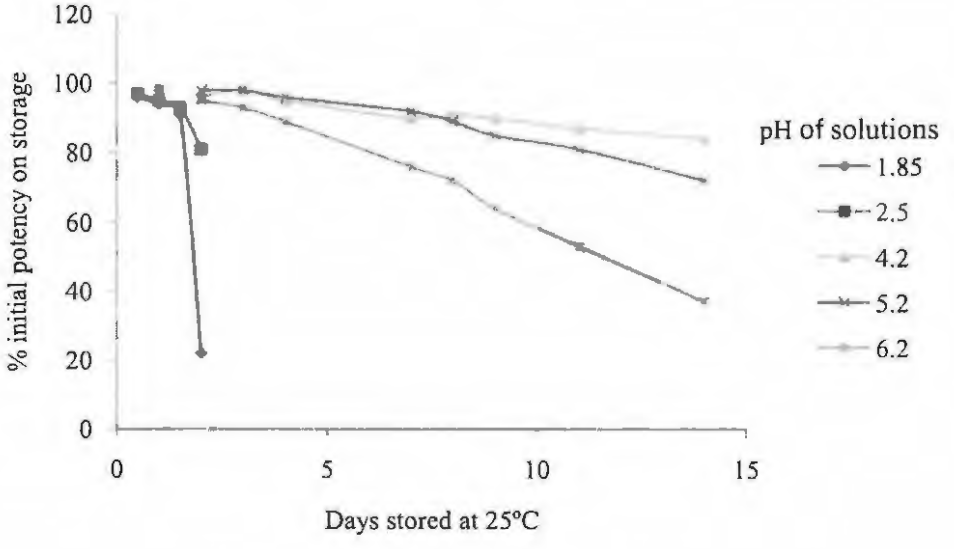


Figure 1.12. Stability data for minocycline solutions (% Initial activity retained)

1.4.3 Oxidative degradation

Minocycline is more susceptible to oxidative degradation when compared to other tetracycline antibiotics as the D-ring of minocycline contains a substituted p-amino-phenol [10]. Minocycline has been associated with cases of pigmentation of the thyroid in patients with a history of chronic minocycline use [28]. The pigmentation is thought to be as a result of the oxidation of minocycline by the enzyme thyroid peroxidase which is required for thyroid hormone synthesis [28]. Although minocycline-induced thyroid hyper-pigmentation has been reported as a rare and harmless side effect, a significant number of patients reported to have this side effect have been found to exhibit signs of thyroid cancer [28].

1.4.4 Structural rearrangement of tetracycline antibiotics

Tetracycline antibiotics readily undergo 4-epimerization and the rate of epimerization differs from analogue to analogue [10]. The tricarbonyl system of ring A of the tetracycline antibiotics allows enolization involving loss of the C-4 hydrogen [29]. Reprotonation of the molecule may occur from the top of the enol, regenerating the tetracycline. However reprotonation may also occur from the bottom of the molecule resulting in the formation of an inactive 4-epitetracycline as depicted in Figure 1.13 [29]. Several methods that have been used to reduce the epimerization of the tetracycline antibiotics have been reported [30]. These methods include forming calcium, magnesium or aluminium salts with tetracyclines to limit epimer formation when manufacturing is performed at basic pH in non-aqueous solutions. Formation of metal complexes under acidic conditions results in the subsequent preparation of a stable solid form of the drug. Maintenance of pH at values greater than 6.0 during processing, avoiding contact with conjugates of weak acids, avoiding contact of the reactants with moisture and aqueous based solutions and working at low temperatures reduces epimer formation in tetracyclines.

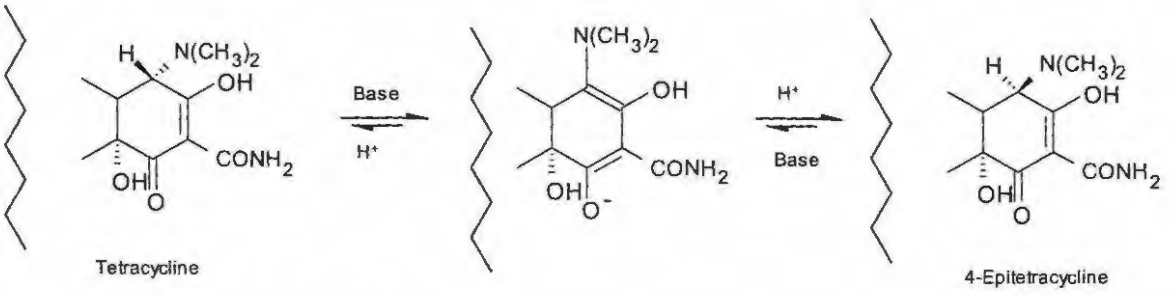


Figure 1.13. Epimerization of tetracycline antibiotics

1.5 CLINICAL PHARMACOLOGY

1.5.1 Mode of action

Minocycline exhibits a similar mode of action as other tetracycline analogues [2] but has proved to have a broader spectrum of activity against both aerobic and anaerobic bacteria due to enhanced tissue penetration activity as compared to other tetracycline derivatives [3].

Tetracyclines are bacteriostatic antibiotic compounds [31] and their anti-microbial activity is a consequence of bacterial protein biosynthesis inhibition [2]. Tetracycline compounds bind to the 30s ribosomal subunit [2] thereby preventing aminoacyl-tRNA binding to the acceptor site of the mRNA-ribosome complex [32]. This mechanism by which the inhibition of protein synthesis occurs is similar for human and bacterial cells; however, bacteria have an energy-dependant active transport process that moves tetracyclines against a concentration gradient to produce appreciable intracellular concentrations [32,33]. The active transport process confers selectivity to bacterial cells on tetracycline antibiotics [33]. Tetracyclines have however, been found to have the potential to interfere with protein synthesis in mammalian cells which in turn may impair wound healing or produce catabolism in patients with renal failure [31].

1.5.2 Spectrum of activity

Tetracyclines, including minocycline, display their antimicrobial action on Gram-negative bacteria, Gram-positive bacteria, anaerobes, intracellular bacteria such as *Rickettsiae*, *Chlamydiae* and *Mycoplasma* and on some protozoa [3, 31,32,34,]. Tetracyclines exert their antimicrobial effects on Gram-negative bacteria including; *Escherichia coli*, *Enterobacter*, *Klebsiella* [2,3,31], *Hemophilus influenza* [2,31], Gonococcus, *Proteus mirabilis*, Meningococcus [31], *Vibrio* and *Campylobacter fetus* [2].

Minocycline has been found to be effective on some Gram-negative bacteria resistant to other tetracyclines. Using disc sensitivity criteria, it was found that 32% of *Serratia* resistant to other tetracyclines was sensitive to minocycline [3]. In a study conducted on strains of *Neisseria gonorrhoea* sensitive to penicillin or ampicillin it was determined that minocycline was the most sensitive of the tetracyclines with 80% of the *N. gonorrhoea* inhibited when using concentrations of less than 0.4 µg/ml [2]. At concentrations of less than 2 µg/ml, minocycline was found to completely inhibit *Pseudomonas* non-*aeruginosa* species in susceptibility tests of these species to aminoglycosides and tetracycline antibiotics [2]. Of the tetracycline analogues, minocycline was shown to have the highest activity against strains of *Legionella* with a reported minimum inhibitory concentration (MIC) of 0.25 µg/ml [2].

Minocycline shows activity towards some Gram-positive strains that are resistant to methicillin and other tetracycline compounds [35]. Whilst other tetracycline analogues have been shown to inhibit only 50% of penicillin-sensitive and penicillin-resistant *Staphylococcus aureus*, minocycline has been shown to inhibit 90% of these in vitro [31]. Minocycline was found to exert greater activity against tetracycline-resistant strains of *Streptococcus pneumoniae* [2]. Although there is increasing resistance to tetracycline, studies have shown minocycline activity against beta-hemolytic streptococci (Group A, B, C, G) and *Clostridium perfringes* [31].

1.5.3 Indications

Minocycline is categorised as a systemic antibacterial and antiprotozoal as well as a systemic agent for the treatment of acne [1]. Tetracyclines (including minocycline) are used in the treatment of rickettsial, chlamydial and vibrio infections, brucellosis, chronic bronchitis and acne [9,36]. Tetracyclines are also primarily used to treat urinary tract infections, shigellosis and Lyme disease [36].

Minocycline has been specifically indicated for the treatment of severe acne and may be of use in forms of acne that are resistant to other tetracycline derivatives [1]. Systemic minocycline is indicated for the treatment of mycobacterial infections and nocardiosis [1]. Minocycline is effective in the eradication of the asymptomatic carrier state of meningococcal infectious disease in the nasopharynx [1,36]. Minocycline may be used as an adjunct for the treatment of amoebic dysentery and periodontitis [37].

Minocycline may be used in cases of penicillin allergy to treat bacterial infectious disease, *Treponema pertenue*, *Listeria monocytogenes*, *Fusobacterium fusiforme*, *Actinomyces israelii* and Clostridium species [37]. Minocycline may be used as a substitute for the treatment of anthrax when penicillin is contraindicated [37].

1.5.4 Role of minocycline in neurology

Animal studies have revealed that minocycline may be effective in alleviating and treating a number of neurological disorders [38]. Neurodegenerative diseases exhibit common symptoms that include apoptotic cell death and production of neurotoxins by the persistent activation of microglia [38]. Experimental neurology has established that minocycline impairs microglial activation, neuroinflammation and apoptosis [38]. An open label blind study for acute stroke, significantly lowered NIH Stroke Scale and modified Rankin Scale, and increased Barthel Index in patients, indicating the potential benefit of minocycline in the treatment of acute ischaemic stroke [39]. Minocycline is currently used in combination with other conventional therapy for the treatment of multiple sclerosis [39]. Phase II

clinical trials in which minocycline in combination with creatine was administered to treat Parkinson's disease have demonstrated the absence of significant safety concerns and currently phase I/II pilot studies to examine the efficacy of this combination in patients with acute spinal cord injury are underway [39].

1.5.5 Resistance

Bacterial resistance to clinically used tetracyclines occurs due to acquired resistance of the organism by acquisition of resistant genes [40]. Chopra et al. [7] have reported that twenty nine tetracycline resistant (*tet*) and three oxytetracycline resistance (*otr*) genes have been characterised and details are summarized in Table 1.6. The two main biochemical mechanisms by which resistance to tetracyclines can occur are as a result of the presence of an efflux system that actively exports tetracyclines from bacterial cells and the presence of ribosomal proteins that protect bacterial ribosomes from the effect of tetracyclines [7,40]. A less important mechanism of resistance to tetracyclines includes enzymatic inactivation of tetracyclines rendering them ineffective against the bacteria being treated [7,40]. Efflux genes which are found in both Gram-positive and Gram-negative bacteria confer resistance to tetracycline but not to minocycline and the glycylcyclines [7]. In contrast, ribosomal protection proteins, which confer a wider spectrum of resistance to tetracyclines except for *tet* (B), protect the ribosomes from the action of tetracycline as well as that of doxycycline and minocycline [7].

Table 1.6. Mechanisms of resistance for characterised *tet* and *otr* genes [7]

Mechanism of resistance	Genes
Efflux	*tet* (A), *tet* (B), *tet* (C), *tet* (D), *tet* (E), *tet* (G), *tet* (H), *tet* (I), *tet* (J), *tet* (Z), *tet* (30), *tet* (31), *tet* (K), *tet* (L), *otr* (B), *tcr*3, *tet* P(A), *tet* (V), *tet* (Y)
Ribosomal protection	*tet* (M), *tet* (O), *tet* (S), *tet* (W), *tet* (Q), *tet* (T), *tet* P(B), *otr* (A)
Enzymatic	*tet* (X)
Unknown	*tet* (U),*otr* (C)

In a study aimed to unravel the genetic basis of resistance in the potentially probiotic strain of *Lactobacillus plantarum* it was revealed that *L. Plantarum* possessed the *tet* (S) gene that conferred resistance to both tetracycline and minocycline with observed MIC values of 512 µg/ml and 256 µg/ml for tetracycline and minocycline, respectively [41]. The presence of *tet*(M), *tet*(O) and *tet*(S) resistance genes, that encoded for both tetracycline and minocycline resistance in *Sterptococcus pyogenes*, the most common cause of bacterial pharyngitis, has also been reported [42].

The inhibition of R-factor resistant *Escherichia coli* by tetracyclines, reveal that lipophilic analogues are generally more active against tetracycline-resistant Gram-negative bacteria [43]. In a study on tetracycline susceptible and resistant *Escherichia coli*, it was reported that although minocycline and tetracycline were exported by the same active efflux system in resistant cells, minocycline entered resistant host cells largely via this system 10-20 times more rapidly than tetracycline with the net efflux of minocycline being low which may partly be the reason for tetracycline-resistant cells being more susceptible to minocycline [43].

1.5.6 Contraindications

Minocycline is contraindicated in patients with hypersensitivity to minocycline, other tetracyclines or any component of a formulation containing minocycline [44]. The use of minocycline is not recommended for patients with renal insufficiency related to hepatotoxicity [44]. Concomitant use of minocycline with isotretinoin is not recommended as there is an increased risk of pseudotumor cerebri [37].

1.5.7 Drug interactions

Minocycline may augment the effects of warfarin [44]. The effect of minocycline is decreased by concomitant use with aluminium, calcium, zinc or magnesium antacids, bismuth salts, sodium bicarbonate, barbiturates, carbamazepine and hydantoins [44]. The concomitant use of dong quai and St John's Wort with minocycline is not recommended as photosensitization reactions may be precipitated [44].

1.5.8 Adverse reactions

Reported adverse reactions to minocycline are similar to those displayed by other tetracycline antibiotics [9]. The common side-effects of tetracycline antibiotics are usually dose-related and include gastrointestinal disturbances such as nausea, vomiting, anorexia and an "unpleasant taste" [31]. Tetracyclines may also alter the density of normal microbial flora causing candiadiasis and enterocolitis [31]. Tetracycline therapy may also cause phototoxicity and tooth mottling [31].

Minocycline and methacycline have been shown to have the least phototoxic reactivity of the tetracycline group of antibiotics [3]. However minocycline has been associated with a blue-grey pigmentation of the skin and pigmentation of acne scars [9]. Minocycline has also been associated with a greyish-brown discolouration in areas of the skin exposed to the sun that may be due to melanin deposition [20]. Skin discolouration due to minocycline use resolves slowly after discontinuation of minocycline [20].

Minocycline may precipitate vestibular toxicity in the form of nausea, vomiting, vertigo and ataxia [3,31] which is more than likely a consequence of its relatively high lipophilicity and which enhances its CNS penetration capacity [2]. In a study conducted by Jacobson et al. [4] 86% of subjects treated with minocycline experienced vestibular toxicity with 84% displaying toxicity after one or two doses of 100 mg and 52% of the subjects stopped treatment due to the severity of these effects. A review of 30 clinical trials in which minocycline was administered for a period of at least 3 days at standard doses to ambulatory patients revealed that the frequency of vestibular toxicity was no more than 20% and could be severe enough to cause alteration of treatment in one third of the patients or one in fifteen patients receiving the drug [3]. Vestibular toxicity symptoms usually occur when maximal serum concentrations have been reached and are resolved 48 hours following withdrawal or discontinuation of minocycline [2].

1.5.9 High risk groups

Minocycline is classified as a Category D risk factor in pregnancy. Animal studies have indicated that there is a possibility of human foetal risk if the drug is used during pregnancy [44].The use of minocycline is not recommended during the second half of a pregnancy since it causes permanent tooth discolouration in the foetus [37]. High doses of minocycline may result in fatty infiltration of maternal liver [9]. Minocycline is not recommended in lactating mothers as it is excreted in breast milk [9,44]. There is evidence to suggest that minocycline may alter milk production and/or composition [37]. Unless other drugs are contraindicated or lack effectiveness, minocycline should not be used in children under the age of eight years of age, as it may be deposited in growing bones thereby causing growth retardation or in teeth causing permanent discolouration [9]. Tetracycline may also cause enamel hypoplasia if used in children during the process of tooth development [9]. Hepatotoxicity and the anabolic effects of the tetracyclines is likely to occur in elderly or frail patients [9].

1.6 PHARMACOKINETICS

1.6.1 Dosage

Minocycline can be administered to children over eight years of age at an initial dose of 4mg (base)/ kg of body weight and then 2 mg/kg 12 hourly for at least 7 days [9]. Children weighing over 50 kg are dosed as recommended for adults [9]. When treating infections in adults, minocycline is administered at an initial dose of 200 mg (base), then 100 mg every 12 hours [9,44,45]. For the treatment of acne a 100-200 mg dose of minocycline is given initially with 50 mg doses used as maintenance doses daily [9]. A dose of 100 mg every 12 hours for at least 4 days is the recommended dose for treatment for gonorrhoea, and for carriers of *Neisseria meningitides* a 5 day course is recommended whereas 6 to 8 weeks of

therapy is required for treating *Mycobacterium marium* infections [1]. In uncomplicated urethral, endocervical or rectal infections caused by *Chlamydia trachomatis*, 100 mg doses are given twice daily for at least 7 days [1]. The maximum amount of minocycline given on a daily basis should not exceed 400 mg/ 24 hr [44].

1.6.2 Absorption

The absorption of minocycline from the gastrointestinal tract (GIT) is rapid and virtually complete following oral administration [2]. An oral dose of 200 mg followed by 100 mg doses every twelve hours is reported to produce plasma concentrations of between 2 and 4 μg/ml [20]. The absorption of minocycline is not significantly affected if administered with food or moderate amounts of milk [20]. In a study conducted by Leyden [46] to determine the effect of milk, food and iron on the absorption of minocycline and tetracycline, it was observed that although the absorption of minocycline was less affected by concurrent administration with food and milk, there was 27% inhibition of minocycline absorption with milk and 13% inhibition of minocycline absorption with food. The absorption of minocycline and tetracycline was significantly decreased following administration with iron with 77% inhibition of minocycline and 81% inhibition of tetracycline absorption.

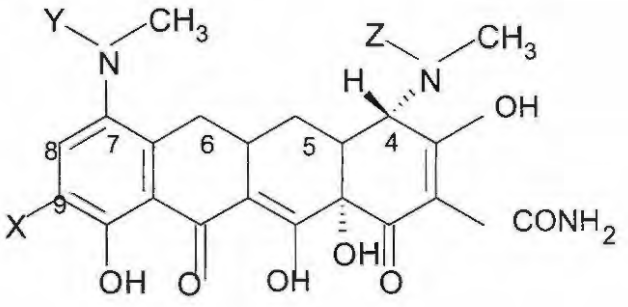
1.6.3 Distribution

Minocycline is widely distributed in body tissues and fluids due to its high lipid solubility relative to other tetracycline derivatives [20]. In a pharmacokinetic study on minocycline in man, peak serum concentrations after a single oral dose of minocycline were achieved more rapidly than with doxycycline, methacycline and demethylchlortetracycline with the absorption half-time of minocycline determined to be less than one hour and approximately 95% of the maximum serum concentration being attained by the first sampling time of one hour [47]. The penetration of minocycline into the cerebrospinal fluid is better than that observed for doxycycline [47]. Minocycline is approximately 75% bound to plasma proteins [20]. A decrease in tissue binding may result in an increase in tissue distribution of the drug. When used in patients with renal failure there is an apparent trend towards increased tissue distribution of minocycline [50].

1.6.4 Metabolism

Minocycline has been demonstrated to be the first tetracycline antibiotic to undergo metabolism in man and this is most likely due to its lipophilicity and the presence of an additional dimethylamino group,

which might specifically be affected by metabolising enzymes and systems [48]. Three major metabolites of minocycline have been isolated, namely: 9-hydroxyminocycline, a hydroxylation product, N_7-demethylminocycline and N_4-demethylminocycline [51]. The metabolic products of minocycline are depicted in Figure 1.14. The relatively low recovery (30.9%) of microbiologically active minocycline at the end of a 72-96 hour interval following a single oral dose indicates that minocycline is partially metabolised to inactive substances [49].



	X	Y	Z
9-Hydroxyminocycline	OH	CH_3	CH_3
N_7-Demethylminocycline	H	H	CH_3
N_4-Demethylminocycline	H	CH_3	H

Figure 1.14. Degradation products of minocycline

1.6.5 Elimination

The plasma half-life of minocycline has been reported to be between 11- 26 hours [20]. Approximately 5-10% of administered minocycline is excreted unchanged in the urine [9,20]. Following oral administration the renal clearance of minocycline between 6 and 12 hours was found to be 9 ml/min [47]. Following a single intravenous dose or repeated oral doses of minocycline to patients with different degrees of renal impairment the observed clearance of minocycline from the circulation appeared to be independent of renal function [50]. Up to 34 % of administered minocycline is excreted in the faeces [20].

1.7 CONCLUSIONS

The structural modifications of the tetracycline backbone, to form minocycline have produced a molecule that has unique properties within the tetracycline group of antibiotics. Specifically, the substitution on position C-7 of the tetracycline molecule with a dimethylamino group, which can be ionized, has produced a molecule that has a higher isoelectric point than its congeners. The isoelectric point of

minocycline at which the zwitterionic form is neutral coincides with physiological pH. Due to its higher lipophilicity at physiological pH, minocycline penetrates cells more readily than other tetracycline antibiotics thereby increasing its spectrum of activity.

Although minocycline has a relatively long plasma half-life of 11-26 hours, modified-release pharmaceutical dosage forms may be essential in reducing the incidence and/or severity of the adverse events, particularly the vestibular adverse effects associated with minocycline, in order to optimise minocycline therapy particularly for long-term therapy such as for acne. Some formulation methods may expose the active component to extreme conditions such as high temperature or extremes of pH. An understanding of the stability of the active drug is therefore critical for one to ensure that the integrity of the drug is maintained during the formulation process.

CHAPTER TWO

2 THE DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR THE ANALYSIS OF MINOCYCLINE

2.1 INTRODUCTION

2.1.1 Overview

The analysis of minocycline has been performed using capillary zone electrophoresis [49], densitometric thin layer chromatography [50] and spectrophotometric methods in which oxidative and diazo-coupling reactions have been applied [51]. These methods have several shortcomings including cost and inherent complexity and require the use of equipment that is not routinely available in all laboratories. High performance liquid chromatography (HPLC) has also been used for the quantitation of minocycline in biological fluids such as plasma [16,52-54], saliva [53] and serum [55]. Several HPLC methods for the quantitation of minocycline are used for the simultaneous analysis of minocycline in the presence of other antibiotics [45,56]. An improved liquid chromatographic method for the determination of minocycline has been used for the analysis of raw material but not for the evaluation of formulations [57]. Perusal of the literature reveals a paucity of information pertaining to the use of stability indicating HPLC methods for the quantitation of minocycline in dosage forms. The objective of this study was to develop a simple, precise and accurate stability indicating reversed-phase HPLC method for the quantitation of minocycline in pharmaceutical dosage forms.

2.1.2 Principles of HPLC

High performance liquid chromatography (HPLC) has been referred to by other terminology including high pressure LC, high speed LC or high efficiency LC and is used as a separative and analytical technique in the pharmaceutical industry [58]. Liquid chromatography is a similar technique to gas chromatography (GC) and refers to any chromatographic method in which the mobile phase used for a separation is a liquid as opposed to a gas as is the case in GC [59]. Liquid chromatography has allowed a wider application in analysis than GC as it is suitable for the analysis of compounds that are not volatile, are thermally unstable and that may decompose under the conditions of separation applied in GC [59]. The separation of samples that are difficult to isolate using GC is more readily achieved using LC as the technique uses two chromatographic phases for selective interaction with the analyte(s) of interest, and offers a greater variety of stationary phases and lower separation temperatures. In addition, a variety of

detectors that have limited use in GC can be used in LC and sample recovery is relatively easily achievable [59]. HPLC has several advantages over other liquid chromatographic methods, which include the fact that HPLC columns are reusable without regeneration, resolution in HPLC is better, reproducibility is superior as HPLC is less dependent on the skill of the operator, the instrumentation of HPLC lends itself to automation and quantitation, analysis times are generally shorter and preparative LC is possible on a much larger scale [58].

The separation of compounds of interest in HPLC occurs as a result of the differential migration of analytes along the HPLC column [59]. Differential migration results from the equilibrium distribution of the different compounds between the stationary phase and the mobile phase [59]. Compounds that interact preferentially with the stationary phase, move through the column slowly and have longer retention times whereas those that interact with the mobile phase move through the column more rapidly and have shorter retention times [59]. Differential migration is dependent on factors that influence the distribution of compounds between the mobile and stationary phases and include the composition of mobile phase, composition of the stationary phase and the separation temperature [59].

Three retention mechanisms/models in HPLC have been mathematically described by Kazakevich [60] in HPLC viz., the partitioning model, the adsorption model and the adsorption-partitioning retention mechanism.

In the partitioning mechanism of retention, Kazakevich [60] proposes that the analyte is distributed between a mobile and stationary phase and that there is instant equilibrium of the analyte between these two phases. At the molecular level, the partitioning mechanism entails a process in which a solute-sized cavity is created in the organic phase after which there is transfer of a solute molecule from the aqueous phase into the organic phase and followed by closing of the cavity created by the solute molecule in the aqueous phase [61]. A weakness of the partitioning mechanism of retention is that the model assumes that the two phases are mutually insoluble, which may not always be the case [61].

The adsorption mechanism for retention has been argued by some to be the better logical mechanism to explain the retention characteristics of a compound in HPLC, as stationary phase material is normally composed of a solid porous matter with an associated high surface area and that is impermeable to both analyte and eluant molecules [60]. In the adsorption model, adsorption occurs at the solid-liquid interface where the solute molecules migrate from the liquid phase to the adsorptive layer where they displace physically adsorbed molecules of solvent [61].

The third mechanism of retention in HPLC is essentially a combination of both the partitioning and the adsorption models of retention in which overall retention is a consequence of superimposition of two consecutive processes [60]. The first process involves the partitioning of analyte molecules from the bulk eluant onto the adsorbed eluant layer and the second stage involves the adsorption of an analyte onto the surface of the stationary phase or column packing matter [60].

HPLC can be divided into four main modes depending on the stationary phase used. These include liquid-liquid chromatography in which the stationary phase is a liquid, liquid-solid chromatography in which the stationary phase is solid, ion-exchange chromatography in which the stationary phase contains fixed ionic groups associated with counter ions of opposite charge and size-exclusion (gel) chromatography in which the stationary phase is composed of a material with pores of a specific size [58].

Modification of liquid-liquid chromatography (LLC) gave rise to bonded phase chromatography (BPC) in which an organic stationary phase is chemically bound to a support, instead of being held mechanically as in liquid phase in liquid-liquid chromatography [59]. BPC columns are the most widely used columns as the chemically bound stationary phase cannot be easily removed or lost during use as is the case with LLC [59]. BPC is further divided into normal phase BPC in which polar packing materials are used and reversed-phase BPC in which relatively non-polar stationary phases such as C_8 or C_{18} hydrocarbons are used in conjunction with polar mobile phases for the separation of a wide range of solutes of lower polarity [59].

The original use of reversed-phase BPC columns was for the separation of non-polar compounds such as hydrocarbons [59]. However, reversed-phase BPC columns have been applied and continue to be used for pharmaceutical and drug analysis in biological fluids, for nucleic acid and nucleosides, in agriculture as well as for characterizing oligomeric mixtures of compounds [59].

2.2 LITERATURE REVIEW

Several HPLC methods have been used for the analysis of minocycline. A summary of the methods published in the literature for the analysis of minocycline is shown in Table 2.1. The majority of these methods have used ultraviolet (UV) detection for the determination of minocycline in biological matrices and have been conducted at low pH.

Table 2.1. High Performance Liquid Chromatographic Analysis of Minocycline

Column	Sample Matrix	Mobile Phase Composition	Detection	Flow rate	Reference
Nucleosil®, C_8 (250x4 mm I.D.; 5 µm)	plasma, saliva	Acetonitrile:methanol:distilled water:0.1% trifluoroacetic acid (25:2:72.9:0.1, v/v; pH=1.76)	UV-350nm	0.9 ml/min	[53]
Symmetry Shields® RP 8 (150x4.6 mm I.D.; 3.5 µm)	plasma	Acetonitrile:methanol:0.01 M KH_2PO_4 (5:20:72.1, v/v) with 0.03 mM Na_2 EDTA and 60% $HClO_4$, pH=2.5	UV-350nm	0.8 ml/min	[62]
Zorbax®, RX-C_8 (150x4.6mm I.D.)	plasma	Acetonitrile:water:TFA (80.0:19.9:0.1)	Mass spectrometry	1 ml/min	[54]
µ-Bondapak®, C_{18} (250x30mm)	tissue, serum	methanol	UV-350nm	1 ml/min	[55]
XTerra® RP-18 (250x4.6 mm I.D.; 5µm)	Drug substance	Acetonitrile:tetrabutylammonium hydrogen sulphate (pH=6.5):0.2 M ethylene diaminetetraacetic acid (pH=6.5);water (20:20:20:40; v/v/v/v)	UV-280nm	1 ml/min	[57]
Vydac®, C_8 (250x4.6 mm I.D.; 5µm)	IV solution	22% acetonitrile in 50 mM K_2HPO_4 (pH=6.5)	UV-254nm	1 ml/min	[27]
Metaphase-Crest-Pak-ODS® (250x4.0 mm I.D.; 5µm)	Drug substance	Tetrabutylammonium hydrogensulphate (0.025 M):methanol:acetonitrile (96:2:2) (pH=3.0)	UV-248nm	1.5 ml/ml	[45]
Nucleosil® 5-CN (125×4.0-mm I.D.)	plasma	methanol and 20 m*M* perchloric acid/4 m*M* triethylamine in water (20:80, v/v; pH approx. 2)	UV-350nm	1 ml/min	[52]
µ-Bondapak®, C_{18} (300x4.6 mm I.D.; 10 µm)	Bulk powders phamaceuticals	KH_2PO_4 (pH=2.5; 0.05 M):acetonitrile (84:16, v/v)	Amperometric detection-1.2 V	1 ml/min	[56]

2.3 EXPERIMENTAL

2.3.1 Reagents

Minocycline hydrochloride was purchased from Sigma-Aldrich (Johannesburg, Gauteng, South Africa). The potency was established as 903 µg minocycline/mg. Methanol (UV cut-off 215nm) and Acetonitrile (UV cut-off 200nm) were purchased from Romil-SpS® (Waterbeach, Cambridge, UK). Potassium dihydrogen phosphate and sodium hydroxide pellets were purchased from Associated Chemical Enterprises (Southdale, Gauteng, South Africa). Polished HPLC grade water was prepared by reverse osmosis using a Milli-RO® 15 Water Purification System (Millipore Co., Bedford, MA, USA) that consisted of a Super-C carbon cartridge, two Ion-X ion exchange cartridges and an Organex-Q cartridge. The HPLC water was filtered through a 0.22 µm Millipak® 40 sterile filter prior to use. Cyclimycin®-50 and Cyclimycin®-100 (Pharmacare Limited, Port Elizabeth, South Africa) capsules containing minocycline hydrochloride equivalent to 50 mg and 100 mg minocycline respectively, were purchased from a local pharmacy (Alpha Pharm, South Africa). All reagents used were at least of analytical grade and were used as received.

2.3.2 Preparation of stock solutions

A stock solution of minocycline hydrochloride was freshly prepared before each analytical run by accurately weighing 25.0 mg of minocycline hydrochloride in a 50 ml A-grade volumetric flask and dissolving in mobile phase. The concentration of the resultant solution was 500 µg/ml. Standard solutions of 5, 10, 20, 60, 100 and 140 µg/ml were prepared by serial dilutions of the stock solution. The stock solution of the internal standard, doxycycline hyclate, was freshly prepared before each analytical run by accurately weighing 12.5 mg of doxycycline hyclate in a 50 ml A-grade volumetric flask and dissolving in mobile phase.

2.3.3 Preparation of buffer solutions

Buffer solutions of concentrations 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM were prepared by accurately weighing appropriate amounts of potassium dihydrogen phosphate into 1 L A-grade volumetric flasks and making up to volume with HPLC grade water. The pH of the solutions was adjusted to the required pH using 1.0 M NaOH. 1.0 M NaOH was made by accurately weighing 4.0 g of sodium hydroxide pellets into a 100 ml A-grade volumetric flask and making up to volume with HPLC grade water.

2.3.4 Preparation of mobile phase

Mobile phase was prepared by adding the required volume of HPLC-grade acetonitrile and methanol and buffer to a glass Duran® Schott solvent mixing bottle (Schott Duran GmbH, Hattenbergstrasse, Germany). The mixture was allowed to equilibrate to room temperature and the mobile phase was filtered through a 0.45 µm Millipore® HVLP filter (Millipore, Bedford, MA, USA) and degassed under vacuum using a Model A-2S Eyela Aspirator (Rikakikai Co., Ltd, Tokyo, Japan) prior to use. The mobile phase was freshly prepared daily and not recycled during analyses.

2.3.5 HPLC system

The modular HPLC system was comprised of a Spectra-Physics, Spectra Series P100 pump (Spectra-Physics, San Jose, CA, USA), a Waters WISP 710B Autosamper (Waters Chromatography Division, Milford, MA, USA), a LINEAR™ UVIS 200 detector (Linear Instruments Co., Irvine, CA, USA) and a Spectra Physics, SP 4290 Integrator (Spectra-Physics, San Jose, CA, USA). Separation was achieved on a Phenomenex® (Torrance, CA, USA) Prodigy® 5 µm ODS, 150 x 4.60 (i.d.) mm column.

2.4 METHOD DEVELOPMENT AND OPTIMISATION

2.4.1 Introduction

HPLC method development should follow a simple and systematic approach with each experiment contributing to an appropriate final result [63]. Prior to selecting the initial conditions for method development, it is important to know the physicochemical properties of the analyte including the chemical structure, molecular weight, pKa values, UV spectrum, solubility in addition to the concentration range to be studied [63]. Two approaches to HPLC method development have been noted, viz., the theoretical approach or the empirical approach [63]. When using the theoretical approach, an analyst matches the "chemistry" of the analyte to HPLC conditions based on past experience and/or based on literature data, whereas when using the second approach, the analyst proceeds directly to the chromatographic procedure without paying attention to the "chemistry" of the analyte [63].

Samples for HPLC analysis may be classified as being "special" or "regular", the latter being composed of small molecules (<2000 Da) [63]. "Regular" samples may be divided further into neutral or ionic samples [63]. The initial conditions recommended for the analysis of ionic samples are shown in Table 2.2 [63].

Table 2.2. Recommended experimental conditions for the HPLC separation of ionic samples

Separation variable	Initial choice
Column	
Dimensions (length, ID)	15 x 0.46 cm
Particle size	5 µm
Stationary phase	C_8 or C_{18}
Mobile Phase	
Solvents A and B	Buffer-acetonitrile
% B	80-100%
Buffer	25 mM potassium phosphate, 2.0<pH>3.0
Temperature	35-45 °C
Flow rate	1.5-2.0 ml/min
Sample size	<25 µl

The initial conditions used for the method development for minocycline in pharmaceutical dosage forms were based on the physicochemical properties of minocycline hydrochloride in addition to data obtained from the literature [27].

2.4.2 Column selection

A stable highly efficient column is essential when developing a rugged, reproducible analytical method [63]. It is important that the analyst has an understanding of the column description and selectivity and the factors that influence this selectivity prior to selecting the column for use in method development. Manufacturers describe reversed-phase HPLC columns in terms of their ligand, particle pore diameter and silica type [64].

Three main column-packing particle types have been described for use in HPLC and these are totally porous microspheres, micro-pellicular particles or perfusion particles [63]. Totally porous microspheres come in a variety of diameters, pore sizes and surface area and are most commonly used in HPLC [63]. Porous microsphere particles may be prepared from silica, alumina, ion exchange material or chemically bonded phases [58]. Although smaller porous particles facilitate faster separations for HPLC, particle sizes of 5 µm are usually recommended as this particle size offers an excellent compromise in terms of column efficiency, back pressure and lifetime [63]. Usually, totally porous particles with pore diameters of 7-12 nm are required for separation of small molecules whereas pore sizes larger than 15 nm are required for the separation of molecules

larger than 10 000 Da with a goal of rapid solute diffusion within the pores whilst retaining column efficiency [63].

Silica-based particles are the most popularly used column materials as they offer several advantages compared to other packing materials. Silica-based packing materials are available in a variety of different particles and pore sizes and can be applied to the analysis of small and large molecules [63]. Silica-based materials are mechanically strong and are therefore durable and column packing is reproducible when using spherical particles. Silica-based packings are compatible with water and most organic solvents allowing for the alteration of column selectivity through use of different mobile phases [63].

Although silica-based packing materials offer several advantages they are not without their disadvantages. At high pH silica packing material may be solubilised leading to collapse of HPLC columns [63]. In a study to provide insight into the use of silica-based bonded-phase column packing materials in aqueous mobile phase of high pH, Kirkland et.al. [65] identified three factors affecting the rate of silica support degradation, viz., organic modifier used in the mobile phase, silica support type and type of silane bonded phase. The study yielded the following conclusions:

- Densely bonded monomeric dimethyl-C_{18} ligands protect the silica support from dissolution better than bulky diisopropyl- and diisobutyl-substituted silanes.
- Failure of silica-based columns at high pH results from loss of silica support and not from the hydrolysis of the Si-O-Si siloxane bond of the bonded silane.
- The nature of the bonded C_{18} stationary phase (monomeric or polymeric) does not significantly influence column degradation at high pH.
- The rate of silica dissolution is a function of the nature of the porous silica support with the most stable columns prepared by use of consolidating silica sols.
- The more highly purified, less acidic Type B silica dissolves more rapidly than the less pure Type A which dissolves more slowly and is more stable at high pH.
- Acetonitrile as the organic modifier often prolongs column life at high pH as compared to methanol.

A further disadvantage of silica-based columns is observed during chromatographic analysis of basic and weakly basic compounds. Analysis of these materials results in the development of

chromatographic separations in which asymmetrical peak shapes, low column efficiencies, poor reproducibility and longer retention times than expected may be observed as a result of the strong interaction between the analyte of interest and the silica support of the stationary phase [66]. Silica based stationary phases that are used for chromatographic separations have been ranked according to their desirability for the separation of acidic or basic compounds [63]. Type A silica refers to the older, lower purity, more acidic silica that may be used for the separation of non-ionisable compounds whereas the newer, highly purified lower acidity materials that are recommended for the separation of ionisable compounds are referred to as Type B silica [63]. In a study in which the acidity of residual silanol groups in HPLC columns with different silica supports was studied [66], the highest degree of silanol acidity was observed for non end-capped columns based on older, lower purity silica. Conversely, the lowest degree of silanol acidity was observed for end-capped columns, in which the stationary phase was bonded to a new generation packing material based on silicon-inorganic hybrid and which had a lower silanol activity allowing use of the material over an extended pH range.

The most widely used reversed-phase columns are those with surface-reacted organosilanes manufactured by one of three processes viz., reaction of surface silanols with chlorodimethylsilane, reaction of surface silanols with trifunctional silane or reaction of surface silanols with trifunctional alkoxysilane [63]. The most commonly used process to produce stationary phases is the former, in which mono functional chlorodimethylsilanes are reacted with surface silanol groups to yield a variety of alkyl and substituted alkyl silicas such as *n*-octadecylsilane (ODS/C_{18}) bonded-phase materials [63]. Since a significant portion of the surface does not react in all reactions, some manufacturers have used a process called "end-capping" to ensure complete reaction of the silica support surfaces occurs in order to minimize any potential and unwanted interactions of residual silanol groups with solutes to be separated [63].

Although stainless steel columns are recommended for most HPLC applications, glass and plastic columns may be used for solutes that have undesirable interactions with steel [63]. Columns with soft outer polymer shells and compression-fitting column types are also commercially available [63]. Columns in which the stationary phases are of particle size 3-10 μm and with internal diameters (i.d.) 0.3-0.46 cm may be used for most HPLC applications [63].

The selectivity of reversed-phase columns has been characterized by models such as the hydrophobic-subtraction model [64]. Five main factors have been found to influence the selectivity of reversed-phase HPLC columns using the hydrophobic subtraction model and include:

i. Hydrophobicity (H)

ii. Steric resistance (S*) to insertion of bulky solute molecules into the stationary phase

iii. Column hydrogen-bond acidity (A), mainly attributable to non-ionized silanols

iv. Column hydrogen-bond basicity (B), hypothesized to result in some columns from adsorbed water in the stationary phase

v. Column cation-exchange (C) activity due to ionized silanols which varies with mobile phase pH

A Phenomenex® (Torrance, CA, USA) Prodigy 5 µm ODS, 150 x 4.60 (i.d.) mm column was selected for the HPLC separation to be developed in these studies.

The performance of the column was assessed based on the theoretical plate number (N) using the equations 2.1 and 2.2. The Center for Drug Evaluation and Research (CDER) recommends the use of columns with at least 2 000 theoretical plates for pharmaceutical applications [67].

$$N = 16\left(\frac{t_R}{W}\right)^2 \qquad \textbf{Equation 2.1}$$

$$N = 5.54\left(\frac{t_R}{W_{\frac{1}{2}}}\right)^2 \qquad \textbf{Equation 2.2}$$

Where,

N = column plate number
t_R = band retention time
W = baseline width
$W_{½}$= bandwidth at half peak height.

2.4.3 UV detection

Methods of detection used in conjunction with HPLC for the analysis of minocycline include the use of ultraviolet (UV), universal, fluorescence, electrochemical detection and mass spectrometric detection [63]. UV detection is the most widely used form of detection in most HPLC method development techniques and is applied to the determination of most compounds that have UV absorptivity [63].

In UV detection methods, absorbance (A) is determined by the concentration (C) of the analyte, the molar absorptivity (ε) of the analyte and the flow cell length (L_{fc}) as shown by Beer's Law shown in Equation 2.3[63].

$$A = C \in L_{fc} \qquad \textbf{Equation 2.3}$$

UV detection at long wavelengths of 350 nm has been used for detection of minocycline in biological fluids whereas shorter wavelengths have been used for detection of minocycline in non-biological matrices [27,45,57]. UV detection at a wavelength of 254 nm was applied in these studies.

2.4.4 Choice of internal standard

The response of a detector to a compound is measured by determining the peak height or peak area of the signal generated in response to the presence of an analyte [63]. The use of an internal standard is widely applied in HPLC in order to compensate for minor variations in separation parameters that affect peak height or area. Such parameters include sample-size fluctuations or variable recoveries of compounds of interest [59]. The construction of calibration curves using an internal standard requires that the internal standard be added at a constant concentration to calibration standards after which the compound to internal standard peak height or area is plotted against the concentration of the compound of interest [59]. Suitable internal standards are generally similar in structure to the analyte of interest, are usually chemically similar to the compound of interest and have similar solubilities and detection responses to the analyte [59].

Doxycycline, also a congener of tetracycline was used at a concentration of 125 µg/ml as the internal standard in these studies.

2.4.5 Mobile phase selection

The properties of the stationary phase depend on the type and concentration of organic modifier used in the mobile phase [68]. Variation of the composition of a mobile phase results in significant changes in the characteristics of the stationary phase and therefore the chemical environment the solute is exposed to when in contact with the stationary phase thereby resulting in changes in the retention time of the analyte [68].

The pH of the eluant is an important factor to consider in the HPLC analysis of ionisable compounds. The pH of an eluant affects the degree of ionisation of the analyte and residual silanols present on the surface of the stationary phase ultimately influencing the interaction between the analyte and the stationary phase and therefore the retention characteristics of the analytes [69]. It is important to buffer mobile phases to maintain a constant pH during analysis of ionisable compounds in order to ensure reproducible and robust analyses are conducted [69].

The preferred buffers for reversed-phase HPLC analyses must be able to control pH to between a pH range of 2-8 and permit the transmittance of light at or below 220 nm to allow low-UV detection, in a concentration range of 10-50 mM as higher concentrations may pose solubility problems when using large volumes of organic modifiers [63]. Phosphate buffers are marginally soluble in solutions that contain high concentrations of organic modifiers and are suitable for use in the pH ranges of 2.1-3.1 and 6.2-8.2 [63]. Potassium dihydrogen phosphate was selected as the buffer of choice for this particular method as the pH of the buffer was 7.0.

The initial selection of the mobile phase composition was based on a previously reported method [27] and was optimized with respect to buffer and organic modifier composition as shown in Table 2.3.

Table 2.3. Mobile phase selection

Mobile phase composition				Retention time (min)		Peak shape
Organic phase (%v/v)		Phosphate buffer				
MeOH	MeCN	% v/v, molarity	pH	Doxy	Mino	
---	20	80, 0.08M	7.0	4.4	8.8	Mino peak sharp and well resolved. Doxy peak sharp but not adequately resolved
---	25	75, 0.08M	7.0	2.4	4.6	As above
---	22	78, 0.08M	7.0	2.8	5.9	As above
40	---	60, 0.08M	7.0	5.2	10.0	Both peaks sharp and adequately resolved but could be sharper
40	---	60, 0.08M	6.4	5.1	8.2	Mino peak sharp and well resolve. Doxy peak sharp but not adequately resolved
45	---	55, 0.08M	7.0	3.5	6.7	As above
35	5	60, 0.08M	6.4	4.8	8.0	As above
30	10	60, 0.08M	7.0	4.3	8.2	Both peaks sharp and adequately resolved but doxy peak could be better resolved
35	5	60, 0.08M	7.0	4.3	8.7	As above
15	15	70, 0.08M	7.0	4.6	9.6	Both peaks sharp and adequately resolved but could be sharper
12	18	70, 0.08M	7.0	3.9	7.6	Both peaks sharp and adequately resolved
13	17	70, 0.08M	7.0	4.1	8.2	Both peaks sharp and adequately resolved

2.4.5.1 Effect of organic modifier

It was desired that the retention times of minocycline and the internal standard be longer than four minutes in order to avoid interference by the solvent front and to elute in not more than ten minutes so that routine analysis is relatively quick. Acetonitrile at a concentration of 20% v/v was selected as the first choice of organic modifier based on a published method [27]. Although, the peak shape and retention time of minocycline was optimised at this concentration, doxycycline was inadequately resolved. At concentrations of acetonitrile greater than 20% v/v, the peak shape of doxycycline improved to a small degree and the peak eluted close to the solvent front. The use of methanol as an organic modifier was therefore investigated. Increasing the concentration of methanol resulted in better peak shapes but with unacceptably short retention

times. The addition of methanol and acetonitrile as organic modifiers to the mobile phase offered the best compromise in terms of optimal peak shape and retention time and a representative chromatogram is shown in Figure 2.1.

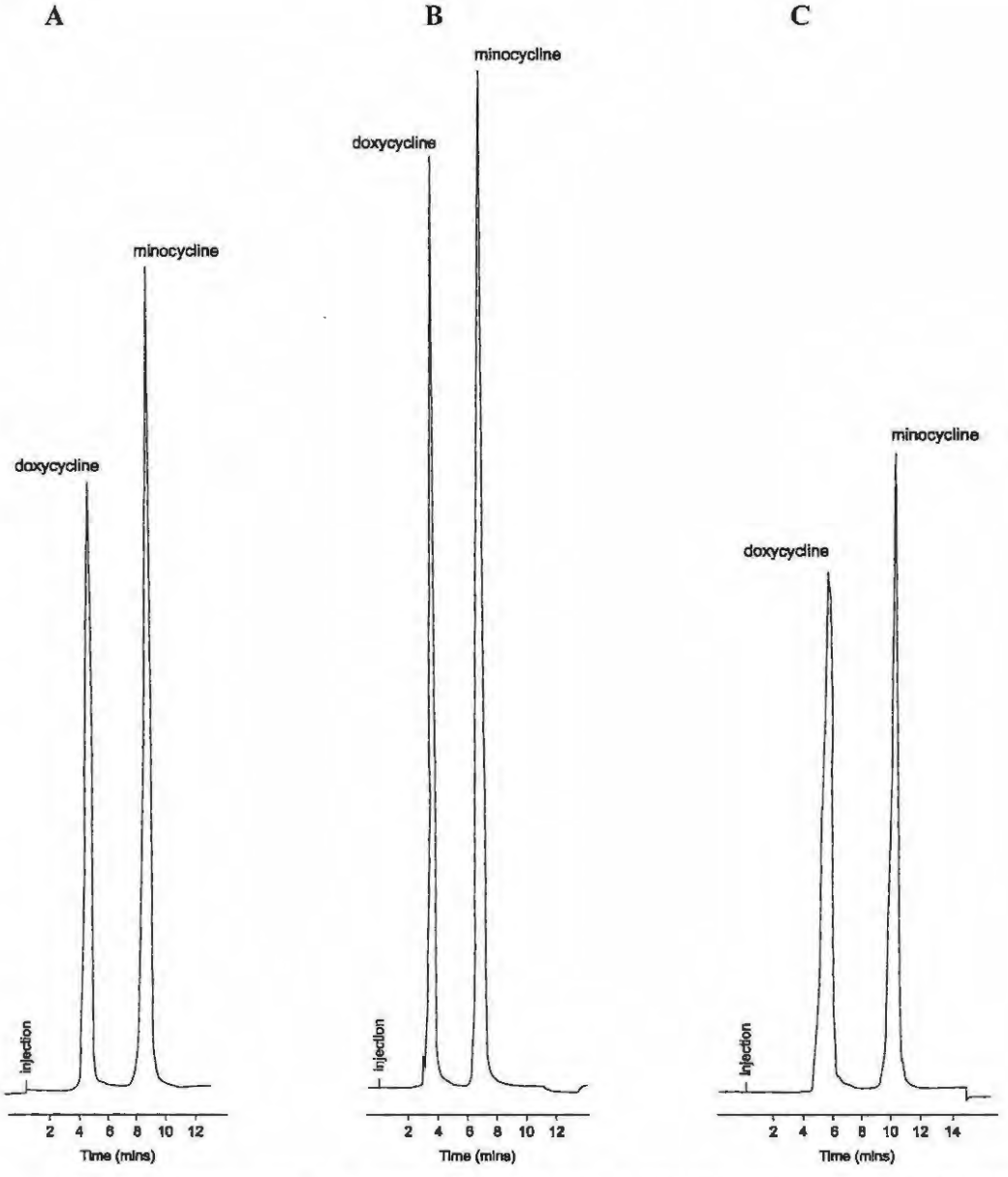


Figure 2.1. Typical chromatograms of doxycycline (125 µg/ml) and minocycline (100 µg/ml) obtained with: A- Acetonitrile (17% v/v) and methanol (13% v/v) as organic modifiers, B-Acetonitrile (22% v/v) as organic modifier and C-methanol (40% v/v) as organic modifier in 70% v/v KH_2PO_4 (80 mM, pH=7)

2.4.5.2 Effect of buffer molarity

The effect of buffer molarity on chromatography was investigated over the range 10 mM to 100 mM for potassium dihydrogen phosphate buffers. No significant difference in peak shape and

retention time was observed for minocycline, however as the molarity was increased more symmetrical and sharper peaks were observed for the internal standard as shown in Figure 2.2. A buffer molarity of 80 mM was therefore used for further optimization of the method.

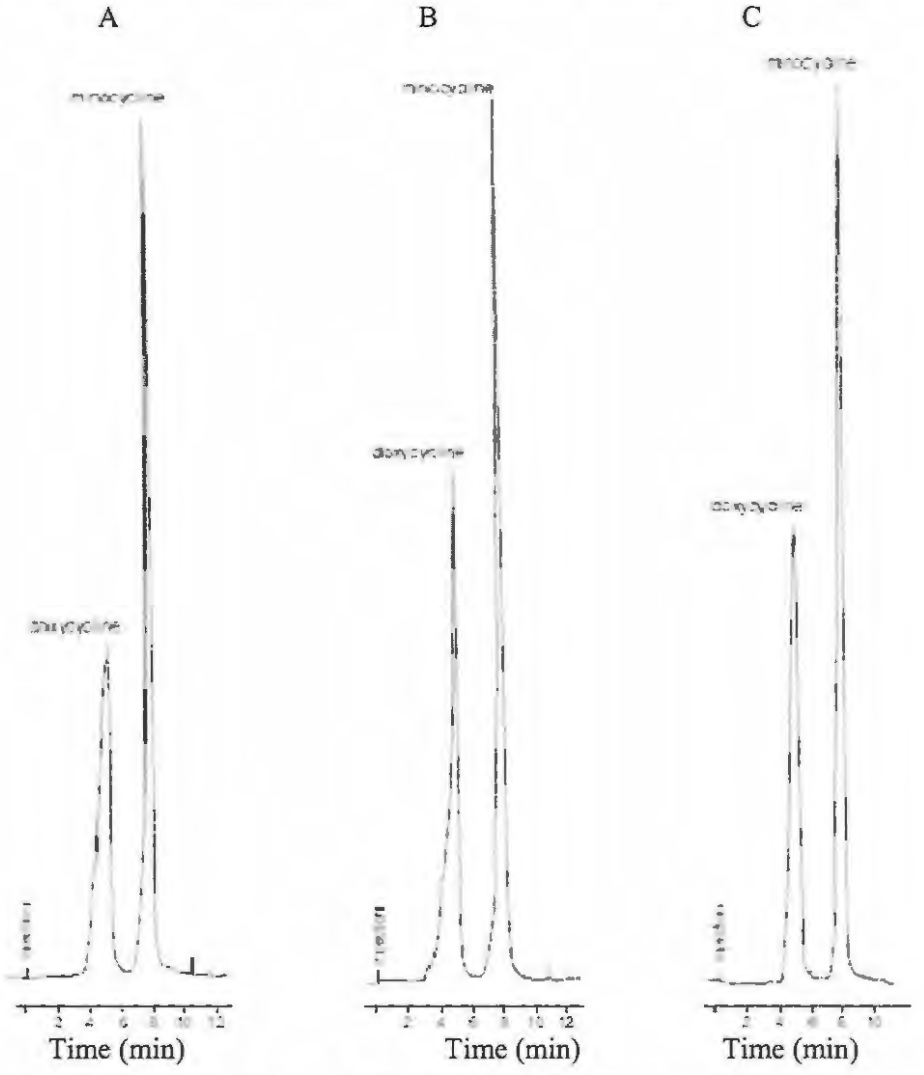


Figure 2.2. The effect of buffer molarity on the separation of minocycline and doxycycline using 70 % v/v KH_2PO_4 (pH=7, A-30 mM, B-50 mM and C-80 mM): 17% v/v MeCN: 13% MeOH

2.4.5.3 Effect of buffer pH

The analysis of minocycline has been performed chromatographically at acidic [45,52,53,56,62] and neutral [27,57] pH. The effect of pH on the retention characteristics of minocycline and doxycycline was investigated over the pH range 2 to 7. At low pH values, in acidic conditions, minocycline and doxycycline peaks were inadequately resolved from each other and the peak shape of doxycycline was distorted. The retention times of minocycline and doxycycline increased and resolution of the two peaks improved with an increase in the pH. A neutral pH of 7

was selected for use as peak shape, retention time and resolution of the peaks were optimal at this pH.

2.4.6 Mobile phase selected

The mobile phase selected for further optimization and validation for the HPLC analysis of minocycline was comprised of 70% v/v potassium hydrogen phosphate buffer (pH 7, 80 mM), 17% v/v acetonitrile and 13% v/v methanol. Peak shapes were assessed by calculating the peak asymmetry factor A_s using Equation 2.4. Peaks of acceptable symmetry have A_s values of 0.95-1.1 although values of up to 1.4 are acceptable [63]. The Center for Drug Evaluation and Research (CDER) recommends that capacity factor (k'), which should be greater than 2, be calculated [67]. The capacity factor was calculated using Equation 2.5 [67]. A typical chromatogram and the typical peak specifications achieved using this mobile phase are shown in Figure 2.3 and Table 2.4.

$$A_s = \frac{B}{A}$$ **Equation 2.4**

Where,

B = the width of the peak to the tailing edge at 10% of the peak height
A = the width of the peak to the leading edge at 10% of the peak

$$k' = \frac{(t_R - t_0)}{t_0}$$ **Equation 2.5**

Where,

t_R = elution time of non-retained components
t_0 = elution time of analyte

Figure 2.3. Typical chromatogram of the separation of minocycline (4.1 min) and doxycycline (8.1 min) in mobile phase

Table 2.4. Peak specifications for typical chromatogram of the separation of minocycline and doxycycline

	Minocycline	Doxycycline
Capacity factor	5.6	2.8
Peak symmetry factor	1.1	1.2
Retention time (min)	8.2	4.1

2.4.7 Chromatographic conditions

The chromatographic conditions selected for the method to be validated are summarised in Table 2.5.

Table 2.5. Chromatographic conditions

Column	**Phenomenex®** (Torrance, CA, USA) **Prodigy 5 µm ODS, 150 x 4.60 (i.d.) mm**
Pump	Spectra-Physics, SPECTRA SERIES P100 pump (Spectra-Physics, San Jose, CA, USA)
Injector	Waters WISP 710B Autosamper (Waters Chromatography Division, Milford, MA, USA)
Detector	LINEAR™ UVIS 200 detector (Linear Instruments Co., Irvine, CA, USA)
Detection wavelength	254 nm
Sensitivity	0.2 AUFS
Recorder	Spectra Physics, SP 4290 Integrator (Spectra-Physics, San Jose, CA, USA)
Integrator speed	2.5 mm/min
Flow rate	1.5 ml/min
Injection volume	20 µl
Temperature	Ambient, 22 °C
Column pressure	1800-2000 psi
Mobile phase composition	MeOH:MeCN:80 mM KH_2PO_4 buffer pH 7.0; (13:17:70,v/v/v)

2.4.8 Conclusions

Using a theoretical approach to HPLC method development [63] a method for the analysis of minocycline in pharmaceutical formulations has been developed by optimising the composition of organic modifiers, buffer molarity and pH buffer. The developed HPLC method displays adequate resolution and has suitable run times of 4.1 and 8.2 minutes for doxycycline (internal standard) and minocycline allowing for its further application in these studies.

2.5 METHOD VALIDATION

2.5.1 Introduction

The validation of an analytical procedure is the process by which it is established by laboratory studies, that the procedure has the performance characteristics to meet the requirements for the intended analytical application [70]. Parameters typically investigated in the early stages of validation studies include specificity, linearity, accuracy and precision with stability studies and ruggedness being conducted at a later stage in the validation procedure [63]. Bodies such as the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [71], The Center for Drug Evaluation and Research (CDER) [67] and the United States Pharmacopoeial Convention [70] have established guidelines or standard procedures for analytical method validation.

2.5.2 Linearity and Range

The linearity of an analytical method is the ability of a method to elicit test results that are directly proportional to the analyte concentration within a given range [72]. The range of an analytical method is the inclusive interval between the upper and the lower levels of analyte concentration for which it has been demonstrated that the analytical method has a suitable level of precision, accuracy and linearity [71].

Linearity is initially analysed visually by determining whether a plot of signals as a function of analyte concentration appear to have a linear relationship [71]. If a linear relationship is observed, test results should be evaluated by use of an appropriate statistical method such as calculation of a regression line by the least squares method [71]. The ICH recommends that linearity be established using a minimum of five concentrations. A method is generally accepted as being linear if the regression coefficient (r^2) is greater than or equal to 0.999. Table 2.6 summarises the validation ranges recommended by the FDA, USP and ICH for validation studies [73].

Table 2.6. Recommended validation ranges for linearity studies

Analysis categories	Recommended validation range (%)
Assay specifications for release	80-120
Assay specifications for check	80-120
Content uniformity tests	70-130
Assay of a preservative in a stability study	40-120
Determination of a degradant in a stability study	0-20

Linearity was evaluated using six calibration solutions (n=6) with concentrations ranging between 5-140 µg/ml. The peak height ratio of minocycline and internal standard was plotted against concentration to produce a calibration curve. The method was linear over the concentration range 5-140 µg/ml, with a representative linear equation of y = 0.0107x - 0.0005 and a regression coefficient (r^2 value) of 0.9999. A typical calibration curve is shown in Figure 2.4.

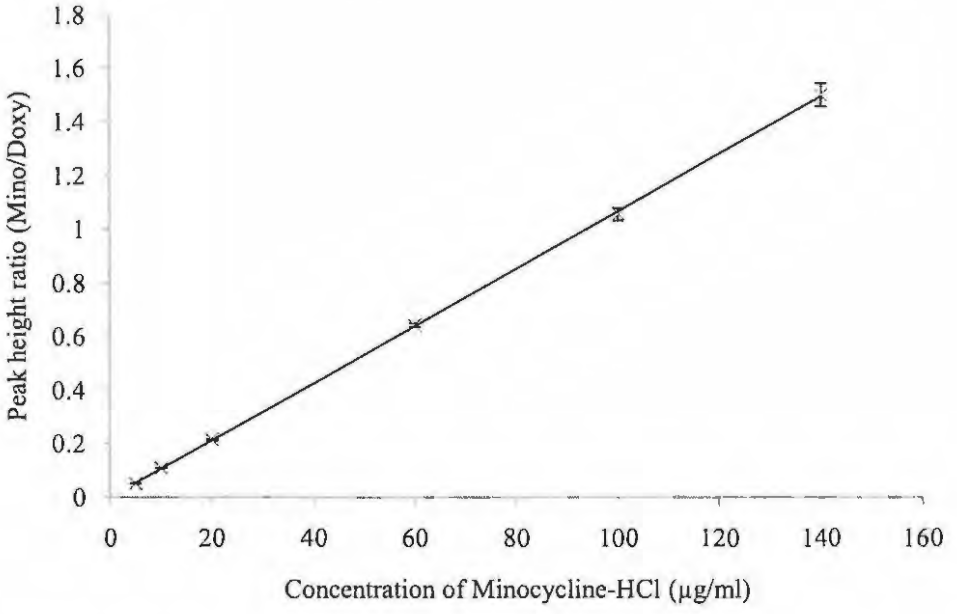


Figure 2.4. Typical calibration curve for minocycline hydrochloride in the concentration range 5-140 µg/ml (n=6)

2.5.3 Precision

The precision of an analytical method expresses the degree of agreement of a series of measurements resulting from multiple sampling of the same homogenous sample under the prescribed conditions [70].The precision of an analytical method is generally expressed as the variation, standard deviation or the coefficient of variation (relative standard deviation) of a statistically significant number of samples [71,72,72]. The ICH considers precision at three levels: repeatability, intermediate precision and reproducibility.

2.5.3.1 Repeatability

The repeatability, also termed intra-assay precision expresses the precision of the analytical method over a short time interval under the same operating conditions, i.e. in the same laboratory by the same analyst with the same equipment [70,71]. The ICH recommends that repeatability be

assessed using a minimum of nine determinations covering the range of the analytical method or using at least six determinations at 100% of the test concentration. For an assay method repeatability should exhibit a % RSD of ≤ 2 % [73].

Replicate analysis (n=6) of samples at low, medium and high concentrations i.e. 10, 60 and 140 µg/ml were performed to assess the intra-assay precision or repeatability of the method. The %RSD values for repeatability were less than 2% and are summarized in Table 2.7.

Table 2.7. Intra-day precision for minocycline hydrochloride in the concentration range 0-140 µg/ml

Concentration (µg/ml)	Peak/Height Ratio (n=6)	Standard deviation	% RSD
10	0.1153	0.0012	1.08
60	0.7372	0.0055	0.74
140	1.6403	0.0098	0.59

2.5.3.2 Intermediate precision

Intermediate precision also know as ruggedness expresses the precision of an analytical method when the method is subject to within-laboratory variations, i.e. when the method is applied by a different analyst, used on different days or used on different equipment [70]. The use of experimental design to monitor effects, if any, of individual variables is encouraged [72]. Tight, but realistic, system suitability specifications should be set as intermediate precision of an analytical method can be partly assured by good system suitability specifications [67].

Replicate analysis (n=6) of concentrations of 10, 60 and 140 µg/ml were performed on three different days to assess the inter-day or intermediate precision of the method. The % RSD values for precision were less than 2% for all determinations and are summarized in Table 2.8 thereby indicating that the method is precise.

Table 2.8. Inter-day precision for minocycline hydrochloride in the concentration range 0-140 µg/ml

Concentration (µg/ml)	Peak/Height Ratio (n=6)	Standard deviation	% RSD
10	0.1148	0.0013	1.12
60	0.7451	0.0099	1.33
140	1.6683	0.0292	1.74

2.5.3.3 Reproducibility

Reproducibility of an analytical method refers to the use of the procedure in different laboratories as in collaborative studies [70,71] and is usually applied to standardization of a methodology [71]. The determination of reproducibility focuses more on the bias of experimental results and not on the difference in precision of the results alone [73]. Although "statistical equivalence" is

normally used as a method to determine reproducibility, the use of "analytical equivalence" in which a range of acceptable results is set for use to judge acceptability of the results, prior to the application of the method in different laboratories, is a more practical approach [73]. The FDA does not expect reproducibility data if intermediate precision has been established [67] hence reproducibility was not performed in these studies.

2.5.4 Accuracy

The accuracy of an analytical method expresses the closeness of an experimental value to that of the true value or that of an accepted reference standard [70,71]. The ICH guidelines recommend that accuracy be established across the specified range of the analytical procedure with a minimum of nine determinations covering the specified range or a minimum of six determinations at 100% of the test concentration [71]. The FDA recommends that accuracy be determined at 80, 100 and 120% levels of label claim for drug substance and drug product analytical methods [67].

Shabir [73] highlighted four techniques used to determine accuracy. The first technique requires a sample of known concentration to be analysed and the measured value is then compared to the known true value. The second approach involves comparing test results from the analytical method being validated to a value obtained by a well characterised method that has been found to be accurate. The third technique is based on the recovery of known amounts of analyte following spiking of blank matrices with the analyte and the fourth technique for determining accuracy is used in cases where a blank matrix cannot be obtained and involves the determination of recovery of the spiked analyte following addition of a standard quantity of analyte to the matrix.

The FDA recommends that the acceptance criteria for accuracy be 100 ± 2% at each concentration studied over the range 80-120% of the target concentration [73]. The extent of deviation from the true value may also be determined as percent bias (% bias). The limit for percent bias for our lab was set at 5%. The accuracy of the analytical method was determined at low, medium and high concentrations i.e. 10, 60 and 140 µg/ml. The percent bias was less than 5% for all solutions tested and the results are summarised in Table 2.9.

Table 2.9. Accuracy for minocycline hydrochloride in the concentration range 0-140 µg/ml

Concentration (µg/ml)	Determined concentration (µg/ml)	% RSD	% Recovery	% Bias
10	9.53	0.21	95.3	4.7
60	57.8	0.30	96.33	3.7
140	144.5	1.28	103.2	3.2

2.5.5 Limits of quantitation (LOQ) and detection (LOD)

The LOQ of an analytical method for quantitative analyses is used to establish the lowest level of a compound in a matrix that can be determined with the requisite precision and accuracy. The determination of LOQ is normally required when an assessment of impurities in bulk material or degradation products in the finished pharmaceutical product are required to be reported [70,71]. The limit of detection (LOD), a characteristic of limit tests [70], is the amount of analyte that can be detected, but not necessarily quantitated using the analytical method under the stated conditions [70,71].

The ICH highlights three approaches to determine the LOQ and LOD of a method and these are summarized in Table 2.10.

Table 2.10. Approaches for determining LOD and LOQ (adapted from [74])

Approach	Limit of detection	Limit of quantitation
Visual evaluation	Minimum level detectable	Minimum level quantifiable
Signal to noise ratio	3:1 or 2:1	10:1
Standard deviation of the response (σ)* and the slope (S)	3.3 X σ/S	10 X σ/S

*Standard deviation of the blank, residual standard deviation of the calibration line, or standard deviation of the intercept

The first approach is based on a visual evaluation and is used with non-instrumental methods but may also be applied to instrumental methods. Using this approach, samples with known concentrations of analyte are used to establish the minimum amount of a detectable analyte (LOD) and the minimum amount that can be quantitated with acceptable precision and accuracy (LOQ). The second approach makes use of the signal to noise ratio method and is applicable only to analytical methods that exhibit baseline noise and makes use of the analysis of samples with known amounts of analyte and then comparing their response to that of blank samples that have been prepared and analysed in the same way. Using the signal to noise ratio method, a signal to noise ratio of 2:1 or 3:1 is considered suitable for establishing the LOD and a signal to noise ratio of 10:1 is considered appropriate for identification of the LOQ. The third approach used to determine LOD and LOQ is based on the standard deviation of response and the slope (S) obtained from the calibration curve of the analyte, with the LOQ and LOD determined using Equations 2.6 and 2.7.

$$LOD = \frac{3.3\sigma}{S} \qquad \textbf{Equation 2.6}$$

$$LOQ = \frac{10\sigma}{S}$$ **Equation 2.7**

The estimate of σ may be established by one of two methods:

1. Using the standard deviation of the response of an appropriate number of blank samples or

2. Construction of calibration curves using samples of the analyte in the concentration range of LOQ and LOD. σ can be estimated from the residual standard deviation of a regression line or from the standard deviation of y-intercepts of regression lines generated for these studies.

Caution should be taken when using the signal to noise ratio method to ensure that baseline noise is not interpreted as extraneous peaks, as undulations may be observed at the void volume if the diluents of the samples are different from, or are in different proportions to the solvents used in the mobile phase [67].

The limit of quantitation (LOQ) may also be determined, from precision studies by analysing samples with decreasing concentration of the analyte until a specified limit of relative standard deviation between the samples is reached; with the concentration at which the limit is reached being deemed the LOQ [74]. As the degree of scatter at low concentrations is considerably higher, a larger number of samples should be analysed using this method [74].

The LOQ has been observed to be more dependent on the equipment used, specific conditions of an analytical method and the method of calculation than any other validation parameter [74]. The FDA has recommended that there be assurance that the detection and quantitation limits be achievable with the test method each time when using low concentrations, in particular with UV detectors that exhibit a loss of sensitivity on ageing. In a nine month experiment to determine the LOQ using five LC-systems, large differences were observed in the value of the LOQ depending on the system used, the method used to calculate the LOQ as well as in repeated analysis for the same sample [74]. The LOQ is important when analytical procedures are to be transferred and for the determination of impurities. When required for these purposes the requirements of the method used for the determination of the LOQ must be taken into consideration [74]. A reliable quantification limit should be three standard deviations away from the specification limit [74].

The limits of quantitation (LOQ) and detection (LOD) were determined based on the signal to noise ratio of the method. The concentrations that produced signal to noise ratios of 10:1 and 3:1

were the LOQ and LOD respectively. The LOQ was found to be 5.0 μg/ml with an associated %RSD of 4.42%, whereas the LOD was found to be 2.5 μg/ml.

2.5.6 Specificity and selectivity

The terms specificity and selectivity are often used interchangeably, however specificity is used to denote the response of a method for a single analyte and selectivity denotes a method that provides a response for a number of analytes that may or may not be distinguished from each other [72]. Application of these definitions implies that the term selectivity would be more appropriate for chromatographic methods; however the term specificity is used to denote the ability of a method to accurately measure the analyte response in the presence of all potential sample components, as indicated by both the USP and ICH Guidelines [72] .

For chromatographic methods, specificity may be demonstrated by a sufficient separation of all substances present. In contrast to the determination of impurities, where each peak is required to be adequately resolved, for assays an appropriate separation requires that the main peak be separated from any potential impurity, placebo or degradant peaks which need not themselves be separated from each other [74]. Care should be taken when using stress testing to demonstrate specificity, that over degradation be avoided as this would result in secondary or higher order degradants which are of no practical relevance being present and separated [74]. It is recommended that samples be exposed to stress conditions that are sufficient to degrade the analyte to between 80 and 90% of the original purity [73].

2.5.7 Stress studies

Stability testing of drug substances and products provides evidence on how the quality of the drug substance or the product varies with time following exposure to different environmental factors such as temperature, humidity and light [75]. Stress testing, which depends on the nature of the drug substance or the type of drug product involved, gives an indication of the degradation pathway and the intrinsic stability of a molecule and validates the stability-indicating power of an analytical procedure that is developed and used [75]. Although the ICH Guideline for Stability Testing of New Drug Substances and Products [75] leaves the approach to conducting stress studies to the discretion of the scientist, it specifies that stress studies should be conducted to include the effect of temperature, humidity, oxidation, photolysis and hydrolysis over a wide range of pH values. Stress studies were performed to determine if the analytical method

developed for the determination of minocycline in pharmaceutical dosage forms was stability-indicating and specific under the conditions described.

2.5.7.1 Photostability studies

The ICH recommends that exposure to light be an integral part of stress testing of pharmaceuticals [76]. It is important to distinguish the effects of light and those of temperature by monitoring temperature or by using a dark sample reference solution [76]. The light sources that are acceptable for use in photostability studies should be designed to produce an output similar to that of D 65, an internationally recognised standard for outdoor daylight or ID 65, an indoor indirect daylight standard emission [76]. Light sources that can be used for photostability studies include artificial daylight fluorescence, lamps combining visible and ultraviolet output and xenon or metal halide lamps [76]. During light induced stress, the drug being tested should be subjected to light exposure of at least 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 Watt hours/square meter [76]. A 10 ml aliquot of 100 µg/ml solution of minocycline hydrochloride in a clear volumetric flask and a control (10 ml of a 100 µg/ml solution of minocycline protected from light) were exposed to direct natural daylight for six hours after which the samples were analysed using the developed HPLC method.

2.5.7.2 Temperature stress studies

The ICH guidelines [75] suggest that temperature stress studies be conducted at 10 °C increments above the temperatures used for accelerated stability testing (e.g. 50 °C, 60 °C etc.). For the purposes of our studies it was necessary to determine if minocycline would degrade at 80 °C, and if so, whether the degradation product would be resolved from the minocycline peak using the analytical method developed in our laboratory. A solution of 100 µg/ml minocycline hydrochloride was prepared as previously described in Section 2.3.2 and heated and maintained at 80°C in a water bath (Laboratory Thermal Equipment, A Searle Company, Greenfield, Oldham, United Kingdom) for two hours and cooled before analysis by HPLC

2.5.7.3 Acid degradation studies

Bakshi and Singh have suggested that the hydrolytic degradation of a new drug be studied in acidic and alkaline conditions by refluxing the drug in 0.1 M HCl/NaOH for eight hours [77]. Forced degradation studies of minocycline in acidic conditions have been studied for the stability-indicating nature of an HPTLC method for minocycline by refluxing minocycline in 2.0 M HCl for two hours at 80 °C [50]. A 5 ml aliquot of a solution of 0.1 M HCl was added to 5 ml of 200 µg/ml solution of minocycline hydrochloride to produce a solution of final concentration of 100

µg/ml. The solution was heated and maintained at 80 °C for two hours in a water bath as previously described and analysed by HPLC.

2.5.7.4 Oxidation studies

In a review of the development and validation of stability-indicating assays [77], Bakshi and Singh suggest the use of hydrogen peroxide in the concentration range of 3-30% to establish the presence of oxidative degradation products. A 2 ml aliquot of 3% v/v hydrogen peroxide solution was added to 8 ml of 120 µg/ml solution of minocycline hydrochloride to produce a 96 µg/ml solution of minocycline hydrochloride. The solution was stored in the dark for 30 minutes after which it was analysed by HPLC.

2.5.7.5 Alkali degradation studies

A 5 ml aliquot of a solution of 0.1 M NaOH was added to 5 ml of 200 µg/ml solution of minocycline hydrochloride to produce a solution with a resultant concentration of 100 µg/ml. The solution was heated and maintained at 80°C for two hours in a water bath as previously described. After cooling, the solution was analysed using HPLC.

2.5.7.6 Results and discussion

As shown in Figure 2.5 (Chromatogram A), no significant degradation of minocycline was observed following six hours of exposure to sunlight.

An additional peak that was fully resolved from that of minocycline was observed after heating a solution of minocycline at 80°C for two hours. The resultant chromatogram is shown in Figure 2.5 (Chromatogram B).

After heating a solution of minocycline in acidic medium, a degradation peak was observed to elute earlier than minocycline (Figure 2.5, Chromatogram C) indicating the instability of minocycline under acid conditions.

Following exposure to hydrogen peroxide for 30 minutes, an additional peak, an oxidative degradant of minocycline, was observed on the chromatogram, as shown in Figure 2.5 (Chromatogram D) confirming the oxidative instability of minocycline reported in Chapter 1, Section 1.4.3 and in a published monograph [10].

As shown in Figure 2.5 (Chromatogram E) evidence of degradation products was observed indicating that more than one degradation product is formed when minocycline in exposed to alkali conditions.

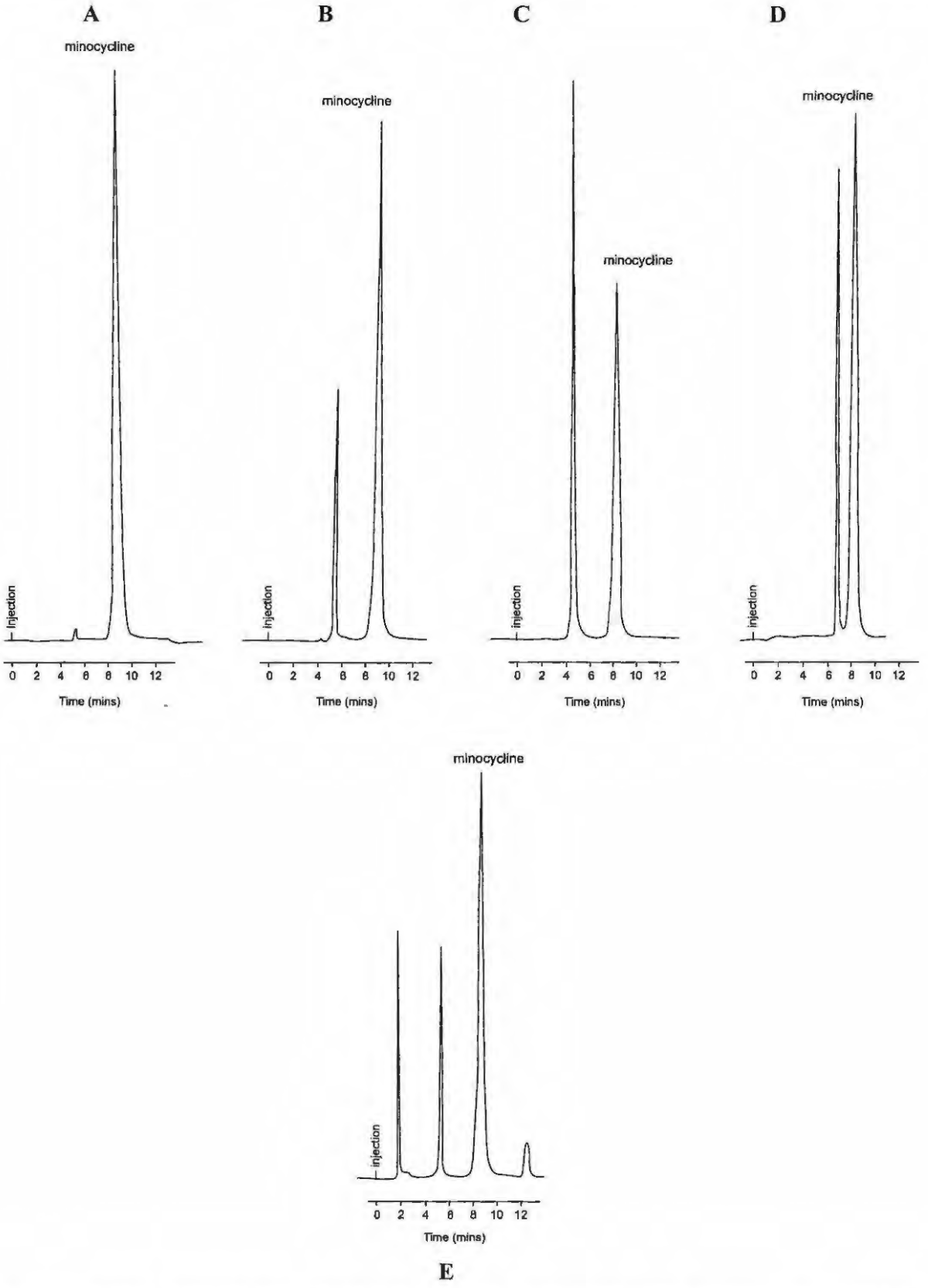


Figure 2.5. Typical chromatograms showing degradation of minocycline after exposure to A-light for 6 hr, B-water at 80 °C for 2 hr, C-0.1 M HCL at 80 °C for 2hr, D-H_2O_2 for 30 min and E-0.1 M NaOH at 80 °C for 2 hr (Refer to Figure 2.3 for chromatogram of untreated minocycline solution)

2.6 ASSAY OF CYCLIMYCIN®-50 AND CYCLIMYCIN®-100 CAPSULES

Samples for assay were prepared based on the monograph for minocycline hydrochloride capsule analysis in the USP [70]. Minocycline hydrochloride powder was removed from 20 capsules and an amount of powder equivalent to the contents of one capsule was removed and dissolved in HPLC grade water with sonication for ten minutes in a 100 ml volumetric flask. A 1 ml aliquot of this solution was filtered through a Millipore Millex-HV Hydrophilic PVDF filter (0.45 µm) and diluted with 5 ml of a 250 µg/ml doxycycline hyclate solution. The solution was made up to 10 ml with mobile phase and analysed using the previously described method in Table 2.5. The analysis revealed that an average of 52.3 mg and 106.8 mg of minocycline were present in the Cyclimycin®-50 and Cyclimycin®-100 capsules, respectively.

Table 2.11. Results for analysis of commercially available Cyclimycin®-50 and Cyclimycin®-100

Minocycline Brand	Expected amount (mg)	Determined amount (Mean ± SD) (mg)	Percentage Recovery
Cyclimycin®-50	50	52.3 ± 1.610	104.6%
Cyclimycin®-100	100	106.8 ± 1.283	106.8%

2.7 CONCLUSIONS

A reversed-phase HPLC method reported in this chapter has been developed and validated for the analysis of minocycline in pharmaceutical dosage forms. The method is simple, linear, and selective and has the necessary accuracy precision and is stability indicating. The method was successfully applied to the analysis of commercially available formulations containing minocycline. The method is also suitable for the evaluation of sustained release and novel dosage forms that will be developed in formulation studies. Perusal of the literature indicates that the developed HPLC method is the first stability indicating HPLC method reported for minocycline in pharmaceutical dosage forms.

CHAPTER THREE

3 FORMULATION DEVELOPMENT AND ASSESSMENT OF MINOCYCLINE SUSTAINED -RELEASE CAPSULE DOSAGE FORMS

3.1 INTRODUCTION

Immediate release dosage forms of minocycline described in the BP and USP are tablets and capsules. Furthermore a suspension is listed in the USP. In addition the BP describes prolonged-release minocycline capsules designed to release the minocycline over a period of several hours. This chapter presents an overview of the formulation development and assessment of minocycline sustained release capsule dosage forms based on matrix technologies.

3.2 ORAL MODIFIED-RELEASE DRUG DELIVERY SYSTEMS

Oral drug delivery is the preferred and most convenient route of drug administration [78]. As a result, most research efforts in searching for new medicines has focused on the discovery of orally active drug substances [78]. Several newly discovered drug entities have limitations associated with poor physicochemical or pharmacokinetic properties, which, even after lead optimization display low oral bioavailability with the result of sub-optimal plasma drug concentration-time profiles [78]. In recent years, the focus has been to develop novel drug delivery systems, with modified-release oral systems holding the major market share [79].

Modified-release formulation technologies provide a means of optimizing drug bioavailability as well as plasma concentration-time profiles for drugs with this limitation [78]. Modified-release formulations offer advantages over immediate release formulations including better efficacy for treating chronic conditions, a reduction in side-effects as well as better patient adherence due to the simplification of dosing schedules [79]. The administration of a drug in a modified-release oral dosage form may enhance the pharmacodynamic profile of a drug compared with administering the drug as a conventional immediate release product [78].

Although several varieties of modified-release formulations have been cited in literature, the majority of modified-release oral dosage formulations are based on one of three technologies, viz., matrix systems, reservoir systems and osmotic systems [79].

3.2.1 Matrix systems

Monolithic matrix systems for modified drug delivery are grouped into two main categories, those in which a drug is dispersed in a water soluble polymeric matrix and conversely those in which the drug is dispersed in a water insoluble polymeric material [80].

Soluble matrix systems, which make up the majority of oral modified drug delivery systems [81], are comprised mainly of hydrophilic colloids that slowly dissolve on contact with water thereby allowing sustained release of the drug [80]. On initial contact with water, the hydrophilic colloid swells to form a hydrated matrix layer allowing further diffusion of water into the matrix device [80]. The drug dispersed in the matrix then diffuses through the hydrated polymer layer and the rate of diffusion of the drug is controlled by the polymeric material [80]. As the outer hydrated matrix layer becomes more dilute, the polymer erodes and the rate of erosion is dependent on the specific type of hydrophilic colloid used to manufacture the dosage form [80]. Insoluble drugs are released from the matrix by erosion and subsequently dissolution of the drug occurs in the dissolution medium [80]. Constant drug release from swellable, soluble matrices may be achieved when the swelling and eroding fronts of the polymer are synchronized [80].

Drug release from hydrophilic matrix devices may be influenced by the physicochemical properties of the gel layer [82], the aqueous solubility of a drug dispersed in the device and the mechanical attrition of the matrix in an aqueous environment [80].

Hydrophilic colloids that have been used to manufacture swellable matrix devices include pH insensitive polymers such as high viscosity grades of hydroxypropyl methyl cellulose (HPMC) and poly (ethylene oxide), as well as pH sensitive polymers such as Carbopol®, xanthan gum and guar gum [81].

Insoluble matrix systems are usually manufactured using lipid matrices that contain drug particles dispersed in that insoluble matrix [80]. The drug is released from these delivery systems by diffusion through fluid filled pores following dissolution in fluid that penetrates the matrix [80]. The rate of drug release from these matrix systems is dependent on the rate of diffusion of the

drug through the fluid filled pores [80,83]. Excipients commonly used to manufacture insoluble matrix devices include hydrophobic polymers such as ethyl cellulose, polyvinyl acetate and various waxes [80]. The rate of drug release from these systems can be modified by changing the porosity and tortuosity of the device [80].

A schematic representation of the process of drug release from diffusion-based and erosion-controlled matrix systems is depicted in Figure 3.1 and Figure 3.2, respectively.

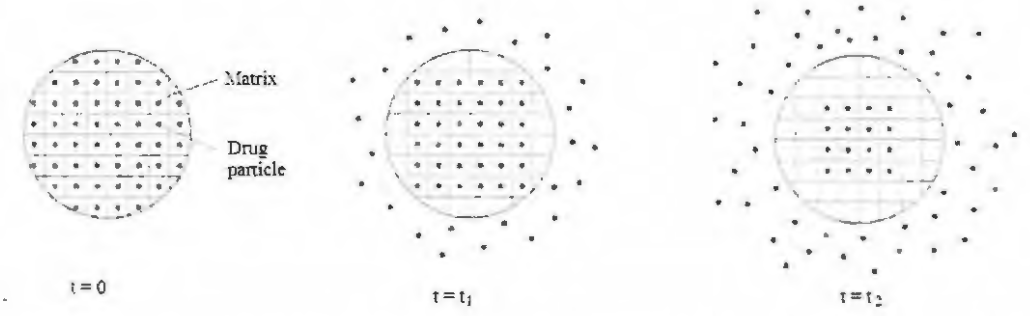


Figure 3.1. Schematic representation of drug release from a diffusion-based matrix system adapted from [80]

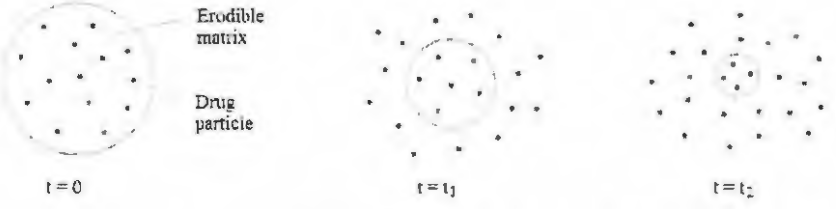


Figure 3.2. Schematic representation of drug release from an erosion controlled matrix system adapted from [80]

3.2.2 Reservoir systems

Reservoir or membrane-controlled drug delivery devices are one of two types of diffusion-controlled systems [83]. Reservoir controlled systems are comprised of a drug core encased by a membrane usually manufactured from a water insoluble polymeric material which is the rate controlling element of the system and through which the drug must diffuse to be liberated. A schematic representation of the process is shown in Figure 3.3 [83].

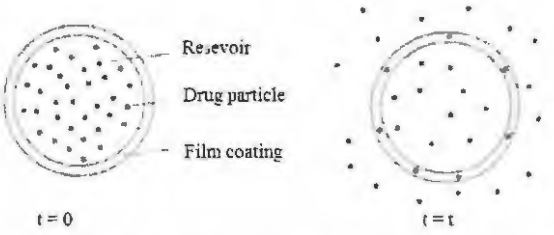


Figure 3.3. Schematic representation of diffusion control of drug release from a reservoir device adapted from [83]

The microencapsulation of drug particles and press coating of whole tablets or particles using materials such as hardened gelatin, methyl and ethyl celluloses, polyhydroxymethacrylate, hydroxypropyl celluloses, polyvinylacetate and some waxes are two methods commonly used to manufacture reservoir devices [84]. The release of drug from a reservoir device involves drug partitioning into the membrane surrounding the reservoir and exchanging the drug with the fluid surrounding the device [84]. Subsequently additional drug enters the membrane, diffuses to the periphery of the membrane and then exchange with the surrounding medium occurs [83]. The release of drug from such systems is governed by Fick's first law of diffusion that is expressed mathematically in Equation 3.1 [83,84]:

$$J = -D\, dC_m/dx \qquad \textbf{Equation 3.1}$$

Where,

J	=	The flux of a drug across a membrane in the direction of decreasing concentration (amount/area-time)
D	=	The diffusion coefficient of a drug in a membrane (area/time)
dC_m/dx	=	The rate of change in concentration of a drug over a distance x

Drug release from reservoir devices follows zero-order release kinetics if a constant area, diffusional path length, concentration and diffusion coefficient are maintained in the device [83]. Important factors to be considered when formulating reservoir devices to ensure constant drug release are the ratio of polymers making up the coating and the thickness of the rate-controlling membrane and in the case of microencapsulation, the method of formulating reservoir devices and the hardness of the wall of the microcapsule [83].

3.2.3 Osmotic systems

Osmotically controlled drug delivery systems use osmotic pressure as the driving force for drug release [79]. The first described osmotically controlled oral delivery system which formed the basis of the Oros® system [78] was the Elementary Osmotic Pump (EOP) [85]. The EOP consisted of an osmotic core containing the drug, surrounded by a semi-permeable membrane with a delivery orifice and the device is illustrated in Figure 3.4 [85].

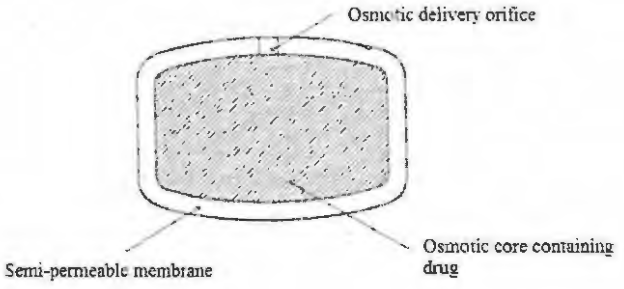


Figure 3.4. Schematic representation of the elementary osmotic pump [85]

On exposure to the aqueous environment of the contents of the gastro-intestinal tract, the osmogenic core draws water into the device at a rate determined by the permeability of the membrane to water as well as the osmotic pressure of the core to form a saturated solution inside the device [85,86]. As the membrane is inflexible the increase in fluid volume in the device, due to water uptake results in the development of hydrostatic pressure inside the device that is relieved by the flow of saturated solution of equal volume to the water uptake, out of the orifice on the device [86]. The release of drug in solution continues at a constant rate for as long as excess solid drug is present in the device but declines parabolically towards no release once the concentration of the solution in the device falls below the saturation concentration for the drug [85].

The release of drugs from EOP and other osmotically driven systems is determined by four main factors viz., the solubility of the drug, osmotic pressure in the device, the size of the delivery orifice and the characteristics of the semi-permeable membrane surrounding the device [79,86]. The rate of drug release from these devices is directly proportional to the aqueous solubility of drug contained within the core [79]. However since the release of drugs from EOP devices declines as solid drug in the core is depleted, highly soluble drugs are not ideal candidates for use

in EOP systems as prolonged constant release cannot be maintained [78]. Poorly soluble drugs on the other hand fail to generate sufficient hydrostatic pressure to ensure drug release, leaving only moderately soluble drugs as the suitable candidates for inclusion in EOP systems [78]. A number of formulation approaches have been used to deliver drugs having extremes of solubility [79]. The release of drugs from an osmotically driven device is directly proportional to the osmotic pressure of the saturated core formulation, with addition of osmotic agents or osmagents to core formulations resulting in faster drug release [79,86]. The size of the delivery orifice in these devices should be within a specified size range to control the release of the drug from these devices [79]. The delivery orifice should be smaller than the maximum permitted size (A_{max}) to minimize solute diffusion through the orifice and be larger than the minimum permitted value (A_{min}) to minimize hydrostatic pressure in the device that would otherwise affect the development of zero-order release rates [86]. The rate of release of drugs is determined by the type and nature of membrane polymer, thickness of the membrane and the presence of other additives [79].

Push-pull osmotic pump systems (PPOP) were designed to overcome the limitations of delivering poorly water soluble drugs in EOP devices [87]. PPOP systems consist of two layers which may be separated by an elastic diaphragm, surrounded by a semi-permeable membrane. One compartment contains the drug and is connected to the outside environment through an orifice whilst the lower compartment which lacks a delivery orifice contains a polymeric osmotic agent and a schematic representation of the device is shown in Figure 3.5 [86].

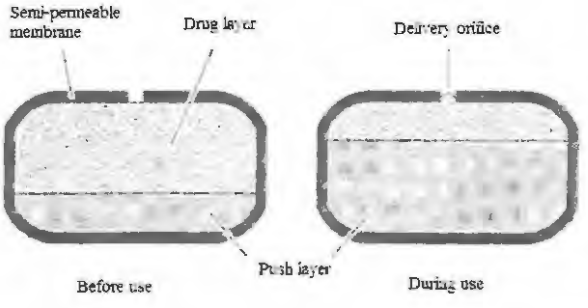


Figure 3.5. Schematic representation of the push-pull osmotic pump before and during operation [86]

Following exposure to an aqueous environment the drug and polymer layers take up water. However, as the polymer compartment lacks an orifice, it expands pushing the diaphragm

towards and into the drug compartment causing a suspension or dispersion of the drug to be released from that compartment as depicted in Figure 3.5 [86].

Waterman et al. [88] have developed a single-layer oval-shaped osmotic controlled-release tablet for the delivery of poorly soluble drugs, that is comprised of a hydroxyethylcellulose, a poorly soluble drug and sugar as an osmagent in the core and that delivers high doses of low solubility drugs through an outlet in the coating at one end of the tablet.

The concept of drug delivery using EOP and PPOP technologies may be combined to achieve patterned release of a drug to produce pulse, ascending and delayed release profiles [78]. Slow hydrating placebo layers which are released from the device, before a pharmaceutically active agent, due to influx of water into the device may be used for the controlled-onset extended release and capsule-shaped osmotically driven tablets that are multilayered and that may be used to achieve ascending and pulsed delivery profiles [78].

The use of osmotically driven systems offers three specific advantages over other modified release systems [85]. The primary advantage is that the delivery of drugs can be designed to follow zero-order kinetics as the fraction of drug delivered at zero-order can be predicted from the solubility of the drug and the core density. A second advantage is that drug release from osmotically driven systems is independent of the pH of the environment, the agitation of the environment and the size of the delivery portal for orifices within the specified size range. A final advantage of drug release from osmotically driven systems is that significant in vitro in vivo correlations are normally achieved [78].

3.3 MINOCYCLINE MODIFIED-RELEASE DOSAGE FORMS

Although minocycline has a relatively long half-life (Section 1.6.5 vide infra) conventional dosage forms and traditional delayed release dosage forms containing minocycline require frequent dosing (every six or twelve hours) on a daily basis resulting in variations in plasma concentrations throughout the course of treatment and ultimately poor patient adherence [89,90]. Therefore the development of a dosage form for minocycline for once-a-day dosing may provide therapeutic benefits and enhanced adherence [89].

A European patent, application number 90115881.6 [89] describes compositions for oral liquid, capsule or tablet dosage forms of minocycline that provide at least minimum therapeutic levels of minocycline in a human subject for approximately 24 hours. The formulation compositions of the dosage forms as described in the patent are composed of a two pulse administration system in which the initial loading dose component, provides a first pulse which releases drug and permits up to 100% absorption in the stomach and a secondary loading component proving a second pulse which is released and absorbed up to 100% in the duodenum and upper part of the small intestine. An international patent, application number PCT/IB2004/002366, describes compositions for minocycline for hard gelatine capsules, soft gelatine capsules, sachet or tablets. The patent describes a non-spheronized, multiparticulate modified release composition that is comprised of a core and a pH sensitive coating that that may be rapidly or completely eroded in a medium of pH in the range of about 4.0 - 7.5 and that releases 95-100% of minocycline after 120 minutes when tested in vitro.

Although rapid dissolution is believed to be essential for the effectiveness of orally delivered tetracycline antibiotics the dissolution rate of minocycline can affect the occurrence and severity of vestibular side effects that is associated with minocycline therapy [5]. The rapid dissolution of minocycline is associated with a higher incidence and greater severity of vestibular effects suggesting that a decrease in the dissolution rate of minocycline may reduce the incidence and severity of such side effects [5]. Although the development of slowly dissolving dosage forms was progressive in reducing adverse effects associated with minocycline therapy there remains a need for treatments for acne that have fewer side effects than observed with the use of conventional minocycline dosage forms [91].

A US patent, patent number 5,908,838 (Solodyn®) [5] describes formulations of minocycline with slower dissolution rates than observed with immediate release capsules. The patent describes the preferred dissolution rate of minocycline and suggests that dosage forms that release 15% of the payload in the first 15 minutes, 35% within 30 minutes, 50% within 45 minutes and 80% within 60 minutes as being suitable. An international patent, application number PCT/US2007/008086 [91] describes compositions for once-daily administration "4-hour" formulations of minocycline with or without a loading dose. The formulations are comprised of minocycline, one or more slow dissolving carriers and one or more fast dissolving carriers and that releases approximately 90% of the minocycline in the dosage form within a range of four - six hours. The formulations have been shown to provide similar or better efficacy for the treatment of acne with a reduced

incidence of at least one adverse effect as compared to the twice-daily administration of Minocin® immediate-release minocycline hydrochloride capsules.

3.4 PHARMACEUTICAL CAPSULES

Pharmaceutically, the term capsule describes oral dosage forms consisting of a container, usually made of gelatin filled with a medicament [92]. Capsules may be classified as "hard" or "soft" capsules [92]. Hard capsules are comprised of two semi-closed cylindrical pieces in which the longer piece, the "body" is filled and fitted into a shorter piece, the "cap" that has a larger diameter [92]. Soft gelatin capsules are one-piece capsules that may come in a variety of shapes [92]. The use of pharmaceutical capsules dates back to the early 19th century with the first recorded patent in 1834 belonging to Messieurs Dublanc and Mothers [93]. The elongated shape of capsules makes capsules easier to swallow as the tongue reflexively lines up the capsule end-on further simplifying the ease with which they can be swallowed [93].

3.4.1 Two-piece Hard Capsules

3.4.1.1 Manufacture of hard gelatin capsules

The manufacturing process of two-piece hard capsules may be summarized into seven steps as listed [93]:

i. Preparation of a gelatin stock solution
ii. Capsule formation
iii. Drying of capsules
iv. Capsule removal and assembly
v. Capsule sorting
vi. Capsule printing
vii. Capsule packing and storage

The first step in the manufacture of two-piece hard capsules involves the production of a viscous 30-40% w/w stock solution of gelatin. The stock solution is made by dissolving gelatin that meets the required specifications of the manufacturer, in demineralised water at 60-70°C. On maturation of the stock solution, aliquots are withdrawn to which additives such as colourants and surface tension modifiers are added so as to meet the requirements of each capsule-forming machine. As the thickness of capsules is dependent on the viscosity of the gelatin solution, the viscosity of the gelatin solution is adjusted before the gelatin is supplied to the machine.

Capsules are formed on stainless steel moulds called "pins", which are designed to produce the required shape for the formation of capsule bodies and caps. The gelatin solution is placed into jacketed, stirred containers called "dip pans" or "dip pots" which maintain the required temperature of the gelatin solution using heat controls. The "pins" are then lowered into the gelatin solution and the quantity of gelatin retained on the pins and the pattern of "run-off" is governed by the viscosity of the gelatin solution. The gelatin is set on the pins by passing the material through a stream of cool air. The capsules are then dried by passing the pins through a series of drying kilns in which the temperature and humidity is controlled in order to strike a balance between drying of the capsules until they are robust enough to withstand handling and not over drying them to a state in which they are brittle.

The dried capsules are removed from the pins using a set of metal jaws, which are placed around each pin. The capsules that are formed are longer than the required length and have to be cut to length before joining the bodies and caps together. The capsules are then checked to ensure that they are the appropriate length for the required size and to ensure that the diameters of the caps and bodies are suitable to ensure that they seal once closed after filling. Capsules with defects, such as thin areas, bubbles, poorly cut edges, splits or holes are discarded.

Capsules that are of appropriate quality are then marked/printed using edible pharmaceutical grade ink, in different designs for ease of identification. Capsules are then stored for use at appropriate temperatures and relative humidities (RH) that are, ideally between 10-30°C and 70% RH, respectively.

3.4.1.2 Capsule properties

The moisture content of hard gelatin capsules on receipt from the manufacturer, is required to be between 13-16% w/w [93,94]. Hard gelatin capsule stability is particularly sensitive to moisture content and concentrations of moisture below 10% w/w result in brittle capsules that fracture on handling whereas moisture levels greater than 18% w/w result in capsules that soften and become distorted in shape [93]. The change in moisture content of hard gelatin capsules can be monitored by a change in diameter of the components of a capsule, with an observed change of 0.5% in diameter for each 1% change in moisture content in the range of 13-16% w/w water content [93]. Heat absorption, moisture content and tensile strength of capsules exposed to different conditions of humidity have been simultaneously measured and found to be affected by the type of gelatin, the overall composition of the capsule as well as the presence of colourants [93]. The stiffness of a gelatine capsule shell has with time been found to be influenced by the water

content and the excipients used in the capsule shell and fill formulations. The optimal performance of capsules during machine-filling is attained when capsules are filled at relative humidity conditions of between 45-55% RH [94].

The solubility of gelatin, which influences the disintegration of hard gelatin capsule shells is affected by temperature [93]. Gelatin is insoluble at 31°C, is readily soluble in biological fluids at body temperature and a 30% change in dissolution rate of gelatin in the temperature range of 35-39°C has been observed [95]. The disintegration of hard gelatin capsule shells is influenced by the hydrophilicity and hydrophobicity of fill materials in addition to the dissolution medium used in testing [93].

Hard capsules are not air tight and gases may penetrate the capsule through the gap between the capsule cap and body, a phenomenon that poses a potential stability concern for compounds susceptible to oxidation [93]. The use of air tight containers significantly reduces this problem [93]. It is important to ensure that hard gelatin capsule formulations are stored appropriately as hard gelatin capsules subjected to the ICH-accelerated storage conditions of 40°C/ 75% RH for six months, have shown significant changes in the dissolution rate of the fill material that has been attributed to a cross-linking reaction of the gelatin [93].

3.4.1.3 Capsule sizes

Two-piece hard gelatin capsules are commercially available in eight different sizes with associated fill volumes that are summarised in Table 3.1 [92]. Sizes larger than 0 are rarely used to treat humans as their large size make swallowing difficult [17]. Small sized capsules, in particular those as small as size 5 are rarely used due to difficulties encountered with automated filling of these [92].

Table 3.1. Sizes of commercially available capsules [92]

Capsule size	000	00	0	1	2	3	4	5
Volume (ml)	1.37	0.95	0.68	0.50	0.37	0.30	0.21	0.13

3.4.2 Alternatives to hard gelatin capsules

Although the majority of two-piece hard capsules are made from gelatin, hydroxypropyl cellulose and potato starch have been used to manufacture two-piece capsules that overcome some limitations of hard gelatin capsules.

3.4.2.1 *Hydroxypropyl methylcellulose (HPMC) capsules*

Two-piece HPMC capsules have been developed as alternatives to hard gelatin capsules and have been commercially available for over two decades [96]. The moisture content of HPMC capsules is less than that of hard gelatin capsules and ranges between 4-7% w/w [93]. As a consequence of this lower moisture content, HPMC capsules are more suitable for formulations containing drugs that exhibit tendencies to degrade in water [97]. The effect of moisture content on HPMC capsules is less pronounced than in hard gelatine capsules and at levels of 1% w/w, HPMC capsules are not brittle [93]. Although the dissolution of HPMC capsules is approximately three times slower than that of hard gelatin capsules in the temperature range of 35-39°C, the dissolution of HPMC capsules is unaffected by temperature changes of between 10-55°C [93].

HPMC capsules offer several advantages over hard gelatin capsules including the fact that they are made from plant derived material rendering them suitable for use in patients with special religious and/or dietary requirements [98]. HPMC, unlike gelatin, lacks chemically reactive groups, which decreases its potential to undergo cross linking reactions over longer periods of storage [97]. In addition HPMC capsules are more suitable for enteric coating as they have a rough surface as compared to hard gelatin capsules, which provides for better adhesion of the coating material to the capsule surface [99]. HPMC capsules have also been observed to have a significantly less pronounced oesophageal sticking tendency in vitro, as compared to hard gelatin capsules [97].

3.4.2.2 *Starch capsules*

Starch capsules are made from starch derived from potatos using injection moulding technology as a method of manufacture and represent a direct alternative to hard gelatin capsules for drug delivery [100]. Hard shell starch capsules consist of a body and a cap that are sealed immediately after filling the capsule body by applying a hydroalcoholic solution to the inner section of the cap prior to its being placed on the capsule body [100]. Hard shell starch capsules are officially recognised in the United Stated Pharmacopeia and National Formulary as a suitable alternative to gelatine capsules [100].

Starch capsules are supplied in five different sizes viz., 0, 1, 2, 3 and 4, all with the same cap size [100]. Starch capsules have a high quality surface finish as they lack a "lip seal" as observed with hard gelatin capsules [100]. Starch capsules are odourless and rigid and display similar dissolution characteristics to hard gelatin capsules [100].

Starch capsules offer several advantages over hard gelatin capsules, which include pH-independent dissolution characteristics and better stability as any moisture content in the capsules shells is bound to the starch [100]. The smooth finish of starch capsules makes them tamper-evident as well as renders them more suitable for enteric coating after filling [100]. Furthermore, starch capsules are produced from a non-animal source which makes them suitable for patients with beef allergies and religious preferences.

3.4.3 Capsule Fill Materials

3.4.3.1 Solids for capsule filling

An increased demand for encapsulated products from the mid 1900's led to the development of automated filling machines for ensuring uniform filling of solid materials into two-piece hard gelatine capsules [101]. Modern capsule filling machines are based on two types of automated systems viz., dosator nozzle or tamp-filling machines [93]. The operation of dosator nozzle machines involves lowering into a powder bed to permit powder to enter a dosing tube following which the powder is compressed prior to injection into a capsule body [93]. The operation of tamp-filling machines relies on pins pushing through a powder bed so that a unit dose is transferred into a dosing disc after which this dose is ejected into a capsule body [102]. It is therefore important to understand and optimize powder flow properties to ensure efficient capsule filling and more importantly accurate dosing of fill materials into capsules.

Solid materials and powders exhibit an inherent resistance to the differential movement between particles when subjected to external stresses such as blending [92]. The magnitude of resistance to particle movement is affected by the surface properties of materials, particle size distribution, shape and geometry of particles in addition to any electrostatic charges that may be induced as a function of the manufacturing process [92]. Powders suitable for filling into capsules should display good flow properties, even packing densities, similar particle size distribution and should be non-cohesive which will prevent sticking to metallic parts of capsule filling machines [93]. A large number of fine particless, i.e. more than 20% of particles of a blend with a diameter of 50 μm or less, has been associated with poor flow of powders whilst coarse powders with the majority of particles being larger in diameter than 150 μm have been shown to display excellent flow properties [93]. Optimal powder flow and packing properties for capsule filling can be accurately determined by determination of flow parameters using Carr's compressibility index, Hausner's ratio in addition to the angle of repose, use of Kawakita's equation and Jenike's flow factor [103].

The use of coated pellets for capsule filling has received recognition for use as sustained release formulations, which may offer advantages over monolithic dosage forms, such as tablets, in that the risk of dose dumping is significantly reduced [104]. The effect of pellet shape, pellet size distribution and pellet shape for capsule filling has been investigated using a three-dimensional computer simulation (Monte-Carlo) technique [104]. Rowe et al. observed that for all sizes of capsules, there was a gradual decrease in fill weight and increasing variability with increasing pellet size [104]. For all capsule sizes increasing polydispersity within a set range normally seen with pellets produced by extrusion and spheronisation had no effect on fill weight or weight variation and that for any pellet shape with aspect ratios of 1.2 and below, there was no difference in fill weight and weight variation [104].

The use of granules to fill hard gelatin capsules to overcome difficulties such as variations in fill weight due to impaired powder flow or stickiness has been shown to be beneficial [105]. Granules prevent fine powder segregation, which is important if the particle size of the drug cannot be matched to those of compatible excipients [105]. Granules can enhance homogeneity of powder mixtures, which is particularly important for the inclusion of low dose drugs in formulations [105].

Different capsule filling mechanisms have facilitated filling of capsules with tablets as well as combinations of powders, pellets and tablets [105].

3.4.3.2 Liquid and semi-solid fill materials for capsule filling

Liquid and semi-solid fill materials for pharmaceutical capsules offer several advantages over solid fill materials, including better control of capsule fill weight and content uniformity, particularly for low dose drugs of relatively higher potency [106]. Furthermore liquid fill formulations for capsules reduce the presence of airborne particles and ensure easy containment of spillages when working with toxic materials [93,106]. In general liquid formulations use fewer excipients and ensure an improvement in the dissolution of poorly water soluble drugs or control drug release with these relatively simple formulations [93,107].

Liquid fill formulations for pharmaceutical capsules are mainly thixotropic gels that undergo shear thinning during the filling process and gel restructuring with an apparent increase in viscosity once dosed into the capsule [108]. In addition thermosoftening systems that are

liquefied by heat to permit filling into capsules followed by rapid solidification on cooling in the capsules, have been used [93,109].

When filling capsules using the abovementioned systems, important factors to consider include the apparent viscosity of the formulation as this influences the efficiency and accuracy of filling, the capsule shell integrity as it is important for gelatin shells to maintain their moisture content balance in addition to the excipients used in relation to thermal stability, rheology and capsule shell integrity [93,110].

3.4.4 Sustained-release capsule formulations

Capsules have made an important contribution to the production of oral controlled release products and to date several formulation techniques have been used to produce controlled-release capsule formulations [111-116]. It is advantageous to fill pellets coated with rate retarding polymers into capsules as this strategy will avoid the damage that may be caused to coating materials during compaction of materials into tablets [93]. Enteric coating of the capsules after filling has been successfully applied to the intestinal targeting of drugs using HPMC capsules and Eudragit® polymers [99]. Formulations of powder blends of active pharmaceutical ingredients and sustained release polymers [93] in addition to liquid and semi solid blends, particularly with lipid materials as retardants of drug release [113,117-120] have been successfully used in capsule formulations to achieve controlled release of the active.

3.5 MATERIALS AND METHODS

3.5.1 Excipients

All excipients used for formulation studies were GRAS (Generally Accepted As Safe) listed and/or are accepted for use as food additives in Europe and/or are included in the FDA Inactive Ingredients guide [121]. A summary of the trade names and sources of all excipients used in formulation studies is summarised in Table 3.2.

Table 3.2. Excipients used in formulation studies

Name	Trade name	Manufacturer/Donor
Minocycline hydrochloride	Minocycline HCL	Aspen Pharmacare, SA
Hydroxypropylmethyl cellulose (HPMC)	Methocel® K100M	Colocorn® LTD, UK
Hydroxypropyl cellulose (HPC)	Klucel® EF	Aspen Pharmare, SA
Glyceryl behenate	Compritol® 888 ATO*	Gattefossé, Germany
Glyceryl palmitostearate	Precirol® ATO 5**	Gattefossé, Germany
Microcrystalline cellulose (MCC)	Avicel® 102	Aspen Pharmacare,SA
Lactose monohydrate USP/NF/BP/EP/JP	Super Tab®	Lactose Co. Hawera, NZ
Sodium starch glycolate	Explotab®	Aspen Pharmacare,SA
Poloxamer 188	Lutrol® micro 68 MP *** Technical	BASF Corporation, USA

Composition

* Glyceryl dibehenate, Tribehenin, Gylceryl behenate

** Tripalmitin, Tristearin

*** Polyoxypropylene-Polyoxyethylene Block Copolymer

3.5.1.1 Hydrophilic matrix-forming polymers

Hydrophilic matrix tablets are regarded as the simplest and most cost effective means of formulating extended release dosage forms [122]. Various hydrophilic or water swellable polymers with high molecular weights, such as hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC) and polyethylene oxide (PEO) have been used as sustained release polymeric matrices [122].

The mechanism of drug release from hydrophilic polymer matrices is complex but may be described as including three main stages viz. dissolution of the drug (if soluble), diffusion of the drug through a hydrated portion of a matrix and erosion of the outer hydrated polymer on the surface of the matrix [122].

Initially drug release from hydrophilic matrix systems involves the formation of a steep concentration gradient at the polymer/water interface resulting in water uptake into the matrix facilitating hydration of the polymer to form a 'gel layer" around the dosage form [82,122]. This process is a glassy-to-rubbery state transition of the polymer [122]. Except for soluble drug on the surface of the dosage form, which may result in an initial burst release, the core of the formulation is essentially comprised of drug at the initial stages of polymer hydration [122]. Following contact of the dosage form with water, drug diffuses out of the system along a concentration gradient with a substantial increase in the diffusion coefficient as water content in the system increases [82]. Dissolved and un-dissolved drug co-exist within the matrix in the case of poorly soluble drug inclusion and un-dissolved drug is not available for diffusion [82]. High

initial drug loadings result in matrix systems that change significantly during the drug release process and they tend to become porous and less restrictive to diffusion as drug is depleted [82]. The final stage of drug release and which may be negligible in cases in which rapid drug release occurs, is the dissolution of the matrix [82,122]. The rate of dissolution of the matrix is dependent on the chain length and degree of the substitution of the matrix used [82,122].

3.5.1.1.1 Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) or hypromellose is commercially available as Methocel® and is a partly O-methylated and O-(2-hydroxypropylated) cellulose [121]. HPMC is an odourless and tasteless, white or creamy-white fibrous or granular powder that is soluble in cold water forming a viscous colloidal solution on hydration [121]. Different grades of HPMC may be distinguished by appending a number indicative of the apparent viscosity, in megapascals (mPa), of a 2% w/w aqueous solution at 20°C [121].

The physicochemical properties of HPMC, such as for example its solution viscosity, are strongly affected by the content of methoxy and the hydroxypropoxy functional group content and the associated molecular weight of the polymer [82]. Four different types of HPMC are distinguished by the USP based on their relative methoxy and hydroxylpropoxy functional group content. The grades reported include HPMC 1828, HPMC 2208, HPMC 2906 and HPMC 2910 where the first two digits denote the approximate percentage content of the methoxy functionality and the last two digits denote the percentage of hydroxypropoxy functionality calculated on a dried basis [82,121].

HPMC may be classified as a coating agent, film-former, rate-controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder and viscosity-increasing agent [121]. High viscosity grades of HPMC may be used at concentrations of between 10-80% w/w to retard the rate of release of drugs from a matrix in tablets and capsules in which it is included [121].

HPMC is the most frequently used and studied hydrophilic polymer for the formulation of sustained release matrices [122-124]. HPMC is readily available and globally accepted as a pharmaceutical excipient. HPMC can be used with various drugs or in combination with other polymers. The stability of HPMC and its non-ionic nature allows for the development of dosage forms that exhibit pH-independent performance [122-124].

3.5.1.1.2 Hydroxypropylcellulose (HPC)

Hydroxypropylcellulose (HPC), also known as hyprolose or Klucel® is a non ionic partially substituted poly (hydroxypropyl) cellulose ether [121]. HPC is a white or slightly yellow-coloured, odourless and tasteless powder that is freely soluble in water below 38°C and that forms a smooth clear colloidal solution on hydration [121].

As the molar substitution of HPC is fixed, the molecular weight is varied by controlling the degree of polymerization of the cellulose backbone [125]. The viscosity of HPC increases as the degree of polymerization increases [125].

HPC may be classified as a coating agent, emulsifying agent, suspending agent, tablet binder, thickening agent or viscosity-increasing agent [121]. Low viscosity grades of HPC are used mainly as tablet binders whereas high viscosity grades of HPC are used in sustained-release matrix formulations [125]. The release rate of drug included in HPC matrices increases with decreasing viscosity of HPC that is included in the formulation [125].

3.5.1.2 Lipophilic matrix-forming agents

Lipid excipients, particularly solid lipids have gained popularity as matrix forming agents for sustained release. The advantages of using lipid or wax matrices for sustained release includes chemical inertness, ease of manufacturing with high reproducibility and low cost [126]. Lipid matrices have been used to formulate dosages forms that contain freely [127] and poorly water soluble [117] compounds.

Considerable attention has been focussed on the Gelucire® family as matrix forming lipids for sustained release technologies. Gelucire® is derived from natural hydrogenated food grade fats and oils [117]. Gelucire® may contain pure gycerides which may be mono-, di- or triglycerides of saturated fatty acids, or may be comprised of mixtures of glycerides and fatty acid esters of polyethylene glycols in varying proportions [117].

Gelucire® is commercially available in different grades that are characterised by their hydrophilic-lipophilic balance (HLB value) and their melting points [128,117]. In vitro release and sustained release characteristics and mechanism of drug release are influenced by the grade and proportion of Gelucire® used in the matrix [117]. A specific grade of Gelucire® is denoted by two sets of digits, of which the first two digits denote the melting point of the material and the

last two denote the HLB value. Different grades of Gelucire® have been used pharmaceutically to ensure sustained release of drugs can be achieved [129]. Lipid matrices are prepared by dispersing a drug into molten lipid, after which the matrix is filled into capsules directly or left to solidify prior to screening through sieves and subsequent compression into granules [117,126,130]. Lipid matrices may also be prepared, by mechanical blending to form physical mixtures, which may then be compressed into tablets [131]. Drug granulation followed by compression at elevated temperature has been used to prepare diprophylline sustained release formulations using glycerol palmitosterate lipid matrices [132].

Drug release from highly lipophilic lipid matrices has been attributed to diffusion through channels formed in the matrices which result from rapid dissolution of soluble drug particles located on the surface of the matrices [117]. Further penetration of aqueous media through the channels that are formed, facilitates further release of drug embedded deeper in the matrices [131]. Drug release from matrices may be further enhanced by the addition of water soluble excipients such as lactose, which promote the formation of pores through which more drug can be released as the excipients dissolve [131]. The addition of surfactants increases the rate of release of drugs from lipid matrices as surfactants reduce the hydrophobic interactions between lipid matrix materials and the drug dispersed in the matrix [130,131]. Drug release from amphiphilic or water dispersible Gelucire® bases has been demonstrated to be predominantly a result of erosion of the matrix [117].

One of the major disadvantages associated with the use of lipid matrices is the instability of lipids over long periods of storage and which is attributed to either the conversion of triglycerides to more stable polymorphic forms or conversion of amorphous to crystalline forms of the lipid bases [133]. Remunan et al. [120], have demonstrated a decrease in the in vitro release rate of nifedipine from lipid matrices over a period of six months and San Vicente et al. [134] showed an alteration of biopharmaceutic parameters of salbutamol sulphate in lipid matrices dependent of the type of Gelucire® and size of the dosage form used.

Glycerol palmito-stearate (Precirol® ATO 5, also known as Gelucire® 54/02) and glycerol behenate (Compritol® 888, also known as Gelucire® 70/02) have been widely used as glyceride bases for preparation of sustained release dosage forms [126]. The esterification of long chain fatty acids and the absence of PEG esters have resulted in Precirol® and Compritol® showing a pronounced hydrophobic character that is expressed by a low HLB value of approximately 2

[135]. The classification and physical characteristics of Precirol® and Compritol® are summarized in Table 3.3.

Table 3.3. Classification of Precirol® and Compritol® [126]

Category	Lipid	Melting point	Description	Acid value
Fatty acid ester of gycerol	Compritol®	~70	Glyceryl behenate	4>
Fatty acid ester	Precirol®	~57	Glyceryl palmitostearate	-

3.5.1.2.1 Glyceryl Palmitostearate (Precirol® ATO 5)

Glyceryl palmitostearate is a white powder with a faint odour and a melting point of 52-55°C [121]. Glyceryl palmitostearate is classified as a coating, gelling, release-modifying and sustained-release agent, tablet and capsule lubricant and diluent and taste masking agent [121].

3.5.1.2.2 Glyceryl Behenate (Compritol® 888 ATO)

Glyceryl behenate is a fine white powder or hard waxy mass with a melting point of 65-77°C [121]. Glyceryl behenate may be classified as a coating agent, tablet binder or a tablet and capsule lubricant and is used as a lipophilic matrix or coating for sustained-release tablets and capsules when used at concentrations greater than 10% w/w [121].

3.5.1.3 Microcrystalline cellulose (MCC)

Microcrystalline cellulose is commercially available as Avicel® PH or Emcocel® amongst others and is a purified, partially depolymerised cellulose that occurs as a white, odourless, tasteless, crystalline powder composed of porous particles [121]. Microcrystalline cellulose is available in different grades based on the method of manufacture, the particle size as well as the moisture content of the material [121]. The larger particle size grades exhibit greater flowability than small particles sizes whilst low moisture content grades have been used to formulate moisture sensitive drugs [121].

Microcrystalline cellulose has been particularly useful pharmaceutically as a result of its low chemical reactivity and its excellent compactibility at low pressures [136]. Microcrystalline cellulose is used pharmaceutically as a binder or diluent in the formulation of tablets and capsules as well as a lubricant and disintegrant in tableting [121].

3.5.1.4 Lactose Monohydrate

Lactose monohydrate is a naturally occurring disaccharide, derived from milk and consists of a single galactose and glucose moiety [121]. Lactose is odourless and slightly sweet tasting and occurs as white to off-white crystalline particles or powder [121].

Several grades of lactose differentiated by different particle size distribution and flow characteristics are available for use as fillers or diluents for tablets and capsules [121]. The selection of suitable grades of lactose for use in the formulation of capsules is determined by the type of encapsulation machinery used and fine grades are regarded suitable for tableting as they permit adequate mixing with other formulation excipients during a wet granulation or milling process [121].

3.5.1.5 Sodium starch glycolate

Sodium starch glycolate is described in the USP-NF23 as the sodium salt of carboxymethyl ether of starch [121]. Sodium starch glycolate occurs as a white to off-white, odourless, tasteless, free-flowing powder [121].

Sodium starch glycolate is used pharmaceutically at concentrations of 2-8% w/w as a disintegrant in tablet and capsule formulations [121]. Disintegration occurs as a result of rapid fluid uptake and swelling of the sodium starch glycolate [121]. The effectiveness of sodium starch glycolate as a disintegrant is determined by its physicochemical properties which are affected by the degree of crosslinking and extent of carboxymethylation of the polymer [121].

3.5.1.6 Poloxamer 188

Poloxamers are commercially available as Lutrol®, Pluronic®, Monlan®, Supronic® or Synperonic®, amongst others and are block copolymers of ethylene oxide and propylene oxide conforming to the general formula $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$ [121]. Poloxamers are non-ionic and are comprised of a hydrophilic polyoxyethylene segment and a hydrophobic polyoxypropylene segment, the compositions of which are included in their nomenclature [121]. The non-proprietary name "poloxamer" is followed by a number, the first two digits, of which when multiplied by 100, corresponds to the approximate average molecular weight of the polyoxypropylene portion of the copolymer and the third digit, when multiplied by 10, corresponds to the percentage by weight of the polyoxyethylene portion [121].

Poloxamers occur as white, waxy free flowing solids and are tasteless and odourless [121]. Poloxamer 188 has a melting point of 52-57°C and is freely soluble in water [121]. Poloxamers

are used as dispersing agents, emulsifying and co-emulsifying agents, solubilising agents, tablet lubricants and as wetting agents [121].

3.5.2 Formulation Composition

Sustained release formulations based on the matrix technology were prepared. Hydrophilic and lipid polymeric matrices were studied. To ensure the selection of optimal formulations in terms of minocycline release pattern, for further studies, the following formulation variables were investigated during formulation development:

i) the effect of hydrophilic polymer type
ii) the effect of different concentrations of hydrophilic polymer
iii) the effect of lipid type
iv) the effect of different concentrations of lipid
v) the effect of using different diluents/capsule fillers
vi) the effect of adding disintegrant to the formulations
vii) the effect of adding surfactant to the lipid formulations

A summary of the composition of formulations manufactured in these studies is shown in Table 3.4.

Table 3.4. Minocycline capsule formulations

Batch #	Release-retarding polymers (%w/w)				Capsule filler	Na-starch gycolate (%w/w)	SAA Pluronic F68
	HPMC	HPC	Precirol®	Compritol®			
MINO-01	10	-	-	-	MCC		
MINO-02	20	-	-	-	MCC		
MINO-03	30	-	-	-	MCC		
MINO-04	50	-	-	-	MCC		
MINO-05	-	-	-	10	MCC		
MINO-06	-	-	-	20	MCC		
MINO-07	-	-	-	30	MCC		
MINO-08	-	-	-	50	MCC		
MINO-09	-	50	-	-	MCC		
MINO-10	-	-	50	-	MCC		
MINO-11	20	-	-	-	LACTOSE		
MINO- 12	-	-	-	30	LACTOSE		
MINO-13	20	-	-	-	MCC	2	
MINO-14	20	-	-	-	LACTOSE	2	
MINO-15	-	-	-	30	MCC	2	
MINO-16	-	-	-	30	LACTOSE	2	
MINO-17	10	-	-	10	MCC	-	-
MINO-18	-	-	-	30	MCC	-	2
MINO-19	-	-	-	30	MCC	-	1
MINO-20	-	-	-	30	LACTOSE	-	1
MINO-21	-	-	-	30	MCC	-	0.5
MINO-22	-	-	-	30	LACTOSE	-	0.5

3.5.3 Formulation of sustained release minocycline hydrophilic matrix capsules

Powder formulations for capsule filling that contained minocycline hydrochloride equivalent to 100 mg minocycline base (per capsule), 10-50% w/w of the hydrophilic polymer under investigation and microcrystalline cellulose or lactose monohydrate as filler were manufactured. The effect of sodium starch glycolate was investigated for selected formulations. Powders were prepared by the geometric dilution method of mixing using a mortar and pestle and passed through a 315 μm sieve before manually filling into size 0 hard gelatin capsules. Each capsule contained 300 mg of powder fill material. A schematic representation of the procedures in the preparation of the formulations is summarised in Figure 3.6. The formula for each capsule is shown in Table 3.5 below. A sample production record for all formulations based on hydrophilic polymer matrix fills is located in Appendix I.

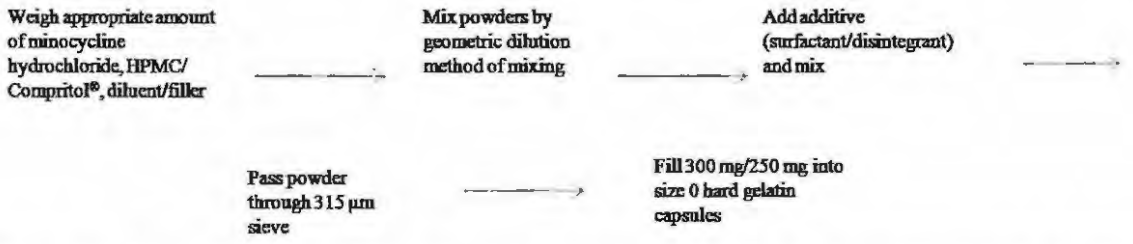


Figure 3.6. Schematic representation of procedure for preparation of minocycline sustained release capsule formulations

Table 3.5. Formula for hydrophilic polymer matrix sustained release minocycline capsules

Minocycline hydrochloride	108 mg
Hydrophilic polymer (HPMC/HPC)	*
Sodium starch glycolate	**
Capsule filler (Microcrystalline cellulose/Lactose monohydrate)	Ad 300 mg
* 10%, 20%, 30%, 40%, 50% w/w	
** 0, 2% w/w	

3.5.4 Formulation of sustained release minocycline lipid matrix filled capsules

Powder formulations for capsule filling contained minocycline hydrochloride equivalent to 100 mg minocycline base (per capsule), 10-50% w/w of the lipid under investigation and microcrystalline cellulose or lactose monohydrate as filler. The effect of sodium starch glycolate and Poloxamer 188 was investigated for selected formulations. Powders were prepared by geometric dilution method of mixing and passed through a 315 µm sieve before filling into size 0 hard gelatin capsules. Each capsule contained 250 mg of powder. A schematic representation of the preparation procedures for the formulations is illustrated in Figure 3.6. The formula for each capsule is shown in Table 3.6. A sample production record for all formulations based on lipid matrices can be seen in Appendix I.

Table 3.6. Formula for lipid matrix sustained release minocycline capsules

Minocycline hydrochloride	108 mg
Lipid (Compritol® / Precirol®)	*
Sodium starch glycolate	**
Poloxamer 188	***
Capsule filler (Microcrystalline cellulose/ Lactose monohydrate)	Ad 250 mg
* 10%, 20%, 30%, 40%, 50% w/w	
** 0, 2% w/w	
*** 0, 0.5%, 1%, 2% w/w	

3.6 DOSAGE FORM ANALYSIS

3.6.1 Pharmacopoeial tests for capsules

To ensure quality dosage forms are released onto the market regulatory authorities and pharmacopoeial bodies require that specific standards of quality are met. The British Pharmacopoeia (BP) requires that pharmaceutical capsules comply with the test for uniformity of content or uniformity of mass [22]. The BP also recommends a suitable dissolution test be used to demonstrate the appropriate release of active pharmaceutical ingredients. In addition the BP requires that hard capsules comply with the test for disintegration of tablets and capsules.

3.6.1.1 Uniformity of mass

The test for uniformity of mass as described in the BP requires the use of ten capsules from each manufactured batch [22]. The entire contents of each capsule are accurately measured after which, the average mass and percent deviation are calculated. For capsules containing less than 300 mg of fill material, the percent deviation should be no more than 10 % whilst for capsules containing 300 mg or more, the percent deviation should not exceed 7.5 % [22].

The exact weight for capsules manufactured in this study was obtained by calculating the difference in weight between the mass of the filled and empty capsules. The mean weight and relative standard deviations were then calculated and recorded.

3.6.1.2 Disintegration

The BP requires that hard capsules conform to the disintegration test for tablets and capsules [22]. The disintegration apparatus is comprised of a basket rack assembly and a 1 litre low form beaker which measures 149 ± 11 mm in height with an inside diameter of 106 ± 9 mm for the immersion fluid [22]. Present in the apparatus is a thermostatic arrangement for heating to ensure a temperature between 35°C and 39°C is maintained, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute, through a distance of 55 ± 2 mm [22]. The immersion fluid may be water and when justified, 0.1M HCl or artificial gastric fluid [22]. The basket rack assembly moves vertically on its axis and there is no significant horizontal movement of the axis from the vertical [22].

Six dosage forms for testing are placed in each of the six tubes of the basket rack assembly [22]. Discs of dimensions that meet pharmacopoeial specifications may be used when permitted for dosage forms that float [22]. On completion of the disintegration test using the specified

conditions, all dosage forms must have disintegrated. If all six dosage forms have not disintegrated, the test should be repeated twice with twelve more dosage units [22]. To pass the test, sixteen of the eighteen dosage forms must have disintegrated [22].

Although it is not a requirement of some pharmacopoeias such as the United States Pharmacopoeia (USP) for tablets or capsules intended as extended-release or delayed-release dosage forms to comply with the disintegration test, disintegration tests were carried out to ensure that the capsule shell disintegrated in order to ensure that the capsule contents were exposed to dissolution media within a short period of time. A summary of the conditions used for the disintegration tests conducted in this study is shown in Table 3.7.

Table 3.7. Conditions for disintegration test for minocycline capsule formulations

Instrument	ERWEKA ZT6 Disintegration Tester, Heusenstamm, Germany
Temperature of disintegration medium	37°C
Volume of disintegration medium	700 ml
Dip rate per minute (dpm)	30 dpm

3.6.1.3 Dissolution

Dissolution tests were carried out to study in vitro release characteristics of minocycline formulations developed and manufactured in these studies. In vitro release profiles of minocycline from manufactured formulations are described in Chapter 4.

3.6.1.4 Assay

Assays for the powder fills were performed for each formulation. An amount of powder equivalent to the contents of one capsule viz., 300 mg and 250 mg for the HPMC based and Compritol® based formulations, respectively, were weighed out and dissolved in 100 ml HPLC grade water and sonicated for ten minutes in a 100 ml A-grade volumetric flask. A 1 ml aliquot of this solution was filtered through a Millipore Millex-HV Hydrophilic PVDF filter (0.45 μm) and diluted with 5 ml of a 250 μg/ml solution of doxycycline hyclate solution. The solution was made up to volume with mobile phase and analysed using the developed HPLC method summarised in Table 2.5.

3.6.2 Results

3.6.2.1 Uniformity of mass

A summary of the average content per capsule in addition to the relative standard deviations for all batches of minocycline sustained release capsules is located in Table 3.8.

Table 3.8. Mean mass and standard deviation for capsule formulations

Formulation	Mean mass (mg) (n=12)	Standard deviation	%RSD
MINO-01	300.27	0.468	0.16
MINO-02	299.63	0.887	0.30
MINO-03	299.33	0.650	0.22
MINO-04	299.87	1.088	0.36
MINO-05	250.85	0.235	0.09
MINO-06	249.70	0.754	0.30
MINO-07	249.62	0.924	0.37
MINO-08	250.58	0.488	0.19
MINO-09	300.17	0.753	0.25
MINO-10	250.48	0.652	0.26
MINO-11	299.63	0.971	0.32
MINO- 12	250.45	0.409	0.16
MINO-13	299.77	0.404	0.13
MINO-14	299.47	0.231	0.077
MINO-15	250.73	0.280	0.11
MINO-16	250.15	0.217	0.087
MINO-17	250.32	0.407	0.16
MINO-18	250.08	0.232	0.093
MINO-19	250.07	0.186	0.074
MINO-20	250.11	0.319	0.13
MINO-21	249.33	0.473	0.19
MINO-22	250.25	0.383	0.15

3.6.2.2 Disintegration

The disintegration of capsule shells when using de-ionised water as the immersion fluid commenced within the first three minutes of the test and was complete within ten minutes for all formulations tested. The disintegration of capsule shells of Formulation MINO-06, MINO-11 and MINO-17 with 0.1M HCl as immersion medium was complete within ten minutes of the commencement of the disintegration test.

3.6.2.3 Assay

Assays of the powder fills for the capsule formulations revealed that all formulations contained between 95.2% and 105.5% of the stated amount of minocycline hydrochloride. Assays for specific formulations may be seen on specific batch record summaries in Appendix II.

3.7 CONCLUSIONS

Although technologies for modified-drug delivery have become more advanced over the years, matrix-based technologies continue to play a significant role in modified-drug delivery. The use of lipid matrices to produce sustained release has been extensively studied over the past few years and has provided an alternative means of formulating matrices, which may display some advantages over conventional matrix forming agents.

The use of capsules in the pharmaceutical and food industry has advanced over recent years, particularly with modernisation of capsule filling machines which have allowed for a huge variety of materials to be filled in capsules. Capsule formulations using powder blends are relatively simple to make and may provide for means of providing modified release dosage forms.

Dosage form analysis is an important part of pharmaceutical formulations, particularly to ensure quality of the products. The manufactured formulations in these studies were assessed according to pharmacopoeial requirements of the BP and the USP. The formulations were of uniform mass and the capsule shells disintegrated within the acceptable time as stated in the BP. The dissolution characteristics of the manufactured formulations are discussed in Chapter 4.

CHAPTER FOUR

4 DRUG RELEASE FROM CAPSULE DOSAGE FORMS

4.1 INTRODUCTION

Drug absorption following administration of a solid dosage form depends on the release characteristics of the API from the dosage form, dissolution rate and/or solubilisation potential under physiological conditions and subsequent transport of the API across the membranes of the gastrointestinal tract [137]. As the first two steps of the process are crucial to the success of the absorption process, in vitro dissolution may be relevant as a predictor of in vivo performance [137]. Dissolution testing is routinely used in Quality Control (QC) and Research and Development (R&D) laboratories [138].

In vitro dissolution test methods for quality control purposes provides information on the consistency of batches, homogeneity of dosage forms within a specific batch in addition to the impact of changes in composition, process or site of manufacture [139]. In vitro dissolution methods are important for the assessment of stability of pharmaceutical products [140] which is in turn important in establishing an appropriate shelf life for pharmaceutical products.

As a tool in Research and Development, the focus of dissolution testing is geared towards providing some predictive estimates of drug release rates and mechanism in respect to the in vivo performance of a drug product [138]. In this instance, it is essential that the in vitro and in vivo dissolution behaviour of a drug product be similar or have a scalable relationship to each other, i.e. an in vitro in vivo correlation has been established [141]. In vitro dissolution test data may be used to evaluate and interpret any possible risk, such as for example dose dumping or food effects on bioavailability and the potential for an interaction with other drugs that has been associated with some pharmaceutical dosage forms [140].

The different approaches to in vitro dissolution testing for Quality Control and Research and Development requires that suitable dissolution media that fulfil the purpose of the dissolution test are selected for use in the test [138]. In addition, one should select an appropriate dissolution test apparatus as the type of apparatus has been shown to influence drug release characteristics [142].

Whilst highly discriminatory methods are suitable for QC purposes, they may not be suitable for the prediction of in vivo performance [138].

4.2 USP DISSOLUTION APPARATUS FOR SOLID ORAL PRODUCTS

The United States Pharmacopoeia (USP) Chapter on dissolution testing; <711> DISSOLUTION, describes the use of four types of apparatus for dissolution testing of immediate-release (IR) and modified-release (MR) solid oral pharmaceutical products. These include Apparatus 1 (Basket Apparatus), Apparatus 2 (Paddle Apparatus), Apparatus 3 (Reciprocating Cylinder) and Apparatus 4 (Flow-Through Cell or BIODIS) [70].

The basket and paddle apparatus are recommended in various guidelines as the first choice for in vitro testing of immediate- and modified-release dosage forms as these apparatus are simple, robust and have been adequately standardised [140,142]. The basket apparatus is comprised of a vessel made of glass or other transparent and inert material, a motor, a metallic drive shaft and a cylindrical basket [70]. The vessel is partially immersed in a water bath of suitable dimensions and that is normally maintained at 37 ± 0.5° C during dissolution testing [70]. There is no significant motion from any part of the assembly except the controlled movement of the smoothly rotating stirring element attached to the basket [70]. The assembly of the paddle apparatus is similar to that of the basket apparatus except that a paddle formed from a blade and a shaft is used as the stirring element [70]. The use of a sinker for floating dosage forms is permissible although the use of sinkers has been shown to influence the in vitro release characteristics of drugs significantly [143]. In general dissolution media are of an aqueous nature and are typically buffers in the pH range 1- 8 and in some cases the use of water is permitted [70,140]. The volume of dissolution medium used in each vessel for the basket and paddle methods should be between 500 and 1000 ml with the use of 1000 ml recommended for new drug products or in the case of a revision of any existing test procedure [140]. The degassing of dissolution media, using a suitable method, prior to dissolution testing is recommended as drug release from some formulations is sensitive to the presence of dissolved gases in dissolution media [144,145]. Agitation in the basket and paddle apparatus is usually achieved by rotation of the basket or paddles at speeds of 50 to 100 rpm and not exceeding stirring speeds of 150 rpm [140].

The basket and paddle apparatus have been criticised due to their "single-container" nature as this does not readily allow for changes in pH or any other changes during dissolution testing [140]. In

addition the use of the basket and paddle apparatus is not suitable for sparingly soluble drugs and aerophilic multiple unit doses that tend to float during the initial stages of testing [140].

A major disadvantage of using the basket or paddle apparatus is the variability of the release data that can be attributed to hydrodynamic artifacts or fluid flow patterns in the dissolution vessel [146]. Contrary to the belief that commercially available dissolution vessels all have the correct physical dimensions for dissolution testing, it has been observed that some commercially available dissolution vessels have irregular inner surfaces, such as for example deviations from circularity for the inner cylinder, deviation from cylindricity for the entire cylindrical shape and deviation from concentricity for the centre of the sphere, that result in an altered liquid flow dynamic or pattern that lead to variable drug release patterns throughout the dissolution test [147]. Furthermore, vessel contour imperfections have been reported to cause variability as a result of altered fluid flow within the vessel [148]. It is important to ensure that the physical parameters including type of dissolution apparatus (basket or paddle), paddle design, basket mesh size, agitation speed, fluid flow velocity and pattern, media temperature, deaeration method and type of sinker used, are carefully monitored and accurately reported, as they have been found to have a significant impact on the hydrodynamics of a dissolution test system and hence dissolution patterns of drug products tested in these systems [146]. A "dead zone" phenomenon or cone formation, whereby large quantities of insoluble excipients form a dense mass at the bottom of the vessel have been observed during dissolution testing of poorly soluble drugs when using the paddle apparatus [149]. Cone formation has also been observed to cause slower dissolution rates [150]. Several modifications to the paddle apparatus that change flow hydrodynamics in dissolution vessels have been proposed to minimise the variability of dissolution data attributed to cone formation [146]. These modifications could be either by displacing the unstirred disintegrated particles/excipients away from the centre of the vessel or by shortening the distance between the drug and the bottom of the paddle, thereby allowing for greater interaction between the surfaces of the drug and the moving dissolution medium.

The shortcomings of USP Apparatus 1 and 2, particularly shaft wobble, location, centering, deformation of baskets and paddles and the effect of dissolved gases in dissolution media have brought about the need for the development of dissolution apparatus that are able to withstand the physical, mechanical and hydrodynamic variations inherent in USP Apparatus 1 and 2 test systems [151]. In addition the increased interest to approach the goal of establishing an in-vitro in vivo correlation (IVIVC) has made it necessary to develop instruments in which the pH, molarity,

presence of cations and anions, buffer type, inclusion of surface active agents and degree of agitation can be altered sequentially during the dissolution test process [151]. The recognition of the above factors led to the development of USP Apparatus 3 [151].

USP Apparatus 3 or reciprocating cylinder apparatus (BIODIS) consists of a set of cylindrical flat bottomed glass vessels, a set of glass reciprocating cylinders, inert fittings and screens that are made of suitable non adsorbing and inert material [70]. The fittings and screens are designed to fit the tops and bottoms of the reciprocating cylinders [70]. USP Apparatus 3 is also fitted with a motor and drive assembly to reciprocate the cylinders vertically inside the vessels or horizontally to another row of vessels [70]. The vessels of the USP Apparatus 3 are partially immersed in a water bath that is usually maintained at 37 ± 0.5°C during dissolution testing [70]. USP Apparatus 3 allows the operator to programme the agitation rate (dips per minute) within the vessels, the time the dosage form spends in each row of vessels as well as the pattern of dissolution media changes, which is of importance when developing dissolution methodology to study IVIVC [151].

The main advantages presented by USP Apparatus 3 include the fact that variability of dissolution data is reduced as this apparatus is less sensitive to changes in geometry of the dissolution vessels and the presence of dissolved air in the dissolution media [151]. The "coning" effect observed in USP Apparatus 2 is absent with USP Apparatus 3 resulting in more precise dissolution results when using this apparatus [151]. USP Apparatus 3 may be applied as an in vitro predictor of bioavailability in the fed and fasted state [151,152].

USP Apparatus 4 or flow-through cell apparatus consists of a reservoir and a pump for the dissolution medium, a flow-through cell and a water bath that maintains temperature at 37 ± 0.5°C during dissolution testing [70]. During testing, the dosage form is placed in the flow-through cell, which is fitted with a filter system to prevent escape of undissolved particles from the top of the cell [70]. The dissolution medium is continuously flushed through the cell during the test at a flow rate of between 4 and 16 ml per minute, providing the mechanical agitation for dissolution of the drug substance [70,140]. The flow-through cell may be run either as a closed or open system with the open system overcoming some of the problems associated with the "single container" nature of the basket and paddle apparatus [140].

The availability of the four compendia dissolution apparatus has ensured that dissolution testing of all oral solid pharmaceutical dosage forms is possible on a reasonable basis [140]. In addition, the use of alternate models of the above four apparatus as well as appropriate modifications are available for the dissolution testing of formulations that cannot be accommodated by use of standard USP Apparatus 1- 4 [140].

4.3 IN VITRO RELEASE STUDIES OF MINOCYCLINE FROM SUSTAINED RELEASE CAPSULE FORMULATIONS

4.3.1 Procedure

There is no pharmacopoeial method for the dissolution of modified-release minocycline in the USP or BP [11,153,154]. Furthermore the monograph for prolonged-release minocycline in the BP does not indicate the desired release pattern for minocycline from prolonged release formulations. The objective of this study was to formulate minocycline dosage forms that released minocycline through a zero-order kinetic process over a 12 hour period. At least 80% of the minocycline in the formulation was expected to have been released at the end of the dissolution test period of 12 hours.

The USP monograph for immediate release minocycline formulations recommends the use of water as the dissolution medium. De-ionised water was therefore used as the dissolution medium for preliminary dissolution studies of minocycline sustained release formulations. The de-ionised water was degassed by filtration through a 0.45 µm Durapore® HVLP membrane filter (Millipore Corporation, Bellerica, MA, USA) with the aid of an Eyela Vacuum pump (Tokyo Rikakikai Co., Tokyo, Japan). The in vitro release pattern for minocycline from the formulations MINO-01 to MINO-22 listed in Table 3.4 were assessed using a fully automated Model SR 8 PLUS dissolution apparatus (Hanson Research Corporation, Chatsworth, CA, USA) fitted with an Autoplus™ Multifill™ and a Maximizer Syringe Fraction Collector (Hanson Research Corporation, Chatsworth, CA, USA). The dissolution studies were conducted using USP Apparatus 1 [70] with USP baskets of mesh size 40 and a basket rotation speed of 50 rpm (n=6). Aliquots of 2 ml were withdrawn at the sampling times summarised shown in Table 4.1 without further replacement of the dissolution medium. Samples were analysed using a validated HPLC-UV method described in Table 2.5 of this dissertation. A complete summary of the dissolution test conditions used in these studies is shown in Table 4.1.

Table 4.1. Summary of dissolution test conditions

Dissolution apparatus	USP Apparatus 1
Dissolution media:	
Preliminary studies	900 ml de-ionised water
USP Delayed-release two-stage test	750 ml 0.1 M HCl followed by addition of 250 ml 0.2 M tribasic sodium phosphate after 2 hr. Resultant pH of dissolution medium = 6.8
Temperature	37°C ± 0.5°C
Basket speed	50 rpm
Basket screen size	40
Filter size	0.45 µm
Volume withdrawn	2 ml
Volume replaced	No replacement
Sampling times	1, 2, 4 , 6 , 8, 12 hours

All formulations were tested within 12 hours of manufacture and the in vitro dissolution test was able to discriminate formulation factors that affected drug release during early formulation development studies of minocycline sustained release formulations.

Several extended-release capsule formulations described in the USP have been assessed using the USP "two-stage" test for delayed-release dosage forms [70,153,154]. The USP "two-stage" test involves exposure of a dosage form to an acidic medium for two hours (usually 0.1 M hydrochloric acid) and then to a buffer (usually 0.2 M tribasic sodium phosphate, pH = 6.8) for the duration of the test [70]. The three test formulations that exhibited "near-zero order" release and released at least 80 % of minocycline in the 12-hour dissolution test were then tested using the USP "two-stage" test to gain an understanding of the type of in vivo release pattern that could be expected, should the dosage form be administered to humans.

4.3.2 Statistical Interpretation of data

Dissolution profiles were compared using statistical and mathematical methods to better understand the release characteristics of minocycline from the test formulations. A one way analysis of variance and the difference (f_1) and similarity (f_2) factors were used to compare dissolution profiles of the formulations displaying "near-optimal" release and the release profile of the target formulation. Zero-order, Higuchi and Korsmeyer-Peppas mathematical models were used to fit data from all formulations tested in order to determine the mechanisms of drug release from the formulations and to establish the effect of formulation variables on the mechanism of minocycline release. The mathematical and statistical comparison of dissolution profiles is discussed in further detail in Chapter 5 of this dissertation.

4.4 RESULTS AND DISCUSSION

4.4.1 The effect of type and concentration of hydrophilic polymer on the rate and extent of minocycline release

The use of hydroxypropyl cellulose (HPC) at a concentration of 50% w/w resulted in almost complete release of minocycline within six hours whereas only 41% minocycline was released from the formulation in which 50% w/w hydroxypropylmethyl cellulose (HPMC) was used, and these profiles are depicted in Figure 4.1. Vuebe et al. [155] observed similar results in an investigation of the influence of cellulose ether polymers on drug release from hydrophilic matrix tablets. Almost complete release of ketoprofen was observed within eight hours from matrices formed using HPC whereas only 60% was released in 20 hours when HPMC was used [155].

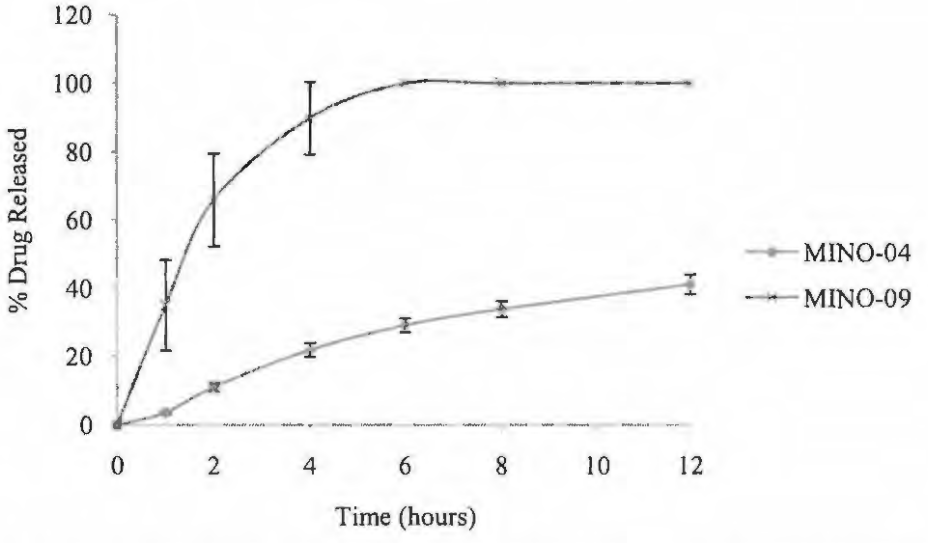


Figure 4.1. Dissolution profiles for batches MINO-04 (50% HPMC w/w) and MINO-09 (50% HPC w/w)

Several factors affecting the release of drugs from hydrophilic polymer matrices have been identified and include the rate of polymer hydration, polymer composition and polymer viscosity [156].

As previously mentioned in Section 3.5.1.1, the initial stage of drug release from hydrophilic matrices involves the hydration of the hydrophilic polymer used in a formulation and the subsequent formation of a gel layer on contact with water. Some authors have described the initial five (5) minutes of contact of the matrix with an aqueous medium as the most important time for the gel structure to form, after which if the structure has not formed, the matrix may erode too quickly to sustain the release of a drug dispersed in that matrix [124,157-159]. An

understanding of the hydration/swelling process of the polymer and factors affecting these phenomena is therefore essential. The important steps in polymer dissolution include the absorption/adsorption of water onto the surface of the material, the rupture of the polymer-polymer linkages with the resultant formation of polymer-water linkages, separation of polymeric chains, swelling and dispersion of polymeric chain in dissolution medium [156]. The hydration rate of hydrophilic cellulose ethers such as HPC and HPMC depends on the nature of the substituent present on the polymer backbone and the degree of substitution of that particular functionality [160]. A comparison between HPMC and HPC in terms of hydrophilicity is difficult to make since they are in different classes of cellulose ethers. However the hydration rate of cellulose ethers increases with an increase in hydroxypropyl content and decreases with an increase in alkyl chain length [160]. The hydrophilic hydroxymethyl group in HPMC positively contributes to the rate of hydration of this polymer [160]. Furthermore Methocel® K polymers hydrate rapidly due to the low concentration of the less hydrophilic methoxy groups [156].

The chemical composition of cellulose ethers is also an important factor that affects drug release from hydrophilic matrices as complex reactions with API that have aldehydes, methylol groups, epoxides, ethylene imine derivatives, sulfones and labile chlorine functional groups may occur [156]. The effect of polymer composition was not considered to be the primary reason for the differences observed in minocycline release as the chemical composition of HPMC and HPC results in similar reactions. In addition minocycline does not possess reactive functional groups that could potentially form complexes with HPMC or HPC and that would affect drug release.

The use of a high viscosity grade polymer in a matrix formulation results in the formation of a gel layer of increased viscosity that results in a slower release rate of an API dispersed in that matrix [156]. Furthermore the more viscous gel is resistant to dilution and erosion thus producing a more sustained release effect [161]. As the viscosity of an HPMC K100M solution is higher than that of HPC [121,162] the faster rate of release observed for minocycline from HPC matrices can be attributed to the formation of a less coherent gel layer by HPC which permits faster diffusion of the minocycline in the matrix. In addition the lower viscosity will facilitate faster erosion of the HPC matrix further increasing the rate of minocycline release.

The effect of the concentration of HPMC on the release of minocycline was investigated in the concentration range 10 – 50% w/w. The resultant release profiles are depicted in Figure 4.2.

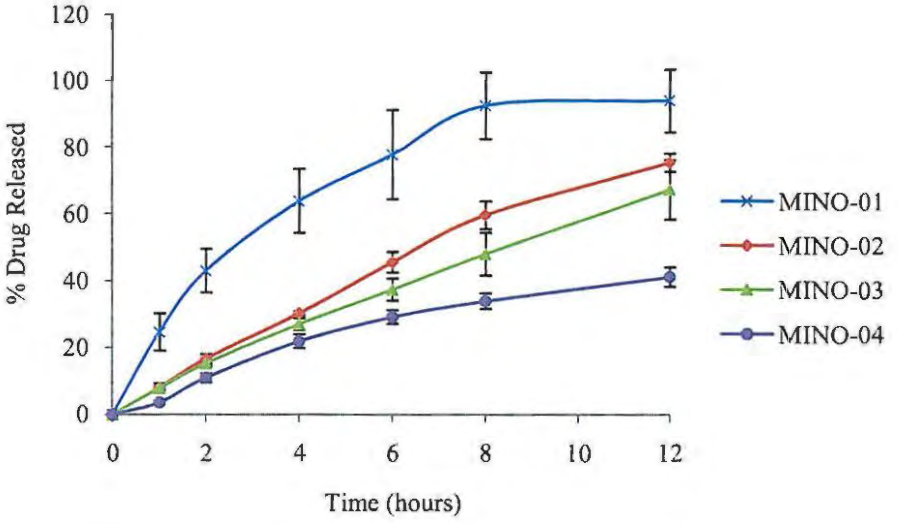


Figure 4.2. Dissolution profiles of batches MINO-01, MINO-02, MINO-03 and MINO-04 (10, 20, 30, 50% w/w HPMC, respectively)

As shown in Figure 4.2 the rate of minocycline release from the matrices was inversely proportional to the concentration of HPMC used to form the matrices. Nearly 100% minocycline release was observed within eight hours of dissolution for the 10% w/w HPMC formulations whereas only 41% of minocycline was released following 12 hours of testing for the 50% w/w HPMC formulation. Similar relationships have been reported where the rate of drug released is slower as the concentration of HPMC used in hydrophilic matrices increases [123,163-165]. This decreased drug release observed with increasing concentrations of HPMC has been attributed to the faster hydration and formation of the gel layer [166] as the solution viscosity of HPMC increases with an increase in the concentration of the polymer [160].

4.4.2 The effect of type and concentration of lipid on the rate and extent of minocycline release

The rate of release of minocycline was found to be faster from Precirol® matrices than those observed from Compritol® matrices when a concentration of 50% w/w of lipid was used as retardant and the release profiles are shown in Figure 4.3. Minocycline was released completely within two hours of the commencement of dissolution testing from Precirol® matrices whereas less than 10% minocycline was released from Compritol® matrices at the end of the 12 hour dissolution test.

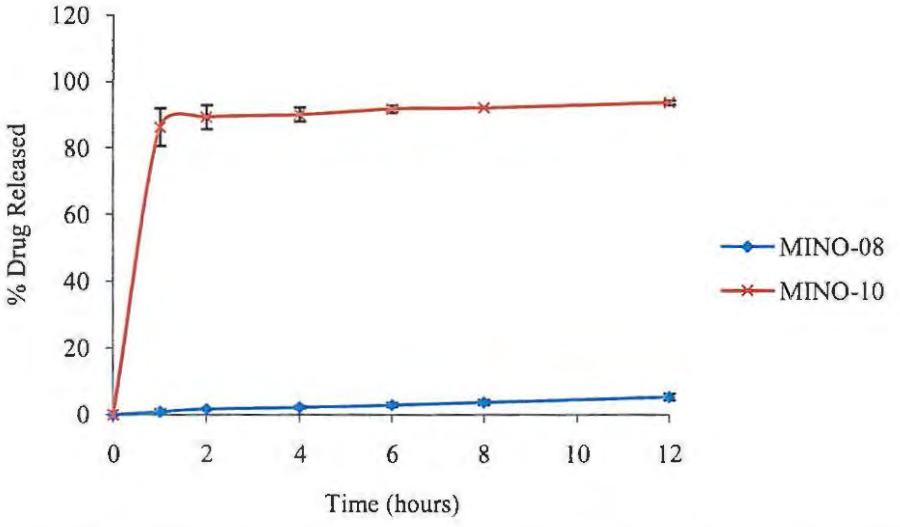


Figure 4.3. Dissolution profiles for batches MINO-08 (50% Compritol® w/w) and MINO-10 (50% Precirol® w/w)

The effect of the lipid on drug release from lipid matrices has been observed to be of utmost importance [167]. The effect of the lipid is primarily dependent on the chemical composition of the lipid, physical properties of the lipid and the composition of the dissolution medium used to test the formulations [167].

Several parameters in particular the acid value, hydroxyl value and saponification value have been used to describe lipids as these relate to the chemical composition and quality of the lipid material [167]. The behaviour of lipids in formulation development and dissolution may be inferred, in part, from these parameters. In a study to evaluate the effect of wax on drug dissolution, Cusimano and Becker [167] observed that dissolution from the lipid matrices they used could be correlated to the acid and hydroxyl values of the lipid used. The release of drug from waxes with high hydroxyl values was observed to be faster in both acidic and alkali dissolution media. This effect can be attributed to the fact that hydroxyl groups in any lipid impart some degree of hydrophilicity to the lipid, such that the matrix as well as the drug particles are easily wetted thereby potentially increasing the rate of drug dissolution from these particular waxes [167]. Cusimano and Becker [167] also observed that drug release from waxes with higher acid values and therefore a higher percentage of free fatty acids, was slower in acidic media and more rapid in basic media and attributed this result to the fact that fatty acids are less soluble in acidic media than they are in basic media. Compritol® and Precirol® have similar hydroxyl values of 102.6 and 106.4, respectively [168] and similar acid values of < 4 and < 6, respectively [126]. Based on these observations it can be inferred that Compritol® due to its lower hydroxyl value,

would retard the release on minocycline to a greater extent than would Precirol® as indicated by the results. However, the difference in hydroxyl and acid values is very small and cannot solely explain the large differences observed in the rate of release from MINO-08 and MINO-10. Higher solubility of a drug in the lipid may result in slower dissolution of the drug out of the matrix. However, as the lipid matrices in this study were not prepared using the fusion method, solubility of minocycline in the lipid matrices was expected to have a negligible effect on the release of minocycline from the formulations. Furthermore, solubility studies of minocycline hydrochloride were conducted during the course of these studies and indicated better solubility of minocycline in Precirol® (4 mg/g) than in Compritol® (2.5 mg/g). If solubility of minocycline in the lipids had a significant effect on minocycline release from the matrix, then release from Precirol® matrices would have been expected to be slower than that from Compritol® matrices.

Ozyazici et al. [126] studied the release of metronidazole from lipid matrices and reported similar results to those observed by Cumimano and Becker [167]. The acid value and hydroxyl value of a lipid contributes to the overall hydrophile-lipophile balance (HLB), which ultimately determines how lipophilic the lipid is. Ozyazici et al. [126] observed the slowest rate of release of metronidazole from lipid matrices manufactured using carnauba wax and the fastest rate of release from matrices manufactured using stearic acid. The carnauba wax is the more lipophilic matrix that does not permit water to penetrate the pores of the matrix structure and hence retards drug release. In contrast the stearic acid is not as lipophilic and permitted hydration of the matrix hence facilitating drug release [126]. The rate of drug release from matrices prepared from Compritol® and Precirol® were between those of carnauba wax and stearic acid [126]. Compritol® and Precirol® have a similar hydrophobic character and identical HLB values of 2 [135] and it is therefore expected that the rate of release from matrices formed from these two lipids would be similar, if all physicochemical considerations are equal. In contrast to the results observed in this study, Ozyazici et al. [135] observed faster drug release rates from Compritol® than from Precirol® matrices that was attributed to the non-ionic surfactant nature of Compritol® [126]. Although other studies have demonstrated slow drug release from Precirol® matrices, comparisons with Compritol® were not included in those studies [127,169].

The in vitro release characteristics of carbamazepine (a less hydrophilic drug compared to minocycline hydrochloride) from lipophilic, amphiphilic and water dispersible Gelucire® matrices has been reported [117]. As expected, drug release was faster from water dispersible bases. Similar lipophilic bases in terms of hydrophobic character were studied i.e. Gelucire® 33/01 and

Gelucire® 39/01. Galal et al. observed that drug release was faster from matrices of Gelucire® 33/01 with 65% carbamazepine released in eight hours compared to only 10% released from Gelucire® 39/01 matrices. This difference in drug release was attributed to the difference in melting point of the Gelucire® bases. The higher amount of drug released from Gelucire® 33/01 may be as a result of the lower melting point of this lipid which results in a high degree of dispersibility of the Gelucire® 33/01 base after melting, providing a large area for surface release of the drug [117]. Wu et al. [170] observed a higher degree of retardation of release of potassium chloride (a more hydrophilic drug than minocycline hydrochloride) from matrices manufactured using Gelucire® bases with high melting points, indicating that the melting point of the Gelucire® used was the most influential factor on potassium chloride release from these bases.

Several factors may contribute to the differences in drug release observed from matrices formed using different lipids. Compritol® and Precirol® have similar hydrophobic characteristics but the major physical difference between these two lipids is their meting points. As all other experimental conditions were similar it can be concluded that the most likely reason for the observed difference between the release profiles of minocycline from batches MINO-08 and MINO-10, is a consequence of the lower melting point of Precirol® (±57°C) compared to that of Compritol® (±70°C). The warm dissolution medium may have caused the softening and consequent dispersibility of the Precirol® matrix, which resulted in the formation of a greater area for the surface dissolution of minocycline hydrochloride from this formulation. The high solubility of minocycline hydrochloride in aqueous medium may also have contributed to its rapid release once a greater surface area was available for dissolution of the drug.

The effect of concentration of Compritol® used in the formulations on the release of minocycline was investigated and the release profiles are depicted in Figure 4.4.

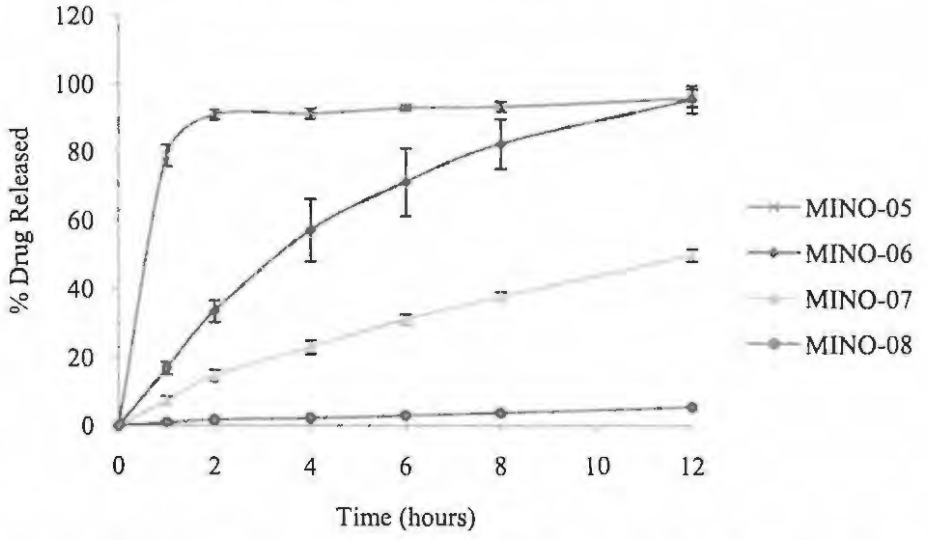


Figure 4.4. Dissolution profiles for MINO-05, MINO-06, MINO-07 and MINO-08 (10, 20, 30, 50% Compritol® w/w, respectively)

The rate and extent of minocycline release from lipid matrices was observed to decrease as higher concentrations of Compritol® were used in the formulation as shown in Figure 4.4. This observation was consistent with that observed by Li et al. [131] who reported a decrease in the rate and extent of sodium ferulate release from Compritol®-based directly compressed tablets as the concentration of Compritol® used was increased. Saraiya and Bolton [127] also observed a correlation between the rate and extent of theophylline release and concentration of lipid used in the matrices. As the concentration of lipid used to form the matrix was increased, the rate and extent of release of theophylline decreased.

As the release of drugs from lipid matrices is largely dependent on the concentration of aqueous medium that penetrates the matrices, the decrease in rate and extent of minocycline release at higher concentrations of Compritol® can, in part be attributed to the fact that more hydrophobic systems result when higher concentrations of Compritol® are used to manufacture formulations. As a consequence it becomes increasingly difficult for the aqueous dissolution medium to penetrate the lipid matrices resulting in minimal dissolution of drug and hence a slower drug release rate. Alternatively, one may view Compritol® as having the ability to "coat" drug particles [131] and therefore infer that at higher concentrations of Compritol® the drug particles are

"coated" to a greater degree with lipid and dissolution in an aqueous medium is thus slower as there is a slower rate of wetting of the drug particles.

4.4.3 The effect of diluent type on the rate and extent of minocycline release

The use of different diluents in the formulation of minocycline sustained release capsules was found to have an impact on the rate of release of minocycline from both the HPMC and Compritol® matrices manufactured and tested in these studies. The rate of release from HPMC matrices with lactose as a diluent was observed to be faster than when microcrystalline cellulose (MCC) was used as a diluent and these profiles are depicted in Figure 4.5.

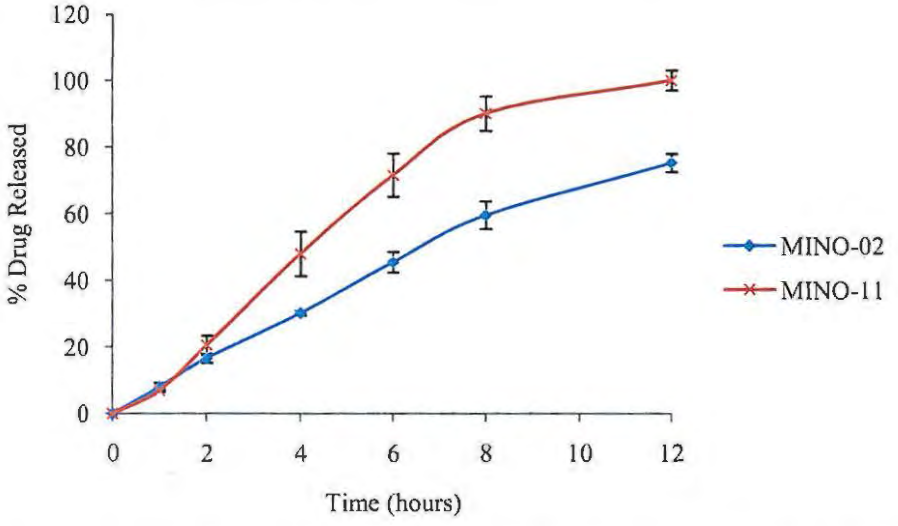


Figure 4.5. Dissolution profiles for batches MINO-02 (20% HPMC w/w - MCC) and MINO-11 (20% HPMC w/w - Lactose)

Almost 100 % of minocycline was released from HPMC matrices in which lactose was added whereas < 80% minocycline was released when MCC was used as a diluent. Similar results were observed by Lapidus and Lordi [171] who reported that the rate of release of chlorpheniramine was shown to increase when a hydrophilic diluent such as lactose was used in a formulation in place of calcium phosphate that is less soluble [171]. Sako et al. [172] showed that although the addition or substitution of hydrophilic diluents did not influence the release of drugs from HPMC matrices in vitro they had a profound effect in vivo and reported an increased rate of drug release from the matrices following administration to volunteers.

The use of lactose as a diluent in place of MCC had the opposite effect for Compritol® matrices to that observed for HPMC matrices. The rate of minocycline release was faster from Compritol®

matrices when MCC rather than lactose was used as a diluent and the release profiles are depicted in Figure 4.6. These data are similar to those reported by Malamataris et al.[132] who observed that the release of diprophylline from Precirol® matrices was faster when MCC was used as a diluent than when lactose was used.

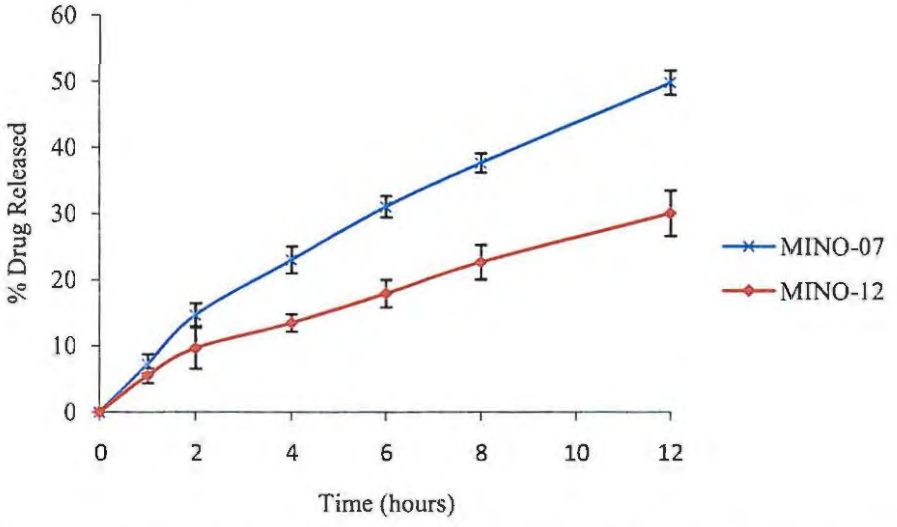


Figure 4.6. Dissolution profiles for batches MINO-07 (30% Compritol® w/w - MCC) and MINO-12 (30% Compritol® w/w - Lactose)

Furlanetto et al. [173] in studies designed to identify formulation variables that affect drug release from hydrophilic polymeric and lipid matrices showed that the overall drug release rates from matrices was as a result of the combined effect of the diluent type, matrix forming polymer diluent ratio, type of matrix and the solubility of drug to be included in the formulation. The use of lactose as a diluent in both hydrophilic and lipid matrices has been observed to increase the rate of drug release due to the hydrophilic nature of lactose which permits dissolution of the carbohydrate in water, producing pores and channels in the matrices through which drug may diffuse and be released [131,165]. However, it has been noted that the use of a hydrophilic diluent with a freely soluble drug may reduce drug release as a result of a competitive effect for dissolution in an aqueous medium in the dosage form [173]. Although MCC is less hydrophilic than lactose and is therefore expected to cause a slower rate of drug release, it has been observed that MCC may actually increase drug release rates due to its ability to swell and draw additional water into the matrix, thereby promoting dissolution of the drug [132,174].

The increased rate of release for minocycline observed when using lactose as a diluent in HPMC matrices can be attributed to more porous matrix being formed following the dissolution of lactose. In contrast the faster release rates observed with the use of MCC as a diluent in

Compritol® base formulations may be attributed to the ability of MCC to draw water into the matrix thereby permitting faster drug dissolution and subsequent release.

4.4.4 The effect of additives on minocycline release

Excipients such as surfactants [130,131,175] and hydrophilic channelling agents [117,169,175-179] have been widely applied in formulation development to optimize the rate of drug release from both hydrophilic and lipid matrices. The effect of incorporating disintegrant on the release of minocycline from both HPMC and Compritol® matrices was investigated. In addition the effect of addition of surfactant on the release of minocycline from Compritol® matrices was studied.

4.4.4.1 The effect of disintegrant on minocycline release

The effect of disintegrant on minocycline release from HPMC and Compritol® based formulations was investigated by incorporating 2% w/w sodium starch glycolate to the formulations. The resultant dissolution profiles are shown in Figure 4.7 to Figure 4.10.

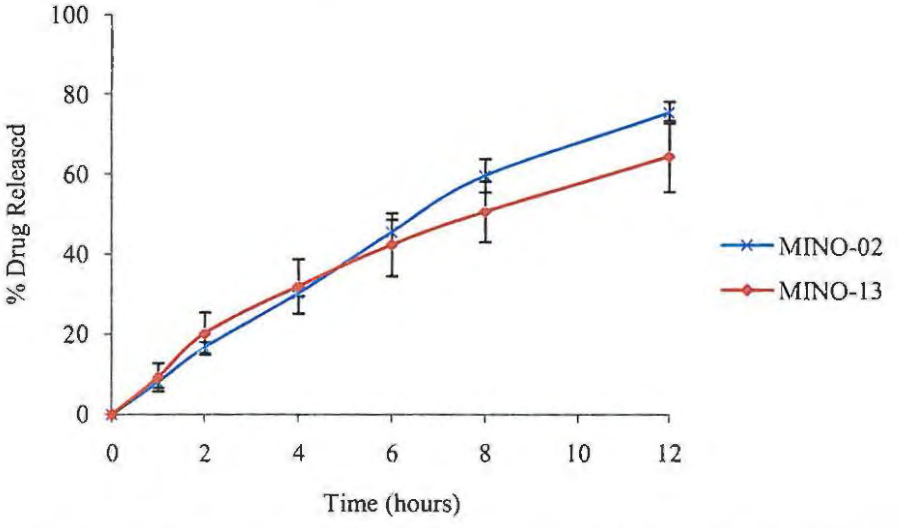


Figure 4.7. Dissolution profiles for batches MINO-02 (20% HPMC w/w - MCC) and MINO-13 (20% HPMC w/w + 2% w/w Sodium starch glycolate - MCC)

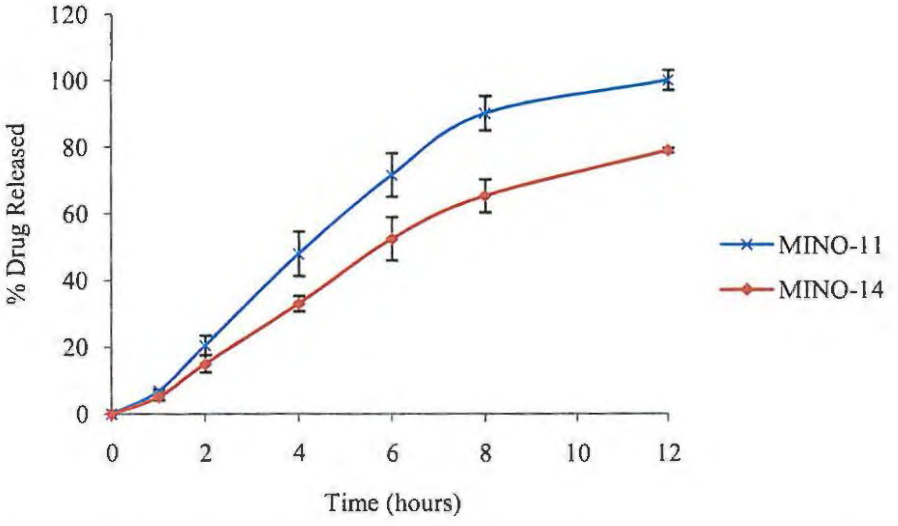


Figure 4.8. Dissolution profiles for batches MINO-11 (20% HPMC w/w - Lactose) and MINO-14 (20% HPMC w/w + 2% starch glycolate - Lactose)

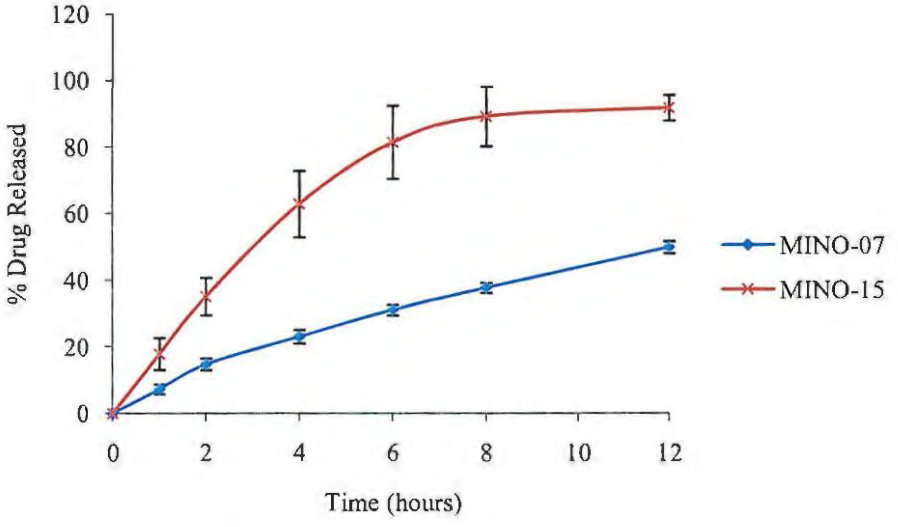


Figure 4.9. Dissolution profiles for batches MINO-07 (30% Compritol® w/w - MCC) and MINO-15 (30% Compritol® w/w + 2% w/w Sodium starch glycolate - MCC)

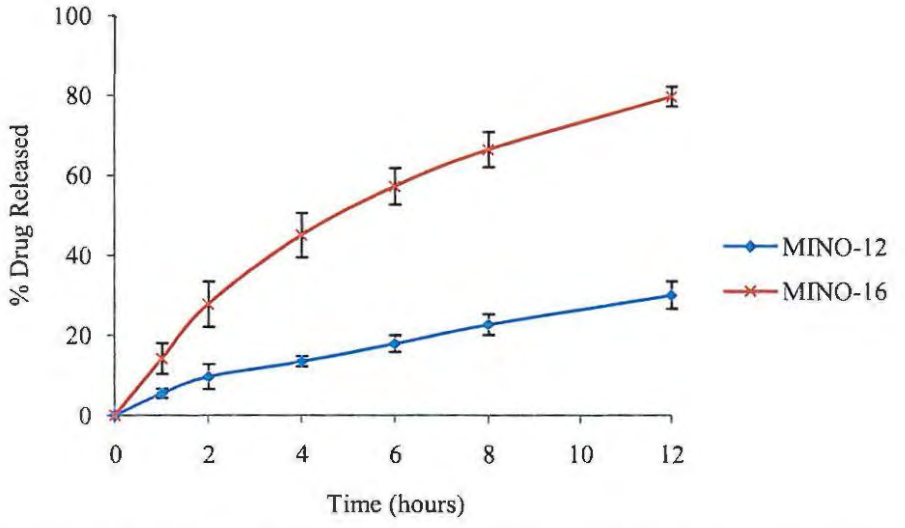


Figure 4.10. Dissolution profiles for batches MINO-12 (30% Compritol® w/w - Lactose) and MINO-16 (30% Compritol® w/w + 2% w/w Sodium starch glycolate - Lactose)

The rate of release of minocycline from HPMC matrices did not improve when sodium starch glycolate was incorporated in the formulation irrespective of the diluent used and these data are depicted in Figures 4.7 and 4.8. However the rate and extent of minocycline release increased when sodium starch glycolate was incorporated into Compritol® matrices irrespective of the diluent used and these data are shown in Figures 4.9 and 4.10.

Drug release has been found to increase [175,176,180] or show no improvement [180] with the incorporation of disintegrant into HPMC matrices. Disintegrants are incorporated into matrix formulations in an effort to increase the rate of release of the API in that formulation [176]. The rapid uptake of water by the disintegrant followed by its swelling leave the matrix highly porous and weak leading to the disintegration of that matrix and subsequent release of the drug formulated into that delivery system [124,176]. In some instances, particularly when lower concentrations of hydrophilic polymers are used, the rapid swelling of the disintegrant on initial contact with water may prevent the formation of a coherent gel structure on the surface of the matrix leading to faster drug release rates [180]. In this study, the addition of disintegrant to the formulation showed no improvement in the rate of release of minocycline. In fact a slightly negative effect on drug release was observed which may be due to the initial rapid uptake of water by the disintegrant thereby permitting faster hydration of HPMC and a more rapid formation of a gel layer which results in a greater degree of retardation of minocycline release.

Galal et al. [117] observed an increase in the rate of drug release from Gelucire® based matrices when disintegrants such as croscarmellose sodium (1-10% w/w) were added to Gelucire® 53/10 and that resulted in an increase in the release of carbamazepine [117]. This increase in drug release was attributed to the increased penetration of dissolution medium into the matrix resulting in the rapid disruption and erosion of the matrices with a subsequent rapid rate of release of the drug. The observed increase in minocycline release from Compritol® matrices with the addition of sodium starch glycolate in our study was attributed to the increase in water uptake by the matrix as a result of rapid swelling of sodium starch glycolate. The presence of additional water in the matrix resulted in faster dissolution of minocycline thereby causing an increase in the overall release of minocycline from the matrix.

4.4.4.2 The effect of surfactant on minocycline release

The effect of surfactant on the release of minocycline from Compritol® based formulations was investigated by incorporation of concentrations of 0.5% and 1% w/w of Poloxamer 188 to the formulations. Dissolution profiles of minocycline from formulations incorporating surfactant are depicted in Figures 4.11 and 4.12.

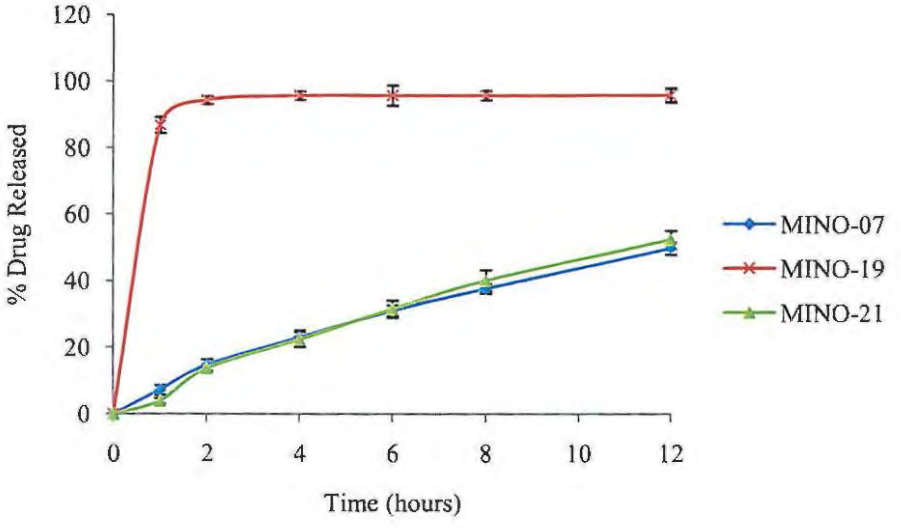


Figure 4.11. Dissolution profiles for batches MINO-07 (30% Compritol® w/w - MCC), MINO-19 (30% Compritol® w/w + 1% w/w Poloxamer 188 - MCC) and MINO-21 (30% Compritol® w/w + 0.5% w/w Poloxamer 188 - MCC)

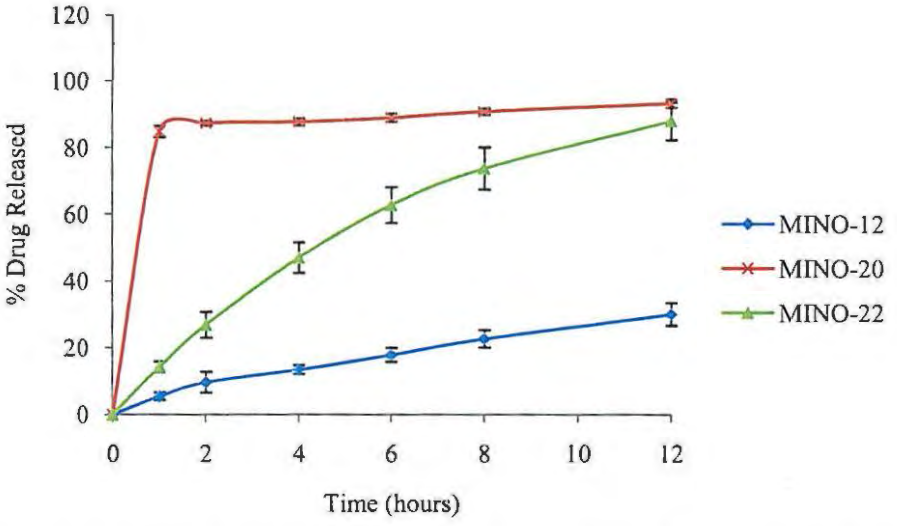


Figure 4.12. Dissolution profiles for batches MINO-12 (30% Compritol® w/w - Lactose), MINO-20 (30% Compritol® w/w + 1% w/w Poloxamer 188 - Lactose) and MINO-22 (30% Compritol® w/w + 0.5% w/w Poloxamer 188 - Lactose)

As shown in Figure 4.11 and 4.12, the rate and extent of minocycline release from Compritol® based formulations increased with the addition of poloxamer 188 to the formulation. As is more clearly illustrated in Figure 4.12, this increase in the rate and extent of release could be correlated to the concentration of the surfactant added, with higher surfactant concentrations resulting in faster drug release.

The results generated in these studies are consistent with those reported by Li et al. [131], who observed an increase in sodium ferulate release from Compritol® based matrices when polysorbate-80 or poloxamer 188 were added to the formulation. Jannin et al. [130] also observed an increase in theophylline release from Precirol® matrices with the addition of Lutrol® F68 and Lutrol® F127 to the formulation.

The HLB value of a formulation is a major determinant of the degree to which water will penetrate that matrix and allow drug dissolution. The addition of surfactant to a lipid matrix increases the HLB value of the formulation thus facilitating the penetration of additional water to the matrix with a resultant faster dissolution rate of drug [130]. Three types of interactions may affect the rate of drug release from matrices and include electrostatic interactions, hydrogen bonding and hydrophobic interactions [131,181]. Hydrophobic interactions and textural properties of matrices are the most important interactions that determine drug release from a lipid matrix [181]. The interface between hydrophobic lipid carrier and hydrophilic drug is thought to

hinder the release of the drug from the matrix [131]. The addition of a surfactant to a formulation is believed to adjust hydrophobic interactions between the drug and lipid matrix resulting in an increased rate of release of the drug [131]. The observed increase in the rate and extent of minocycline release from formulations MINO-19, MINO-20, MINO-21 and MINO-22 in which poloxamer 188 was incorporated may be attributed to the resultant increase in the HLB of the system. This may have resulted in an increased uptake of water by the formulation with the consequent faster dissolution and faster diffusion of minocycline into the dissolution medium.

Careful consideration of the type of surfactant to be added to formulations is important so as to attain the desired rate of drug release from a specific formulation. As the overall HLB value of a formulation is a major determinant of drug release from such formulations in aqueous medium, it is therefore easy to assume that surfactants with higher HLB values will promote the rate of drug release from lipidic matrices. However Jannin et al. [130] observed that at surfactant concentrations greater than 25% w/w, the less hydrophilic Lutrol® F127 produced a more pronounced increase in theophylline release from Precirol® based matrices than the more hydrophilic Lutrol® F68 (Poloxamer 188). This result was attributed to the fact that Lutrol® F127, as it is less water-soluble swelled to a greater extent than Lutrol® F68, creating pores in the matrix through which more drug could be dissolved [130]. At surfactant concentrations of 2% w/w, Li et al. [131] observed a more pronounced increase in the release of sodium ferulate from Compritol® based matrices using the less hydrophilic surfactant polysorbate-80 as compared to that from the more hydrophilic surfactant, Poloxamer 188. This difference in effect was attributed to the higher solubility of polysorbate-80 in the lipid matrix which may have aided the diffusion of dissolved drug out of hydrophobic pores or channels in that matrix [131].

4.4.5 The effect of drug release from a matrix combination of HPMC and Compritol®

The addition of hydrophilic polymers to lipid matrices has been applied in formulation development to optimise drug release rates. Parab et al. [169] observed that the rate and mechanism of release of theophylline from Precirol® based matrices differed when both mannitol and HPMC were added to the formulation, from when either one of the two was used separately in the formulation. As shown in Figure 4.13 the combination of Compritol® and HPMC had an additive effect on the retardation of minocycline release although this effect was small. The combination of HPMC and Compritol® resulted in less variable release of minocycline from these dosage forms.

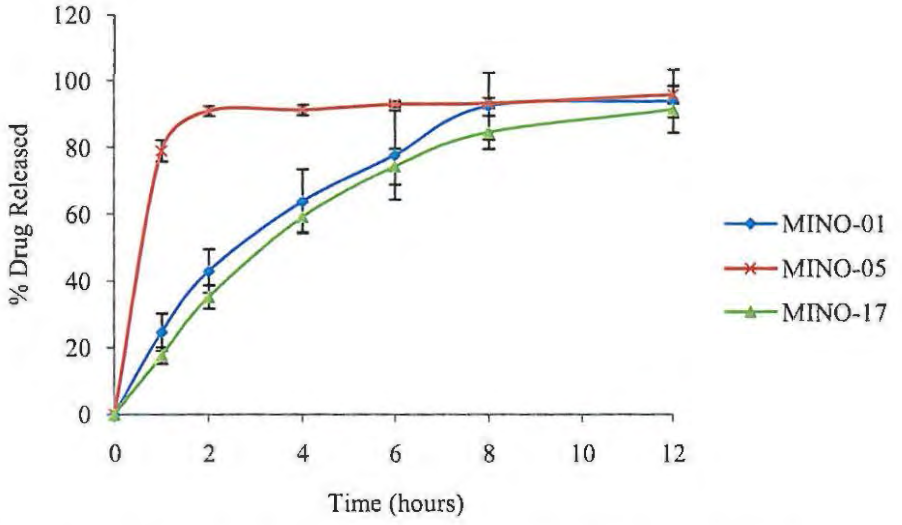


Figure 4.13. Dissolution profiles for batches MINO-01 (10% w/w HPMC - MCC), MINO-05 (10% w/w Compritol® - MCC) and MINO-17 (10% Compritol® w/w + 10% HPMC - MCC)

4.4.6 USP Delayed-release dissolution test for selected formulations

Resultant dissolution profiles of minocycline from formulations MINO-06, MINO-11 and MINO-17 that released at least 80% of minocycline following "near-zero order" kinetics based on visual analysis were tested using the USP conditions for delayed release formulations and the profiles are depicted in Figures 4.14 to 4.16.

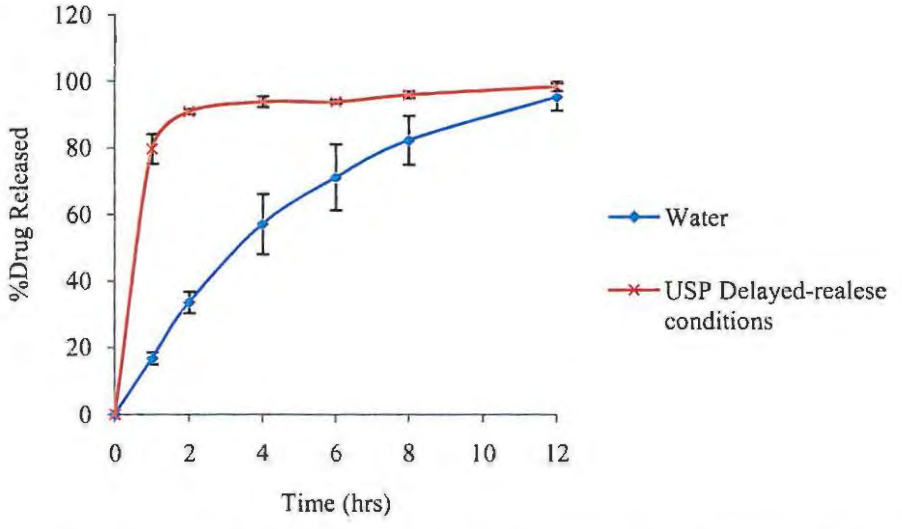


Figure 4.14. Dissolution profiles for batches MINO-06 (20% Compritol® – MCC) under study dissolution conditions and USP delayed-release test dissolution conditions

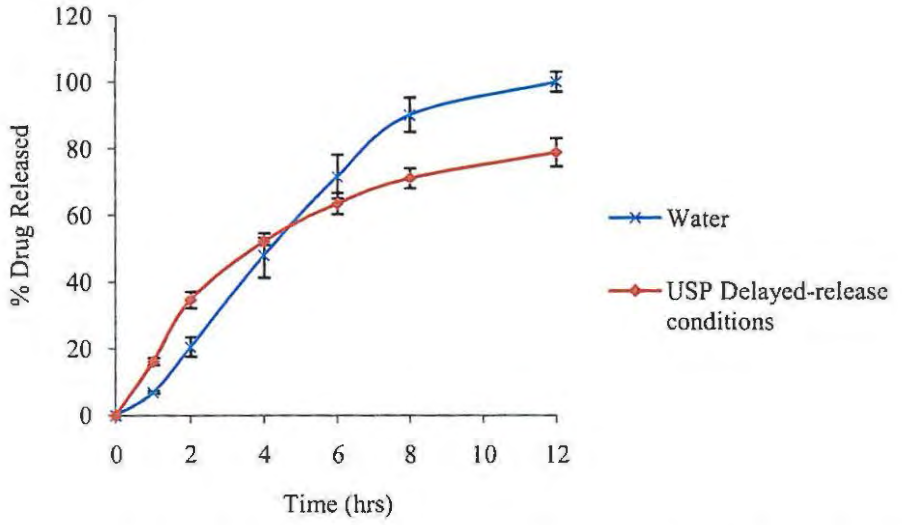


Figure 4.15. Dissolution profiles for batches MINO-11 (20% HPMC – Lactose) under study dissolution conditions and USP delayed-release test dissolution conditions

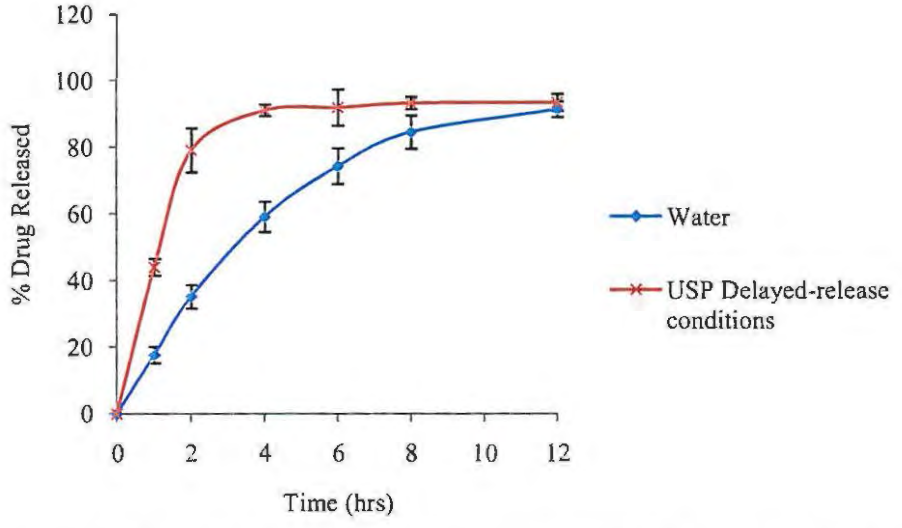


Figure 4.16. Dissolution profiles for batches MINO-17 (10% Compritol® + 10% HPMC – MCC) under study dissolution conditions and USP delayed-release test dissolution conditions

The use of the USP two-stage test conditions for dissolution testing of delayed release formulations, revealed that the minocycline formulations developed in these studies and based on lipidic matrices did not adequately sustain drug release. Batch MINO-06 was comprised of 20% w/w Compritol® yet 95% of the drug was released within two hours of the commencement of dissolution testing as shown in Figure 4.14. Similarly for Batch MINO-17 that was comprised of 10% w/w of each of Compritol® and HPMC revealed that 95% of the minocycline was released within four hours as shown in Figure 4.16.

Drug release from formulations based on lipid or wax matrices has been observed to be dependent on the pH of the dissolution medium due to the chemical composition of the lipid bases [167]. Compritol® is an ester of behenic acid [121] and may undergo hydrolysis in aqueous acid media [182]. Hydrolysis of the ester yields a less hydrophobic product, thereby facilitating the penetration of water into the lipid matrices promoting the subsequent dissolution and faster release rates of minocycline. As formulation MINO-17 was comprised of both Compritol® and HPMC the release of minocycline was less rapid than that of MINO-06 which was comprised only of Compritol® as the retardant, as the HPMC swells and provides a sustaining effect despite the hydrolysis of Compritol® in the acid medium.

The rate and extent of drug release from MINO-11 that was comprised of 20% w/w HPMC and lactose as the diluent was slower when tested using the USP two-stage test conditions than with water as the dissolution medium and as is shown in Figure 4.15. HPMC and lactose are pH-independent materials [172] and any change in drug release can be attributed to other formulation variables. The ionic state of a drug in matrix formulations is an important factor in in vitro testing of drugs [124,183]. Drug molecules that exist in their ionised state are more soluble in aqueous dissolution media than unionised species, resulting in faster in vitro release rates from matrices [183]. Minocycline has four reported pKa values or dissociation constants of 5 and 9.5 for the amine functional groups and 2.8 and 7.8 for the hydroxyl groups as reported in Chapter 1. The overall slower rate of release of minocycline under the USP two-stage test conditions may be attributed to the ionisation state of minocycline which may be less favourable for its dissolution under the pH ranges used in the USP "two-stage" dissolution test.

4.5 CONCLUSIONS

The objective of preliminary dissolution studies was to determine the effect of formulation variables on the development of minocycline sustained release dosage forms. The use of the USP Apparatus 1 and de-ionised water as dissolution medium was successfully applied to these assessments and provided a means to discriminate between the different minocycline formulations manufactured. Following dissolution testing a hydrophilic polymer matrix comprised of 20% w/w HPMC and a lipid matrix of 30% w/w Compritol® were identified for further optimisation. The target profile for minocycline release was that drug release was to follow zero-order kinetics and that at least 80% of the minocycline in the formulation was to be released in a 12 hour period.

The optimisation of minocycline release from the lipid and hydrophilic matrices was achieved by using different diluents and the addition of different hydrophilic excipients to the formulations. The use of different diluents and the addition of disintegrant and surfactant was found to affect minocycline release from both hydrophilic and lipophilic matrices.

The three formulations with dissolution profiles similar to that of the target dissolution profile were tested using the USP "two-stage" test for delayed-release dosage forms. This test gave a better indication of the potential in vivo release behaviour of the dosage forms as it is comprised of an acid stage with a pH similar to that found in the stomach and a buffer stage with a pH similar to that found in the intestines. All formulations behaved differently in this test due to the different chemical characteristics of the excipients and the ionic state of minocycline in the different dissolution media. Based on this information it may be beneficial to conduct preliminary dissolution investigations using the USP delayed release conditions as opposed to water to avoid such situations in which formulations that showed optimal release patterns fail in media that are better indicative of the conditions in vivo.

Based on the obtained results, HPMC based formulations may be more appropriate for further investigation of minocycline sustained release dosage forms as they are more resistant to changes in pH of dissolution media. However, Compritol® was successfully used to sustain minocycline when water as used as the dissolution indicating its potential for use as a polymer for sustaining drug release. Further studies focussing on protecting fatty esters from extremes of pH may be of benefit for the successful application of lipids as sustained release polymers.

CHAPTER FIVE

5 MATHEMATICAL MODELING AND COMPARISON OF IN VITRO DISSOLUTION PROFILES

5.1 INTRODUCTION

The comparison of dissolution profiles plays an important role in the course of product development [184,185]. The comparison of dissolution profiles provides a basis for establishing the final dissolution specifications for a pharmaceutical dosage form for the purposes of quality control and to demonstrate the batch to batch consistency of a product [184,185]. Furthermore the comparison of dissolution profiles provides a basis for establishing the similarity of pharmaceutical dosage forms for which the composition, manufacturing site, scale of manufacture and manufacturing processes may have changed within specifically defined limits [186]. The methods used for the comparison of dissolution profiles may be classified as exploratory data analysis, statistical, model-independent and model-dependent methods and/or models [184].

5.2 EXPLORATORY DATA ANALYSIS

Exploratory data analysis methods are useful as initial means of assessing dissolution data from a graphic or numerical perspective [184]. Graphically the dissolution curves to be compared, i.e. a test and reference(s) product or formulations are plotted on the same set of axes with error bars at the same time points and that extend to twice the standard error of estimates [184]. As the standard error bars are considered to represent an approximate 95% confidence interval, the formulations can be considered to be significantly different to a reference formulation if the error bars for the time points under consideration do not overlap [184].

Graphical comparisons of dissolution profiles may be complemented by presenting the mean and standard deviation of dissolution data for a test and reference formulation at each time point, numerically [184]. Dissolution profiles may be considered significantly different at a 5% level of significance if the 95% confidence interval for the mean difference ($reference-test$) at a specific time point does not represent a numerical value of zero [184].

The use of exploratory methods of data analysis is not comprehensive due to the difficulty of concluding if dissolution profiles are different in cases where the error bars for measurements overlap at some of the times data are accumulated [184]. Furthermore, it is challenging to interpret results when comparing more than two formulations as graphical depiction or illustration of the data becomes cluttered and the summary table for that data becomes too large [184].

5.3 STATISTICAL METHODS

The comparison of in vitro dissolution profiles has been conducted using statistical methods based on the analysis of variance (ANOVA-based methods) [184,187,188]. Statistical methods test the statistical difference between dissolution profiles in terms of the shape and size of the curves and provide probability values related more to statistical equivalence than to pharmaceutical similarity [177].

ANOVA-based methods may be further differentiated as one way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) methods [189,190]. One way analysis of variance methods execute separate statistical comparisons of mean dissolution data at each time point and are equivalent to t-tests when dissolution data for only two formulations are compared [184]. In cases of the comparison of more than two data sets, posteriori comparisons such as Tukey's Multiple Range Test, Scheffé Method, Newman-Keuls Test and Dunnett's Test may be used to establish where, if any, differences arise [191]. Multivariate analysis of variance methods assess the difference between the mean of dissolution data sets in multiple time point dissolution tests [189].

One way analysis of variance methods have been criticised as they ignore the correlation between the data at the sample time points within a single dissolution profile [184]. The risk of incurring a Type I error is relatively high when using one way analysis of variance methods and when statistical differences are observed at some but not all time points, it is difficult to conclude if the profiles are different [184]. Despite the criticisms of one way analysis of variance methods they provide more informative and easier to interpret data compared to MANOVA methods of data analysis [187].

ANOVA-based methods have also been reported to be overly discriminating and not useful for the analysis of pharmaceutical data [190]. The use of ANOVA-based methods is not recommended and there in no mention of these methods in FDA guidance documents [184].

5.4 MODEL-INDEPENDENT METHODS

Model-independent methods used for comparison of dissolution profiles do not require the use of a preconceived or fitted model [177]. Model independent models may be further divided into ratio tests and pair-wise procedures [189].

5.4.1 Ratio tests

Ratio tests involve the comparison of parameters obtained at the same time point from the release profiles of a test and reference and formulation [189]. The comparison may be expressed as the ratio of the percent drug released from each formulation at specific time points, ($t_{x\%}$), as a ratio of the area under the curve (AUC) of the two formulations or the ratio of the mean dissolution time (MDT) for each formulation [189]. The mean dissolution time may be calculated using Equation 5.1:

$$MDT = \frac{\sum_{j=1}^{n} t_j \, \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \qquad \textbf{Equation 5.1}$$

Where,

j = sample number
n = number of dissolution sample times
t_j = time at midpoint between t_j and t_{j-1}
ΔM_j = additional amount of drug dissolved between t_j and t_{j-1}

Ratio tests, particularly the time point approach, ($t_{x\%}$) have been criticised for their inadequacy to completely characterise dissolution profiles as they do not take into account profiles that do not follow a single path or which cross over [192]. Although such a cross over may be insignificant for immediate release products as the time scale of release is short, such occurrences with controlled release products may have significant implications for quality assurance and the establishment of in-vitro-in-vivo correlations [192].

5.4.2 Pair-wise procedures

Two commonly used pair-wise procedures used for the comparison of dissolution profiles are the difference (f_1) and similarity (f_2) factors [193], and the Rescigno index [194].

5.4.2.1 The difference and similarity factor

The difference and similarity factors, first described by Moore and Flanner [193], constitute the most widely known model-independent approach for the comparison of dissolution profiles [177]. The difference factor, f_1, is the sum of the absolute values of vertical distances between the mean values for a test and reference product at each dissolution time point and is expressed as the percentage of the sum of the mean fraction released from the reference formulation at each time point as depicted in Equation 5.2 [184]. The similarity factor, f_2, is a logarithmic transformation of the average of the squared vertical distances between the mean test and the reference dissolution time points, which may be multiplied by an appropriate weighting factor, usually set as 1 and is calculated using Equation 5.3 [184].

$$f_1 = \frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100 \qquad \textbf{Equation 5.2}$$

$$f_2 = 50 \times log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t|R_t - T_t|^2\right]^{-0.5} \times 100\right\} \qquad \textbf{Equation 5.3}$$

Where,

n = number of dissolution time points
R_t = percent dissolved of reference product at each time point, t
T_j = percent of dissolved of test product at each time point, t
w_t = optional weight factor

The difference and similarity factors have been adopted by the Center for Drug Evaluation (FDA) and may be used for the comparison of dissolution profiles if at least three dissolution time points are available for use in computing the factors [137,186,195]. The similarity factor in particular has been widely recognised as a valid criterion to assess the similarity between two dissolution profiles and should ideally be used following testing of at least twelve individual dosage form units [137]. Values of f_1 between 0 and 15, and of f_2 between 50 and 100 indicate similarity or sameness of the two dissolution profiles under comparison.

It has been recommended that only one data point after 80% of the drug has been released should be included in the calculation of the difference and similarity factors, as the region of interest for comparability normally lies between 0 and 80% of dissolution, i.e. before the plateau region of the dissolution profile is reached [185].

The main advantage of using the difference and similarity factors for the comparison of dissolution of profiles is that they are easy to compute and provide a single number to describe the comparison of dissolution profiles [184].

Despite the useful nature of the difference and similarity factors for the comparison of dissolution profiles there are several limitations. In particular, interchanging the reference and test formulations in the calculation for the difference factor will result in a different value for the difference factor being calculated [184,189]. The similarity factor is insensitive to the shape of the dissolution profile and does not take into account information relating to unequal spacing between sampling points [196]. Furthermore as the similarity factor is a function of the mean difference between curves, it does not take into account variability within the test or reference formulations, making careful interpretation of data for batches with high inter-batch variability, necessary [197]. As the similarity factor is a sample statistic that cannot be used to formulate a statistical hypothesis for assessment of dissolution similarity, it is difficult to access the Type I and Type II error rates that may be associated with its use [196,197].

5.4.2.2 Rescigno Index

The Rescigno indices, first described by Rescigno [194] as bioequivalence indices to measure the difference between a test and reference product based on blood plasma concentration functions may also be applied to the comparison of dissolution profiles based on drug concentrations in dissolution samples [184,189]. The Rescigno indices are denoted by $\xi_i\,(i = 1,2)$, and may be calculated using Equation 5.4 [184].

$$\xi_i = \left(\frac{\int_0^{t_n} |R_t - T_t|^i dt}{\int_0^{t_n} |R_t + T_t|^i dt}\right)^{\frac{1}{i}} \quad i = 1,2 \qquad \textbf{Equation 5.4}$$

Where,

R_t = mean percent drug dissolved for reference product at time (t)
T_t = mean percent drug dissolved for test product at time (t)
t_n = final dissolution time point
t = time

A high value of i for the Rescigno index, implies a higher weighting of the magnitude of change in concentration than in the duration of that change [189]. The indices which may take up values between 0 and 1 inclusively measure the difference between two dissolution profiles [189]. A value of 0 or close to 0 indicates similarity between the mean dissolution profiles being compared and a value of 1 is obtained if one of the two mean dissolution profiles is zero at each dissolution time point [184]. The Rescigno indices, ξ_1 and ξ_2 are approximately analogous to the difference and similarity factors, respectively [184].

An advantage of using the Rescigno indices is that unlike the difference factor the value of the indices remains unchanged if the test and reference dissolution profiles are interchanged [184]. However the Rescigno indices are more difficult to compute compared to the difference and similarity factors and it is not clear how close their values should be to 0 in order for one to conclude that the mean dissolution profiles for the test and reference products are similar [184]. The use of the Rescigno indices is not included in any of the guidance documents published by the FDA [184].

5.5 MODEL-DEPENDENT METHODS

5.5.1 Zero order model

Zero-order kinetics can be used to model dissolution data from pharmaceutical dosage forms that do not disaggregate and release drug slowly over an extended period of time [189]. The dissolution profiles of pharmaceutical formulations that follow zero-order kinetics release the same amount of drug per unit time and are ideal for ensuring a prolonged action of the drug in the dosage form [189]. The zero-order model can be expressed using Equation 5.5:

$$Q_1 = Q_0 + K_0 t \qquad \textbf{Equation 5.5}$$

Where,

Q_1 = amount of drug dissolved in time t
Q_0 = initial amount of drug in the solution
K_0 = zero-order release constant
t = time

Zero-order kinetics have been successfully used to describe drug release from modified-release dosage forms based on matrices manufactured using polyethylene oxide and polyethylene glycol

polymers of various molecular weights [199,200]. Zero order kinetics are usually the preferred release kinetics for sustained release dosage forms.

5.5.2 First order model

First order kinetics were first applied to the evaluation of dissolution data by Gibaldi and Feldman [201] and can be expressed mathematically using Equation 5.6:

$$lnQ_t = lnQ_0K_1t \qquad \textbf{Equation 5.6}$$

Where,

Q_t = amount of drug released in time t
Q_0 = initial amount of drug in the solution
K_1 = first order release constant
t = time

Pharmaceutical dosage forms that display first order kinetics, particularly those with water soluble drugs in porous matrices [202] release the API in proportion to the amount of drug remaining in the dosage form and the rate of drug release declines as the amount of drug remaining in the dosage form diminishes [189]. The release of hydrophilic drugs from modified-delivery systems manufactured by poly-(ethylene glycol-b-(DL-lactic acid-co-glycolic acid)-b-ethylene glycol) has been shown to follow first order kinetics [203].

5.5.3 The Higuchi model

The Higuchi model was developed to study the release of water soluble and low solubility compounds incorporated in semi-solid and/or solid matrices [204,205]. Although the model was initially used to describe the dissolution of drugs in suspension in an ointment, it is now applied to other types of dissolution from other pharmaceutical dosage forms [189]. The Higuchi model is based on Fick's first law of diffusion where the fraction of drug released is proportional to the square root of time [82,189]. A simplified expression of the Higuchi model is shown in Equation 5.7 [189].

$$Q_t = K_Ht^{1/2} \qquad \textbf{Equation 5.7}$$

Where,

Q_t = amount of drug release at time t
K_H = Higuchi dissolution constant
t = time

5.5.4 Korsmeyer-Peppas model

The Korsmeyer-Peppas model or power law [206] is an empirical expression that relates the fraction of drug released over time as an exponential. The model incorporates a constant, k, that takes into account the structure and geometry of the dosage form and a release exponent, n, that is indicative of the mechanism of drug release [189]. A simplified expression of the Korsmeyer-Peppas model is shown in Equation 5.8 and in the log-transformed state in Equation 5.9:

$$\frac{M_t}{M_\infty} = kt^n \qquad \textbf{Equation 5.8}$$

$$log\left(\frac{M_t}{M_\infty}\right) = \log k + n\, logt \qquad \textbf{Equation 5.9}$$

Where,

$\frac{M_t}{M_\infty}$ = released fraction of drug at time t
k = constant incorporating structural and geometric characteristics of the dosage form
t = time
n = release exponent indicating drug release mechanism

The use of the release exponent, n, to characterise drug release mechanisms from dosage forms of different geometries was described by Peppas [207]. For a slab, Peppas concluded that values of n = 0.5 indicated Fickian diffusion and values of $0.5 < n < 1.0$ indicated mass transfer following a non-Fickian model. For a cylinder it was concluded that values of n = 0.45 indicated Fickian diffusion whilst values of $0.45 < n < 0.89$ represent drug release via non-Fickian diffusion mechanisms. A summary of the interpretation of the release exponent, n for release mechanisms from a slab is summarised in Table 5.1.

Table 5.1. Interpretation of the release exponent

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5 < n < 1.0$	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero-order release
> 1.0	Super Case-II transport	t^{n-1}

The Korsmeyer-Peppas model is generally used to characterise drug release from polymer based dosage forms in addition to dosage forms that display more than one type of release mechanism [189]. The Korsmeyer-Peppas model has been extensively used to model drug release from hydrophilic [82,208,209] and lipid matrices [210].

The application of the Korsmeyer-Peppas model requires that only the portion of the curve that displays up to a maximum of 60% drug release be used for model fitting [189].

5.5.5 Weibull model

The Weibull function was first adapted to model drug release processes and dissolution by Langenbucher [211] and is an empirical model that can be used for the characterisation of drug dissolution from a variety of dosage forms [212]. The log transformed expression of the Weibull model is shown in Equation 5.10:

$$log[-ln(1-m)] = b\ log(t - T_i) - log\ a \qquad \textbf{Equation 5.10}$$

Where,

m = accumulated fraction of drug in solution at time t
a = scale parameter definitive of the time scale of the process
b = shape parameter
T_i = location parameter representative of the lag time before the onset of the release process
t = time

From the expression in Equation 5.10 a linear relationship can be obtained for a log-log plot of $-ln(1-m)$ versus time (t). The shape parameter, b is obtained from the slope of the line and may be equal to, greater than or less than 1 depending on the shape of the curve as summarised in Table 5.2 [189]. The ordinate value, a, is estimated from the ordinate value ($1/a$) at time t = 1 [189].

Table 5.2. Interpretation of shape parameter, *b* in Weibull model

Value of *b*	Curve description
b = 1	Case (1), exponential
b > 1	Case (2), sigmoid, S-shaped, with an upward curvature followed by a turning point
b < 1	Case (3), parabolic with higher initial slope and after that consistent with exponential

The Weibull model lacks any kinetic basis and as a result has several limitations, particularly in that it does not adequately characterise the dissolution kinetics or intrinsic dissolution of a drug [189,213].

Despite the criticisms of the Weibull method it has been used successfully to describe drug release from fractal matrices [214]. The Weibull function is linearly related to the exponent, n of the power law derived from the analysis of the first 60% of dissolution profiles [214]. The value of b, can be used as an indicator of drug release from the matrices with values of $b \leq 0.75$

indicating that Fickian diffusion drives drug release. Values of b in the range $0.75 < b < 1$ indicate that drug release is a combination of Fickian diffusion and Case-II transport and values of $b > 1$ indicate that a complex release mechanism predominates [214].

5.6 SELECTION OF BEST-FIT MATHEMATICAL MODEL

Although the Higuchi, zero order, Weibull and Korsmeyer-Peppas models are the most widely used models to describe drug release [189], it is important that an analyst selects a model that best fits and describes their data.

A commonly used method to determine the 'best fit" model for the description of in vitro release data is the coefficient of determination, R^2 [189]. The value of R^2 tends to increase as the number of model parameters increases, posing a problem of inconsistency in selecting the "best fit" model [189]. The use of an adjusted coefficient of determination ($R^2_{adjusted}$) is regarded as more meaningful when comparing fitting models with different numbers of parameters to experimental data [189]. The adjusted coefficient of correlation is calculated using Equation 5.11 [189]:

$$R^2_{adjusted} = 1 - \frac{(n-1)}{(n-p)}(1 - R^2) \qquad \textbf{Equation 5.11}$$

Where,

n = number of dissolution data points
p = number of parameters in the model
R^2 = coefficient of determination

Several other methods may be used to assess the suitability of a mathematical model to fit experimental data. These methods include using the sum of squares of residuals (SSR), the mean square error (MSE), the Alaike's Information Criterion (AIC) and the F-ratio probability [189].

As only one parameter for all mathematical models used i.e. time (t) was manipulated in the dissolution studies, the coefficient of determination, R^2 was adequate for determining the model that best fitted minocycline dissolution profiles generated from formulations manufactured in these studies . Values of R^2 greater than 0.950 were considered acceptable for the comparisons investigated and higher values of R^2 indicated that the mathematical model used fitted the data better.

5.7 MATHEMATICAL TREATMENT OF IN VITRO RELEASE DATA

The primary objective of these studies was to manufacture formulations that released approximately 80% of minocycline at a constant rate over a 12 hour period. In order to elucidate if manufactured dosage forms followed this pattern of release, the difference and similarity factors [193] were used to compare all dissolution profiles of minocycline for all prepared formulations to the reference profile described in Section 4.3.1. Formulations with dissolution profiles similar to the reference profile based on the difference and similarity factors were further analysed using one way analysis of variance to elucidate the specific points at which differences and similarities occurred in the dissolution profile. Furthermore formulations MINO-06, MINO-11 and MINO-17 with dissolution profiles which appeared visually, to be similar to the target dissolution profile, were analysed using the one way analysis of variance method to determine if the profiles were indeed similar to the reference profile.

All in vitro dissolution profiles obtained in Section 4.4 were modelled by the Korsmeyer-Peppas model which was used as the primary mathematical model to investigate the effect of formulation variables i.e. polymer concentration, diluent type, addition of disintegrant and surfactant on the mechanism(s) of minocycline release. The zero order and the Higuchi model were also used to establish which model best described drug release from these formulations.

5.8 RESULTS AND DISCUSSION

5.8.1 Difference and similarity factors

The in vitro release profiles for minocycline obtained in the dissolution studies in Section 4.4 were compared to a target dissolution profile described in Section 4.3.1 using the difference (f_1) and similarity (f_2) factors. The results showed that only three of the formulations were similar to that of the target profile and the results are summarised in Table 5.3.

Table 5.3. Difference and similarity factors values for minocycline batches using the target profile (Section 4.3.1) as a reference

Batch #	Fit Factors	
	f_1	f_2
MINO-01	125	21.7
MINO-02	**11**	**71.5**
MINO-03	**15**	**59.5**
MINO-04	41.6	39.2
MINO-05	*	*
MINO-06	52.5	42.0
MINO-07	30.2	44.1
MINO-08	*	*
MINO-09	169.8	17.2
MINO-10	*	*
MINO-11	83.1	24.2
MINO- 12	55.9	33.7
MINO-13	24.1	54.7
MINO-14	**9.8**	**76.2**
MINO-15	113.3	22.5
MINO-16	31.8	52.5
MINO-17	52.7	41.1
MINO-18	*	*
MINO-19	*	*
MINO-20	*	*
MINO-21	30.5	45.6
MINO-22	32.3	51.7

* The difference and similarity factors were not applicable as the amount of minocycline released was greater than 80% before the second sampling point

The target minimum amount of minocycline to be released from the manufactured dosage forms was set at 80% within or at 12 hours. Visual analysis of the dissolution profiles of MINO-06, MINO-11 and MINO-17, indicated that the amount of minocycline released from these formulations was greater than 80% at 12 hours. Furthermore, a significant portion of each of the dissolution profiles appeared to follow zero order kinetics resulting in the selection of these dosage forms for further dissolution testing under the USP "two-stage" test for the assessment of delayed-release formulations. However, the importance of using statistical methods for the comparison of dissolution profiles was emphasised when the difference and similarity factors indicated that MINO-06, MINO-11 and MINO-17 were in fact different to the target release profile. MIN0-02, MINO-03 and MINO-14 which were not selected for further dissolution studies as the release of minocycline from these formulations was less than the required minimum of 80% in 12 hours were the only profiles that were regarded similar to the target profile when using the similarity and difference factors. Whilst MINO-06, MINO-11 and MINO-17 were selected for further dissolution testing as they released more than 80% of minocycline, the similarity and difference factors considered only the closeness of minocycline release from MINO-02, MINO-03 and MINO-14 to 80% regardless of whether this was above or below 80%.

Furthermore, the value of the similarity and difference factors is influenced by the actual pattern of drug release. It is therefore of utmost importance that a formulation scientist understands the factors that influence the results generated using different statistical methods of analysis, in particular the extent and pattern of release. The selection of an appropriate reference profile is vital, as it should allow the formulation scientist to generate reliable results relating to the desired pattern and extent of release from test formulations.

5.8.2 Analysis of variance

Although the interpretation of the similarity and difference factors is easier than other analyses as these factors are a single value that describes the similarity or difference between any two profiles, ANOVA-based methods may be more informative in formulation development studies in order to match a reference product as closely as possible since they show the exact point(s) of difference between two formulations [187].

The one way analysis of variance results for dissolution profiles of minocycline obtained for MINO-02, MINO-03 and MINO-12 and MINO-06, MINO-11 and MINO-17 in Section 4.4 were tested against the target profile and are summarised in Table 5.4 and 5.5, respectively.

One of the challenges of ANOVA-based methods is the overall conclusions of similarity or difference between any two profiles when the difference is significant at some but not all dissolution points. As observed in Table 5.4, the difference between the target profile and MINO-02 is significant at three of the six sampling points, whereas the difference between the target profile and MINO-03 is significant at only two of the six sampling points. The differences between the target profile and MINO-14 is significant at four out of six sampling points. Based on these observations it is clear that formulations that are similar (based on the similarity and difference factors) may in reality be different when using ANOVA-based methods for data analysis. Such scenarios highlight the need for regulatory bodies to stipulate which methods take precedence over others. These results indicate the over-discriminatory power of ANOVA-based methods which may explain their lack of suitability for data analysis in the pharmaceutical sciences [190].

Using ANOVA analysis to compare MINO-06, MINO-11 and MINO-17 to the target profile, it can be clearly observed that the release of minocycline from these formulations was significantly

different from that of the target profile as significant differences in minocycline release were observed at all but one dissolution time point for MINO-11.

Table 5.4. Summary of ANOVA analysis for in vitro release profiles of MINO-02, MINO-03 and MINO-14 versus the reference profile

Time (hours)	Comparison	Mean difference	95% Confidence interval to mean difference		P value	Summary
1	MINO-02	3.097	-2.302	0.2810	P > 0.05	Not significant
	MINO-03	2.707	-2.175	0.4081	P > 0.05	Not significant
	MINO-14	5.758	0.5873	3.171	P < 0.01	Significant
2	MINO-02	5.740	-4.540	-0.8341	P < 0.01	Significant
	MINO-03	2.764	-3.147	0.5590	P > 0.05	Not significant
	MINO-14	2.137	-2.853	0.8527	P > 0.05	Not significant
4	MINO-02	4.491	-4.117	-0.2597	P < 0.05	Significant
	MINO-03	2.040	-0.9344	2.923	P > 0.05	Not significant
	MINO-14	10.28	-6.938	-3.080	P < 0.001	Significant
6	MINO-02	2.801	-8.403	1.439	P > 0.05	Not significant
	MINO-03	3.738	-0.2730	9.568	P > 0.05	Not significant
	MINO-14	8.400	-15.36	-5.522	P < 0.001	Significant
8	MINO-02	2.092	-10.19	3.143	P > 0.05	Not significant
	MINO-03	4.790	1.402	14.74	P < 0.05	Significant
	MINO-14	5.513	-15.96	-2.619	P < 0.01	Significant
12	MINO-02	4.598	1.216	16.25	P < 0.05	Significant
	MINO-03	8.853	9.295	24.33	P < 0.001	Significant
	MINO-14	2.687	-2.412	12.62	P > 0.05	Not significant

Table 5.5. Summary of ANOVA analysis for in vitro release profiles of MINO-06, MINO-11, MINO-17 versus the target release profile

Time (hours)	Comparison	Mean difference	95% Confidence interval to mean difference		P value	Summary
			Lower limit	Upper limit		
1	MINO-06	15.83	-12.32	-7.390	P < 0.001	Significant
	MINO-11	0.06097	-2.426	2.502	P > 0.05	Not significant
	MINO-17	17.13	-13.12	-8.197	P < 0.001	Significant
2	MINO-06	17.26	-24.09	-15.10	P < 0.01	Significant
	MINO-11	5.756	-11.03	-2.042	P < 0.001	Significant
	MINO-17	18.62	-25.64	-16.65	P < 0.001	Significant
4	MINO-06	11.76	-38.92	-19.32	P < 0.001	Significant
	MINO-11	8.067	-29.78	-10.17	P < 0.001	Significant
	MINO-17	12.54	-40.85	-21.25	P < 0.001	Significant
6	MINO-06	10.98	-39.61	-18.61	P < 0.001	Significant
	MINO-11	11.15	-40.08	-19.08	P < 0.001	Significant
	MINO-17	12.14	-42.70	-21.70	P < 0.001	Significant
8	MINO-06	12.54	-34.54	-17.97	P < 0.001	Significant
	MINO-11	16.26	-42.33	-25.75	P < 0.001	Significant
	MINO-17	13.57	-36.71	-20.13	P < 0.001	Significant
12	MINO-06	11.78	-15.09	-7.498	P < 0.001	Significant
	MINO-11	16.69	-19.79	-12.21	P < 0.001	Significant
	MINO-17	7.554	-11.03	-3.447	P < 0.001	Significant

5.8.3 Application of Korsmeyer-Peppas model

Several drug delivery devices in which HPMC has been used exhibit different mechanisms that control API release including polymer swelling and subsequent erosion, drug dissolution, drug diffusion or a combination of these [82]. Important factors that influence drug release from hydrophilic polymer matrices such as HPMC include the water solubility of the drug dispersed in the dosage form [123,161,161,215,216] and/or drug loading in the system [215]. The release of water soluble drugs from hydrophilic matrices is predominantly a consequence of the dissolution of the drug in the aqueous medium that penetrates the matrix and the subsequent diffusion of the drug from the matrix system [38]. In contrast the release of poorly water soluble compounds occurs predominantly as a result of the erosion of the matrix [217]. Formulation variables such as the shape of the matrix device [123] and the addition of diluents such as lactose may alter the penetration rate of water into the matrix system and therefore may influence the mechanism of drug release from these hydrophilic matrix systems [123,161].

Although several mathematical models have been used to describe the release mechanisms of drugs from hydrophilic matrices, the most frequently used methods have been the Higuchi [204,205] model and the Korsmeyer-Peppas [207] model [82,161,215,216,218].

Several mechanisms of drug release from lipid matrices may be observed and are dependent on the HLB value and composition of the lipid [219,220]. As with hydrophilic matrices, drug release from lipid matrices may be due to drug diffusion across the matrix into the dissolution medium, or following erosion of the matrix or as a result of the combination of both mechanisms [219,220]. Drug release from Gelucire® bases with low HLB values occurs as a result of a simple diffusion mechanism whilst drug release from Gelucire® bases with high HLB values occurs predominantly as a result of lipid erosion [219,221]. The mechanisms of drug release from lipid matrices may also be influenced by formulation variables such as the concentration of lipid used in the matrix or by the addition of hydrophilic additives [220]. In addition, drug release from lipid matrices may be affected by the temperature and the pH of the dissolution medium used to monitor drug release [220].

Several mathematical models such as the first-order and Higuchi models have been applied in describing release mechanisms of drugs from lipid matrices [222]. In addition modifications of

the Korsmeyer-Peppas and Higuchi models have been used to demonstrate the contribution of both diffusion and erosion mechanisms to drug release from lipid matrices [219,221].

As the release of drugs from both hydrophilic and lipid matrices is mainly a consequence of diffusion of the drugs from, or erosion of matrices or as a result of both mechanisms, the Korsmeyer-Peppas model is an ideal model to use to evaluate the effects of formulation variables on the release of minocycline from the manufactured dosage forms. The exponent, n, has a value of 0.5 when applied to Higuchi ($\sqrt{time}$) kinetics or a value of 1.0 for a zero-order kinetic model [123]. It is therefore possible to identify the predominant mechanism of drug release from the matrices judging by how close to 0.5 or 1.0 the release exponent is after modeling experimental data. A slab geometry was assumed in these studies for the interpretation of the release exponent.

In vitro release profiles of minocycline obtained in Section 4.4 for all prepared formulations were modelled using the Korsmeyer-Peppas model and are summarised in Table 5.6.

Table 5.6. Summary of Korsmeyer-Peppas best-fit parameters

Batch #	$\frac{M_t}{M_\infty}$	N	K (%/min)	R^2
MINO-01	0.6376	0.6864	0.252872	0.9906
MINO-02	0.5952	0.9542	0.08213	0.9987
MINO-03	0.4793	0.8594	0.081077	0.9987
MINO-04	0.4116	0.9656	0.046644	0.9511
MINO-05	*	*	*	*
MINO-06	0.571161	0.8804	0.173061	0.9944
MINO-07	0.497627	0.7562	0.079013	0.9926
MINO-08	0.05361	0.6495	0.009849	0.9814
MINO-09	*	*	*	*
MINO-10	*	*	*	*
MINO-11	0.479745	1.3923	0.072377	0.9952
MINO- 12	0.30044	0.6634	0.056494	0.994
MINO-13	0.643351	0.7614	0.104978	0.9834
MINO-14	0.524427	1.2869	0.054967	0.9935
MINO-15	0.627994	0.9083	0.181009	0.9983
MINO-16	0.57197	0.7757	0.14983	0.9888
MINO-17	0.590479	0.8707	0.181677	0.9935
MINO-18	*	*	*	*
MINO-19	*	*	*	*
MINO-20	*	*	*	*
MINO-21	0.524017	0.9931	0.051487	0.9578
MINO-22	0.627029	0.8265	0.146487	0.8265

* The Korsmeyer-Peppas model was not applicable as the amount of minocycline released was greater than 60% before the second sampling point

5.8.3.1 Effect of HPMC and Compritol® concentrations on mechanism of drug release

In order to elucidate the impact of concentration of rate retarding polymers on the mechanism of drug release, the change in value of the release exponent, *n*, was monitored as the concentration of the polymer in the formulation changed. A plot of the release exponent versus concentration of HPMC and Compritol® in the formulation is depicted in Figure 5.1.

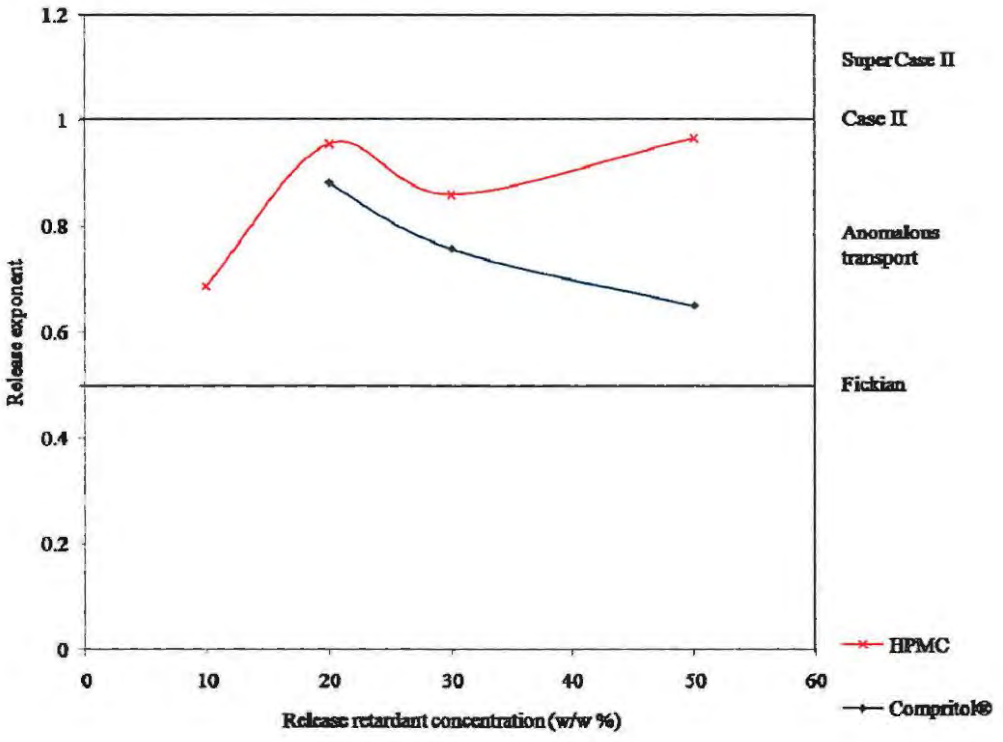


Figure 5.1. Effect of polymer concentration on the value of the release exponent

The value of *n* at all concentrations of HPMC used was found to be between 0.5 and 1.0 indicating that the mechanism of minocycline release was a result of both diffusion and erosion. Minocycline release occurred by anomalous transport kinetics at all concentrations of HPMC. Similar results indicating the somewhat independence of the release exponent from HPMC concentration were observed by Fu et al. when tablets containing water soluble and slightly water soluble drugs comparable to minocycline, particularly theophylline were tested [216]. Although the overall release kinetics were unchanged over the HPMC concentration range studied the value of *n* increased from a 0.6864 when 10% w/w HPMC was used in the formulation to a value of 0.9656 when the polymer content was increased to 50% w/w. It is important for a pharmaceutical scientist to consider the solubility of the drug, the coherence of the matrix in addition to the extent of drug release when interpreting the value of the release exponent *n*. A value of *n* of 0.9656 at 50% w/w HPMC is very close to 1.0 and thus indicates minocycline release follows a zero-order kinetic model. Although the release kinetics using 50% w/w HPMC were ideal it is important to note that only 41% (versus the targeted minimum of 80%) of minocycline was released from these delivery systems. At the other extreme, a value of *n* of 0.6864 using 10% w/w HPMC was an indication that diffusion was the predominant mechanism controlling release. At a concentration of 10% w/w HPMC, a gel layer formed by HPMC is not as coherent as when

higher concentration of HPMC are used and this layer is more likely to erode than at the higher HPMC concentrations. Consequently erosion is likely to be the more dominant mechanism of release. However, minocycline hydrochloride is readily soluble in water and can diffuse through channels of the matrix into the dissolution medium at a faster rate than matrix can erode. As a result the overall dominant mechanism of release observed when this concentration of polymer was used is diffusion as is clearly indicated by the value of *n*.

Similarly the overall mechanism of minocycline release from the lipid matrices at the concentrations of Compritol® studied was found to be independent of the concentration of Compritol® used in the formulation. Minocycline release occurred as a result of both diffusion and erosion mechanisms. It was observed as is clearly depicted in Figure 5.1 that the value of *n* decreased as the concentration of Compritol® used in the matrix increased with the lowest value of *n* (0.6495) observed when a Compritol® concentration of 50% w/w was used in the formulation. As the concentration of Compritol® used in the formulation increases, diffusion became the more dominant mechanism of drug release. Similar results were observed by Kopcha et al. [220]. As a higher concentration of Gelucire® with a low HLB value was added to the lipid matrix drug release using anhydrous theophylline and D&C Yellow No.10 as model drugs, was observed to be predominantly diffusion controlled [220]. Furthermore drug release from more hydrophobic lipids has been observed to be controlled predominantly by diffusion [219,221]. As the concentration of Compritol® used in these studies increased the formulation became more hydrophobic resulting in minocycline release becoming predominantly diffusion controlled.

The impact of concentration of the polymer used on the rate of release was evaluated by monitoring the change in the kinetic constant, *k*, as the concentration of polymer changed. Plots of the kinetic constant versus concentration of HPMC and Compritol® in the formulations are depicted in Figure 5.2.

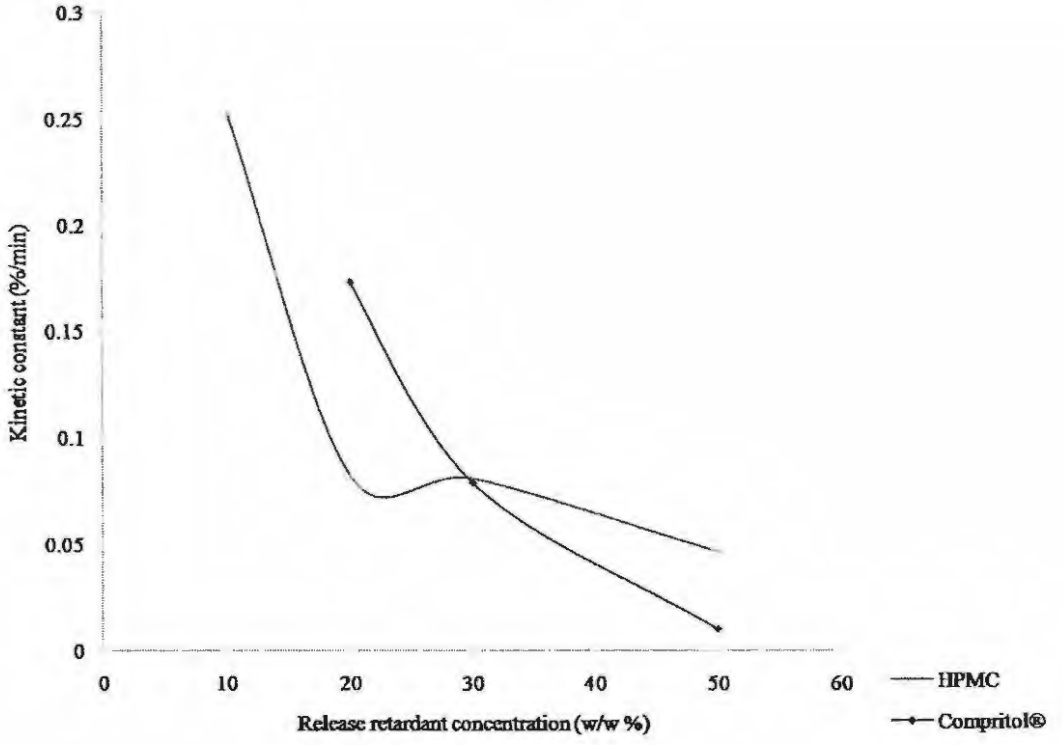


Figure 5.2. Effect of polymer concentration on the kinetic constant

The kinetic constant *k* was observed to decrease as the concentration of HPMC used in the formulation increased although this observation was less significant between polymer concentrations of 20% w/w and 30% w/w. A correlation between the release constant and the rate and extent of minocycline released was observed. When lower kinetic constants were generated correspondingly lower rates and extents of minocycline release were observed. Ku et al. [216] observed similar results in their investigation of the release of water soluble and slightly water soluble drugs from HPMC matrices.

As the concentration of Compritol® used in the formulation increased, the value of *k* decreased in an almost linear manner as can be seen in Figure 5.2. It is interesting to note that there is a clearly defined relationship between the concentration of Compritol® used and the value of *k* and *n* in addition to the rate and extent of minocycline release. The values for of *k* and *n* in addition to the rate and extent of minocycline release decrease as the concentration of Compritol® used in the formulation increases.

5.8.3.2 *Effect of diluent type on mechanism of drug release*

To investigate the influence of diluent type on the mechanism of minocycline release from the test formulations, microcrystalline cellulose (MCC) and lactose representing a slightly soluble and a freely soluble diluent, respectively, were studied. A plot of *n* versus the diluent used is depicted in Figure 5.3.

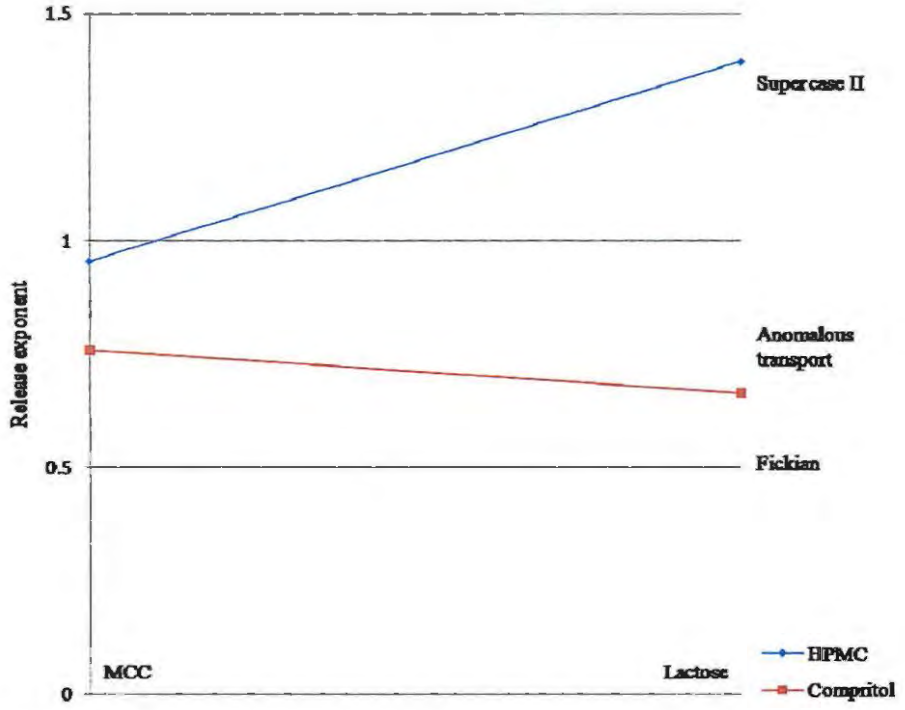


Figure 5.3. Effect of diluent type on release exponent

As can be seen in Figure 5.3, the mechanism of minocycline release from HPMC matrices was affected by the type of diluent used in the test formulations. When MCC was used as a diluent, the mechanism of release of minocycline from the matrix was readily described by an anomalous transport mechanism with both erosion and diffusion playing an integral part in the release of minocycline. However, when lactose was used as the diluent, the release of minocycline from test formulations could be easily explained by Super Case II transport mechanisms. Super Case II diffusion mechanisms, in most cases display a phenomenon in which the concentration fronts are sharp with a flux into the phase change boundary less than the flux out of the phase change boundary [223]. Similar Super Case II release mechanisms have been observed for the release of theopylline from alginate capsule formulations [224] and for the release of diclofenac sodium from natural gum capsule formulations [225]. This change in release mechanism from the HPMC matrices was associated with an increase in the rate of release of minocycline when lactose was used as a diluent than when MCC was used as a diluent.

Unlike the effect of diluent type on minocycline release mechanisms from the HPMC matrices, there was no change in the release mechanism from Compritol® matrices. The release of minocycline from HPMC matrices followed anomalous transport mechanisms when either of the two diluents, MCC or lactose was used in the formulations. However, when lactose was used as the diluent in the Compritol® based formulations, minocycline release occurred primarily as a result of diffusion rather than erosion. The diffusional release was associated with a lower extent of minocycline release after the 12 hour dissolution period, with a release of approximately 30% observed versus 49% when MCC was used as the diluent. As mentioned is Section 4.4.3, this may be explained by the more dominant "water-drawing" effect of MCC than the "channel-creating" effect of lactose in the Compritol® matrices. When MCC was used as a diluent the release of minocycline was predominantly controlled by erosion due to the greater uptake of water by the formulation that increased the dispersibility and hence deformation of the matrix.

5.8.3.3 Effect of addition of disintegrant on mechanism of drug release

The effect of disintegrant addition on the mechanism of minocycline release from the manufactured formulations was investigated by studying the release of minocycline from formulations with and without sodium starch glycolate. A plot of the release exponent versus the amount of sodium starch glycolate added to the HPMC matrix based formulations is shown in Figure 5.4.

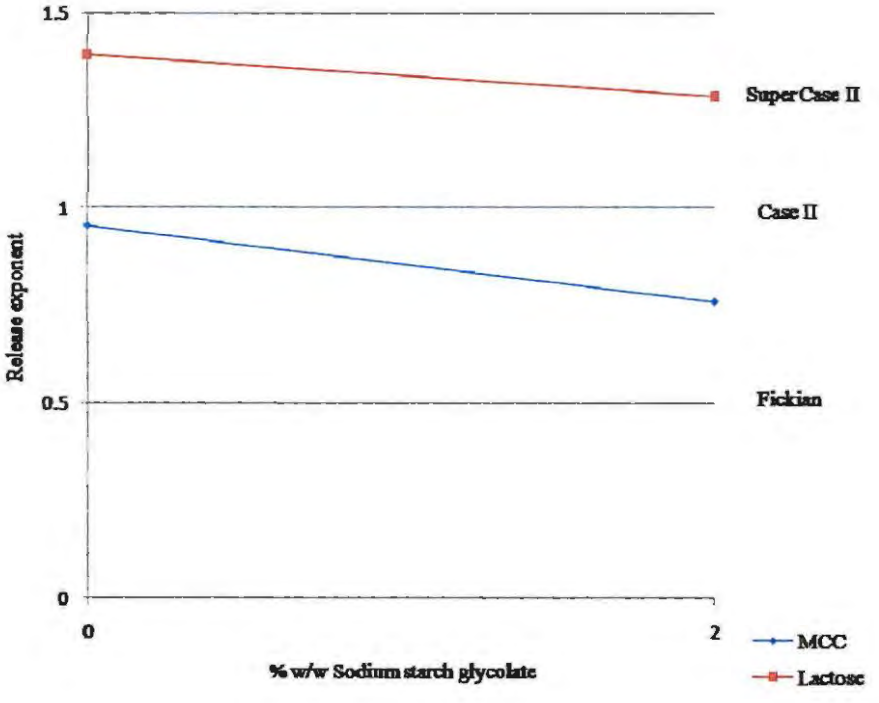


Figure 5.4. Effect of disintegrant on release exponent for HPMC matrix formulations

The addition of disintegrant to HPMC matrices using either MCC or lactose as a diluent had no significant effect on the mechanism of release of minocycline in both instances. When MCC was used as a diluent the release of minocycline was controlled by anomalous transport kinetics with or without the addition of disintegrant whereas the release of minocycline from formulations incorporating lactose as the diluent were better described by Super Case II kinetics with or without the addition of disintegrant. In both cases the inclusion of disintegrant, with MCC or lactose resulted in a decrease in the release exponent from 0.9542 to 0.7614 for MCC and from 1.3923 to 1.2869 for lactose formulations. This decrease in the value of the release exponent was associated with a decrease in the extent of minocycline release after a twelve hour dissolution period indicating that erosion contributed to faster drug release when MCC was used as the diluent. The addition of disintegrant when MCC was used as the diluent resulted in an immediate and increased water uptake by the formulation which may have caused the formation of a coherent HPMC gel layer more rapidly, as discussed in Section 4.4.4.1, thereby decreasing the contribution of erosion to minocycline release from the test formulations and making diffusion more dominant. The faster formation of a coherent HPMC gel layer with the addition of disintegrant to test formulations with lactose may have contributed to the decrease in the value of the release exponent observed.

A plot of the release exponent versus the amount of sodium starch glycolate added to Compritol® based formulations is shown in Figure 5.5.

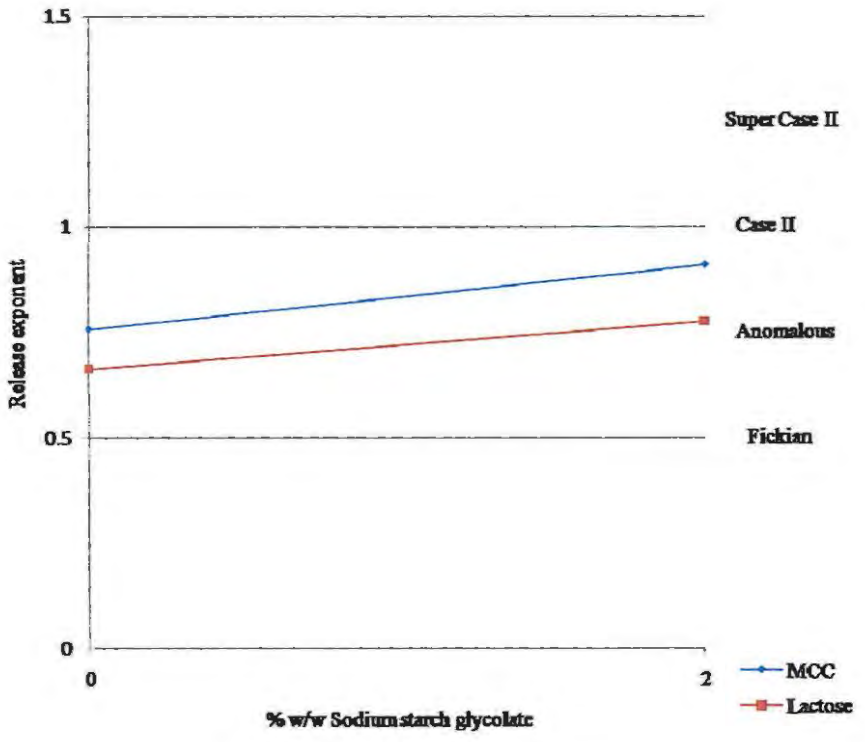


Figure 5.5. Effect of disintegrant on release exponent for Compritol® matrix formulations

As was observed for the HPMC matrix based formulations the addition of disintegrant had no effect on the general mechanism of minocycline release from Compritol® based formulations. The release of minocycline with or without the addition of disintegrant, using MCC or lactose occured via anomalous transport mechanisms. However, the addition of disintegrant to the Compritol® based formulations had an effect opposite to that observed in the HPMC based formulations in terms of the change in value for the release exponent. When either MCC or lactose was used the value of the release exponent increased with the addition of disintegrant. In both instances the release of minocycline from the formulations was controlled by erosion, approaching zero-order release, particularly for the formulations with lactose as the diluent. As discussed in Section 4.4.1, the addition of disintegrant increased the water uptake into the formulation which may have resulted in a higher dispersibility/erosion of the Compritol® matrix. It was observed during dissolution studies that the Compritol® matrix maintained its integrity i.e. a shape similar to that of the capsule shell was retained throughout the twelve hour duration of the dissolution test. The integrity of the matrix was not observed when disintegrant was added to the formulations. The resultant increase in the value of the release exponent corresponding to the predominance of erosion of the mechanism of release was associated with a higher extent of minocycline release. The addition of disintegrant resulted in an increase in release from 49% -

91% for formulations containing MCC and from 30% - 79% for formulations containing lactose. From these observations, it may be concluded that erosion resulted in a higher release of water soluble drugs at a more constant rate from lipid matrices.

5.8.3.4 Effect of addition of surfactant on mechanism of drug release

The effect of surfactant on the mechanism of release of minocycline from lipid matrices was investigated by comparing dissolution profiles of formulations with and without Poloxamer 188 added. A plot of the release exponent versus the concentration of Poloxamer 188 added into the formulations is depicted in Figure 5.6.

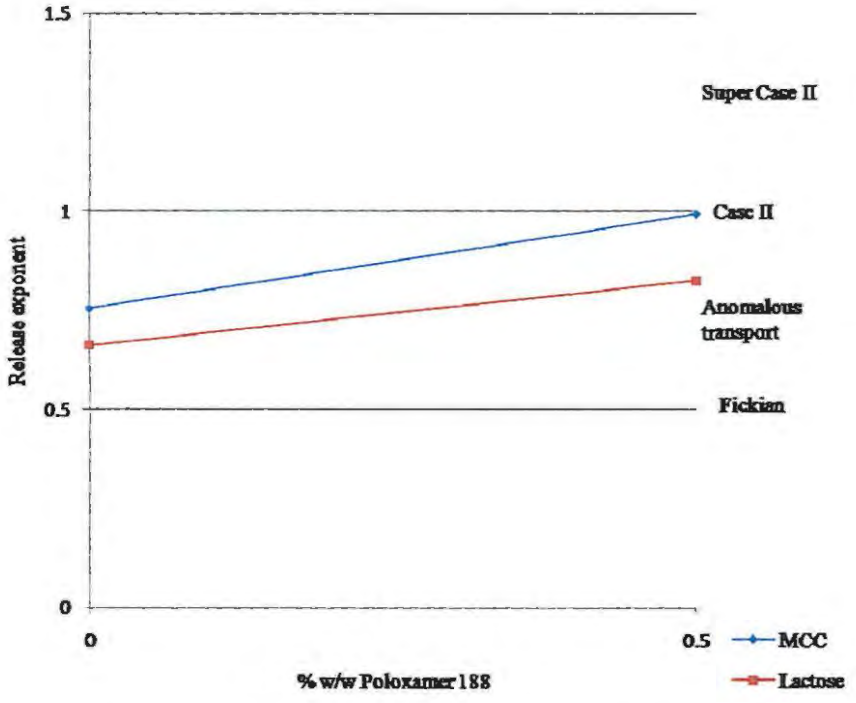


Figure 5.6. Effect of surfactant on the release exponent for Compritol® formulations

The addition of 0.5% w/w Poloxamer 188 to the Compritol® based formulations had no effect on the overall release of minocycline from the formulations and release was controlled by anomalous mechanisms with or without the addition of 0.5% w/w Poloxamer 188 for formulations containing lactose or MCC as the diluent. The addition of surfactant to the Compritol® based formulations had similar effects to the addition of disintegrant to the Compritol® based formulations. The addition of surfactant to the test formulations increased the water uptake by the formulations. Following the addition of the surfactant, the value of the release exponent increased from 0.7562 to 0.9931 and from 0.6634 to 0.8265 for formulations with MCC and lactose, respectively, indicating the predominance of erosion as the controlling mechanism of drug

release. It is particularly interesting to note that Case II or zero-order kinetics were observed for formulation MINO-21, which had MCC as a diluent and 0.5% w/w Poloxamer 188 with a resultant high value of 0.9931 for *n*. Similar to the effects observed when disintegrant was included erosion predominated as the release controlling mechanism and a greater rate and extent and rate of release of minocycline was observed, albeit at a constant rate.

5.8.4 Application of other mathematical models

As the mechanism of release from the matrix systems tested in these studies occurs by diffusion, erosion or a combination of both, it was necessary to further evaluate the release mechanisms using other models, viz., Higuchi and a Zero-order model. The results obtained using the Higuchi and the zero-order models are summarised in Table 5.7.

Table 5.7. Summary of mathematical model parameters

Batch #	Zero-order model		Higuchi model	
	R^2	K_0	R^2	K_H
MINO-01	0.8384	8.2249	0.9712	32.196
MINO-02	0.9831	6.9662	0.9473	24.87
MINO-03	0.9938	5.9705	0.9479	21.206
MINO-04	0.9398	3.8464	0.9604	14.142
MINO-05	0.3412	5.1141	0.6222	25.115
MINO-06	0.9101	8.5013	0.982	32.117
MINO-07	0.974	4.37	0.9749	15.901
MINO-08	0.9761	0.4452	0.9604	1.6061
MINO-09	0.6535	8.0174	0.8832	33.896
MINO-10	0.294	4.3449	0.5654	21.914
MINO-11	0.9363	9.8753	0.9344	35.875
MINO- 12	0.9737	2.5668	0.9765	9.3488
MINO-13	0.9597	5.6954	0.9797	20.928
MINO-14	0.9647	7.6335	0.935	27.331
MINO-15	0.8364	8.4732	0.9505	32.849
MINO-16	0.9276	7.0225	0.9869	26.342
MINO-17	0.8715	8.2348	0.9715	31.62
MINO-18	0.4027	5.4133	0.692	25.809
MINO-19	0.2858	4.8246	0.5623	24.612
MINO-20	0.3059	4.7046	0.5753	23.465
MINO-21	0.9788	4.7969	0.9532	17.216
MINO-22	0.9403	7.9279	0.9797	29.431

As shown in Table 5.7, most formulations fitted better to the Higuchi model compared to the zero-order model indicating the predominance of diffusion as the overall mechanism of drug release from the manufactured formulations. However some formulations i.e. MINO-02, MINO-03, MINO-11, and MINO-14 with HPMC as the release retarding polymer and MINO-08 and MINO-21 with Compritol® as the release retarding polymer were better modelled by the zero-order model. Minocycline release from these formulations was less than 80% after twelve hours of dissolution; hence the portion of the dissolution profile that was mathematically modelled would be expected to follow zero order kinetics than Higuchi kinetics, as the drug release plateau that deviates from zero order kinetics had most likely not been reached.

5.9 CONCLUSIONS

Using statistical, model-independent and model-dependent mathematical models the comparison of dissolution profiles and the elucidation of mechanism of minocycline release from the manufactured dosage forms was achieved.

The comparison of dissolution profiles against a target or reference profile was successfully performed using the difference and similarity factor approach and a one way analysis of variance. When using the difference and similarity factors, three formulations, viz., MINO-02, MINO-03 and MINO-14 were identified to be similar to the target release profile. On further analysis of these formulations using one way analysis of variance, differences were observed at some dissolution points indicating the highly discriminative nature of ANOVA based methods. The importance of setting limits and selecting methods for comparing dissolution profiles cannot be overlooked as different methods may result in different conclusions being drawn.

The Korsmeyer-Peppas model was successfully used to elucidate the mechanisms involved in the release of minocycline from the test formulations. The release mechanism for most formulations was a combination of erosion and diffusion. The contribution of diffusion and erosion to the release of minocycline was influenced by the type of diluent used and the addition of disintegrant or surfactant to the formulations. Super Case II transport mechanisms were observed when lactose was used as the diluent for HPMC matrix based formulations. In all cases it was noted that as the value of the release exponent increased, an associated higher overall rate and extent of minocycline release was observed.

By means of application of the Higuchi and the zero-order models to investigate mechanisms of release, it was noted that diffusion was the more dominant controlling mechanism than erosion for the release of minocycline from the manufactured test formulations.

The formulations that displayed similar release profiles to that of the target profile viz. MINO-02, MINO-03 and MINO-14, were all based on hydrophilic matrices. This may be an indication of the relative ease with which drug release from hydrophilic matrices can be manipulated compared to lipid matrices.

Based on the information obtained, the insight gained from mathematical and statistical modeling in formulation development cannot be undermined. Mathematical modeling forms a crucial component in formulation development particularly when an appropriate reference product is available. It is however vital that the formulation scientist has an understanding of the assumptions of each model as well as its limitations to ensure that information gained is beneficial in the formulation process.

CHAPTER SIX

6 CONCLUSIONS

Minocycline is a tetracycline antibiotic that is indicated for the treatment of a broad range of systemic infections. The broad spectrum of activity of minocycline can, in part, be attributed to the physicochemical properties of the compound, in particular its lipophilicity at physiological pH. The use of minocycline has been associated with vestibular side effects that are likely to reduce adherence to treatment by patients. Sustained release formulations of minocycline that ensure slower dissolution of the API, once administered, may be beneficial in improving adherence to minocycline therapy by a patient.

A sensitive, selective and accurate HPLC method for the analysis of minocycline in pharmaceutical dosage forms was developed and validated following ICH guidelines. The method was linear over the range 5 – 140 μg/ml. Furthermore the method was accurate and precise with % Bias and % RSD values of less that 5% for accuracy, intra-day and inter-day precision, which was within the limits set in our laboratory. In addition, the method was stability indicating as chromatographic analysis of minocycline samples exposed to stress conditions of extreme pH, temperature, light and exposure to a 3% v/v solution of hydrogen peroxide, revealed appropriate resolution of minocycline from degradant peaks.

Sustained release capsule formulations of minocycline were manufactured using hydroxypropyl methylcellulose (HPMC) and Compritol® as rate retarding polymers. Following initial studies, the use of hydroxypropyl cellulose (HPC) and Precirol® was discontinued as HPC and Precirol® containing formulations failed to exhibit extended release profiles for minocycline even when used at concentrations as high as 50% w/w. Hydrophilic and lipophilic powder fill materials for formulations were manufactured using the geometric dilution method for powder mixing.

To investigate the effect of polymer concentration on the rate and extent of minocycline release the composition of HPMC and Compritol® in fill formulations was varied within the range of 10-50 % w/w. Furthermore the use of other excipients was also investigated to optimise the release of minocycline from the capsule formulations that had been manufactured. These studies were conducted to establish the impact of diluent and disintegrant on formulation performance.

Furthermore the impact of incorporating different concentrations of Poloxamer 188 on Compritol® based formulation performance, was also investigated.

Uniformity of mass and capsule shell disintegration tests were conducted on all formulations and the results confirm that the dosage forms complied with BP specifications. In addition, the analysis of hydrophilic and lipophilic capsule fill material was conducted and all dosage forms were found to contain 95.2 – 105.5% of the stated 108 mg of minocycline hydrochloride, equivalent to 100 mg minocycline base.

In vitro dissolution testing was performed on all capsules using USP Apparatus I with water as the dissolution medium. The dissolution method was used to differentiate the dissolution rate profiles of experimental minocycline formulations tested. The rate and extent of minocycline release was influenced by the concentration of polymer used in the formulations with faster rates and greater extents of drug release being observed when low concentrations of polymer were incorporated into formulations. Formulation MINO-02 in which 20% w/w HPMC and MINO-07 in which 20% w/w Compritol® were used, released approximately 75.3% and 49.8% minocycline after twelve hours, respectively. These formulations were also used to investigate the effects of other excipients on the performance of formulations in order to further optimise minocycline release from these dosage forms.

Microcrystalline cellulose (MCC) and lactose were evaluated as potential diluents during further formulation optimisation studies. For HPMC based formulations minocycline release was faster when lactose (Batch MINO-11) as opposed to MCC (Batch MINO-02) was used as the diluent. Less than 80% minocycline release was observed from Batch MINO-02 whereas almost 100% release was observed for Batch MINO-11 after twelve hours. This difference was attributed to the hydrophilic nature of lactose which promoted the uptake of water by the formulation resulting in faster dissolution and subsequent diffusion of minocycline through the polymeric matrix. In contrast for Compritol® based formulations faster release of minocycline was observed when MCC (Batch MINO-07) as opposed to lactose (Batch MINO-12) was used as the diluent. Whilst approximately 50% minocycline was released from Batch MINO-07 only 30% was released from Batch MINO-12. The greater extent of release of minocycline from Batch MINO-07 was attributed to the significant ability of MCC, due to its hygroscopicity, to draw water into the lipid matrix thereby enhancing the dissolution of the API with a consequent faster diffusion of minocycline through the matrix.

The inclusion of sodium starch glycolate to formulations in which HPMC was used, resulted in slower release of minocycline, whereas for Compritol® based formulations faster release of minocycline from the formulations was observed. The resultant slower release of minocycline from HPMC based formulations was attributed to a higher initial uptake of water by the formulation, which resulted in the early formation of a more coherent HPMC gel layer that impedes minocycline release more effectively. Formulations in which Compritol® was used do not include polymers that form gels on contact with water. Consequently an increased uptake of water may enhance dissolution and subsequent diffusion of minocycline through the matrix and hence faster release is observed.

The addition of Poloxamer 188 at concentrations of 0.5 and 1% w/w to Compritol® based formulations was investigated. An increased rate of minocycline release was observed following addition of surfactant. This increased release was attributed to the overall increase in the HLB value of formulations which would have resulted in greater water uptake thereby promoting dissolution and subsequent release, of minocycline. Furthermore the addition of surfactant to the formulation may reduce hydrophobic interactions between minocycline and Compritol® thereby increasing the rate of minocycline release.

The effect of combining hydrophilic and lipid polymers was investigated and 10% w/w HPMC and 10% w/w Compritol® were included in a formulation (Batch MINO-17). The dissolution results generated indicate that there is no significant advantage in the use of this combination as the release pattern of minocycline was similar to that observed for Batch MINO-01, in which 10% w/w HPMC was used. However, further investigations using different proportions of hydrophilic and lipophilic polymers in combination are necessary to allow for adequate characterisation of such formulations.

Three formulations, viz., Batches MINO-06, MINO-11 and MINO-17 released more than 80% minocycline after twelve hours and, based on visual analysis, displayed "near zero order" release kinetics. Consequently these formulations were subjected to further in vitro dissolution testing using the USP test for delayed release dosage forms. The test is comprised of two stages, viz., a two hour acid exposure (pH=1.2) stage followed by a second stage in which the dosage form is exposed to buffer (pH=6.8) for the duration of the dissolution test. For Batches MINO-06 and MINO-17, in which Compritol® was used as the polymer, sustained release of minocycline was not observed and 95% was released within four hours of the commencement of dissolution

testing. Compritol® is comprised of fatty acid esters that undergo hydrolysis in acid conditions rendering Compritol® less lipophilic thereby reducing the capability for sustaining drug release. Minocycline release from Batch MINO-11 occurred at a slower rate than when water was used as the dissolution medium most likely as a result of a change in the ionisation state of minocycline, which in turn may have altered its solubility and hence its dissolution characteristics.

The disparity in dissolution results observed when different dissolution media are used raises important questions as to which medium is suitable for dissolution testing of experimental dosage forms, in general and for modified release delivery systems, in particular. As both the USP and BP do not specify dissolution test conditions for modified release dosage forms of minocycline, water was selected as the dissolution medium as it is recommended for use for immediate release dosage forms of minocycline. In addition water is recommended as a dissolution medium of choice for several modified release capsule dosage forms listed in the USP, including phenytoin sodium, phenylpropanolamine hydrochloride, pseudoephedrine, diltiazem hydrochloride and trihexyphenidyl hydrochloride. As modified release dosage forms will be exposed to different conditions in the gastro-intestinal tract (GIT) the use of dissolution media of pH similar to those likely to be encountered in the GIT would be more appropriate than water for preliminary formulation development studies. Furthermore the use of USP Apparatus 3 should also be considered as it may be possible to establish in vitro in vivo correlations (IVIVC) relatively easily more so than when using other official dissolution apparatus. Moreover, it is important to consider excipient properties when selecting dissolution test apparatus and conditions as several excipients are sensitive to pH changes.

Statistical, model-dependent and model-independent mathematical tests were used to analyse the in vitro dissolution profiles generated following testing of minocycline formulations. The difference and similarity factors, f_1 and f_2, were used for the comparison of all experimental formulations to a target or reference profile. Only three formulations, MINO-02, MINO-03 and MINO-14 were identified as being similar to the target profile. One way analysis of variance (ANOVA) studies indicated that there were several differences, at specific dissolution points, between experimental formulations and the reference formulation. Formulations MINO-06, MINO-11 and MINO-14 that had been observed to show optimal release patterns following visual inspection of dissolution profiles were regarded as different from the target profile when tested using the difference and similarity factors and ANOVA analysis. The use of mathematical models for comparing dissolution profiles is thus an important step during formulation

development studies. However, formulation scientists should have a deep understanding of the limitations of such methods and in particular the discriminatory potential and nature of each model prior to using them. Furthermore it is important that reference profiles that show the required pattern of release in addition to an appropriate extent of drug release are selected for the comparison.

Mathematical models were used to elucidate the mechanism(s) of drug release and determine whether the use of additional excipients in formulations had an impact on the release mechanism for the formulations manufactured in these studies. The Korsmeyer-Peppas model was used as the primary model to describe minocycline release from formulations. The Korsmeyer-Peppas release exponent, n, indicated that minocycline release occurred as a consequence of diffusion and erosion based mechanisms, in most cases. The value of n increased or decreased indicating a change in the dominant mechanism of release from a formulation as water uptake by the matrix system changed. In addition the uptake of water by matrix formulations was influenced by the excipients incorporated in those formulations.

Despite the fact that only three formulations were regarded as being similar to the target or reference profile and that most formulations followed Higuchi type release kinetics as compared to zero order kinetics, this study forms a strong basis for further formulation development of minocycline sustained release dosage forms. The study has shown the applicability of lipids as potential polymers for sustaining the release of minocycline as Compritol® was able to sustain release when water was used as the dissolution medium. As sustained release formulations are subjected to pH conditions in which lipid esters may be susceptible to hydrolysis, further studies that include an investigation into the use of acid-resistant coatings when lipids are included as sustained release polymers may be beneficial. Furthermore, it is important to investigate manufacturing variables such as for example the use of the fusion method to manufacture matrices when lipids are incorporated as sustained release polymers in dosage forms. In addition the compression of lipophilic powder material into tablets may well be an important approach for the optimization of such formulations. Furthermore a comparison of dissolution methods is crucial to determine the most appropriate approach and conditions to evaluate minocycline release from sustained release formulations accurately.

Based on these studies formulations MINO-02 and MINO-14 may be considered as appropriate starting compositions for minocycline sustained release dosage forms as 75% and 78%

minocycline was released from these formulations in twelve hours using water as the dissolution medium. Furthermore the release profiles are better fitted to a zero order kinetic model, which is an important consideration when formulating sustained release dosage forms.

The research undertaken has highlighted the importance of selecting the appropriate composition and test conditions for the development and assessment of sustained release dosage forms and lays the foundation for the further development of a minocycline sustained release formulation that may well improve adherence through side-effect minimisation.

APPENDIX I

SAMPLE PRODUCTION RECORDS

Only a sample of one production record for a hydrophilic matrix based formulation and one of a lipophilic based matrix formulation are included. The production records for the other formulations manufactured and assessed in these studies are available on request.

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BATCH PRODUCTION RECORD

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride Capsules
Batch #	MINO-02
Batch size	6 g
Date of Manufacture	20 August 2009

ORIGINAL AND WORKING FORMULAE

Name	Original Formula (mg)	Working Formula (mg)	RM #	Dispensed By	Checked By
Minocycline hydrochloride	108	2 160	RM000225	TS	FC
Methocel® K100M	60	1 200	RM000062	TS	FC
Microcrystalline cellulose (MCC)	132	2 640	XO61882	TS	FC

EQUIPMENT VERIFICATION

Description	Type	Verified By	Confirmed By
Balance	Mettler Toledo Model AG 135	TS	FC
Mortar and pestle	Porcelain	TS	FC
Sieve	315 µm	TS	FC

MANUFACTURING PROCEDURE

Step	Procedure	Time	Done By	Checked By
1	Weigh appropriate amounts of minocycline hydrochloride and actives: -Minocycline hydrochloride -Methocel® K100M -MCC	9:07 9:10 9:12	TS TS TS	FC FC FC
2	Mix powders by geometric dilution method of powder mixing using a mortar and pestle	9:25	TS	FC
3	Pass powder fill material through 315 µm sieve	9:27	TS	FC
4	Weigh empty capsule body and cap	10:39	TS	FC
5	Weigh 300 mg of powder fill material and fill into capsule body and cover with cap			
6	Weigh filled capsule			

SIGNATURE AND INITIAL REFERENCE

Full Name	Signature	Initials	Date
Tinotenda Sachikonye	Sach	TS	20/8/9
Faith Chaibva	Chaibva	FC	20.08.2009

RHODES UNIVERSITY

FACULTY OF PHARMACY

BATCH PRODUCTION RECORD

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride
Batch #	MINO-06
Batch size	5 g
Date of Manufacture	26 August 2009

ORIGINAL AND WORKING FORMULAE

Name	Original Formula (mg)	Working Formula (mg)	RM #	Dispensed By	Checked By
Minocycline hydrochloride	108	2 160	RM000225	TS	FC
Compritol®	50	1 000	RM000201	TS	FC
Microcrystalline cellulose	92	1 840	XO61882	TS	FC

EQUIPMENT VERIFICATION

Description	Type	Verified By	Confirmed By
Balance	Mettler Toledo Model AG 135	TS	FC
Mortar and pestle	Porcelain	TS	FC
Sieve	315 µm	TS	FC

MANUFACTURING PROCEDURE

Step	Procedure	Time	Done By	Checked By
1	Weigh appropriate amounts of minocycline hydrochloride and actives:			
	-Minocycline hydrochloride	9:36	TS	FC
	-Compritol® K100M	9:39	TS	FC
	-MCC	9:41	TS	FC
2	Mix powders by geometric dilution method of powder mixing using a mortar and pestle	9:56	TS	FC
3	Pass powder fill material through 315 µm sieve	9:57	TS	FC
4	Weigh empty capsule body and cap			
5	Weigh 250 mg of powder fill material and fill into capsule body and cover with cap	10:49	TS	FC
6	Weigh filled capsule			

SIGNATURE AND INITIAL REFERENCE

Full Name	Signature	Initials	Date
Tinotenda Sachikonye	Tsachi	TS	26/8/09
Faith Chaibva	Chaibva	FC	26.08.09

APPENDIX II

BATCH RECORD SUMMARIES

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FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-01
Batch size	6 g
Date of Manufacture	19 August 2009

Formula:

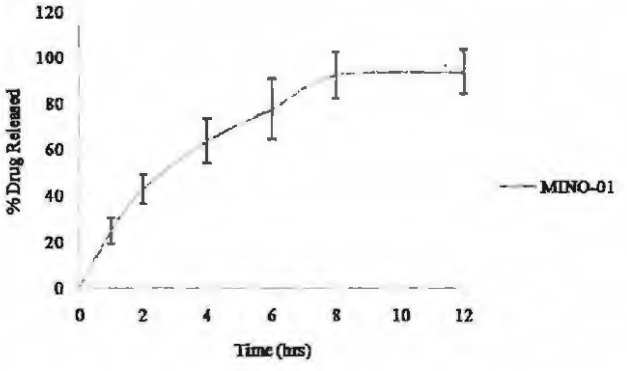
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	30	600	RM000062
Microcrystalline cellulose	162	3 240	XO61882

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	300. 27 ± 0.47
Assay (%)	95.79 ± 0.29

IN VITRO RELEASE PROFILE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

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FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-02
Batch size	6 g
Date of Manufacture	20 August 2009

Formula:

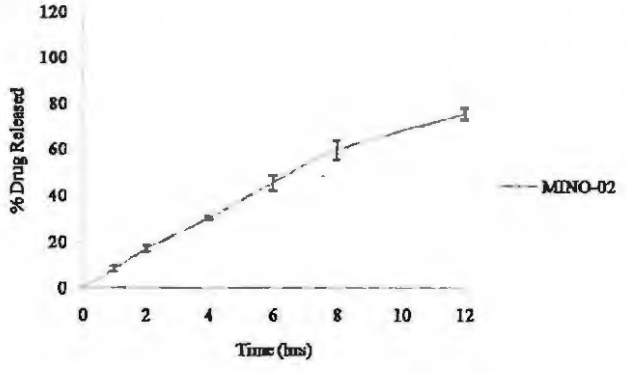
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	60	1 200	RM000062
Microcrystalline cellulose	132	2 640	XO61882

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	299.63 ± 0.89
Assay (%)	95.87 ± 0.61

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

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FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-03
Batch size	6 g
Date of Manufacture	21 August 2009

Formula:

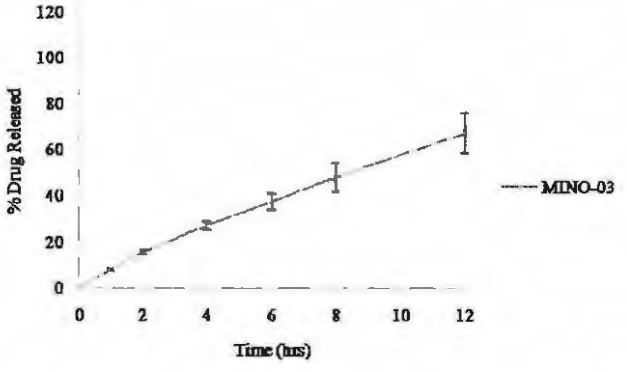
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	90	1 800	RM000062
Microcrystalline cellulose	102	2 040	XO61882

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	299.33 ± 0.65
Assay (%)	100.44 ± 0.48

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-04
Batch size	6 g
Date of Manufacture	24 August 2009

Formula:

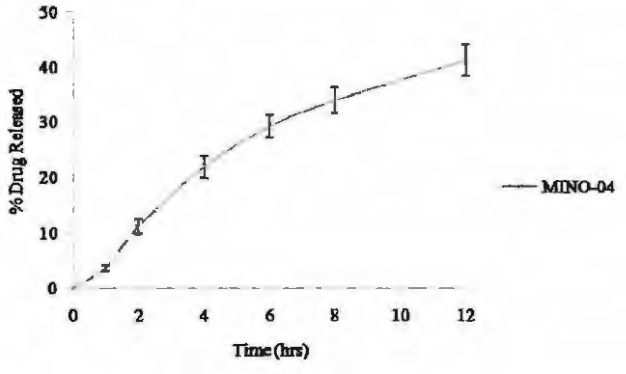
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	150	3 000	RM000062
Microcrystalline cellulose	42	840	XO61882

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	299.87 ± 1.089
Assay (%)	96.77± 0.28

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-05
Batch size	5 g
Date of Manufacture	25 August 2009

Formula:

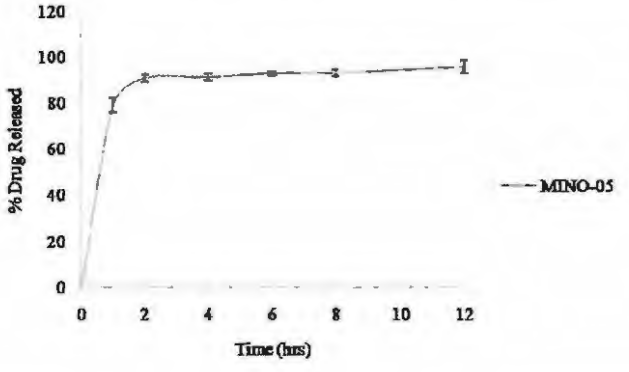
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	25	500	RM000201
Microcrystalline cellulose	117	2 340	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.85 ± 0.24
Assay (%)	98.47± 0.50

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-06
Batch size	5 g
Date of Manufacture	26 August 2009

Formula:

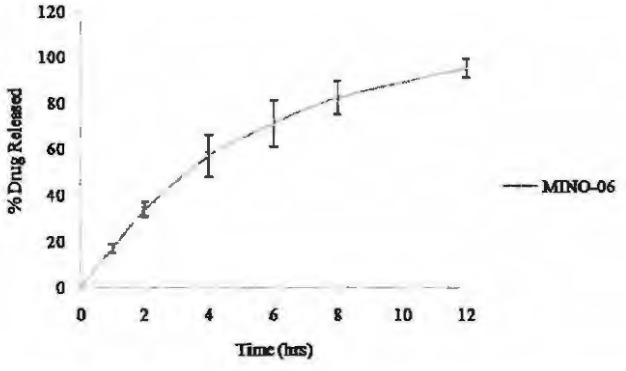
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	50	1 000	RM000201
Microcrystalline cellulose	92	1 840	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	249.70 ± 0.75
Assay (%)	96.37 ± 0.90

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-07
Batch size	5 g
Date of Manufacture	27 August 2009

Formula:

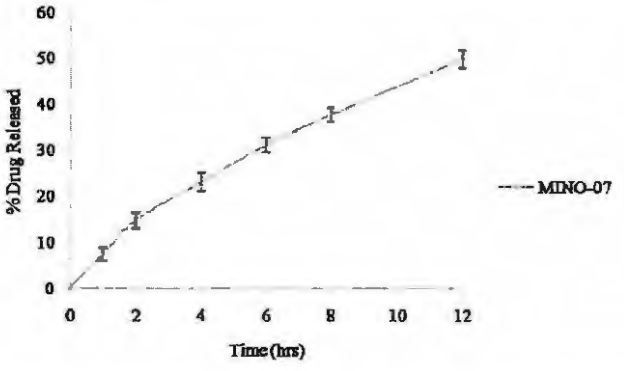
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Microcrystalline cellulose	67	1 340	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	249.62 ± 0.92
Assay (%)	95.20 ± 0.79

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill materials indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-08
Batch size	5 g
Date of Manufacture	28 August 2009

Formula:

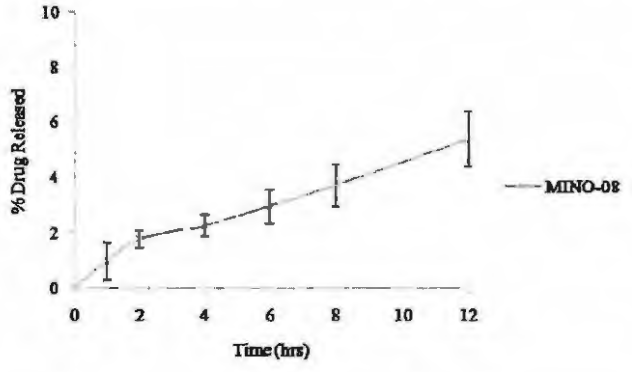
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	125	2 500	RM000201
Microcrystalline cellulose	17	340	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.58 ± 0.49
Assay (%)	104.03 ± 0.87

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-09
Batch size	6 g
Date of Manufacture	31 August 2009

Formula:

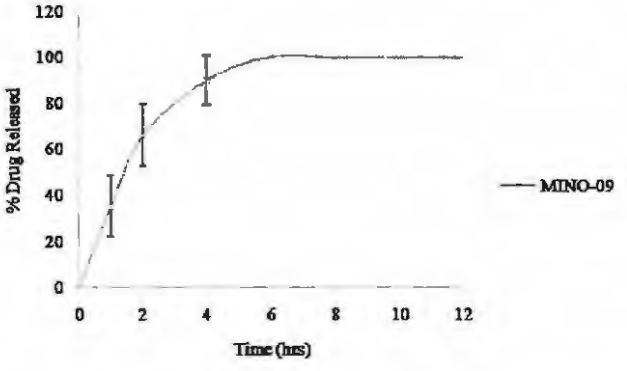
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Hydroxypropyl cellulose	150	3 000	XO54558
Microcrystalline	42	820	XO61882

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	300.17 ± 0.75
Assay (%)	101.19 ± 1.63

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-10
Batch size	5 g
Date of Manufacture	1 September 2009

Formula:

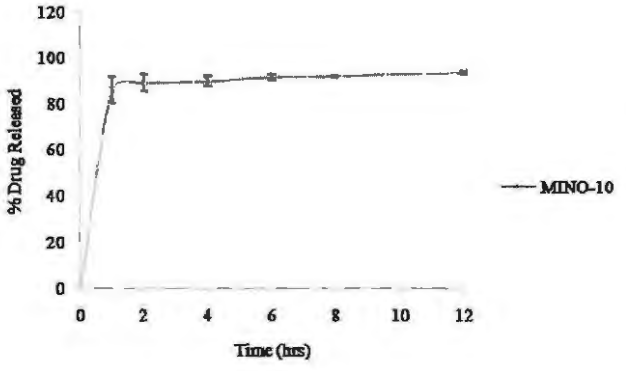
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Precirol®	125	2 500	RM000200
Microcrystalline cellulose	17	340	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.48 ± 0.65
Assay (%)	102.63 ± 1.17

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-11
Batch size	6 g
Date of Manufacture	2 September 2009

Formula:

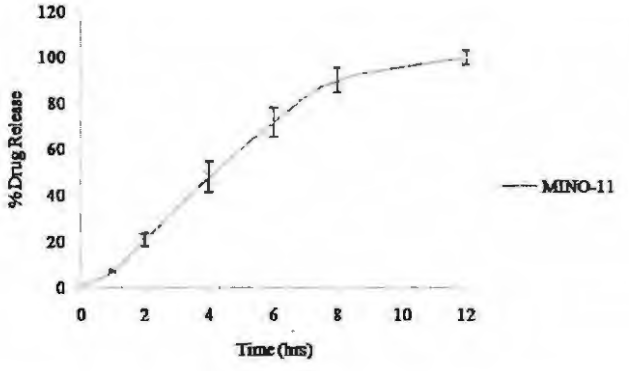
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	60	1 200	RM000062
Lactose monohydrate	132	2 640	RM000013

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	299.63 ± 0.97
Assay (%)	105.50 ± 1.11

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-12
Batch size	5 g
Date of Manufacture	3 September 2009

Formula:

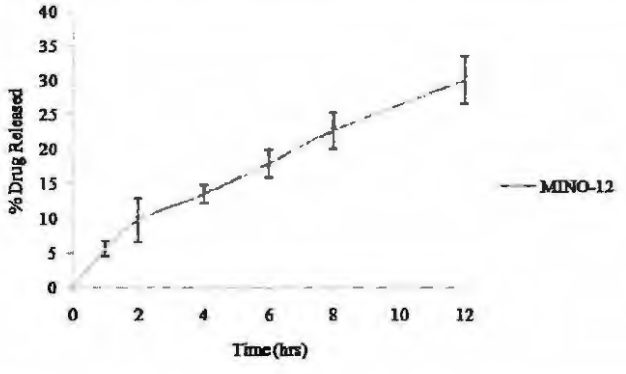
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000062
Lactose monohydrate	67	1 340	RM000013

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.45 ± 0.41
Assay (%)	101.36 ± 0.55

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-13
Batch size	6 g
Date of Manufacture	4 September 2009

Formula:

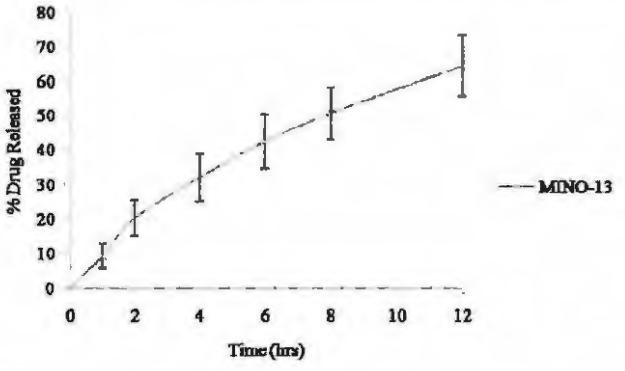
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	60	1 200	RM000062
Sodium starch glycolate	6	120	X070074
Microcrystalline cellulose	126	2 520	XO61882

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	299.77 ± 0.40
Assay (%)	101.27 ± 1.12

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-14
Batch size	6 g
Date of Manufacture	7 September 2009

Formula:

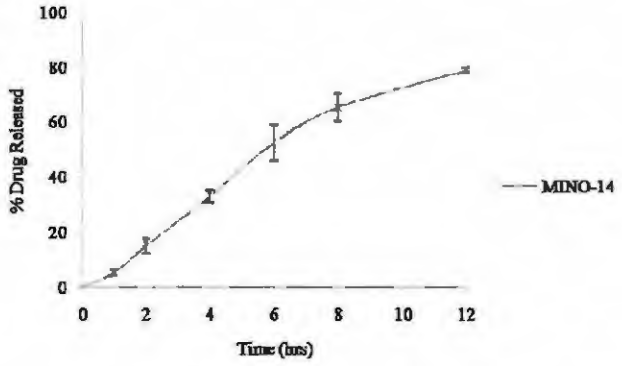
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	60	1 200	RM000062
Sodium starch glycolate	6	120	X070074
Lactose monohydrate	126	2 520	RM000013

Target capsule fill weight: 300 mg

Tests performed:

Weight (mg)	299.47 ± 0.23
Assay (%)	103.00 ± 0.40

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-15
Batch size	5 g
Date of Manufacture	8 September 2009

Formula:

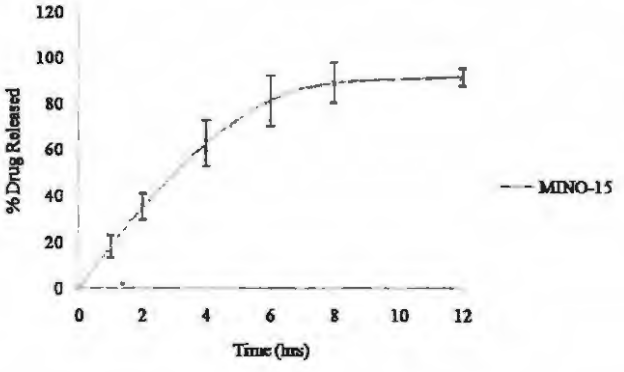
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Sodium starch glycolate	5	100	X070074
Microcrystalline cellulose	62	1 240	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.73 ± 0.28
Assay (%)	100.26 ± 0.62

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-16
Batch size	5 g
Date of Manufacture	9 September 2009

Formula:

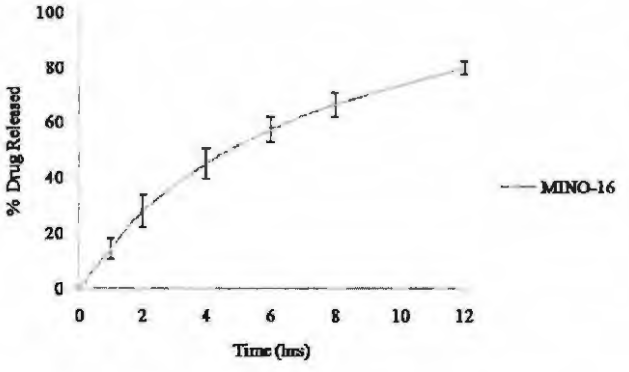
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2160	RM000225
Compritol®	75	1 500	RM000201
Sodium starch glycolate	5	100	X070074
Lactose monohydrate	62	1 240	RM000013

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.15 ± 0.22
Assay (%)	104.65 ± 0.71

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-17
Batch size	5 g
Date of Manufacture	10 September 2009

Formula:

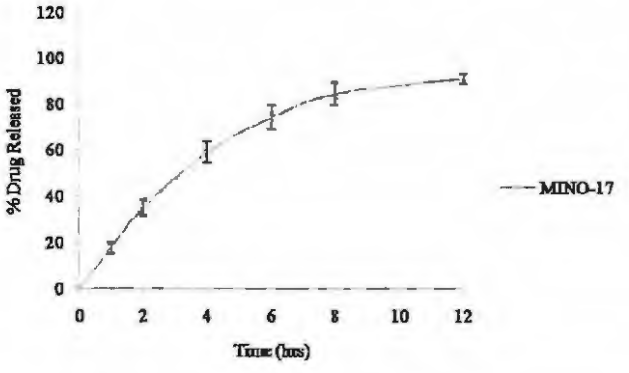
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Methocel® K100M	25	500	RM000062
Compritol®	25	500	RM000201
Microcrystalline cellulose	92	1 840	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	550.32 ± 0.40
Assay (%)	105.45 ± 1.03

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-18
Batch size	5 g
Date of Manufacture	11 September 2009

Formula:

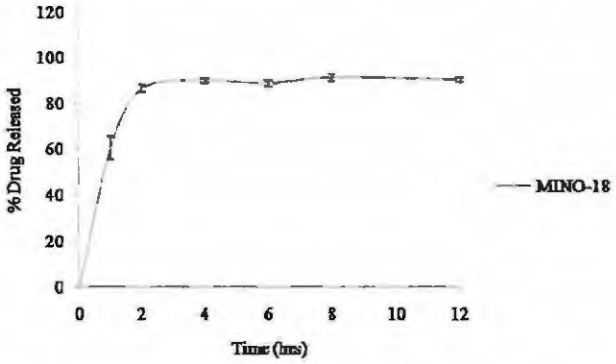
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Poloxamer 188	5	100	RM000196
Microcrystalline cellulose	62	1 240	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.08 ± 0.23
Assay (%)	99.97 ± 0.25

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-19
Batch size	5 g
Date of Manufacture	14 September 2009

Formula:

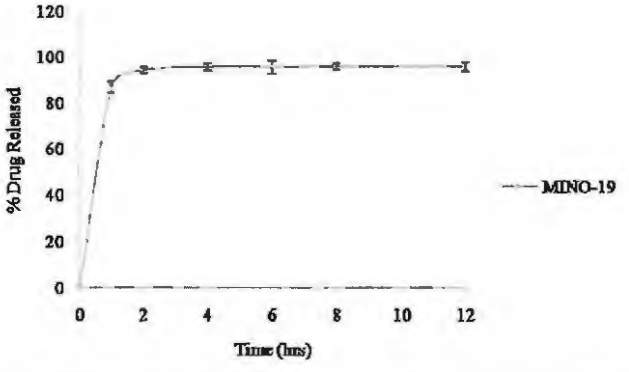
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Poloxamer 188	2.5	50	RM000196
Microcrystalline cellulose	64.5	1 290	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.07 ± 0.19
Assay (%)	104.78 ± 0.85

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-20
Batch size	5 g
Date of Manufacture	15 September 2009

Formula:

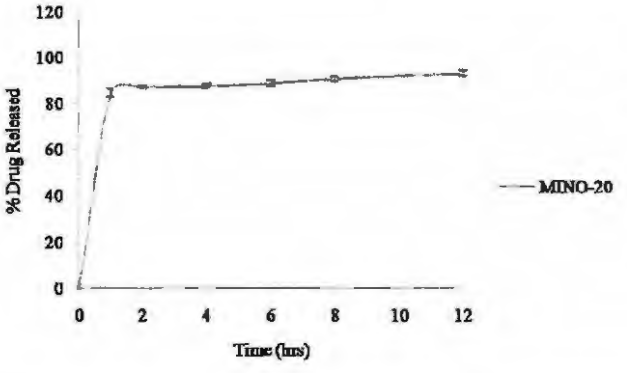
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Poloxamer 188	2.5	50	RM000196
Lactose monohydrate	64.5	1 290	RM000013

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.11 ± 0.32
Assay (%)	102.21 ± 0.78

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-21
Batch size	5 g
Date of Manufacture	16 September 2009

Formula:

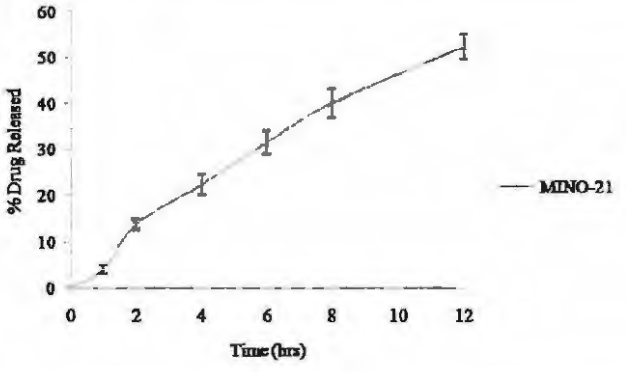
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Poloxamer 188	1.25	25	RM000196
Microcrystalline cellulose	65.75	1 315	XO61882

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	249.33 ± 0.47
Assay (%)	103.85 ± 1.16

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

RHODES UNIVERSITY

FACUTLY OF PHARMACY

BATCH RECORD SUMMARY

Formulator	Tinotenda Sachikonye
Product	Minocycline hydrochloride capsules
Batch #	MINO-22
Batch size	5 g
Date of Manufacture	17 September 2009

Formula:

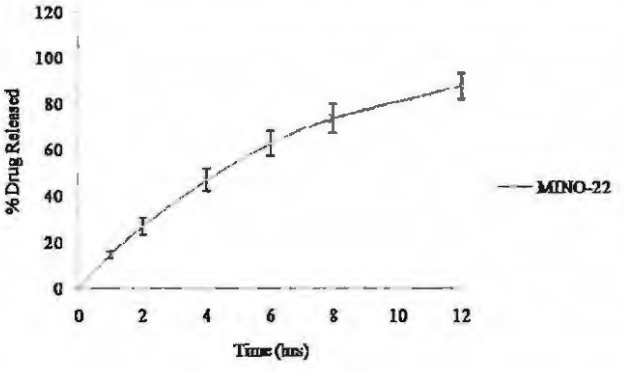
Name	Original Formula (mg)	Working Formula (mg)	Rhodes Batch Number
Minocycline hydrochloride	108	2 160	RM000225
Compritol®	75	1 500	RM000201
Poloxamer 188	1.25	25	RM000196
Lactose monohydrate	65.75	1 315	RM000013

Target capsule fill weight: 250 mg

Tests performed:

Weight (mg)	250.25 ± 0.38
Assay (%)	105.08 ± 0.84

IN VITRO RELEASE AND COMMENTS/OBSERVATIONS



- Fine pale yellow-coloured powder fill material
- No white specs in powder fill material indicating homogenous mixing of excipients
- Satisfactory powder flowability

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