DEVELOPMENT AND ASSESSMENT OF AZITHROMYCIN PAEDIATRIC SUPPOSITORY FORMULATIONS

By

Happiness Mollel

A Thesis Submitted to Rhodes University in Fulfillment of the Requirements for the degree of

MASTER OF SCIENCE (PHARMACY)

January 2006

Faculty of Pharmacy
Rhodes University
Grahamstown
South Africa

ABSTRACT

The use of the oral route of administration for the treatment of young children with antibiotics can at times be problematic since, factors such as nausea, vomiting, taste and/or smell, in addition to the challenges associated with the administration of suspensions, may contribute to poor patient compliance. In such cases, the use of the rectal route of administration may be appropriate. Therefore, suppositories containing 250 mg azithromycin (AZI) were manufactured and assessed for potential as an antibiotic suppository dosage form.

Suppositories, containing AZI dihydrate were manufactured by the fusion method, using different grades of PEG, Witepsol® and Suppocire® bases. The rate and extent of AZI release was evaluated using USP apparatus I, and samples were analyzed using a validated HPLC method.

Differences in the rate and extent of AZI release were observed with the greatest amount of AZI being released from PEG formulations. The rate and extent of AZI release from formulations manufactured using fatty bases were influenced by physicochemical properties, such as melting rate and hydroxyl value, of the bases. In addition drug partitioning appeared to favor the lipid phase and had a negative impact on AZI release characteristics.

Two different formulation approaches were used in an attempt to increase the rate and extent of AZI release from fatty base formulations. The use of surfactants significantly increased AZI release from formulations manufactured with fatty bases with high hydroxyl values. The use of urea or Povidone K25 in combination with AZI as a physical mixture or solid dispersion did not increase the rate and extent of AZI release from the fatty suppositories, to any significant extent.

The mechanism of drug release was evaluated using several mathematical models, including the Higuchi, Korsmeyer-Peppas, Zero and, First order models. In addition, *in vitro* dissolution profiles were characterized by the difference and similarity factors, f_1 and f_2 and by use of the Gohel similarity factor, S_d.

AZI release kinetics were best described by the Higuchi and Korsmeyer-Peppas models and the values of the release exponent, *n*, revealed that drug release was a consequence of the combined effects of AZI diffusion, rate of melting of the base and partitioning of the drug which can be considered to be anomalous release.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to the following people:

My supervisor, Prof. Roderick. B. Walker, for his patience and guidance throughout this project. Also for providing laboratory facilities and financial support and for showing confidence in me.

The Dean and Head, Prof I. Kanfer, and the staff of the Faculty of Pharmacy, for the use of the facilities in the Faculty.

Mr T. Samkange, for technical support throughout the duration of this project and also Mr. Leon Purdon for his assistance in various ways.

The Andrew Mellon Scholarship Funds and National Research Foundation for financial support.

The Bergen Trading C.C (Südafrika, Germany), Gattefossé Corp. (Paris, France), Aspen Pharmacare (Port Elizabeth, South Africa) and Zhejiang Huayi Pharmaceutical Co., Ltd (Yiwu, Zhejiang**,** China) for the donation of excipients.

My colleagues in the Biopharmaceutics Research Laboratory, S.M.M. Khamanga, R.N.O. Tettey-Amlalo, B.V. Chamboko, S. Patnala and V.D.P. Nair, thank you so much for the support and guidance throughout my project.

My parents, especially my late father Mr Jacob Mollel, and my family, whose love and support have encouraged and motivated me, not only in this project, but throughout my life. To my uncle, Mr Lot Mollel, for supporting me financially throughout my studies, thank you.

Dickson Chida, for his support, encouragement and particularly for his patience throughout this period.

STUDY OBJECTIVES

Bacterial infectious diseases, particularly those of the respiratory tract, such as pharyngitis/tonsillitis, skin and soft tissue infections, acute otitis media and community-acquired pneumonia, are common in children [1, 2]. The antimicrobial activity of azithromycin (AZI), a semi-synthetic derivative of the macrolide antibiotic erythromycin, against pathogens commonly implicated in paediatric community-acquired bacterial infections has been reported [1]. AZI is considered the drug of choice for the treatment of upper and lower respiratory tract infectious diseases, uncomplicated skin and soft tissue infections, as well as community-acquired pneumonia in children [1, 3]. Although several studies have been reported to investigate the release of AZI from various dosage forms such as tablets, capsule and parenterals, studies on the release of AZI from suppository dosage forms have not yet been reported.

The objectives of this study were:

1. To develop and validate a suitable High Performance Liquid Chromatographic (HPLC) method with the necessary sensitivity and selectivity to accurately and precisely quantitate AZI dihydrate in aqueous solutions and pharmaceutical dosage forms.
2. To develop a 250 mg AZI paediatric suppository in both water soluble and fatty bases.
3. To assess and evaluate the rate and extent of AZI release from the suppositories, using an appropriate dissolution method.
4. To determine the effects of aging of selected suppository formulations on AZI release.
5. To study the dissolution kinetics and release mechanism for selected AZI suppository formulations manufactured using fatty bases.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1. AZITHROMYCIN DIHYDRATE

1.1 INTRODUCTION

Azithromycin (AZI), (Figure 1.1, **1**) a semisynthetic derivative of erythromycin A, belongs to a new class of azalide antimicrobials [4]. The term azalide was originally introduced to represent a group of ring-expanded derivatives of erythromycin A (Figure 1.1, **2**) that contain an additional basic nitrogen at the 9a-position of the macrocyclic framework [5]. AZI consists of a 15-membered macrocyclic lactone ring onto which two sugar moieties are linked. An amino sugar D-desosamine is attached through a β-glycosidic bond to the C_5 position of the lactone ring, and a neutral sugar L-cladinose is attached via a α-glycosidic linkage to the C_3 position of the lactone ring.

The development of AZI arose from an interest in expanding the antimicrobial spectrum, improving the tolerability and the pharmacokinetic profile of the prototype macrolide antibiotic erythromycin A. AZI was discovered in the 1980s by the team of Pliva researchers and patented by Pliva in 1981, followed by the licensing of the patent to Pfizer in 1986 [6]. Pliva brought their AZI onto the market under the brand name, Sumamed® in 1988 and Pfizer launched Zithromax® in 1991 [6]. AZI was first approved by the Food and Drug Administration (FDA) for clinical use in 1992 [7, 8].

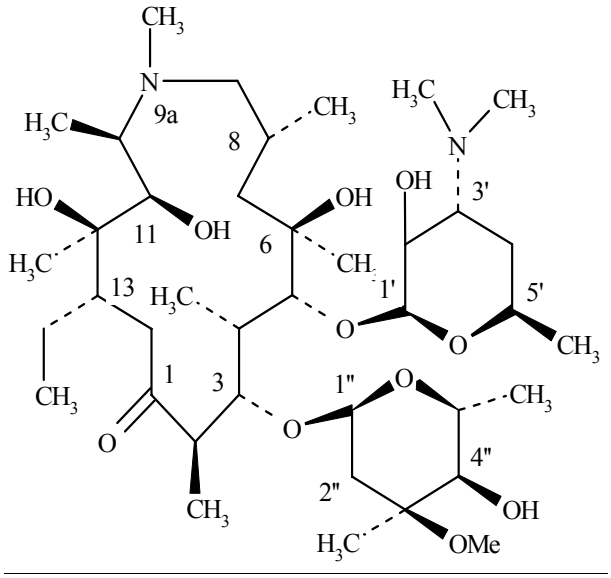
AZI is now widely used for the treatment of a range of adult and paediatric infections, including those of the upper and lower respiratory tract, skin and soft tissues, as well as sexually transmitted diseases [8, 9]. AZI is used as the dihydrate form in oral dosage products and as the sodium salt in parenteral dosage forms.

1.2 DESCRIPTION

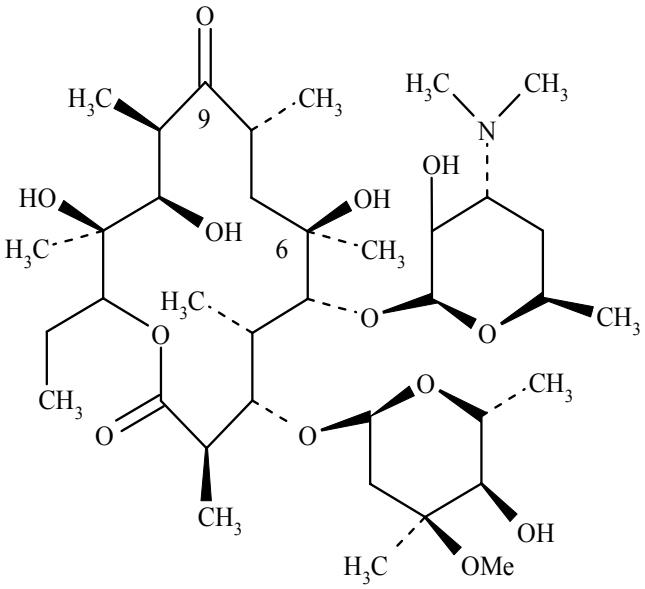
1.2.1 Name, formula, molecular mass

The chemical name for AZI is 13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[3,4,6-trideoxy-3-(dimethylamino)-*β*-*D*-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one dihydrate or 9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A [10, 11].

AZI occurs as a white to off white fine crystalline dihydrate ($C_{38}H_{72}N_2O_{12}\cdot 2H_2O$) powder with a molecular weight of 785.02 and in the amorphous anhydrous form ($C_{38}H_{72}N_2O_{12}$) with a molecular weight of 748.99.



1. Azithromycin

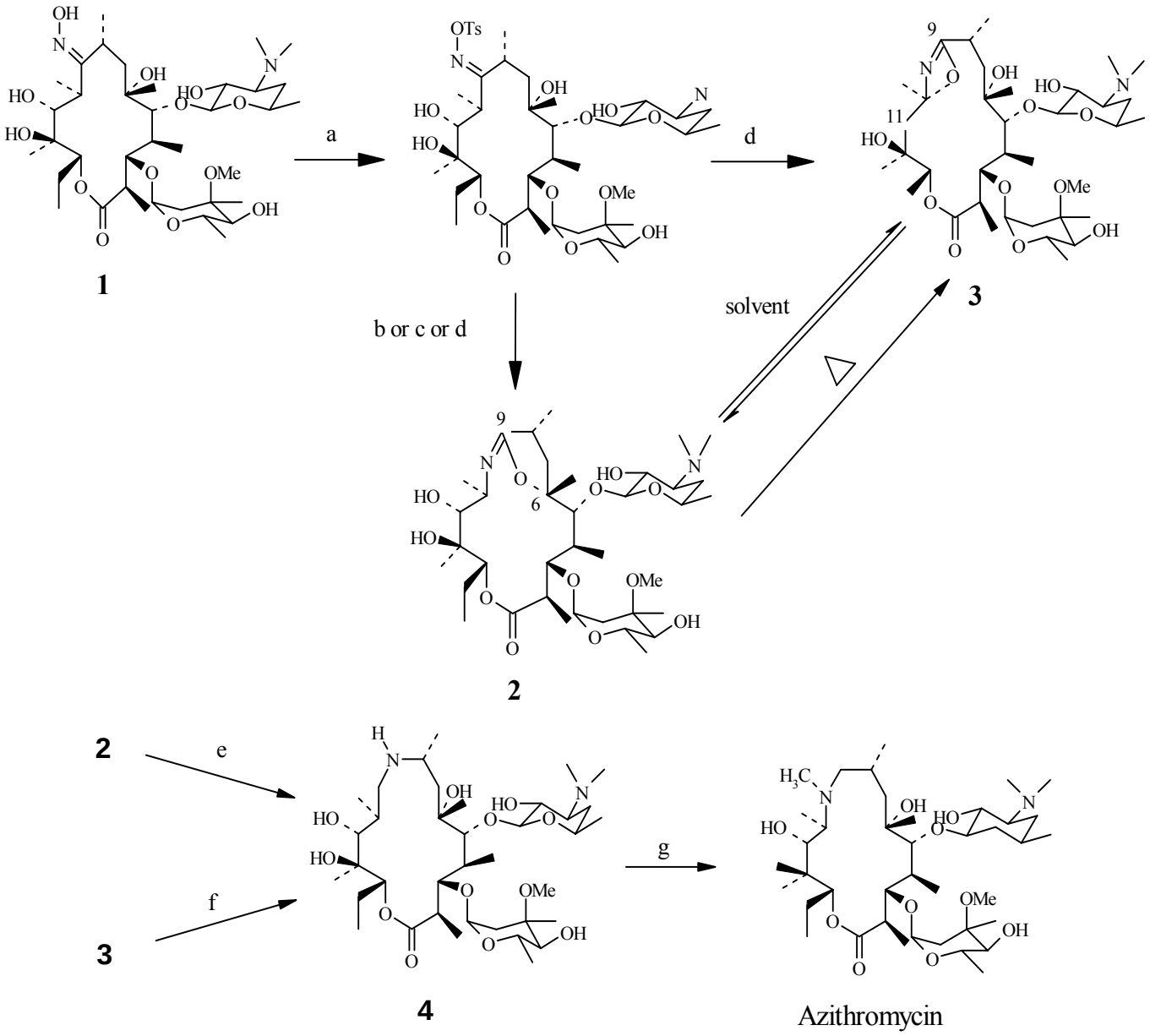


2. Erythromycin A

Figure 1.1: Chemical structures of AZI and erythromycin A

1.3 SYNTHESIS

AZI is prepared through Beckmann rearrangements of the corresponding 9(E)-erythromycin A oxime (Figure 1.2, **1**) [5, 12]. Oximation of erythromycin A (Figure 1.1, **2**) with hydroxylamine hydrochloride, provided 9(E) oxime (Figure 1.2, **1**) [12]. 9(E)-erythromycin A oxime undergoes several Beckmann reactions to form two isomers 6,9-iminoether (Figure 1.2, **2)** and 9,11-iminoether (Figure 1.2, **3)**. Reduction of either of the iminoethers by catalytic hydrogenation or sodium borohydride reduction, at room temperature under acidic conditions yields 9-deoxo-9a-aza-9a-homoerythromycin A (Figure 1.2, **4)** [5, 12]. This amine undergoes reductive N-methylation under Eschweiler-Clarke conditions to generate AZI [5, 12].



Key: (a) TaCl, acetone, $NaHCO_3$; (b) 2N HCl; (c) TsCl, $NaHCO_3$, aqueous. acetone; (d) TsCl, pyridine, ether, -45ºC, (e) H_2, PtO_2, HOAc or $NaBH_4$, MeOH, room temperature, acidic condition; (f) H_2, PtO_2, HOAc; (g) HCHO, HCO_2H, [12]

Figure 1.2: The synthesis of AZI

1.4 STEREOCHEMISTRY AND STRUCTURAL ACTIVITY RELATIONSHIP

In aqueous solution, AZI exists as a single isomer [13]. The absolute stereochemistry of AZI is shown in Figure 1.3.

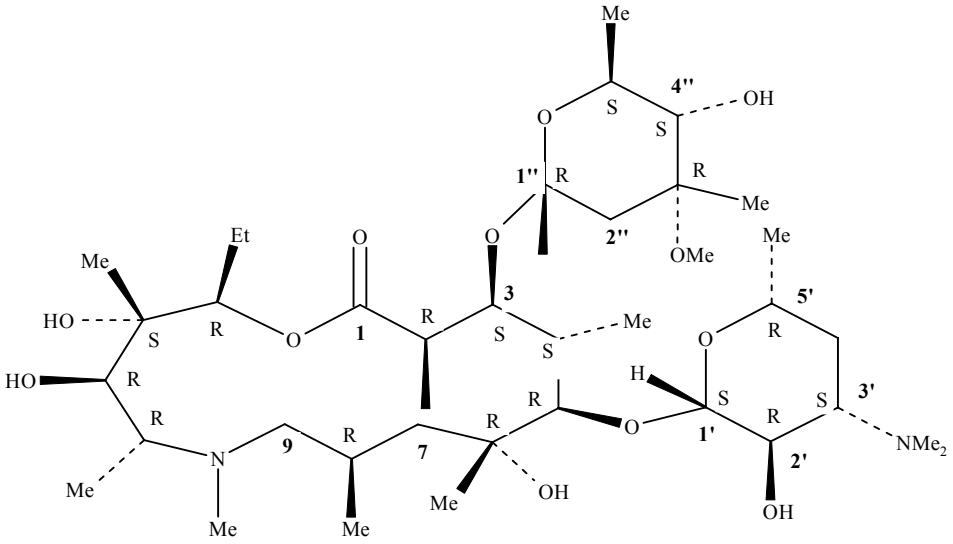


Figure 1.3: Stereochemistry of AZI

Chemical modification of AZI has been reported to affect its activity against bacterial pathogens in addition to its pharmacokinetic and pharmacodynamic properties. It has been shown that cleavage of the macrolide ring structure results in a significant loss in bactericidal activity of AZI [14]. The antibacterial activity of novel azalides decreases with an increase in the number of methyl groups substituents of the macrocyclic backbone [15]. The effect of methyl group substituent addition was shown in a series of experiments, in which the antibacterial activity of O-methylazithromycin derivatives was evaluated [15].

Similarly, the activities of these macrolide agents are greatly reduced if the tertiary amine group is removed from the desoamine sugar at position $C_{3'}$ (Figure 1.3) [14]. The discovery of the ketolides has disproved a long held belief that the cladinose sugar at position C_3 (Figure 1.3) was an essential component for the antibacterial activity of these compounds [12]. In the ketolide family, the cladinose sugar at position C_3 is replaced by a ketone group, and these have been shown to exhibit enhanced antibacterial potency when compared to erythromycin [12].

Conversely, increased potency of these compounds has also been observed when the original basic macrolide ring of erythromycin A was modified by the addition of a methyl-substituted nitrogen at position C_9 (Figure 1.3). This modification has rendered AZI two to eight fold more active against *Haemophilius influenzae* than erythromycin [8, 9, 14]. The addition of a methylamino group on the lactone ring (position 9a, Figure 1.3) has extended the range of antimicrobial activity of the macrolides against gram negative organisms whilst retaining activity against gram positive microorganisms.

The replacement of the 9a carbonyl in the aglycone ring with a methyl substituted nitrogen, in addition to ring expansion to a 15 membered ring, resulted in improved acid stability of AZI as compared to erythromycin [4, 16]. In addition to increased acid stability, formation of an anhydrohemiketal derivative, which has been associated with the gastrointestinal toxicity observed with erythromycin in acidic media is markedly reduced or even avoided [16]. These changes also increase the lipid solubility of the molecule, thus conferring unique pharmacokinetic characteristics and microbiocidal properties to AZI [4]. The increased lipid solubility may be a reason for the improved antibacterial activity.

1.5 PHYSICAL PROPERTIES

1.5.1 Solubility

The solubility of both AZI dihydrate and AZI monohydrate in water at 37°C were reported to be 1.98 ± 0.11 mg/ml and 1.80 ± 0.081 mg/ml respectively [17]. AZI is highly soluble in ethanol and methylene chloride [18]. Solubility studies of AZI dihydrate in phosphate buffer (0.1 M, pH 6, 7.2, and 8) and in water were conducted by the addition of AZI dihydrate to saturation into the appropriate solvent and shaking at room temperature (22ºC) at 200 rpm using a Junior Orbit Shaker (Lab-line Instruments Inc., Melrose Park, ILL, USA) for 24 hours. The samples were analyzed after 24 hours of shaking using a validated HPLC method (§ 2.3, Chapter 2). The results of these solubility samples are summarized in Table 1.1, which list the solubility of AZI in phosphate buffer and in water.

Table 1.1: Solubility of AZI

Solvent	Solubility (mg/ml)
Water	0.14 ± 0.6075
pH 6.0	27.03 ± 0.0503
pH 7.2	9.05 ± 0.0729
pH 8.0	1.92 ± 0.0035

1.5.2 Dissociation constant and partition coefficient

Apart from a basic sugar with a tertiary ionazible amine group located at position $C_{3'}$ (Figure 1.3), AZI has a second basic tertiary amine at position C_{9a} that makes it a dibasic macrolide. This results in AZI having both a low (pK_{a1}) and high (pK_{a2}) dissociation constant. A summary of the pK_a of AZI as reported in the literature is depicted in Table 1.2.

Table 1.2: pK_a values for AZI

PK_{a1}, PK_{a2}	Reference
8.74[a], 9.45[b]	19
8.85[a]	20
8.6[a], 9.5[b]	21
8.1[a], 8.8[b]	22

Key: [a] lowest measured pK_a value, [b] highest measured pK_a value

The lipophilicity of a molecule is one of the core properties that may be used to estimate the absorption, distribution and transportation potential of a drug in biological systems [23]. Lipophilicity is a molecular parameter that describes the distribution equilibrium of solute molecules between an aqueous and various water immiscible, lipid-like organic solvents or other solubilizing media, such as, for example, membrane systems [23, 24]. In most cases, lipophilicity is measured as the Log $P_{o/w}$, which is the logarithm of the partition ratio of a drug between octanol and water.

The Log $P_{o/w}$ value of AZI, determined potentiometrically at 25ºC, has been reported as 4.02 [19], which is similar to that of 4.04 reported by Chanteux, *et al,* [22] that was determined by high-performance liquid chromatography. The Log P value is one of the most important factors that can be used during dosage form development to predict the potential partitioning

of AZI between a lipophilic medium such as a semi-synthetic suppository base and an aqueous environment, during dissolution testing and subsequently *in vivo*.

1.5.3 pH of solution

The pH of AZI (2mg/mL) in a mixture of methanol and water (1:1) was found to be in the range of 9.0 to 11.0 [10].

1.5.4 Specific rotation

The specific optical rotation of AZI (20mg/mL) in dehydrated alcohol is between 45º and -49º (t=20ºC) [10].

1.5.5 Differential scanning calorimetry (DSC) and melting range

DSC themograms of azithromycin dihydrate and monohydrate were recorded using a Mettler Star Differential Scanning Calorimeter in a sealed aluminium pan and are depicted in Figure 1.4 [17]. The melting of AZI crystals was preceded by the loss of water. Azithromycin dihydrate had a melting range of 134.65-141.35ºC, whereas the monohydrate form had a melting range of 139.88-156.31°C [17].

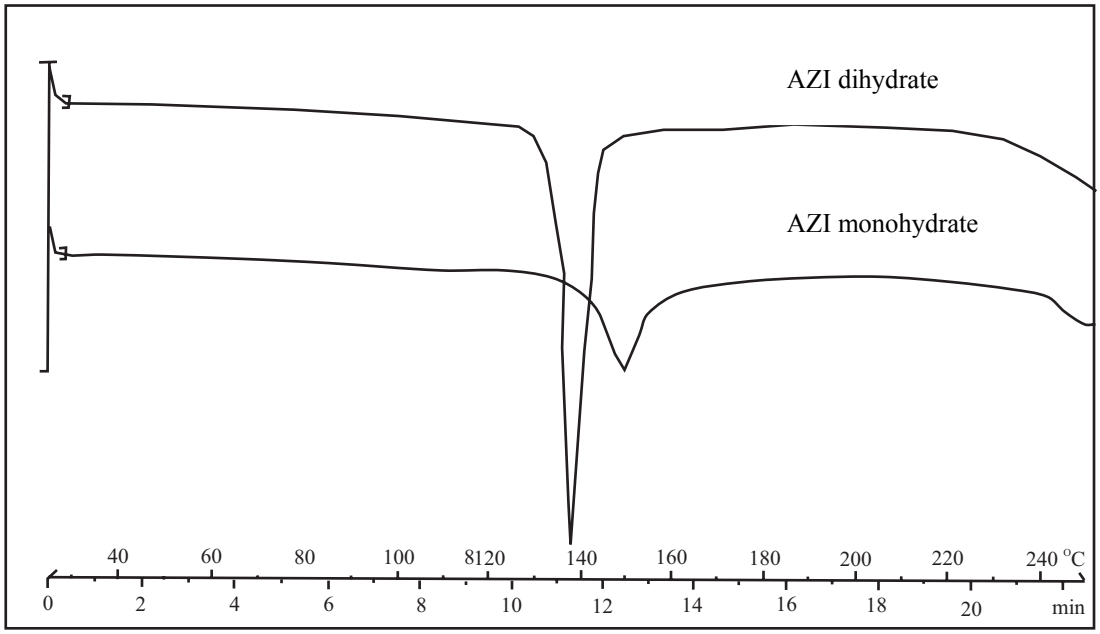


Figure 1.4: DSC thermograms of the different forms of AZI [17]

1.5.6 Infrared spectrum (IR)

There are three main methods for the preparation of solids for IR scanning. These methods include the use of Nujol® mull, potassium bromide (KBr) press disks or glassy film deposits [25]. The KBr method was used to obtain the IR spectrum of AZI in the range of 4000-400cm^{-1} using a Perkin-Elmer FT-IR spectrum 2000 spectrophotometer (Perkin-Elmer LTD, Beaconsfield, Bucks, England). The IR spectrum of azithromycin dihydrate is shown in Figure 1.5 and the relevant band assignments are shown in Table 1.3 and were determined using the theoretical concepts previously described by Silver *et al,* [25].

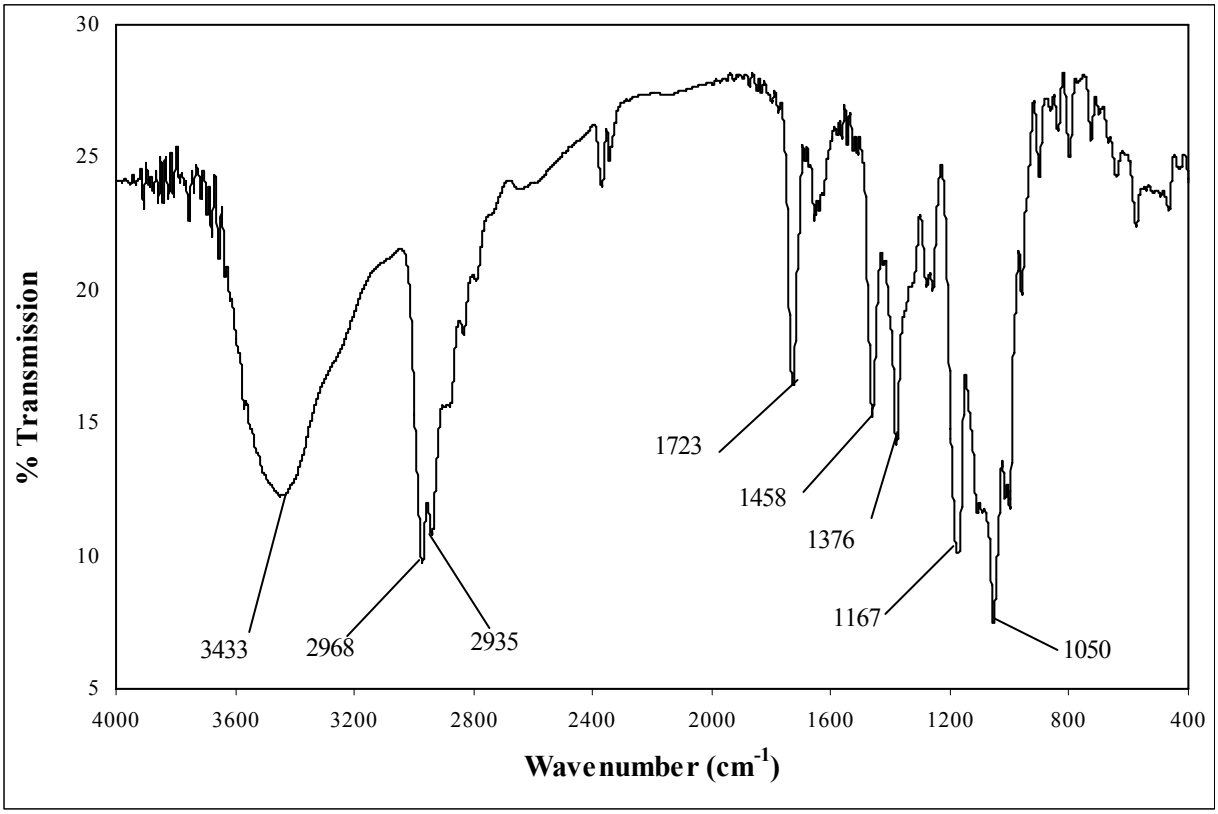


Figure 1.5: Infrared spectrum of AZI dihydrate in KBr

Table 1.3: Characteristic band assignment of AZI infra red spectrum

Frequency	Assignment
3433	γOH stretching (broad-intermolecular hydrogen bonding)
2968-2935	γC-H (aliphatic) stretching vibration
1723	γC=O Carbonyl ester stretch
1458	δCH_3-O (alkyl ether)
1376	δCH_2-O (alkyl ether)
1167	C-O-C asymmetrical stretching (aliphatic ethers)
1050	C-O-C symmetrical stretching (aliphatic ethers)

1.5.7 Ultraviolet spectrum

The ultraviolet spectrum of azithromycin dihydrate in mobile phase (methanol:acetonitrile: phosphate buffer pH 6.7:tetrahydrofuran, 15:25:60:2.5v/v) and that in methanol at a concentration of 0.3mg/ml are shown in Figures 1.6 and Figure 1.7 respectively. Spectra were obtained using a double beam Model GBC 916UV VIS spectrophotometer (GBC Scientific Equipment Pty Ltd, Melbourne, Victoria, Australia). The spectra obtained in both mobile phase and methanol showed one shoulder at 199.2 nm and 201.6 nm respectively. Since AZI is insoluble in water, the UV spectrum of AZI in water was not developed. UV spectral studies were performed in order to obtain the λ_{max} for AZI, which can be used in high performance chromatographic method development for the quantitation of AZI in pharmaceutical dosage forms using UV detection.

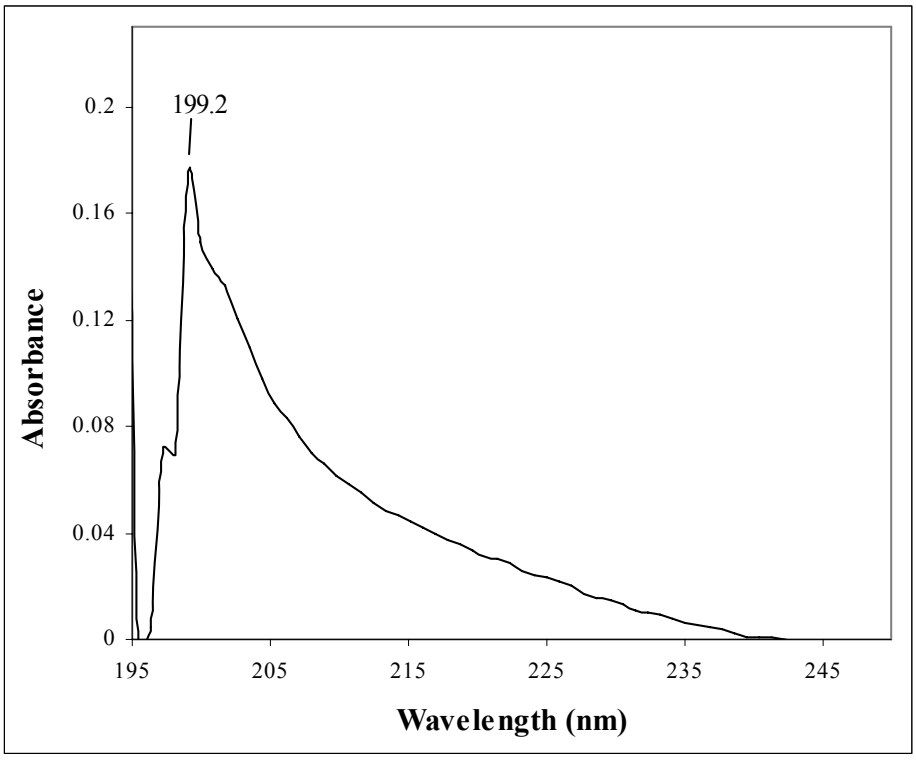


Figure 1.6: The ultraviolet spectrum of AZI dihydrate in mobile phase

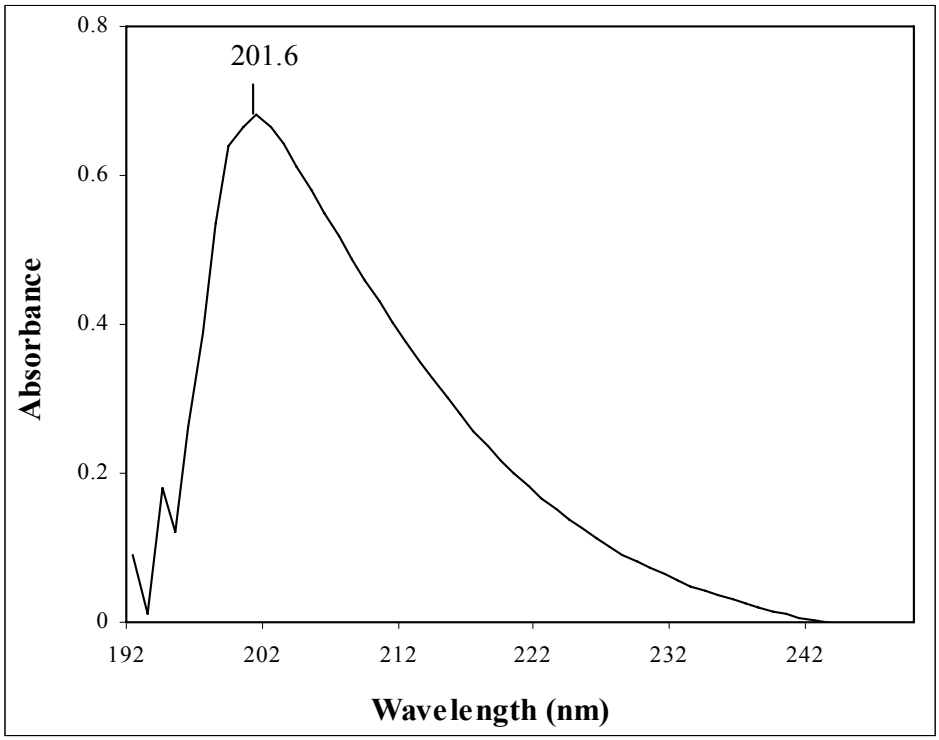


Figure 1.7: The ultraviolet spectrum of AZI dihydrate in methanol

1.5.8 Proton magnetic resonance spectrum

The ^{1}H spectrum of AZI was acquired at ambient temperature using a Bruker AVANCE 400 MHZ spectrometer (Bruker, Grerman) calibrated using solvent signals at 7.25 ppm. The spectrum in chloroform-d_1 ($CDCL_3$) obtained from Merck (Darmstadt, Germany) is shown in Figure 1.9 with the chemical shifts (δ) in ppm listed in Table 1.4 and correspond to the proton assignment shown in Figure 1.8. The NMR studies are of value when elucidating degradation products of AZI in future studies that are beyond the scope of this project.

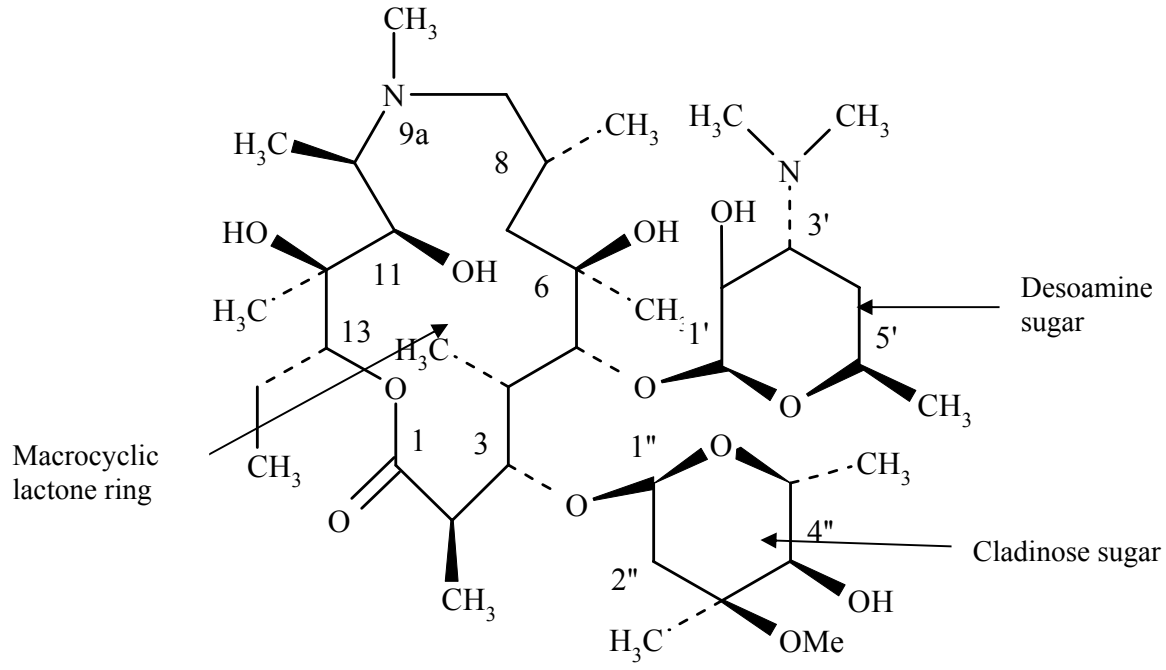


Figure 1.8: AZI proton assignment

Table 1.4: ^{1}H spectra of AZI in $CDCl_3$

Macrocyclic lactone ring						**Desoamine and Cladinose sugar**		
H (site)	δ ppm	Multiplicity	H (site)	δ (ppm)	Multiplicity	H (site)	δ ppm	Multiplicity
2	2.71	m[a]	14eq	1.90	dd	1'	4.44	d
3	4.25	dd	14ax	1.45	dd	2'	3.51	dd
4	1.91	m	14Me	0.90	t	3'	2.14	d
5	3.63	d	2Me	1.25	d[b]	4'eq	1.60	d
7eq	1.78	d	4Me	1.02	d[c]	5'	3.51	m
7ax	1.25	dd[b]	6Me	1.42	s	5'Me	1.24	d
8	2.01	m	8Me	1.02	d[c]	3'NMe_2	2.97	s
9eq	2.53	d	10Me	1.09	d[d]	1"	5.15	d
9ax	2.03	dd	12Me	1.10	d[d]	2"eq	2.34	d
10	2.70	m[a]	9aNMe	2.41	s	2"ax	1.55	dd
11	3.66	d				4"	3.26	d
11OH	5.02	s				5"	4.05	m
12OH	2.99	s				5"Me	1.31	d
13	4.70	d				3"Me	1.22	s
						3"OMe	3.30	s

Key: [a, b, c, d] ^{1}H resonance coincident. d-doublet, t-triplet, m-multiplet

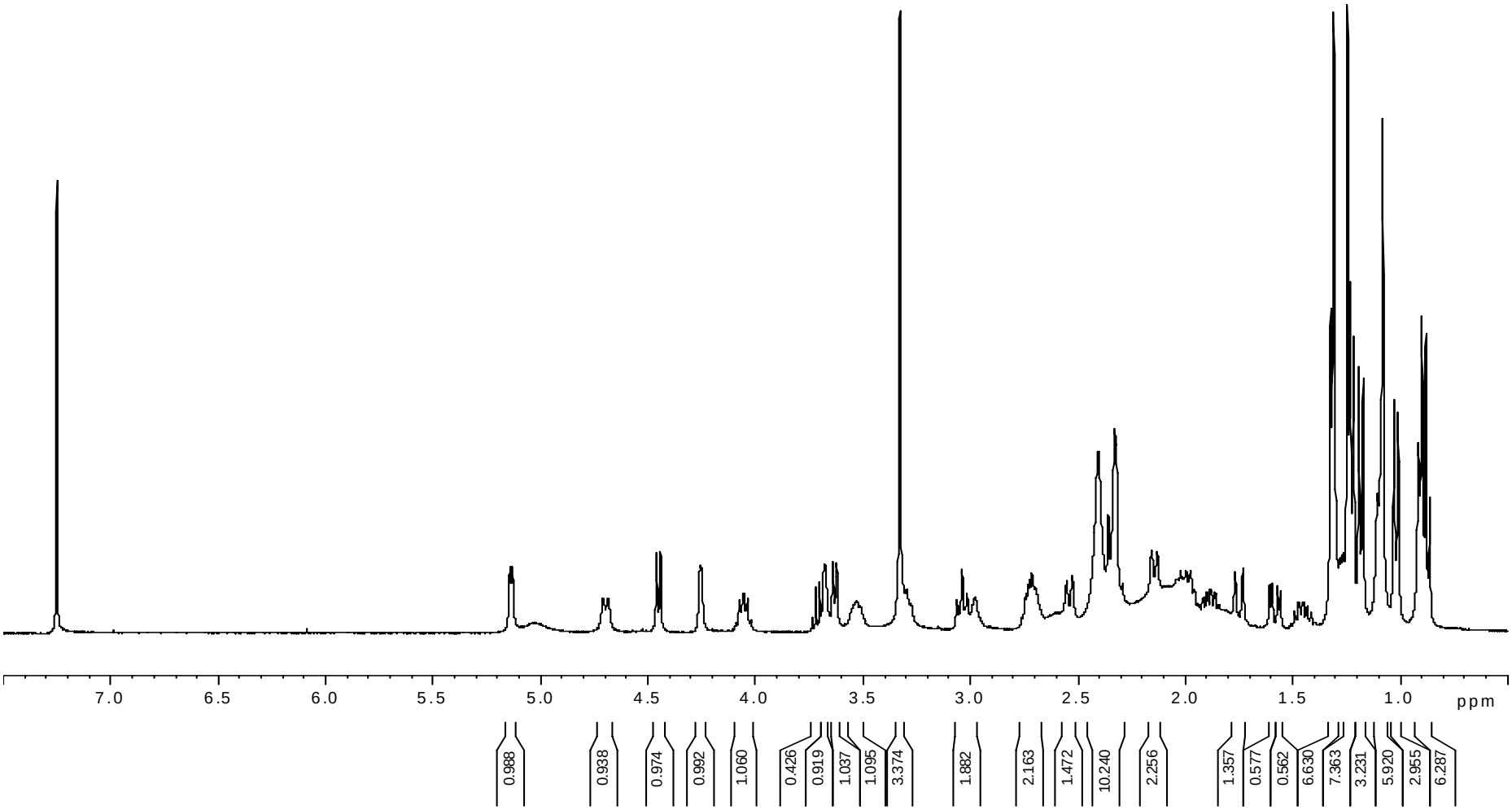


Figure 1.9: Proton magnetic resonance spectrum of AZI in $CDCl_3$

1.6 STABILITY

The structural modification of erythromycin A has resulted in the development of the macrolide antibiotic, AZI, which is more stable in aqueous acidic solutions than erythromycin A. The insertion of a 9a methyl-amino group at position C_{9a} (Figure 1.1, **1**) and ring expansion, blocks potential internal acid hydrolysis to form a hemiketal product, as is produced by erythromycin under acidic conditions due to the presence of a carbonyl group at position C_9 (Figure 1.1, **2**). Acid hydrolysis of the ether bond at position C_3 (Figure 1.1, **1**) is the main decomposition pathway of AZI [16]. The mechanism proposed for this reaction involves proton attack on the ether oxygen followed by cleavage to form a secondary alcohol [16]. The resultant degradation product, the pseudo-aglycone of AZI, is shown in Figure 1.10. The degradation of AZI is pH dependant and the rate and extent of acid hydrolysis increases in solution of decreasing pH [16].

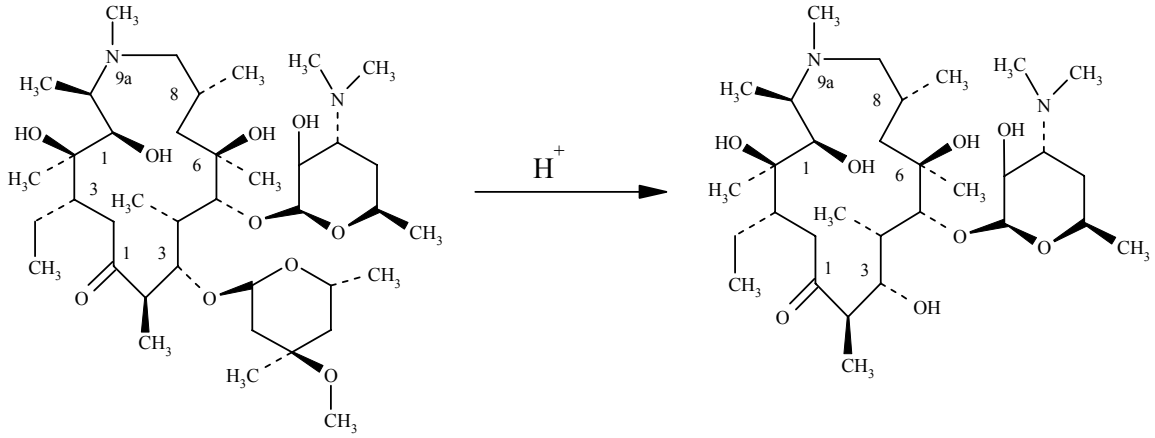
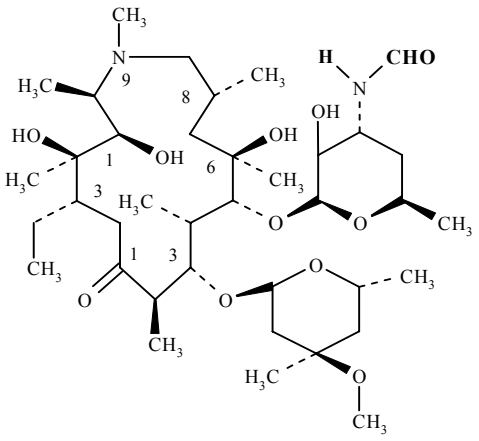


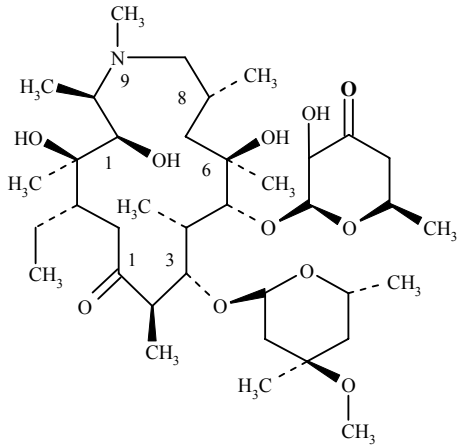
Figure 1.10: Decomposition pathway for AZI in aqueous acidic medium

Apart from its acid instability, AZI is also poorly water-soluble, which is one of the major challenges in formulating AZI parental products. The solubility of AZI in water improves when the solution pH is adjusted to pH 5.0. The change in pH can be effected by adding citric acid, but this solution is not stable and precipitates are seen over time [26]. Solutions may be stabilized by the addition of sodium salts such as sodium hydroxide, thereby changing the pH from 5.0 to 7.0 [26]. Solutions of AZI containing both citric acid and sodium hydroxide, when diluted with sterile water are chemically and physically stable for 24hrs when stored at or below 30ºC. These solutions are also stable for 7 days if stored under refrigerated conditions (5ºC).

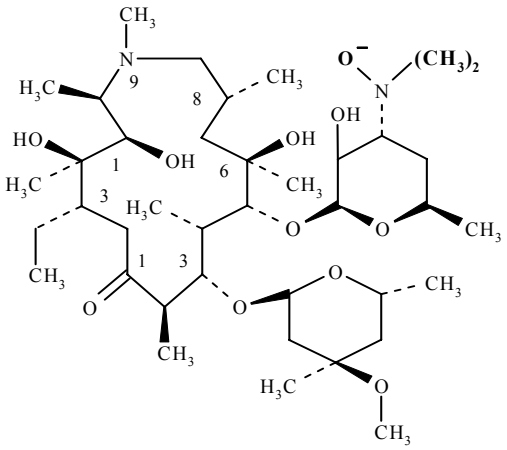
Solid-state stability studies are particularly valuable for assessing the potential instability of an active compound that may occur during formulation, manufacturing and/or storage. The amine functionality of AZI located at position $C_{3'}$ (Figure 1.3) is susceptible to oxidation and other degradation reactions if exposed to elevated temperatures and/or air during the development and manufacturing of AZI containing dosage forms [27, 28]. Michael *et al,* [27] performed degradation studies on AZI at an elevated temperature of 55°C for a period of 3 months and used HPLC/MS-MS, NMR and UV-spectrophotometry to identify degradation products formed under these stress conditions. The chemical structures of the degradation products formed during the aforementioned studies are shown in Figure 1.11. The knowledge of AZI stability in elevated temperatures is vital for this study, since AZI is added to a suppository base that has been melted at 50°C. The presence of the degradation products may lead to deviation of the product from regulatory purity requirements before the product even reaches the patient [27].



N-Formyl-N-di(dimethyl)-
Azithromycin
Mwt = 749.5, λ_{max} = 200.1nm



Azithromycin
Mwt = 720.5, λ_{max} = 202.5nm



Azithromycin-N-oxide
Mwt = 765.5, λ_{max} = 200.1nm

Figure 1.11: Degradation product of AZI formed at 55°C [27]

AZI has a tendency to undergo oxidative degradation or decomposition under normal storage conditions, which may result in unacceptable levels of impurities being present in dosage forms [28, 29]. The susceptibility of AZI to degradation results in the need to develop an optimal formulation composition and method of manufacture in which the stability of AZI is not compromised or is enhanced, resulting in the production of a high quality, stable, safe and effective dosage form. A variety of strategies including the use of antioxidants [29] and of gas impermeable laminated aluminium packaging [28] have been shown to enhance the stability of AZI during manufacturing and storage. This is important in the design of the new pharmaceutical dosage forms with respect to storage and packaging of the product with the aim of minimizing the presence of unacceptable levels of impurities at the time of administration.

1.7 CLINICAL PHARMACOLOGY

1.7.1 Mode of action

AZI differs from other macrolide antibiotics in terms of its pharmacokinetic profile rather than in the mechanism of action and antimicrobial spectrum. As with other macrolide antibiotics AZI, exerts its antibacterial activity by interfering with the ribosomal function of bacteria. AZI binds reversibly to the 50S component of the 70S ribosomal subunit in the bacterial cell, thereby inhibiting the transpeptidation/translocation process, causing the premature detachment of incomplete peptide chains, resulting in subsequent cell death [8, 14]. It has been suggested that the higher ribosome binding affinity of AZI may account for its enhanced activity against gram-negative microorganisms [8]. However, if the ribosome is derived from a resistant strain of bacteria with an *erm* type gene that results in modification of the 50S ribosomal subunit, the inhibitory activity of AZI is partially lost [14, 30].

1.7.2 Spectrum of activity

AZI exerts its antimicrobial activity against a large number of gram-positive and gram-negative aerobic and anaerobic species that are associated with respiratory, skin and sexually transmitted infections. AZI is of major importance in the treatment of intracellular pathogens such as *Chlamydia pneumoniae, Chlamydia trachomatis, Mycloplasm pneumonia and Legionella pneumonia* [8], as well as enteric pathogens such as *Salmonella typhi, Shigella flexnen, Shigella sonnei* and *Shigella dysentenae* [30]. Although slightly less potent than erythromycin against gram-positive microorganisms, AZI demonstrates superior activity *in vitro* against a wide variety of gram-negative bacteria, including *Haemophilus influenzae*, *Moraxella catarrhalis* and *Haemophilus parainfluenzae* [8, 30].

In vitro potency testing of AZI is sensitive to the pH of the bacterial growth medium and a reduction in pH will result in the ionization of AZI, which alters its ability to penetrate into bacterial cells with a subsequent increase of the minimum inhibitory concentration (MIC) of the antibiotic [31]. Hoepelman, *et al,* [8] and Harold *et al,* [9] have listed *in vitro* activity and MIC's of AZI against both gram-positive and gram-negative species, in addition to anaerobic bacteria and intracellular pathogens. The MIC of AZI ranges between 0.12-0.5 mg/l compared to values of 3-8 mg/l for erythromycin against both β-lactamase-positive and β-lactamase-negative strains [8]. AZI is more potent against *Neisseria gonorrhoeae* compared to other macrolides such as erythromycin, roxithromycin or clarithromycin [8].

In addition, AZI is highly effective in inhibiting clinically significant intracellular pathogens such as *Chlamydia trachomatis* and *legionella* [8, 9]. Its activity profile also includes efficacy against *Mycoplasma* species such as *Mycoplasma hominis*, that are resistant to both erythromycin and tetracycline antibiotics [8]. AZI has also shown good *in vitro* activity against a number of non-bacterial intracellular pathogens such as the protozoan organism, *Toxoplasma gondii* [8, 9, 11].

AZI is either bactericidal or bacteriastatic, depending on the concentration of the molecule *in vivo*. A number of *in vitro* studies have shown AZI to possess bactericidal activity against both gram-positive and gram-negative organisms, such as *Streptococcus pyogenes*, *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Moraxella*

catarrhalis, Escherichia coli, Klebsiella pneumoniae, Bordetella pertussis, Legionella pneumophila, Chlamydia trachomatis and *Borrelia burgdorferi* [8, 9]. In addition, prolonged tissue concentrations above the MIC at the site of infection for several days [32] suggest that AZI may possess bactericidal effects *in vivo*. AZI is inactive against methicillin-resistant *Staphylococci* and most strains of *Enterococcus fecalis*, as well as gram-positive strains that are known to be resistant to erythromycin [7-9].

1.7.3 Indications

AZI is indicated for clinical indications that include the treatment of respiratory infections such as acute *Streptococcus pyogenes* pharyngitis, tonsillitis, otitis media, sinusitis, community-acquired pneumonia, acute bronchitis, acute exacerbations of chronic bronchitis and acute bacterial exacerbations of chronic obstructive pulmonary disease [30]. In addition, AZI can be used as an alternative antibiotic for patients that show penicillin sensitivity.

AZI can also be used for the treatment of skin and soft-tissue infections caused by pathogens such as *Streptococcus pyogenes, Streptococcus agalactiae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and bite organisms such as *Pasteurella multocida* and *Eikenella corrodens* [8, 9]. The treatment of urogenital and other sexually transmited infections caused by *Chlamydia trachomatis, Neisseria gonorrhoeae*, *Hemophilus ducreyi* and *Ureaplasma urealytecum* can be achieved by the use of a single one-gram dose of AZI [8].

AZI is the first or second choice antibiotic for the treatment of legionnaires' diseases [8, 33]. AZI has been approved by the FDA as the macrolide antibiotic that can be used for the prevention of disseminated *Mycobacterium avium* complex (MAC) disease in patients with advanced Human Immunodeficiency Virus (HIV) infections [34]. It can be used in combination with ethambutol, for the treatment of *Mycobacterium avium* intracellular complex infection in patients with advanced HIV infections [35], and also for prophylaxis alone or in combination with Rifabutin® against MAC. In addition AZI may also be used for the treatment of typhoid fever [9, 36], trachoma [8, 36], diseases caused by protozoal organisms such as cryptosporidiosis and toxoplasmosis [9, 36, 37], as well as gastrointestinal infectious diseases caused by pathogens, such as those caused by the *Shigella* and *Salmonella* species [9, 38].

1.7.4 Resistance

The clinical significance of AZI resistance has been the subject of ongoing research. Koch *et al* [39] proposed that most of the AZI administered orally leaves the body unchanged, in the faeces, which suggests that a significant amount of active AZI may be introduced into wastewater treatment plants. The presence of active AZI in sewage treatment plants could be a factor in promoting the development of antimicrobial resistance to AZI in species that are present in the wastewater treatment plants and those that may be exposed to the compound after the water has been released into the general environment [39].

It has been suggested by some researchers [40, 41] that low serum concentrations of AZI that persist after the completion of a course of AZI, could act as a trigger for the emergence of resistant strains amongst pathogens. However, the pharmacokinetic and pharmacodynamic properties of AZI suggest that patients who have been treated successfully, would be completely clear of any residual pathogens, thus preventing the potential emergence of resistant populations of bacteria [31].

Despite its unique pharmacokinetic/pharmacodynamic properties, AZI resistance is more prevalent in gram-positive than in gram-negative bacteria [8, 14, 42-44]. In the case of gram positive organisms harboring the type *erm* gene, bacterial resistance is thought to result from the induction or constitutive production of an enzyme, *erm* 23S-rRNA methyltransferase, which catalyses methylation of adenosine residues in the bacterial ribosome [8, 14, 30, 42]. Methylation of the adenosine moiety is responsible for the transfer of resistance to erythromycin and other 14-membered macrolide antibiotics [8]. Since AZI lacks the ability to inhibit methylated ribosomes, it also shows cross-resistance with erythromycin resistant gram-positive organisms [8]. Examples of bacteria having the *erm* gene mechanism of resistance include *Streptococcus pneumoniae (erm*B gene) and *Staphylococcus aureus* (*erm*A or *erm*C gene) [14].

Another possible method by which resistance is thought to be induced appears to be associated with the presence of an energy dependent efflux pump mechanism that is present in some organisms and appears to be specific for 15- membered ring macrolide antibiotics [14, 42, 44]. Examples of microorganisms that show energy dependent efflux resistance mechanisms include *Streptococcus pneumoniae* encoded with *mefE* gene [14, 42] and *Staphylococucs pyogenes* encoded with *mefA* gene [14].

The mechanism of resistance involving the production of β-lactamase alone does not appear to influence the *in vitro* activity of AZI. Evidence for this is provided by the *in vitro* activity of AZI against β-lactamase-producing strains of *Haemophilus ducreyi, Haemophilus influenzae, Moraxella catarrhalis* and *Neiseria gonorrhoeae* [8, 44].

1.7.5 Contraindications

AZI should not be administered to patients with a known hypersensitivity to AZI or any other macrolide antibiotics [37]. It should also not be co-administered with ergot derivatives due to known interactions between ergot alkaloids and the early macrolides, such as, for example, triacetyloleandomycin [8]. In a clinical study in which a single oral dose of 500 mg of AZI was administered to patients with class A or B liver cirrhosis no differences in the pharmacokinetic profile of AZI were observed when compared to those obtained following AZI administration to healthy volunteers [45]. These findings suggest that AZI can be used without changing the dose in patients with liver disease [45]. However, it should be avoided in patients with severe hepatic diseases, since the liver is the principal route of AZI metabolism and thus excretion [37]. Similarly, there was no significant differences in the urinary excretion of AZI in elderly and young volunteers, suggesting that dosage adjustments are unnecessary when treating elderly patients who may present with mild renal impairment [46].

1.7.6 Drug interactions

Unlike 14-membered ring macrolide antibiotics, AZI does not significantly inhibit or induce cytochrome P450 enzyme systems [47]. Pharmacokinetic studies in healthy volunteers have demonstrated no evidence of interactions between AZI and theophylline, tefenadine [8], cimetidine and zidovudine [48]. In a study in which ambulatory patients were co-administered AZI and warfarin, no significant drug interaction was observed [49]. Co-administration of AZI with aluminum and magnesium containing antacids has been shown to reduce the peak plasma serum levels of AZI, but not the extent of absorption, as indicated by AUC [11].

1.7.7 Adverse reactions

Clinical studies have shown that AZI is well tolerated and has a low incidence of adverse events, when compared to many other antibiotic agents [50]. Adverse effects of AZI are mainly gastrointestinal in nature and occur less frequently than with erythromycin. However, rashes, headache and dizziness may occur when AZI is administered [11].

1.7.8 High risk groups

In a study in which rats and mice were given AZI at doses of between two and four times that of a normal human dose of 500 mg, no teratogenic or other effects were reported [33]. In addition, there were no reported effects on the fertility of these animals [33]. Consequently AZI falls under the FDA risk factor B category, which suggests that animal studies have not shown a foetal risk, but are no controlled studies that have been carried out on pregnant women. Therefore both the patient and the prescriber should be aware that the use of AZI during pregnancy may involve safety and toxicity risks [51]. The safety of use of AZI during lactation has, as yet, not been established and it should be avoided when treating breastfeeding mothers [51].

1.8 PHARMACOKINETICS OF AZITHROMYCIN

1.8.1 Dosage

The interrelationship between pharmacokinetics and pharmacodynamics determines the dose rate, duration of dosing and the dose required for optimal efficacy of AZI [52]. A review of the pharmacokinetic and pharmacodynamic characteristics of AZI supports its use in monotherapy for the treatment of infection [52]. The usual adult dose of AZI is 500 mg as a single daily dose for three days. A three-day oral regimen of once daily AZI has been shown to be as effective as a five to ten day course of other more frequently administered antibacterial agents such as erythromycin, amoxicillin/clavulanic acid and penicillin V in patients with acute exacerbations of chronic bronchitis, pneumonia, sinusitis, pharyngitis, tonsillitis and otitis media [53].

Uncomplicated sexually transmitted diseases may be effectively treated by use of a single one-gram (1g) dose of AZI [8]. It has been suggested that single-dose therapy of AZI for the treatment of uncomplicated urethritis or cervicitis offers a great advantage from the patient compliance point of view over the other use of antibiotics [4, 7-9]. A single two-gram dose (2g) may be used for the treatment of uncomplicated gonorrhea. A 1.2 g weekly dose of AZI may be prescribed for the prophylaxis of disseminated MAC infections in immuno-compromised patients [11, 35, 36]. In children over one year old a total dose of 30 mg/kg, given as a10 mg/kg once daily dose for three days may be used for the treatment of pneumonia or otitis media [36, 54], whereas a 12 mg/kg once daily dose for five days may be given to children with pharyngitis caused by *streptococcal* species.

The FDA has approved the use of intravenous AZI and has permitted market authorization for 1 mg/ml or 2 mg/ml solutions such that the administration of 500mg intravenously would require the use of either 250 ml or 500 ml of compatible fluid formulations for administration [55]. Intravenous AZI may be administered as a 2 mg/ml solution as a bolus dose over a 60 minute administration period [55]. Azithromycin dihydrate may be given initially by intravenous infusion in doses equivalent to 500 mg of AZI as a single daily dose for at least two days for the treatment of community-acquired pneumonia and pelvic inflammatory disease [56]. Intravenous therapy should be followed by azithromycin via the oral route and usually as a single daily dose of 500 mg to complete a 7- to 10- day course of therapy for community-acquired pneumonia and a daily dose of 250 mg to complete a 7- day course of therapy for pelvic inflammatory disease [56].

1.8.2 Absorption

The oral bioavailability of AZI following administration of a single 500 mg oral dose to healthy male volunteers in the fasted state was estimated to be 37%, based on comparisons of area under the plasma concentration-time curve (AUC) calculated up to 24, 48 and 72 hours after dosing. The peak serum concentration of 0.4 to 0.45 µg/ml occurred approximately two hours after the dose [30, 32, 44, 57]. The administration of an AZI capsule with a large meal has been reported to reduce the peak serum concentration by up to 50% and the AUC by 43% [30, 57]. Therefore the manufacturer recommends that AZI capsules should be taken one hour before or two hours after meal, in order to ensure that the dose has been administered on an empty stomach [58]. Oral bioavailability of the tablets and oral suspension are not affected by meals [7].

1.8.3 Distribution

Following oral absorption, AZI is rapidly distributed to the tissues such as tonsils, prostate and gynaecological tissues, reaching high and sustained concentrations of up to 10-100 times greater than those found in serum [32]. AZI is highly concentrated in a number of different cell types following absorption including leukocytes, monocytes, alveolar cells, polymophonuclear lymphocytes, fibroblasts and macrophages [33, 53, 59]. The presence of

high concentrations of AZI in phagocytic cells allows for the release of the antibiotic at local sites of infection and inflammation. The penetration of AZI into eukaryotic and prokaryotic cells may be responsible for the expanded spectrum of activity of AZI, particularly against these intracellular infective organisms [9, 60].

The transport of AZI into cells by both passive and active processes is probably due to its dibasic amphiphilic nature [61]. The concentration of AZI in most tissues was found to be between 1 mg/kg and 9 mg/kg between 12 and 24 hours after a single oral dose of 500 mg [32, 61]. This tissue concentration largely exceeds the MIC_{90} for most relevant pathogens. Several studies have shown that levels of AZI in tonsil and prostate tissue were sustained and remained at greater than 2 mg/kg for 12 and 24 hours after administration of a 500 mg dose and for three days after a loading dose of 500 mg on day one, followed by 250 mg administered daily on days two to five [31, 61].

Foulds *et al,* [32] have reported that four days after a single 500 mg oral dose, the concentration of AZI in prostate tissue was between 0.8 mg/kg and 2.8 mg/kg; in pulmonary tissue was between 2.3 mg/kg and 8.1 mg/kg and in gynecological tissue was between 0.27 mg/kg and 1.48 mg/kg [62]. The extensive tissue distribution, intracellular accumulation and the relatively long half-life of between 2 and 4 days allows for a reduced dosing period, yet still achieves highly efficient antimicrobial activity at the site of infection. The large apparent volume of distribution of 23 l/kg, and the relatively low plasma serum level of about 0.4 μg/ml achieved following oral administration of a single 500 mg dose, suggests that AZI is subject to extensive tissue distribution and intracellular accumulation [30, 53, 61].

The fraction of AZI bound to plasma proteins is variable depending on the concentration of the compound in the blood. However, protein binding does not appear to influence the distribution of AZI, since serum concentrations do not adequately reflect the availability of AZI in the tissues [32, 61]. The serum protein binding of AZI is concentration dependent, with 50% at 0.05 mg/l declining to 12% at 0.5 mg/l [32]. Protein binding occurs primarily to α- and β- globulins, but not to serum albumin [30]. Protein binding of AZI also correlates to α_1-acid glycoprotein levels, thus patients with elevated levels of α_1-acid glycoprotein have an increase in AZI protein binding [30, 32].

1.8.4 Metabolism

Most of the dose of AZI that is absorbed is not metabolized [61]. Metabolism that does occur is primarily located in the liver and is a result of N-demethylation of the desosamine sugar to form N-desmethylazithromycin or at the 9a position of the macrolide ring to form 9a-N-desmethylazithromycin [8, 44, 61]. One of the metabolites of AZI, 9a-N-desmethylazithromycin has been shown to be active against many strains of bacteria [63]. Other pathways of metabolism include O-demethylation, deconjugation of the cladinose sugar and hydroxylation of the desosamine or aglycone ring. Hunter *et al,* [64] conducted a study to determine the profile of metabolites of AZI in plasma, bile, liver, lung, kidney and skin tissues using the Ball python snake as the animal model. Fifteen metabolites of AZI together with their structures and possible pathways of metabolism have been described [64] and the major sites of metabolism are shown in Figure 1.12.

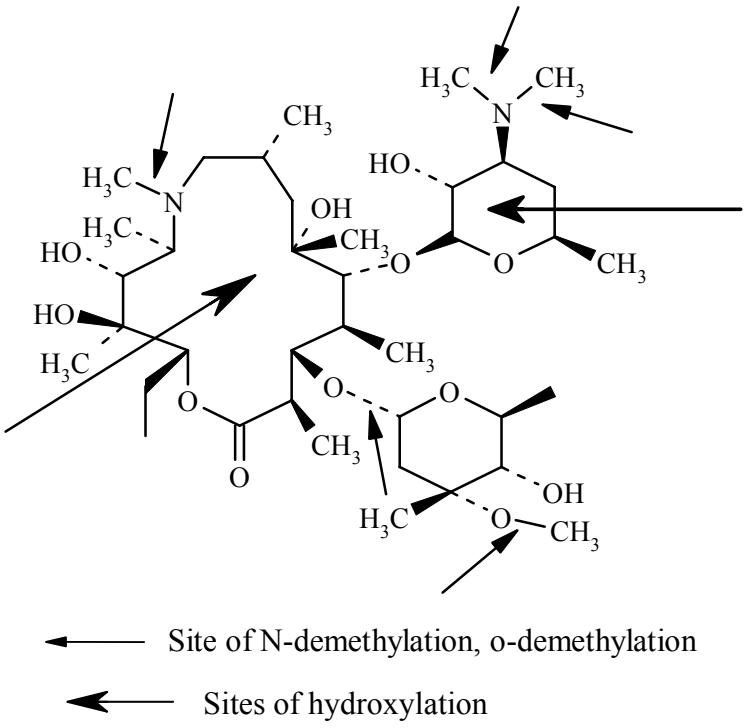


Figure 1.12: Major sites of AZI metabolism

Bile secretions in human have been found to contain as many as 10 metabolites of AZI and these are thought not to have any significant antimicrobial activity [8, 61]. Studies in rats have shown that, after administration of a 200 mg/kg dose daily for seven days, significant hepatic accumulation and elevation of N-demethylase activity occurred [47]. However, there was no evidence of hepatic cytochrome P 450 induction or inactivation via the formation of a cytochrome-metabolite complex [47]. In addition, auto-induced metabolism of AZI by this pathway has not been detected [8].

1.8.5 Elimination

AZI elimination from the plasma occurs in a biphasic manner. A rapid decline in drug plasma concentration implies a rapid redistribution phase into tissues following administration that is then followed by a second distribution component, which in turn is followed by biliary excretion, the major pathway of elimination [30, 61]. AZI is largely excreted in the bile as unchanged drug and a small percentage of between 4.5% and 12.2% appearing unchanged in the urine following oral and intravenous administration of 500mg respectively [8, 11, 32, 61]. The reported renal clearance of AZI is between 100 ml/min -189ml/min [61]. Extensive enterohepatic recycling of the drug may also occur [4]. The average elimination half-life of AZI is approximately 68 hours [4].

1.9 CONCLUSIONS

Structural modification of the commonly used macrolide antibiotic erythromycin has led to the discovery of a broad-spectrum antibiotic, AZI. AZI possesses unique pharmacokinetic properties that necessitate further research on potential drug-drug and drug-herb interactions in both animal and human subjects. A deeper understanding of the stability of AZI is crucial for the development of pharmaceutical dosage forms since AZI is known to degrade under conditions that may occur during manufacture. The presence of air, heat and acidic environments may precipitate degradation reactions, which might cause the formation of impurities that may subsequently present in the final dosage form and that could be harmful to patients. The potency of AZI, in particular, its beneficial pharmacological properties in the treatment of most common childhood infections has promoted research into the development

of a rectal dosage form for paediatric use. Rectal administration of erythromycin has been reported to produce plasma levels that compare favourably with those obtained following intravenous [65, 66, 67] and oral [68] administration of the compound. Since AZI is similar in chemical structure to erythromycin, it may be a suitable candidate for rectal administration. In addition, AZI has several physicochemical properties that are crucial for successful rectal absorption, including but not limited to, possessing an intermediate partition coefficient, appropriate lipid solubility and that it exists as a weak base.

CHAPTER TWO

2. *IN VITRO* ANALYSIS OF AZITHROMYCIN

2.1 INTRODUCTION

The quantitative determination of AZI in pharmaceutical dosage forms and raw materials has been accomplished using a variety of different analytical techniques such as microbiological [69-71], voltametric [72-75], spectrofluorimetric [76] and chromatographic assay methods [77-83]. In addition, high performance liquid chromatography (HPLC) with electrochemical [84-88], fluorimetric [82, 89] or voltametric detection [72] has been used for the analysis of AZI in biological fluids.

Recently a technique using a chemiluminescence reaction and flow injection has been reported for the ultra-sensitive analysis of AZI in dosage forms and biological fluids [90]. Of the reported techniques, HPLC has been shown to be superior with respect to specificity, rapidity of analysis, precision and ease of use of different kinds of detection systems depending on the minimum concentration to be quantified and the need for simultaneous analysis of related compounds.

A summary of the techniques that have been used for the analysis of AZI in pharmaceutical dosage forms, raw materials and biological samples is reported in § 2.1.1

2.1.1 Analysis of AZI in pharmaceutical dosage forms, raw materials and biological samples

2.1.1.1 Microbiological assay methods

Microbiological assays can be used for potency determination of antibiotics in biological fluids and quantitation of the *in vitro* antibacterial activity of these agents in pharmaceutical dosage forms. In most microbiological assay methods, potency determination is performed by comparing a dose of an antibiotic that is known to inhibit the growth of an appropriate selection of microorganisms with a dose of a standard sample of known potency and that is able to produce the same degree of inhibition.

Lingerfelt *et al,* [69] used a zone of inhibition assay to determine the activity of AZI where *Staphyllococcus aureus* was used as the reference microorganism. A microbiological diffusion assay using an eight by eight (8×8) Latin Square design was used to determine AZI potency with different microorganisms and conditions, so as to standardize the method and to select a suitable test organism for the analysis of AZI in formulations and biological fluids [70]. *Sarcina lutea, Bacillus pumilus* and *Escherichia coli* are the recommended test organisms for the analysis of AZI, since they responded with satisfactory precision and accuracy for such analyses [70]. The USP [10] lists an official method for the determination of erythromycin potency using the test organism *Sarcina lute.* For potency determination by diffusion method on 8×8 Latin Square and Petri dishes, the British Pharmacopoeia (BP) [91] suggests the use of the test organism *Bacillus pumilus.* As AZI and erythromycin belong to the same antibiotic group, the results obtained by Turčinov *et al,* [70] that make use of the test microorganisms recommended by the pharmacopoeias for erythromycin potency, showed that the test microorganisms used were similarly sensitive to both of them.

A validated microbiological assay using *Micrococcus luteus* as the test microorganism was used to quantitate AZI in pharmaceutical dosage forms [71]. In addition, Turčinov *et al,* [70] and Breier *et al,* [71] demonstrated that a microbiological assay using an 8×8 Latin Square design or a microbiological cylinder-plate assay can be used as an alternative method for routine quality control of AZI in pharmaceutical formulations.

Although microbiological assays are inexpensive to perform for the estimation of effective drug concentrations, they do not distinguish between the activity of an API and any active metabolites [88]. In addition, microbiological assays have a long turn-around time and are tedious. Most drawbacks to microbiological testing arise from the lack of accuracy and precision, since the bioassay is largely dependent on culture techniques, in addition to the physiological state of the indicator microorganism [88].

2.1.1.2 Voltametric analysis

Electroanalytical techniques may be used for estimating the potency of pharmaceutical compounds that have an electroactive functionality in their structure. Amino functional groups, of which there are two in AZI, are easily oxidized, thus oxidation of amines forms the basis for the voltametric determination of AZI. Mandić *et al,* [21] investigated the behavior of electrochemical oxidation of AZI and its derivatives and found that AZI is electroactive only in its non-protonated form and that the process of oxidation is an irreversible one and is pH dependent [72, 73]. Nigović *et al,* [73] compared an electro-analytical assay for the determination of AZI in pharmaceutical formulations using the HPLC method described in the USP [10] and determined that there was no significant difference between the performance of the two methods in terms of selectivity, accuracy and precision.

A validated method for the determination of AZI in its pharmaceutically active form, in dosage forms such as powders for oral suspension and capsules, and in biological samples, using square-wave anodic adsorptive stripping voltametry has recently been reported [75]. This method was reported to be more sensitive than previously reported voltametric methods [72, 74]. Adsorptive stripping involves the electrochemical pretreatment of a carbon electrode before differential pulse voltametric measurements are made. AZI can be adsorbed and accumulated on the pretreated carbon electrodes, which accounts for the high sensitivity of this technique for the determination of AZI [75].

Voltametric methods can be used as an alternative for the analytical determination of AZI, as it is simple, fast, low cost, and has the necessary precision, accuracy, sensitivity and selectivity. It is valuable in stability studies of AZI in solutions of different pH [72] and in biological sample analysis, in which a low limit of quantitation must be achieved [75].

2.1.1.3 Spectrofluometric analysis

The use of the spectrofluorimetric method as an analytical tool relies on the presence of a fluorophore in the compound of interest or the analytical procedure must be designed in such a way that an oxidizing agent is used to facilitate the formation of a fluorescent product. The spectrofluorimetric method has been used for the analysis of macrolide antibiotics [76]. The basis of fluorimetric analyses depends on the oxidation of a macrolide antibiotic with ceric ammonium sulphate in the presence of sulphuric acid and the measuring the resultant fluorescence of cerium (Ce) III and cerium IV. This is the first method that has described a fully validated spectrofluorimetric procedure for the assay of macrolide antibiotics in pharmaceutical dosage forms without interference from common dosage from excipients.

2.1.1.4 Chemiluminescence analysis

Song *et al,* [90] developed a chemiluminescence method combined with a flow injection technique for the analysis of AZI. The primary aim of the method was to improve sensitivity and shorten analytical run times. Luminol and hydrogen peroxide were used to generate chemiluminescence. The chemiluminescence of AZI is a result of the luminal-hydrogen structure. The chemiluminescence intensity was linear over the range of 0.1 pg/ml to 1.0 ng/ml. This method is the most sensitive method reported thus far and was successfully applied to the analysis of AZI in pharmaceutical dosage forms and in biological matrices such as plasma and urine.

2.1.1.5 Thin layer chromatography (TLC)

TLC, an open-bed version of adsorption chromatography, was introduced in the early 1950s [92]. TLC can be used to great advantage in conjunction with modern liquid solid chromatography (LSC) or HPLC techniques. TLC is well suited for exploring the best combination of the mobile phase and stationary phase for a given sample matrix. Furthermore, TLC can be used as a preliminary test for the visualization of unknown compounds that might not be detected by liquid chromatographic (LC) detectors [92]. Visualization is made possible by the use of specific spray reagents that highlight the sample bands separated by TLC.

Attempts to analyze AZI in pharmaceutical dosage forms using a validated TLC method have been reported [77]. In these studies, AZI, related AZI impurities and degradation products were selectively and accurately estimated in raw material and marketed products using a pre-coated silica-gel 60F254 TLC plate. A mixture of *n*-hexane:ethylacetate:diethylamine (75:25:10) was used as a developing system and modified Dragendorff's solution was used for the visualization of the TLC bands. The method was accurate and precise for purity testing, stability indication and the determination of content uniformity of commercially available AZI products.

2.1.1.6 HPLC

Liquid chromatography refers to any chromatographic procedure in which the mobile phase is liquid, in contrast to the moving gas phase as described in gas chromatography. Various terms have been used to describe liquid chromatography, including high-speed liquid chromatography (HSLC), high efficiency liquid chromatography (HELC) and high-pressure liquid chromatography (HPLC). High performance liquid chromatography (HPLC) is the generally accepted terminology for this analytical technique [93]. HPLC is particularly suitable for the separation of compounds that are of high polarity and/or molecular weight, exhibit thermal instability and for compounds that have a tendency to ionize in solution [93].

Most HPLC separations of pharmaceutical compounds are carried out on surface reacted or chemically bonded organic stationary phases or bonded-phase chromatographic columns BPC [92]. Polar BPC column packing materials, are used for normal phase (NP) HPLC (NP-HPLC) separation of compounds of moderate to strong polarity, whereas relatively non-polar BPC column packing is used for reversed phase (RP) HPLC (RP-HPLC) separation of less polar compounds [92, 94]. Unlike NP-HPLC, stationary phase interactions play a minor role in overall solute retention in RP-HPLC [94]. The high polarity of RP-HPLC solvents makes the separation of compounds possible through polar interactions [94]. In addition, RP-HPLC columns have higher stability, lower equilibration times and higher retention time reproducibility than NP-HPLC columns [94].

RP-HPLC coupled with different types of detection systems, is the most extensively used chromatographic method for the analysis of macrolide antibiotics and related substances [83]. The principles of RP-HPLC involve the use of a non-polar stationary phase and polar mobile phase. Successful separations are usually achieved by partition, adsorption or ion-exchange processes depending on the type of stationary phase and polarity of the mobile phase used [92, 94].

The development of an HPLC method requires a systematic approach in order to ensure that an accurate, simple and reproducible method is developed in a short time. The successful use of an HPLC method for a given compound requires the appropriate combination of operating conditions such as, for example, type of column packing, length and diameter of column, mobile phase composition and flow rate, separation temperature, sample volume and most importantly method of detection.

The measurement of peak area for the purposes of quantitation of compounds of interest is popular in HPLC [95], however, well resolved nearly symmetrical peaks or those of a Gaussian nature may be more precisely and accurately quantitated using peak height rather than peak area measurements [95]. Peak height measurements were used for quantitation of AZI in pharmaceutical dosage forms and stability estimation in these studies.

AZI has a low molar absorptivity since it lacks a suitable chromophore, thus selective and sensitive UV detection of this compound is difficult to achieve. However, analysis of AZI using low UV wavelengths of between 210 and 215 nm have been reported [78, 80, 81]. The use of wavelengths lower than 210nm in order to achieve higher sensitivity can result in potential interference from sample impurities, especially when analysing AZI in complex matrices such as biological fluids and/or tissues [95]. To overcome the challenges associated with the use of low wavelength detection, other methods of detection should be considered. Most of the HPLC methods developed for the analysis of AZI in complex matrices use electrochemical [79, 85-88], fluorescence [59, 82, 89] or mass spectrometry (MS) [96-98] detection systems.

Although HPLC/MS is sensitive and capable of quantitation of impurities and degradation products of pharmaceutical compounds at very low concentrations [98], it requires careful tuning of the instrument and such sensitivity is not required for the analysis of AZI in dosage forms [88]. The use of HPLC coupled with either amperometric of coulometric electrochemical detectors has been reported as the standard method for the sensitive detection of macrolides, since most macrolide antibiotics contain electrochemically sensitive tertiary amines [79, 85, 87, 88]. Electrochemical methods of detection require several optimization steps, including pre-concentration of samples prior to the analysis of the accumulated analyte [72]. In addition, a clear understanding of the mechanism of oxidation and oxidation byproducts is critical in order to optimize the method [21]. HPLC methods with fluorescence detection have been reported [76, 82, 89], however derivatization of an analyte is necessary prior to detection.

The aim of this study was, therefore, to develop and validate a simple HPLC method using conventional materials, reagents and equipment for the determination of AZI in suppository dosage forms.

2.2 HPLC METHOD DEVELOPMENT

RP-HPLC with UV detection was selected as the preferred method of analysis for AZI in pharmaceutical dosage forms. The decision to use RP-HPLC was based on the frequency of use of methods described in literature, in which the quantitative analysis of AZI from a variety of sample matrices has been successfully performed using RP-HPLC, and on the availability of equipment in our laboratory. The chromatographic conditions and details relating to the analysis of AZI in raw material, dosage forms and biological samples that have been reported in the literature are listed in Table 2.1. The preliminary HPLC conditions used for the development of a method of analysis were selected, based on those reported in the literature.

Table 2.1: HPLC conditions used for the analysis of AZI

Column	Sample matrix	Mobile phase composition	Detection	Temperature	Reference
Gamma- alumina	Raw material	phosphate buffer-acetonitrile, adjusted to pH 11.0 with potassium hydroxide	Amperometric guard: +0.70V screen: +0.85V	Ambient	10
LiChroCART® C_{18}, 5µm	Raw material	phosphate buffer-acetonitrile-methanol, adjusted to pH 8.0 with phosphoric acid	UV – 215nm	Ambient	78
Nova-Pack C_{18}, 4µm	Raw material	ammonium acetate-acetonitrile-methanol - tetrahydrofuran, mobile phase pH 7.2-7.4	Amperometric guard: +0.7V screen: +0.8V	Ambient	79
XTerra RP C_{18}, 5µm	Raw material	phosphate buffer-water-acetonitrile, adjusted to pH 6.5 with potassium hydroxide	UV-215nm	70°C	80
Phenomenex Synergi® C_{18}, 4µm	Raw material, dosage forms	gradient elution, phosphate buffer-acetonitrile-methanol, adjusted to pH 7.0 with potassium hydroxide	UV-210nm	Not stated	81
YMC-Park ODS-AP C_{18}, 5µm	Rat's plasma	phosphate buffer-acetonitrile, adjusted to pH 7.2 with potassium hydroxide	Amperometric detect: +0.95V	30°C	85
Nova-Pack C_{18}, 4µm	Human tears and plasma	phosphate buffer-sodium perchlorate-acetonitrile-methanol, adjusted to pH 7.0 with phosphoric acid	Amperometric guard: +0.7V screen:+0.85V	Not stated	87
Radial-Pak Resolve Silica cartridge, 5µm	Rat's Blood plasma, serum and Human urine	Ammonium acetate-acetonitrile-methanol, adjusted to pH 7.0 with acetic acid	Coulometric guard +0.90V	Not stated	88

2.2.1 Experimental

2.2.1.1. Reagents

All chemicals used in these studies were at least of analytical grade. The HPLC grade organic solvents used include, Methanol (MeOH) with a UV cutoff of 205nm from Fluka Chemie® (Johannesburg, South Africa), Acetonitrile (CH_3CN) with the UV cutoff of 200nm from Romil-SpS® (Waterbeach, Cambridge, UK), and Tetrahydrofuran (THF) with the UV cutoff of 212nm from HiPerSolv for HPLC™ (VWR International Ltd, Poole, England). Azithromycin ethanolate dihydrate was kindly supplied by Aspen Pharmacare (Port Elizabeth, South Africa) and Zhejiang Huayi Pharmaceutical Co., Ltd. (Yiwu, Zhejiang, China). Clarithromycin was purchased from Ranbaxy Laboratories Ltd (New Delhi, India). Sodium hydroxide (NaOH) pellets and ortho-phosphoric acid (85% v/v) were purchased from Merck® Chemicals (Midrand, South Africa). Potassium dihydrogen phosphate and hydrochloric acid (32% v/v) were purchased from Associated Chemical Enterprises (Southdale, South Africa).

HPLC grade water for phosphate buffer preparation, and chromatography was prepared by purification 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 (Millipore Co., Bedford, MA, USA) prior to use.

2.2.1.2 HPLC systems

Two modular HPLC systems, System A and System B, were used for the *in vitro* analysis of AZI.

System A

Optimisation of the chromatographic conditions with respect to wavelength of detection, mobile phase composition and choice of internal standard, were performed using System A. The modular HPLC system that comprised System A, consisted of a Waters model M 6000A dual piston solvent delivery module (Waters Associates, Milford, MA, USA), a Rheodyne® Model 7125 manual injector (Rheodyne, Reno, NV, USA) fitted with a 20 µl fixed volume loop, a Model 290 strip-chart recorder (Linear Instrument Co., Irvine, CA, USA) and a Linear UV/VIS-500 Model 6200-9060 detector (Linear Instrument Co., CA, USA). Separation was achieved on a Nova-Pak® C_{18} 60 Å 4 µm, 3.9× 150 mm HPLC cartridge column (Waters Associates, Milford, MA, USA).

System B

System B was used for the validation of the developed HPLC method and for the analysis of all *in vitro* dissolution and other analyses. The modular HPLC differed from System A in that it consisted of a model P100 dual piston solvent delivery module (ThermoSeparation Products, San Jose, CA, USA), a Model AS100 autosampler (ThermoSeparation Products, San Jose, CA, USA) fitted with a Rheodyne® Model 7010 injector (Rheodyne, Reno, Nevada, USA) fitted with a fixed volume 20 µl loop and a GASTIGHT® 250 µl Model 1725 syringe (Hamilton Co., Reno, NV, USA) and a SpectraPhysics SP 4600 integrator (ThermoSeparation Products, San Jose, CA, USA). All other components of the modular system were the same as those used for System A.

2.2.1.3 Column selection

The selection of a high-performance column is essential for the development of a rugged and reproducible HPLC method for drug analysis [99]. The choice of an analytical column is based on the physicochemical properties of the analyte(s) to be analysed, such as, for example, solubility, molecular weight and/or its ionic nature. The retention of a drug on an HPLC column is also a function of the column packing material and column dimensions [95]. The efficiency of a packed column increases as the particle size of the stationary-phase decreases and therefore, the smaller the particles, the better the resolution and sensitivity that can be achieved using that specific column [100].

Typical particle size ranges for HPLC columns are between 3 μm-10 μm. Smaller particles will result in a higher theoretical plate number and better sensitivity, however, higher column backpressures will be evident than those seen with the column packing materials of larger particle size [100]. The sample capacity of a column increases with volume of the stationary phase, length and internal diameter of the column. A 3.9 mm i.d x 150 mm HPLC column with 4 μm particle was selected for use in these studies.

Almost all current applications of modern HPLC make use of two adsorbent types of packing materials, *viz.* silica or alumina. The retention times and separation efficiency of these two adsorbent phases are generally similar with polar drugs being preferentially retained [92]. In particular, the retention of a compound on silica-based columns is primarily due to hydrophobic and silanol interactions [99]. Silica columns are mildly acidic with an approximate pH of 5 [92]. At low pH, the silanol groups are fully protonated and thus at low pH, basic compounds are well resolved due to reduced solute-silanol ionic interactions [99]. At higher pH, the silanol groups tend to dissociate and become negatively charged and thus protonated bases are retained strongly as is evidenced by pronounced peak tailing.

AZI is a weakly basic drug that is unstable at low pH and thus the selection of silica-based columns may be in appropriate for analysis. A useful alternative is to develop a method in which the separation is achieved on a column packed with alumina adsorbents. Alumina columns allow for the use of high pH without compromising the stability of AZI. The USP [10] has described a separation using a Gamma alumina column with a mobile phase of pH = 11.0.

Silica columns have many advantages over Gamma alumina columns among which are, a higher sample loading capacity, a low potential for unwanted reactions during separation and availability in a wide range of chromatographically useful forms [92]. Alternatively, stationary phases in which high purity silica, polymer encapsulation, dibentate or where phases are embedded with polar groups, or are base-deactivated, may be used [99]. Base-deactivated columns are packed with stationary phases that are fully hydroxylated and are of high purity and thus have reduced silica acidity. Base-deactivated columns are claimed to be superior to standard silica based stationary phases as a result of the potential reduced silanophilic-solute interactions [101].

It has been shown that silica-based columns have the ability to provide better separation by the manipulation of mobile phase composition and the use of intermediate pH [78,-81] than alumina-based column with mobile phases of extreme pH. As previously mentioned, the main difficulty when analysing AZI is the interaction that occurs between the protonated amine functional group and the free silanol functional groups present in the packing material. The protection of the silanol groups with other organic functional groups, such as an alkyl group, has shown improved peak symmetry [99, 100]. The peak symmetry of AZI was markedly improved when the silanol groups were replaced with either amino or methyl functional groups [81]. Similar results were obtained in studies in which AZI and its related substances were analysed in bulk active pharmaceutical ingredients (API), using a stationary phase in which silanol groups were partly replaced by methyl functionalities [80].

A Nova-Pak® column, packed with dimethly octadecylsilyl bonded amorphous silica, was chosen for the analysis of AZI and its degradation products. These columns have been used to successfully separate AZI and two of its metabolites in human tear and plasma samples [79, 87].

2.2.1.4 UV detection of azithromycin and internal standard

The choice and operation of detector may affect the relative response of sample components and can potentially interfere with the sensitivity, selectivity and base line noise of a specific application. The importance of adequate detection sensitivity relies on careful selection of the wavelength for analysis. The choice of a suitable wavelength requires knowledge of the UV-spectrum of individual sample components. The UV-spectrum should be developed prior to HPLC method development and is usually generated during preformulation studies. A UV spectrum of AZI is shown in Figure 1.7 and reveals a wavelength of maximum absorption (λ-max) of 201.6nm.

Since AZI does not have a specific chromophore, detection can only be achieved at low wavelengths. It is well known that many endogenous compounds absorb UV irradiation at low wavelengths and that these may interfere with the analysis of the compound of interest [95]. Based on the UV-spectrum of AZI, it was necessary to select an alternate wavelength of 215 nm to monitor AZI at 215nm, since at 215 nm AZI could be selectively analyzed with sufficient sensitivity in pharmaceutical dosage forms. The detection of AZI at this wavelength has previously been accomplished [78, 80]. The detection of an internal standard does not have to occur at the λ-max of the compound of interest, as concentrated solutions of the internal standard may be used to obtain an adequate response.

2.2.1.5 Choice of internal standard

An internal standard (IS) is a compound usually structurally similar, but different from the analyte of interest, which is added in a known amount to a sample matrix [100]. The reasons for the use of an IS are mainly to minimize system and procedural deviations, thus minimizing variations in precision as a function of sample size or instrumental response [100]. Hammarstrand [102] has reported that the use of IS ensures accuracy in analysis and that varying injection volume and day-to-day instrumental changes are easily compensated for. The IS that is selected for use should have physicochemical properties and chromatographic behavior similar to that of the analyte of interest [95]. By implication, a method that is developed for the analyte of interest should be appropriate for the quantitation of both the analyte of interest and the IS. Compounds that have been used as an IS in the

assay of AZI include roxithromycin, clarithromycin [85, 86, 89] and N-propylazitromycin [87]. In effect, it is likely that any macrolide antibiotic could be used as an IS since they have similar absorption spectra and chemical structures to AZI. Clarithromycin was chosen as an IS for the analysis of AZI in our laboratory

2.2.1.6 Mobile phase selection

Liquid solid chromatographic separations are usually achieved using silica as the adsorbent stationary phase. Therefore, separations can be optimised through manipulation the mobile phase composition [92]. Consequently, the development of a satisfactory separation in HPLC is highly dependent on the selection of an appropriate mobile phase composition. When selecting a mobile phase for HPLC, certain qualities of the constituent components that must be considered include purity, reactivity, boiling point, viscosity and cost, amongst others [93].

The selection of solvents of high purity is crucial to prevent the unnecessary detection of trace impurities in samples, in particular, when it is necessary to use low wavelengths for successful quantitation of the analyte of interest. Column backpressure is proportional to mobile phase viscosity [94] and therefore viscosity of solvents and mobile phase compositions can be easily identified by measuring column back pressure. As an analytical columns ages and particles accumulate on the inlet frit, column backpressure will rise. If the initial column pressure is high due to solvent viscosity, the upper pressure limit of the chromatographic method will be reached sooner. Thus as a column ages, the column life span is shortened.

There are numerous ways of improving the resolution of an analytical method through the manipulation of pH, ionic strength and choice of organic modifier used in a mobile phase. In general, retention times in RP-HPLC are shorter when using mobile phase compositions with a strong eluting power, for example where the ratio of organic modifier to the aqueous components of a mobile phase is high [95]. Stronger eluting solvents facilitate the partitioning of a solute into the mobile phase and consequently the retention time for the dissolved analyte is shortened.

The eluting strength of a solvent or mobile phase is dependent on the choice of organic modifier, the proportion of that modifier in a mobile phase and buffer strength. The analysis of ionic compounds requires the inclusion of a buffer in the mobile phase and when control of mobile phase pH is essential for the retention or separation of the compound of interest. In addition, the use of a buffer may assist in achieving high reproducibility for a specific separation.

The retention time of weak acids will decrease with an increasing pH, while conversely the retention time of weak bases will increase with increasing pH [95]. The solute retention of an ionised drug is lower when compared to that of unionised species as ionised molecules are more likely to interact with a polar mobile phase than with a hydrophobic stationary phase [98]. In cases where a solute is completely ionised, a further change in pH would have no effect [94]. The selection of an appropriate buffer for mobile phases requires that buffer capacity, UV absorbance, solubility in organic solvent, interaction with the sample and/or column and potential corrosion of the HPLC system be considered [95].

The mobile phase compositions used in published HPLC methods for the quantitation of AZI are listed in Table 2.1. These compositions were evaluated and used as a starting point for mobile phase development in these studies. None of the reported mobile phase compositions proved suitable without major alteration, therefore an original mobile phase composition was developed.

It has been reported that the use of acetate buffers results in relatively low column efficiencies, which is more than likely a result of the formation of nonpolar complexes between acetate ions and cationic solutes [94]. In addition, ammonium acetate buffers tend to be volatile and with time evaporate, which may result in variations in retention time, resolution and selectivity. Phosphate buffers are known to produce more stable analytical conditions, in addition to providing a buffering capacity over a wide range of pH as a result of having three pKa values for the acid, H_3PO_4, pKa = 2.21 and phosphate ions, $H_2PO_4^-$, pKa = 7.21; and HPO_4^{-2}, pKa = 12.67 [85]. The UV transparency of phosphate buffers is well below 200 nm [103] and therefore their usefulness in low wavelength UV applications is obvious. Potassium dihydrogen-phosphate with pKa = 7.21 was used for these studies since it has a buffering range between pH 6.2 and 8.2 [103].

2.2.1.7 Preparation of buffers

Phosphate buffers containing 50, 65, 75, and 80 mM were prepared by accurately weighing 6.80, 8.85, 10.21 and 10.90 g respectively of potassium dihydrogen phosphate into a 1L volumetric flask and making up to volume with HPLC grade water. The pH of the buffer was adjusted with 1.0 M NaOH to a required pH. 1.0 M NaOH was prepared by dissolving exactly 4.0 g of sodium hydroxide pellets into a 100 ml volumetric flask and making it up to volume with the HPLC grade water.

2.2.1.8 Preparation of mobile phase

The mobile phase was prepared by pipetting specific volumes of MeOH, CH_3CN and/or THF into a 1L A-grade volumetric flask and making it up to volume with phosphate buffer of a specific pH and molarity.

The pH of the buffer solution was measured and adjusted before the organic solvents were added using a Model GLP21 Crison pH meter (Crison Instruments, Barcelona, Spain). The measurement of the pH of a mobile phase that contains organic solvents has been reported to be imprecise, since electrode responses tend to drift in the presence of organic modifiers [95]. Although the addition of an organic solvent after the buffer pH has been adjusted can change the pH, the effect is much less important than the poor reproducibility of altering a specified mobile-phase pH when the pH is measured after the addition of an organic solvent [103].

The mobile phase was degassed under vacuum using a Model A-2S Eyela Aspirator (Tokyo Rikakikai Co., Tokyo, Japan) and filtered through a 0.45 μm Durapore® HVLP membrane filter (Millipore Co., Billerica, MA, USA) prior to use. Degassing of the solvents is necessary to remove any dissolved gases, in particular oxygen, which may lead to the formation of bubbles in the flow cell of a detector or in connecting tubing and in turn may thus affect the reproducibility and sensitivity of the UV detector [93]. In addition dissolved oxygen can cause significant changes in the level of absorbance at 210 nm with methanol, which may in turn cause base line drifts and random noise [93].

A mobile phase that consisted of a phosphate buffer (50 mM, pH 6.5) and CH_3CN in different proportions was used initially for method development purposes and a summary of the mobile phase compositions used in these studies appears in Table 2.2. The mobile phase was then adjusted to increase the retention time of the peak of interest to approximately 4 minutes, such that it was well resolved from the solvent front. The peak tailing was resolved through manipulations of the mobile phase components, i.e. the introduction of the organic modifier (THF) and an increase in buffer molarity. The resultant peak tailing was maintained at acceptable values as specified by the USP [10]. Manipulations of the mobile phase components to achieve well resolved peaks for both AZI and IS are discussed further in § 2.2.2.2.

Table 2.2: Retention times and peak shape of AZI and IS using various mobile phase compositions

Mobile phase composition					**Retention time (min)**		**Peak shape**
Organic modifier (% v/v)			**Phosphate buffer**				
CH_3CN	**MeOH**	**THF**	**% v/v, Molarity**	**pH**	**AZI**	**CLAR (IS)**	
40	-	-	60, 50 mM	6.3	2.0	*	AZI peak is sharp and close to solvent front
35	-	-	65, 50 mM	6.3	2.6	*	AZI peak is sharp and close to solvent front
30	-	-	70, 50 mM	6.3	3.0	*	AZI peak is sharp
30	-	-	70, 50 mM	6.5	4.0	24.0	AZI peak is sharp, IS peak is broad with tailing
30	-	-	70, 65 mM	6.5	3.4	14.8	AZI peak is sharp, IS peak is broad with tailing
30	-	-	70, 75 mM	6.5	2.8	8.2	AZI peak is sharp and close to the solvent front, IS peak is broad with tailing
30	-	-	70, 80 mM	6.5	2.0	6.4	AZI peak is sharp and close to the solvent front, IS peak is broad with tailing
30	5	-	65, 80 mM	7.0	7.6	16	AZI peak is broad with peak tailing, IS peak is broad with tailing
35	5	1.5	60, 80 mM	6.7	2.4	5.2	AZI peak is sharp and close to the solvent front, IS peak well resolved with reduced tailing
30	10	1.5	60, 80 mM	6.7	3.0	8.2	AZI peak is sharp, IS peak well resolved with reduced tailing
25	15	2.5	60, 80 mM	6.7	4.0	11.2	Peaks well resolved, no tailing

* Internal standard (IS) was not included in a test mixture

2.2.1.9 Preparation of stock solutions

Stock solutions of azithromycin dihydrate (1 mg/ml) and clarithromycin (0.5 mg/ml), the internal standard, were prepared by accurately weighing approximately 50 mg of AZI and 25 mg of IS using a Model AE163 Mettler top-loading analytical balance (Melter Instruments, Zurich, Switzerland). AZI and the IS were each quantitatively transferred into a 50 ml A-grade volumetric flask, and dissolved in 50 ml mobile phase with the aid of ultrasonification for one minute (Model B-12 Ultrasonic bath, Branson Cleaning Equipment Co., Shelton, Conn., USA). The stock solutions were serially diluted with the mobile phase to obtain concentrations of AZI at 25, 50, 150, 300, 400, 500 µg/ml and 150 µg/ml IS.

2.2.2 Optimisation of the chromatographic conditions

2.2.2.1 Wavelength (λ)

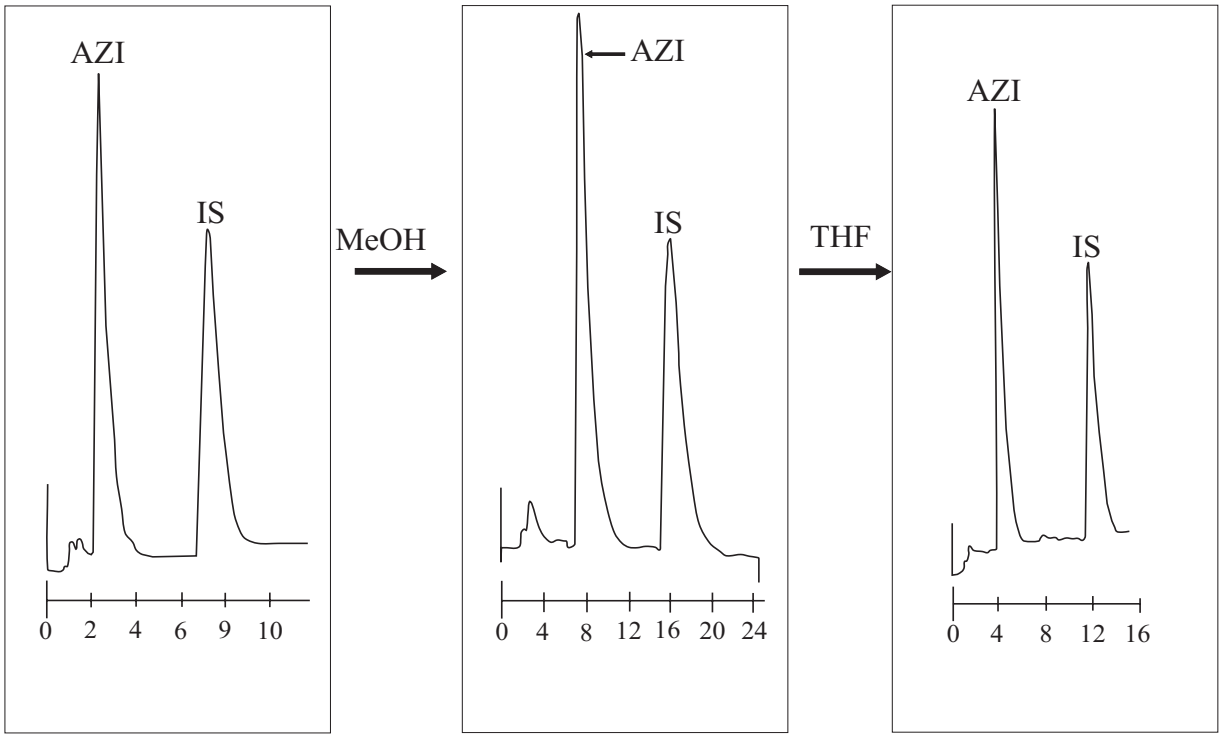
AZI has a λ_{max} of 200 nm, as seen in the UV absorption spectrum in Figure 1.6. However, at this λ a high degree of short-term background noise, which may be attributed to either the detection system or electronic noise of the integration, was observed [95]. The presence of noise makes the determination of the limit of detection (LOD) for a given analytical method extremely difficult. Similar results were obtained when monitoring AZI at 210 nm. Finally, a detection λ of 215 nm was determined to be suitable for the analysis of AZI. The use of UV detection at 215 nm has been reported to be sensitive enough for the purposes of detecting of AZI in *in vitro* experiments [78, 81, 87].

2.2.2.2 Mobile phase selection

2.2.2.2.1 Effect of organic solvent composition

Initially, a binary mobile phase consisting of phosphate buffer (50 mM, pH 6.3) and CH_3CN was selected as a starting point for these studies. An increase in the CH_3CN content produced sharp well resolved peaks for both AZI and IS, but the retention time (R_t) of AZI was short and the drug was eluted close to the solvent front. As the proportion of CH_3CN was reduced the R_t of AZI increased and peak resolution was incomplete. The effect of reducing the CH_3CN content in the mobile phase was more pronounced for the IS than for AZI. This is more than likely due to differences in the solute-stationary phase interaction between IS and AZI, as a consequence of the differences in their physicochemical properties.

As binary compositions did not result in an effective separation for AZI and the IS, tertiary mobile phase compositions were then used. Initially, MeOH was used as a second organic modifier, with the aim of increasing the R_t of AZI. The addition of MeOH to the binary mobile phase resulted in an increase in the R_t of AZI, but resulted in excessive and unacceptable peak tailing, in particular for the IS. In an effort to minimize peak tailing, the buffer molarity was increased without success. Finally THF was added as an additional organic modifier. THF has a low polarity of approximately 4.0 when compared to that of 5.8 for CH_3CN and 5.1 for MeOH [95]. The addition of THF resulted in a reduction in peak tailing, shorter retention times and well resolved sharp peaks for both AZI and the IS. Typical chromatograms showing the effect of the organic modifier on retention times and peak shape are shown in Figure 2.1.



CH_3CN, 80mM phosphate buffer pH 6.50 (35: 65)

MeOH: CH_3CN:80mM Phosphate buffer pH 6.70 (5:30:65)

THF:MeOH: CH_3CN:80mM Phosphate buffer pH 6.70 (2.5%:15:25:60)

Figure 2.1: The effect of the organic modifier on retention times and peak shape of AZI and IS

2.2.2.2.2 Effect of buffer molarity and pH

The impact of buffer concentration on the R_t of basic analytes can be profound, especially in situations where the silanol functionality of a stationary phase is predominantly in a state of ionisation, usually when the pH of the mobile phase is > 5 [95, 100]. The ionised silanol functional groups are able to strongly retain protonated bases or other cations by means of an ion-exchange process, which results in pronounced peak tailing. One way of reducing this effect is to increase the buffer concentration, which has the effect of selectively decreasing the retention of cationic solutes, as a consequence of increasing competition of buffer cations with the protonated sample for adsorption onto the ionized silica reacting material [94, 95].

The pH at which an optimal separation, based on peak resolution, was achieved, was 6.7. At this pH, both AZI and the IS are predominantly ionised, and thus peak tailing, due to solute silanol interactions, is masked. The buffer concentration was increased to 80 mM to further eliminate peak tailing. As the buffer concentration increased, the R_t of AZI and IS was

reduced since less analyte was retained as a result of interactions with the silanol functional group of the stationary phase as can be seen in Figure 2.2.

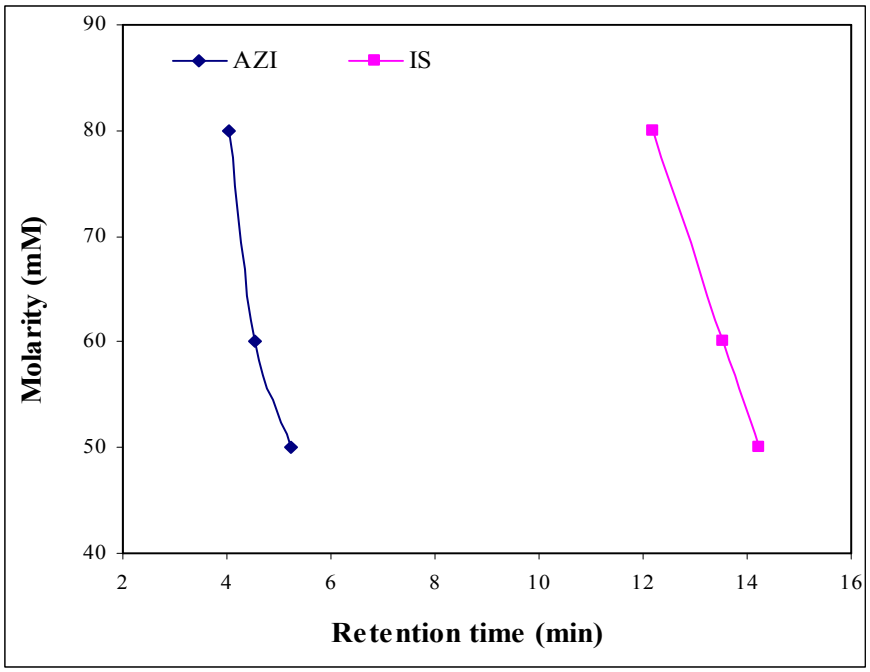


Figure 2.2: The effect of buffer molarity on the retention times of AZI and the IS

A further potential method for reducing peak tailing of basic compounds is to use mobile phases with low pH, as previously explained in section 2.2.1.4. However, due to the acid instability of AZI, mobile phases of pH < 5 could not be used. Therefore the option of using pH to resolve peak tailing is not viable. Nevertheless, the effect of decreasing buffer pH on retention times was investigated and it was observed that as buffer pH decreased the R_t of both AZI and the IS decreased. The observed effect on R_t is a more than likely due to the fact that protonated basic compounds are less hydrophobic and tend to be adsorbed less onto stationary phases than unionized species [94, 104]. The pH was adjusted to pH = 6.7 to avoid silica dissolution and to obtain a lower degree of ionisation of the amine functional groups of both AZI and IS, in an effort to reduce peak tailing.

2.2.2.2.3 Mobile phase selected

The final quaternary mobile phase selected for the analysis of AZI included the solvents THF, MeOH, CH_3CN and 80 mM phosphate buffer pH = 6.7, in proportions of 2.5:15:25:60, respectively. This composition of the mobile phase produced well-resolved peaks with retention times of 4.3 min. and 12.04 min. for AZI and the IS. A typical chromatogram of the separation achieved using this mobile phase is depicted in Figure 2.3.

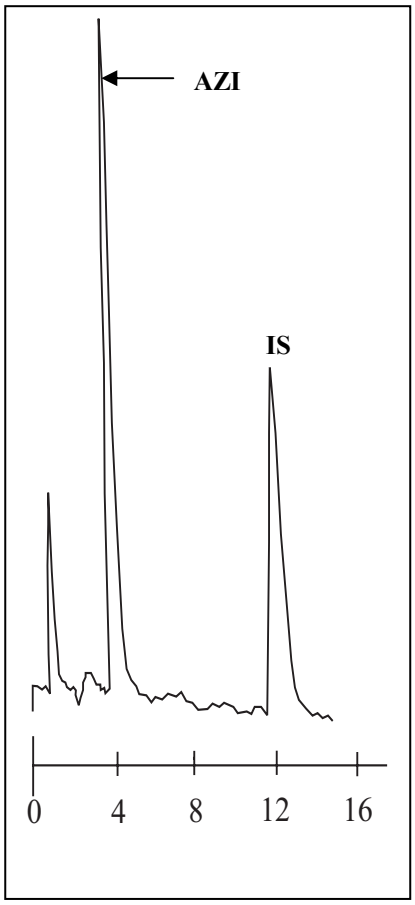


Figure 2.3: Typical chromatogram of the separation of AZI (R_t = 4.3min) and the IS, clarithromycin (R_t = 12.04min)

2.2.2.3 Chromatographic conditions

The final HPLC chromatographic conditions selected for the analysis of AZI are summarised in Table 2.3.

Table 2.3: Chromatographic conditions

Column	C_{18} Nova-Pak® (4 µm). Length 150 mm, I.D. 3.9 mm
Flow rate	1.0 ml/min
Injection volume	20 µL
Detection wavelength	215 nm, 0.005 AUFS
Temperature	Ambient
Column pressure	1700 psi
Recorder	SpectraPHYSICS SP 4600 Integrator
Integrator speed	0.25 mm/min
Recorder input	10 mV
Mobile phase composition	THF:MeOH:AcN:80 mM phosphate buffer pH 6.7 (2.5:15:25:60, v/v)

2.2.3 Conclusions

The manipulation of the composition of a mobile phase, the pH of the buffer, the organic solvent and the buffer molarity are undoubtedly the most powerful means available to an analyst to adjust both the retention time and peak resolution of an analyte. Silica-based columns can be used for the separation of basic compounds using an intermediate pH, provided that conditions for the method are carefully controlled to achieve reproducibility. The choice of wavelength for a specific analysis is also an important factor and is vital to achieve the desired sensitivity and selectivity for the method. The proposed HPLC detection conditions have shown higher sensitivity for the detection of AZI in pharmaceutical dosage forms using UV detection compared to the one reported in the literature that also made use of UV detection and silica-based columns for AZI separation [78].

2.3 METHOD VALIDATION

2.3.1 Introduction

Current Good Manufacturing Practice (cGMP) regulations require that test methods that are to be used for the assessment of compliance of pharmaceutical products with established specifications, must meet the necessary standards of accuracy and reliability [10]. Accuracy and reliability of the analytical method results are of great importance in ensuring the quality, safety and efficacy of pharmaceuticals. Therefore before an analytical method can be implemented for routine use, it must first be validated using specific guidelines to demonstrate that it is suitable for its intended use. Different organisations including, the United States Pharmacopoeial Convention (USP), the FDA and the Tripartite International Conference on Harmonization (ICH) [105, 106] have developed guidelines for the appropriate validation of an analytical method.

The USP guideline [10] for category I analyses or analytical methods for the quantitation of major components of a bulk drug substance or active ingredients in dosage forms were used for the validation of the method. The guideline [10] requires that accuracy, precision, specificity, linearity and range are assessed in order to ensure that the method is reliable. In addition to the parameters listed in the guideline, the limit of quantitation (LOQ) and limit of detection (LOD) were also determined. The efficiency and long term reliability of an analytical method is dependent on establishing whether or not the analyte of interest is stable in an aqueous solution during the entire period of sample collection, processing, storage and analysis [107]. Therefore, the stability of AZI in an aqueous solution was also determined.

2.3.2 Linearity

The linearity of an assay implies that there is a directly proportional relationship between a response and the concentration of an analyte within a given range [10, 106]. The range of an analytical method is the interval between the upper and lower levels of analyte that can be determined with a suitable level of precision, accuracy, and linearity [10, 106, 107].

The ICH guideline [108] specifies that a minimum of five concentration levels are used in the assessment of linearity. Linearity was assessed by analyzing six standard sample solutions (n=5) of different concentrations, i.e. 25, 50, 150, 300, 400 and 500 μg/ml. The peak height ratio of drug to IS was calculated and a calibration curve was plotted in order to establish whether a correlation between response and analyte concentration existed. An example of a typical calibration curve obtained for these studies is depicted in Figure 2.4. The linearity of the method was established from the correlation coefficient (R^2) of the best fit least squares linear regression curve, which was obtained by plotting peak height ratio versus known AZI concentrations. For these studies, an R^2 value of > 0.990 was considered appropriate to demonstrate the linearity of the analytical method [107]. The calibration curve was found to be linear over the concentration range stated, with an R^2 of 0.9998 and the equation for the line of $y = 0.0038x - 0.0046$.

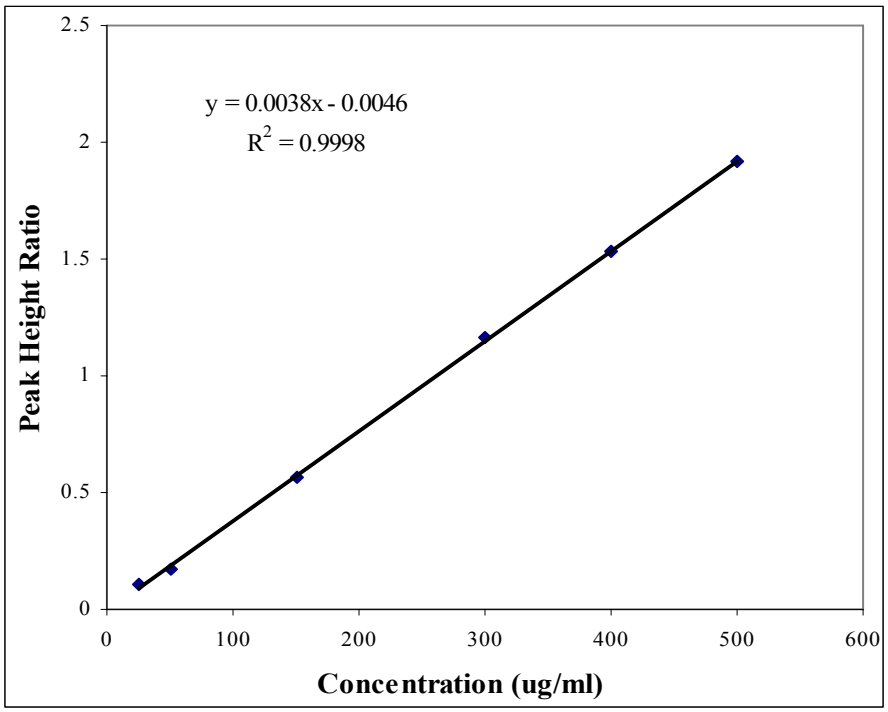


Figure 2.4: Typical calibration curve obtained for the analysis AZI

2.3.3 Precision

The precision an analytical method is defined as the ability of a method to produce precise analytical results from a series of measurements of the same homogenous sample under prescribed assay conditions [107]. According to the ICH guideline [108], precision can be further subdivided into three components, *viz.* repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (between laboratories precision). The standard deviation (SD) or percentage relative standard deviation (% RSD) of a series of measurements is usually used to assess the precision of an analytical method [109]. The % RSD is calculated using equation 2.1.

$$RSD = \frac{\sigma}{\overline{X}} \times 100 \qquad \text{Equation 2.1}$$

Where,

σ = Standard deviation around the mean of a set number of samples (calculated using nonbiased or n-1 method)

$\overline{X}$ = Mean of the peak height ratio responses for a set number of samples

The precision of the method was considered at two levels, *viz*. repeatability and intermediate precision. A value for % RSD of < 5% was set in our laboratory as the test limit.

2.3.3.1 Repeatability or intra-day precision

The repeatability of an analytical method is the precision of the analytical method in which the variability in experimental conditions is kept within a narrow range over a short time interval [107]. Repeatability assessment of an analytical method is performed in one laboratory by one analyst using the same equipment on the same day. It has been suggested [107] that repeatability be determined by the analysis of a minimum of three different concentrations covering the low, medium and high range of a calibration curve with a minimum of five replicates analyzed per concentration level. However, according to the ICH guideline [108], repeatability can be assessed using a minimum of three determinations at three different concentration levels or a minimum of six determinations at 100% of the test concentration.

The repeatability results obtained for three replicates of the three concentration levels of standard solutions of AZI that were analysed on three different days are shown in Table 2.4.

Table 2.4: Intra-day precision data for the analysis of AZI

Theoretical Concentration (µg/ml)	Actual concentration determined (µg/ml)	Standard deviation	Precision (% RSD)
300	299.49	0.012	0.74
150	151.06	0.020	1.68
50	48.92	0.016	2.63

The results reveal that % RSD values were within the limit set in our laboratory thus the method is repeatable and the intra-day precision is appropriate for the analysis of AZI.

2.3.3.2 Intermediate precision or inter-day precision

Intermediate precision or inter-day variability expresses the within laboratory variation and is tested on different days or by using a different analyst or equipment within the same laboratory [10]. The inter-day variability of this method was assessed over a period of three days at three different concentrations, using three replicates of AZI standards using the same equipment as described in § 2.2.1.2. Samples were prepared in the same manner as described in § 2.2.1.7 and the results of these studies are listed in Table 2.5.

Table 2.5: Inter-day precision data for the analysis of AZI

	Theoretical Concentration (µg/ml)	Actual concentration determined (µg/ml) ± SD	Precision (% RSD)
Day 1	300	296.44 ± 0.017	1.46
	150	149.03 ± 0.012	2.03
	50	48.27 ± 0.005	2.66
Day 2	300	298.00 ± 0.006	0.57
	150	147.31 ± 0.014	2.58
	50	47.52 ± 0.005	2.72
Day 3	300	301.35 ± 0.013	1.09
	150	149.23 ± 0.013	2.11
	50	47.80 ± 0.005	2.46

The results show that all % RSD values fell below 5%, which is within the limit set in our laboratory and therefore the method may be precise.

2.3.3.3 Reproducibility

The reproducibility of an analytical method refers to the ability of an analytical procedure used in different laboratories to produce the same results [10, 106] and in which the documentation includes the SD, % RSD, coefficient of variation and confidence intervals of such methods [106]. Reproducibility studies were not conducted as repeatability results were sufficient to suggest that the method was precise and usable. For most purposes, repeatability is the primary criterion of concern in USP analytical procedures [10] and reproducibility is mainly considered during standardisation of a procedure before it is submitted to a Pharmacopoeia for referencing [108].

2.3.4 Accuracy

The accuracy of an analytical method describes the extent to which test results deviate from the expected results and it is a measure of the exactness of an analytical method [106]. Accuracy together with precision is set to determine the error associated with an analytical method and they are therefore important criteria for the evaluation of the suitability of an analytical method for the task for which it is intended [107]. Accuracy may be reported as the percentage recovery of a known added amount of analyte to a sample or as the difference between the mean value obtained and the accepted true value of a sample, together with an associated confidence interval [10]. A two sided t-test can also be used to assess accuracy and to reveal whether any significant difference exists between the mean data and the true value at a 95% level of confidence [107].

The % RSD as well as % bias was used to determine the accuracy of this method. The % bias assesses the influence of an analyst on the performance of a method. A tolerance of 2% for % RSD was set in our laboratory for accuracy. The 2% limit is that set by pharmaceutical industries in the United Kingdom and that was reported in a survey published by the Pharmaceutical Analytical Sciences Group [110]. A % bias of < 5 was set in our laboratory as the test limit. Accuracy studies were determined by making repeat measurements of two samples of a high concentration of 500 μg/ml and low concentration of 150 μg/ml in replicates of five and the results are listed in Table 2.6.

Table 2.6: Accuracy data for the analysis of AZI

Theoretical concentration (μg/ml)	Actual concentration determined (n=5)	% Recovery	%RSD	% Bias
500	490.92 ± 0.007	98.18%	0.40	-1.85
150	148.50 ± 0.009	99.00%	1.57	-1.01

The resultant values for % RSD and % Bias fell below limits of 2% and 5% respectively, indicating that the method is accurate for the determination of AZI.

2.3.5 Limit of quantitation (LOQ)/ Limit of detection (LOD)

The LOQ and LOD are often reported in the literature as a means to indicate the sensitivity of an analytical method. The LOQ can be defined as the lowest amount of analyte that can be detected with acceptable precision and accuracy under the stated experimental conditions and the LOD as the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions [10]. The LOD can also be defined as the lowest concentration that can be distinguished from background noise with a certain degree of confidence [107].

The LOQ and LOD of an analytical method can be determined in different ways [107, 108, 111] and some of the common methods to establish these parameters are listed in Table 2.7.

Table 2.7: Different techniques for the determination of LOQ and LOD

Methods	**Set limits**	
	LOQ	**LOD**
Signal to noise ratio	10:1	3:1 or 2:1
Standard deviation of the noise and the slope of the calibration curve	$=\frac{10\,\sigma}{\text{Slope}}$	$=\frac{3.3\sigma}{\text{Slope}}$
Calculated confidence intervals (CI) around a calibration curve	The concentration at 95% CI that does not overlap the confidence level of the blank matrix standard	Not used for LOD
Background interferences and reproducibility of a response	mean response ≥ 3 SD	Not used for LOD
Based on the percentage relative standard deviation (%RSD)	RSD≤5.0%	0.3 × LOQ

The LOQ of the reported method was determined, based on a precision of ≤ 5% and was found to be 25 µg/ml (2.79 % RSD). A common approach that is listed in the ICH guideline [108] was used for the determination of LOD. This approach makes use of the signal to noise ratio method for the determination of LOD. The peak height of a sample of known low concentration is measured and compared to the noise response observed following the introduction of a blank sample into the HPLC system. A signal to noise ratio of 3:1 was used as the test limit and the LOD was found to be 15 µg/ml for this method.

2.3.6 Specificity and selectivity

A method is considered to be specific if it produces a response for a single analyte and since it is impossible to develop an assay for a drug, in a matrix that responds to only the compound of interest, the term selectivity is a more appropriate description for an analytical method [107]. Reputable international authorities such as IUPAC [109] have used the term selectivity and reserved the term specificity for procedures that are totally selective. In this case, selectivity of the method is therefore considered as the ability of an analytical method to produce a response for an analyte that is distinguishable from all other potential responses, such as those that may be attributed to the presence of impurities, degradation products and matrix components.

In order to demonstrate the selectivity of this analytical method, the method was applied to the analysis of AZI in commercially available Zithromax® tablets. In addition, forced degradation studies were also performed on API to demonstrate the selectivity of the method in the presence of degradation products. The resultant chromatograms following exposure to stress conditions were then compared with that of a standard freshly prepared solution, to establish selectivity.

2.3.6.1 Preparation of tablets for analysis

Five Zithromax® tablets were accurately weighed and powdered in a mortar and pestle. An amount of powder equivalent to the weight of one tablet was weighed and dissolved, with the aid of sonication (15 min), in 50 ml of acetonitrile in a 50 ml A-grade volumetric flask. These analyses were performed in triplicate. 5 ml of the solution were filtered through a Millipore Millex®-GV hydrophilic PVDF 0.22µm syringe filter (Millipore Co., Billerica, MA, USA). A 1 ml aliquot of the filtered solution was added to a 25 ml A-grade volumetric flask containing 150 µg/ml IS and made up to volume with mobile phase prior to injection.

2.3.6.2 Forced degradation studies

In order to insure that the assay was stability indicating, AZI active pharmaceutical ingredient (API) powder was stressed under various conditions in order to force degradation. All degradation studies were performed on solutions. Solutions were prepared to yield a starting concentration of 1 mg/ml by accurately weighing 50 mg of API into a 50 ml A-grade volumetric flask and made up to volume with the medium specific for that degradation study.

2.3.6.2.1 Acid degradation studies

The solutions for the acid degradation studies were prepared in 0.1M HCl and protected from light. The solutions were stored at room temperature for 24 hours. Samples were removed for analysis after 3 hours and 24 hours and neutralised with mobile phase prior to qualitative analysis by HPLC.

2.3.6.2.2 Alkali degradation studies

As AZI is a basic compound, it is relatively insoluble in an alkaline solution. Therefore, a dilute 0.02 M NaOH solution was used to expose the drug to alkali conditions. These solutions were protected from light and stored at room temperature for 24 hours. Samples were removed for analysis after 3 hours and 24 hours and analysed by HPLC. The stability of AZI was also challenged with a more concentrated 0.1 M NaOH solution heated at 50°C for 6 hours in a water bath Model 132, equipped with a Model 102 temperature controller (Scientific Engineering CC, Johannesburg, South Africa), after which a sample was removed , neutralised with mobile phase and analysed by HPLC.

2.3.6.2.3 Oxidation

Solutions of AZI for oxidation studies were prepared in H_2O_2 (10% v/v) (Saarchem, Port Elizabeth, South Africa), protected from light and stored at room temperature for 24 hours. Samples were withdrawn after 24 hours, neutralised with mobile phase and analysed by HPLC.

2.3.6.3 Results and discussion

The chromatograms obtained from the tablet assay were well resolved from the solvent front or void volume and the formulation components did not appear to interfere with the analysis of AZI. The results of the assay revealed that, as expected, the drug content of the commercially available product was within the pharmacopoeial limits described in the USP [10]. The results are summarised in Table 2.8.

Table 2.8: Assay of AZI in tablets

Brand	Label claim	Recovery (mg) ± SD	% Recovery	%RSD
Zithromax®	500mg/Tabs	503.8 ± 0.010	100.76	3.20

The results of the forced degradation studies indicate that the method has a high degree of selectivity for the determination of AZI in the presence of degradation products. Following exposure of AZI API to acidic conditions, a golden yellow solution resulted when compared to the colorless control solution. The resultant chromatograms following exposure of AZI API to acidic conditions are depicted in Figure 2.5. It is evident that the degradation of AZI API is significant under acidic conditions. One of the major degradation products reported to be found after low pH acid degradation of AZI is pseudo-aglycone azithromycin [16], which may be the compound represented by the unknown peak (A) that has a retention time of approximately 1.7 minutes.

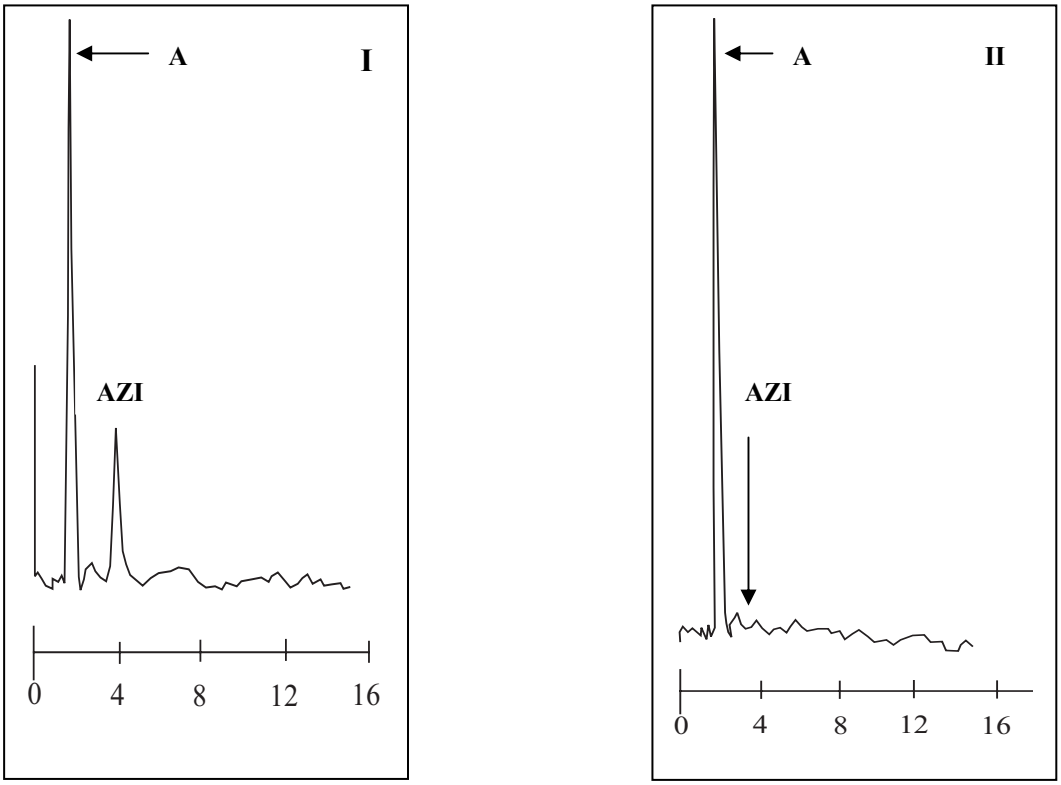


Figure 2.5: Typical chromatograms obtained following the acid degradation of AZI after exposure for 3 hours (I) and 24 hours (II) showing an unknown peak (A) and AZI.

AZI was found to be relatively stable when stored under alkaline conditions for 24 hours at room temperature. When AZI was treated with a 0.02 M NaOH solution, no degradation products were observed after 3 hours and 24 hours of storage. However, following exposure of AZI for to a 0.1 M NaOH solution for 6 hours at 50°C, an unknown degradation peak with a retention time of approximately 5 minutes was observed as can be seen in Figure 2.6. It has been previously reported [77] that AZI was found to be relatively stable following exposure to a 0.1 N NaOH solution for 30 minutes at 60°C

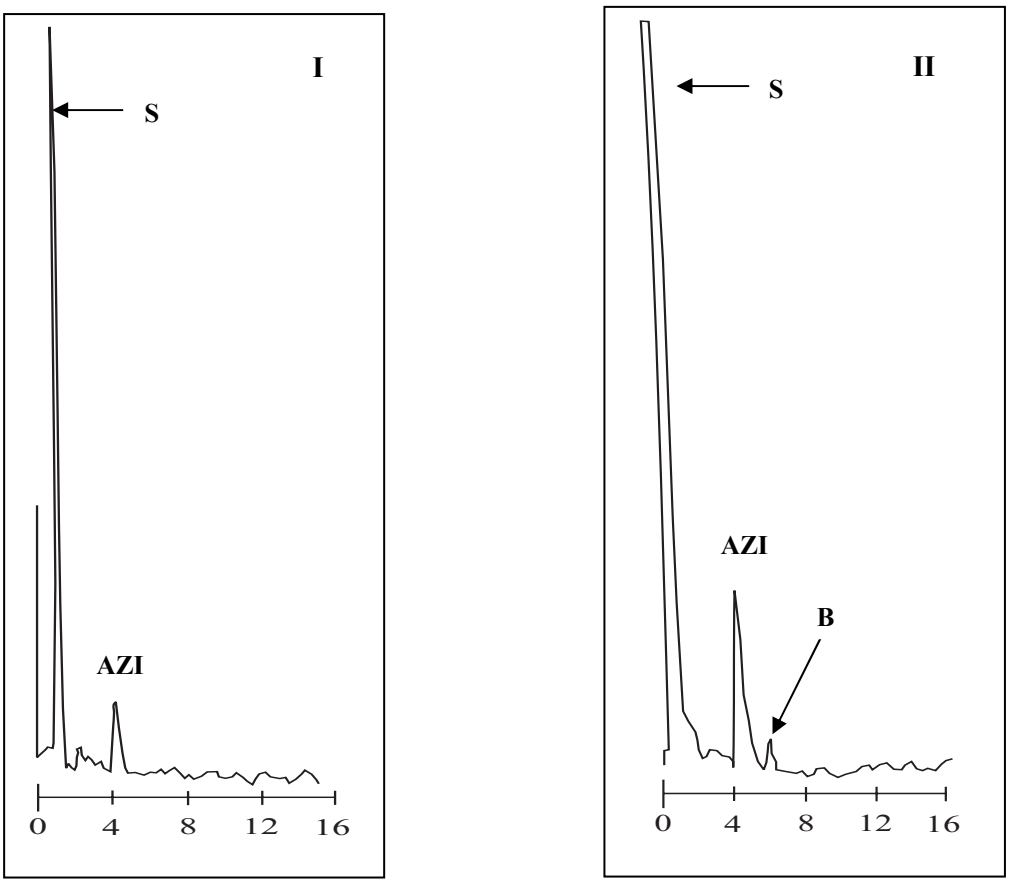


Figure 2.6: Typical chromatograms obtained following the exposure of AZI to 0.02M NaOH for 24 hours at room temperature (I) and 0.1M NaOH at 50°C for 6 hours (II) showing the solvent front (S), AZI and an unknown product (B)

Following exposure of AZI to a solution of 10% v/v H_2O_2 at ambient temperature, a peak that eluted at a similar retention time to that of clarithromycin was observed. The resultant chromatograms of these oxidation studies are shown in Figure 2.7. Therefore, in order to establish the presence or absence of any possible interference with the internal standard, the IS should not be included in samples of AZI intended to be subjected to forced degradation studies.

AZI has been reported to be susceptible to degradation under oxidative conditions [28]. A particular example of oxidative degradation that has been reported is the oxidation of the exocyclic amine functional group of azithromycin [28] and this may be the reason for the presence of unknown peaks in the resultant chromatograms following exposure of AZI to H_2O_2 solutions.

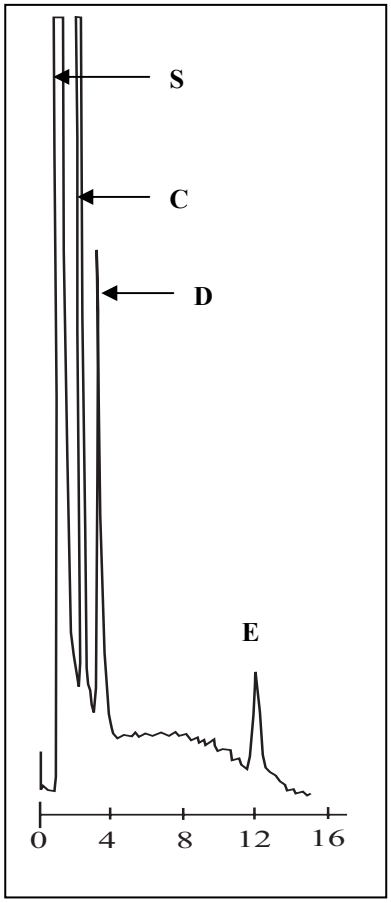


Figure 2.7: Typical chromatogram following exposure of AZI to a 10% v/v H_2O_2 solution showing the Solvent front (S) and unknown products (C, D and E).

2.3.7 Application of the analytical method

The validated method was subsequently applied to the quantitative determination of AZI during *in vitro* dissolution testing of commercially available Zithromax® tablets using the procedure described in USP 28th Edition [10].

The USP recommends that, for the establishment of linearity and selectivity purposes for dissolution testing, a minimum of ± 20% of the specified range must be considered [10]. A limit of up to 75% in 45 minutes is set for dissolution testing of AZI containing pharmaceutical dosage forms, [10]. The results indicate that approximately 70% of the drug was dissolved in 45 minutes, as shown by the mean dissolution profile depicted in Figure 2.8. Thus the assay was within the limit set, according to the USP guidelines [10].

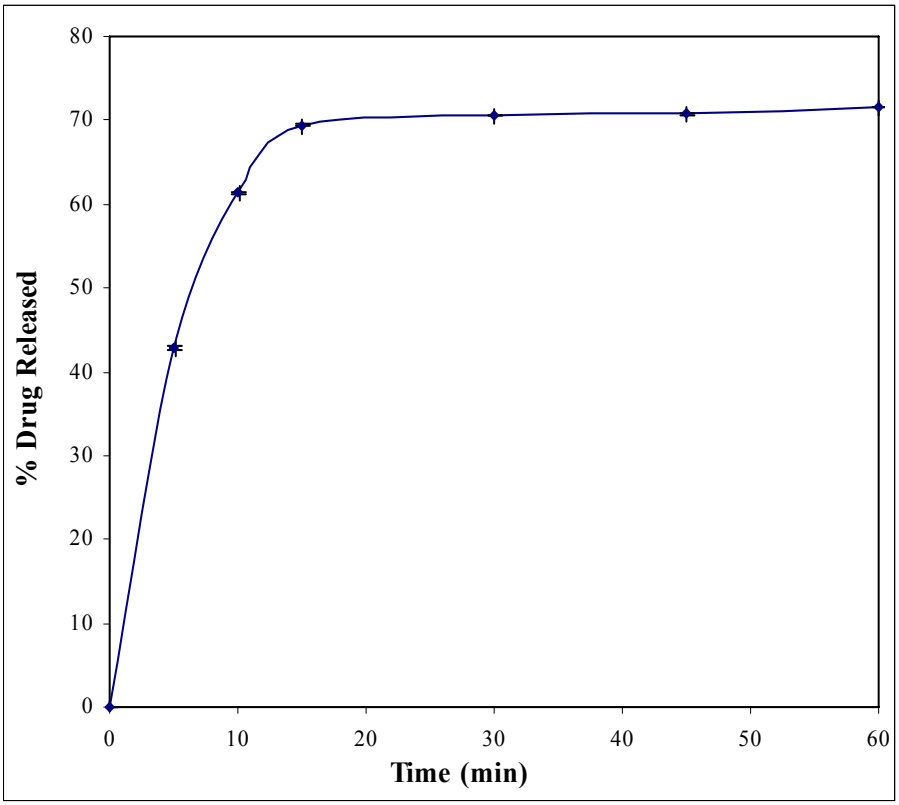


Figure 2.8: Dissolution profile of Zithromax® mean ± SD (n=6).

2.3.8 Stability of the analyte

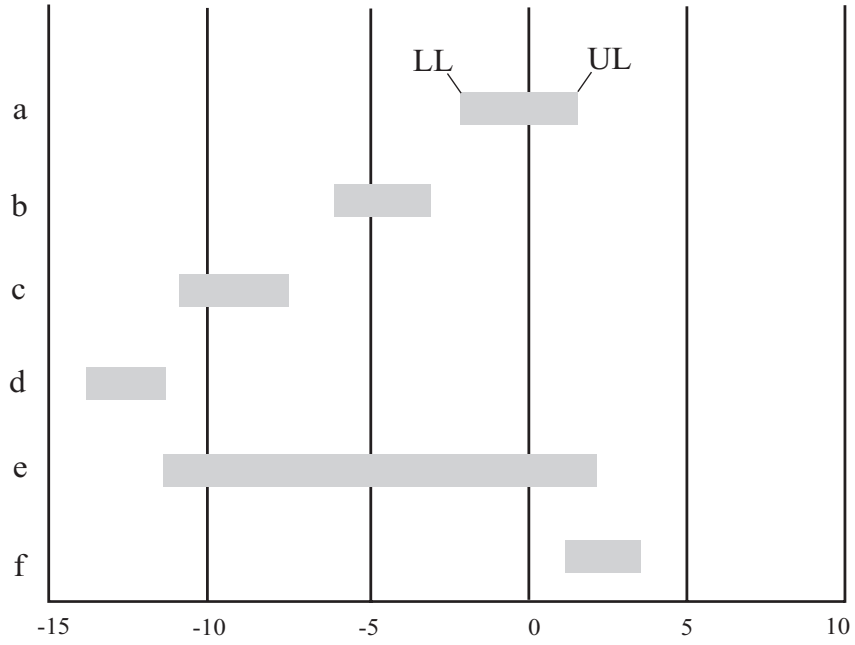
It is important to determine the stability of an analyte for a specified period, in solvents that will be used for an assay for any analytical procedure. An analyte is considered to be stable under certain conditions during a certain period of time when the resultant responses compared to freshly prepared samples do not change significantly over time [107]. The stability of AZI in CH_3CN and phosphate buffer pH 6.0 has been investigated [79] and AZI was found to be stable at 4°C and 25°C for up to 5 days. There are however no data available to establish the stability of AZI in a mobile phase comprised of THF, MeOH, CH_3CN and phosphate buffer. Therefore a stability study of AZI in the aforementioned mobile phase that was used in the validated analytical method was performed over 14 days.

The aim of the stability study was to detect any potential degradation of AZI during sample collection, processing, storage and analysis [107]. The stability study was conducted at both room temperature (22°C) and at 4°C. AZI stock solutions were prepared as previously

described in § 2.2.1.9, and stored at the specified temperatures protected from light using foil. 5 ml and 3 ml samples were withdrawn from the stock solution, added to a 10 ml A-grade volumetric flask and made up to volume with a freshly made mobile phase, so as to obtain a high concentration of 500 µg/ml and a low concentration of 300 µg/ml respectively. Samples were withdrawn and analysed after storage for 1, 2, 3, 7, and 14 days. Aliquots of each sample were mixed with freshly prepared internal standard solution (150 µg/ml) and injected.

The statistical method proposed by Timm *et al,* [112] for the investigation of the stability of drugs in biological samples, was adopted to determine whether the resultant differences in concentrations constituted a relevant and/or significant change. The proposed statistical method [112] takes into account the quality of the experimental procedure i.e. the precision of the method and the number of replicates. The statistical interpretation of the stability data is based on the construction of a 90% confidence interval for the percentage change (Δ) in concentration between the stored and freshly prepared samples. The true change in response on storage is enclosed by the lower limit (LL) and the upper limit (UL) of the confidence interval, with a probability of 90% certainty. In other words, there exists a probability of 5% that the Δ is lower than LL and a probability of 5% that the Δ is higher than UL [112].

Timm *et al,* [112] proposed that a change may only be relevant if both the upper and lower limits of the confidence interval are either > 10% or < -10%. Figure 2.9 shows the possible scenarios that could be obtained when this method is used to analyse the stability of the stored samples.



The bars above the axis represent the 90% confidence intervals for the Δ% between stored and freshly prepared samples

(a) change of response, not significant and not relevant

(b) decrease of response, significant, but not relevant

(c) decrease of response, significant and possibly relevant

(d) decrease of response, significant and relevant

(e) decrease of response, not significant, but possibly relevant

(f) increase of response, significant

Figure 2.9: Statistical interpretation of the stability data, as described by Timm *et al*, [112]

The measurements of the peak height ratios of AZI with IS are referred as the response in this study. The measured percentage difference in response between stored samples and freshly prepared samples was calculated and results are presented in Figure 2.12.

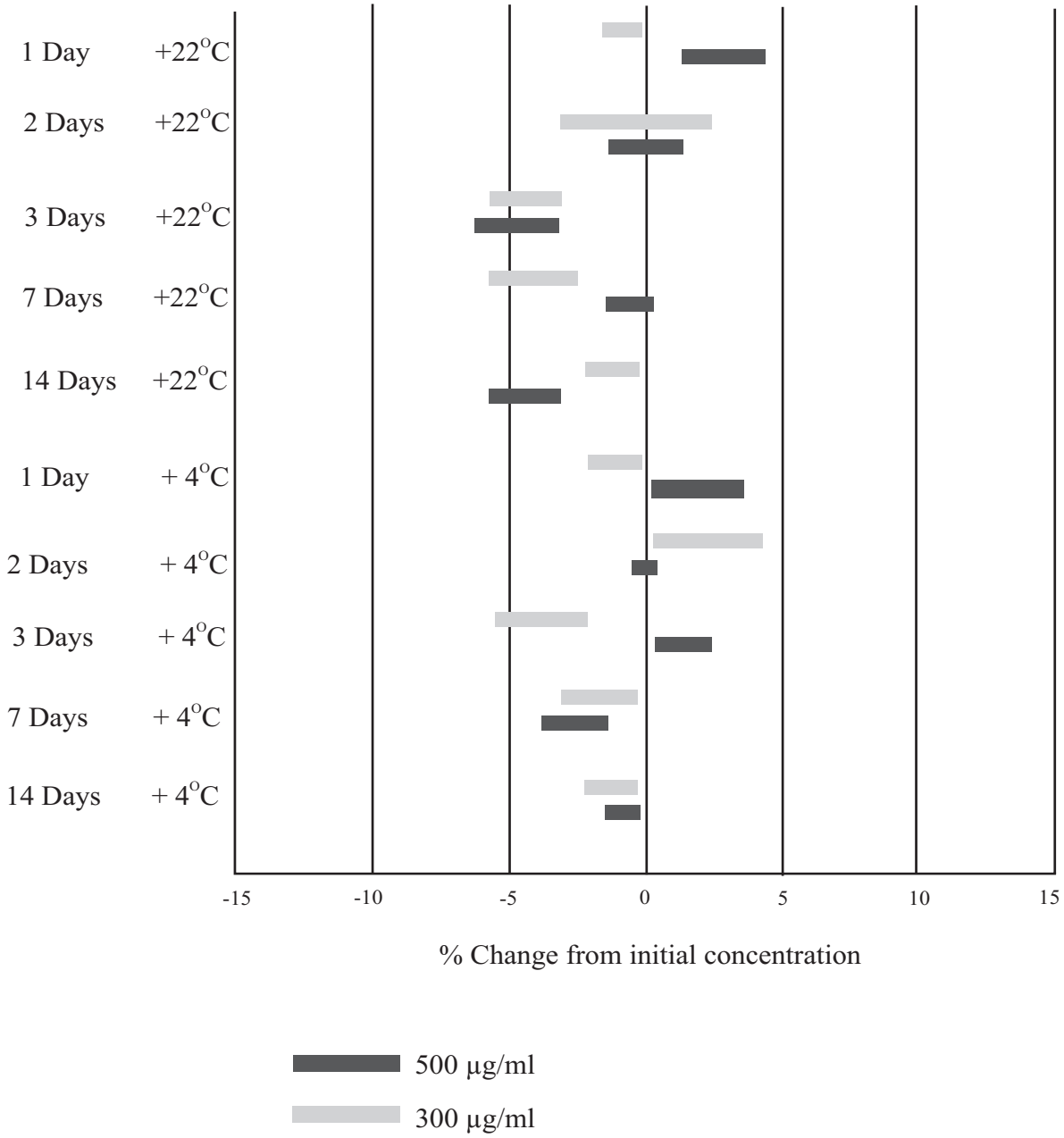


Figure 2.10: Stability of AZI in mobile phase at two different concentrations (300 and 500 µg/ml) stored at +4ºC and +22ºC for 1, 2, 3, 7, and 14 days

Following the procedure described by Timm *et al,* [112], the stability of AZI was evaluated at two concentration levels (300 and 500 µg/ml), temperatures (22ºC and 4ºC) and storage times as indicated in Figure 2.10. From the graphical presentation it is evident that no significant decrease of concentration was detected when AZI was stored in the mobile phase for 14 days at specified temperatures. However, the 300 µg/m samples at day 1 (4ºC and 22ºC) and day 3 (4ºC), as well as the 500 µg/ml samples at day 2 (4ºC) showed an increase in response that could possibly be due to the experimental systematic errors. A significant increase in response is conceivable only in the extremely unlikely instances of the compound producing a degradation product with the same retention time as the parent compound, but having a higher response to the detector system [112]. Therefore, AZI can be considered to be stable under the specified storage conditions for the period of testing.

2.4 CONCLUSIONS

A reversed phase HPLC method with UV-detection for the *in vitro* quantitation of AZI has been developed, validated and subsequently applied to the assessment of pharmaceutical dosage forms. The method was optimized by the manipulation of the mobile phase composition, type and quantity of organic modifier, buffer pH and molarity. The components of the mobile phase that may influence the retention time and resolution of AZI and the internal standard, clarithromycin, or that may have affected the baseline resolution of the peaks of interest, were assessed. It was found that the acetonitrile and tetrahydrofuran content, in addition to the buffer molarity, had a pronounced effect on the retention time and resolution of both AZI and clarithromycin. Consequently, these parameters were manipulated to achieve a separation in which well resolved peaks with desired retention time and baseline resolution were obtained. The analytical method was validated with respect to linearity, accuracy, precision, selectivity, LOQ and LOD and by the assessment of analyte stability in the mobile phase. The HPLC method developed is an improvement on the methods presented in the literature [78, 80, 81] and that listed in the USP monograph for AZI. The described method is selective, accurate, precise, and linear and can therefore be used for the assessment of *in vitro* performance of AZI in pharmaceutical dosage forms.

CHAPTER THREE

3. FORMULATION AND ASSESSMENT OF AZITHROMYCIN SUPPOSITORY DOSAGE FORMS

3.1 INTRODUCTION

3.1.1 Rectal dosage forms

Functionally, suppositories can be described as solid dosage forms of varying weight and shape, intended for the administration of medicines via the rectum, vagina, or urethra for topical or systemic drug delivery [113]. They consist of a dispersion of an active ingredient in an inert matrix, which is generally composed of a rigid or semi-rigid base [113]. These dosage forms melt, soften or dissolve in the relevant body cavity prior to releasing the active ingredient [114]. Rectal suppositories are conventionally bullet, torpedo or conically-shaped with a rounded apex. Suppositories can be used to administer drugs for use as protectants or for palliative care of local tissues at the point of introduction or as a carrier for therapeutic agents where they are intended to exert localized or systemic effects.

Historically, the rectal route of administration was primarily used for the delivery of local anaesthetics, anti-haemorrhoidal, anti-pruritic, antiseptic or laxative agents [114, 115]. More recently, many articles have been published demonstrating that some natural and semi-synthetic drugs can be formulated into rectal suppositories for the purposes of eliciting a systemic therapeutic effect [116-127]. More specifically, such dosage forms have included furosemide [116], chloroquine [117], salbutamol [118], ethosuximide [119], allupurinol [120], β-lactams, erythromycin [121-123] and anti-cancer agents, such as tamoxifen and fluorouracil [123]. Furthermore, the use of the rectal route for the purpose of sustaining drug action has also been reported for a number of drugs, including morphine [124], metoclopramide HCl [125], sodium diclofenac [126] and indomethacin [127].

The use of the rectal route for drug administration is certainly not the route of first choice due to poor patient acceptability and psychological biases [121]. However, the use of rectal delivery is often appropriate in situations where a patient is unwilling or unable to make use

of the oral route of drug administration. This may occur in cases where the administration of a drug via the oral route results in intolerance, nausea and vomiting or associated gastric pain [128]. In addition, in cases where patients are uncooperative, unconscious or lack lucidity or when access to the intravenous route is compromised, as is the case, for example, with children or patients in intensive care units. Rectal dosing may also be of value in achieving appropriate therapeutic outcomes for patients needing multiple drug therapy or continuous intravenous fluid infusion, where treatment is difficult or when there are few undamaged veins available for catheterisation [121].

The ideal suppository should be easy to administer without pain on insertion and should remain at the administration site for a reasonable period of time. Conventional solid suppositories often give patients a feeling of alien discomfort and subsequently their refusal to use such delivery devices may lead to poor patient compliance. Furthermore, if the solid suppositories lack sufficient muco-adhesivity, they may traverse up the rectal cavity and reach the end of the colon, with the result that the drug delivered in this area may be absorbed into the venous blood system, thereby increasing the potential for the compound to undergo hepatic first-pass metabolism, the avoidance of which is one of the potential advantages of suppository use [129].

In order to alleviate the challenges and difficulties associated with the production and use of conventional solid suppositories, attempts have been made to develop *in situ* gelling agents that form liquid suppositories [130-134]. These formulations consist of mucoadhesive polymers that exist as a liquid at ambient temperatures, but instantly turn into a gel at physiological temperatures and pH [130-134]. By use of an appropriate mucoadhesive agent, thermo-reversible liquid suppositories remain in the lower part of the rectum and there is minimal or no leakage and thus drug absorption directly to the general circulation is achieved with the avoidance of drainage into the hepatic portal system [132]. Conventional solid suppositories, however, provide a convenient means of drug administration with respect to patient self-administration as compared to liquid suppositories, since liquid suppositories require a device to administer a specific volume to control the dose. Yahagi *et al,* (1999, 2002) [135, 136] demonstrated that the use of the polymer, Carbopol®, together with white beeswax in a conventional double-phased suppository permitted, and in fact, facilitated lower rectal absorption of an active pharmaceutical ingredient. In addition, leakage of the suppository from the rectum during and following melting was also avoided.

3.1.2 Rectal absorption

The mechanism of absorption of systemically active drugs from the rectum involves drug release from the suppository into the rectal cavity, diffusion of the drug through rectal fluids to the rectal mucosa, followed by absorption across the rectal tissues and subsequent transport into the general circulation. The mechanism of absorption is similar to that that occurs in the gastrointestinal tract, which in turn involves two main routes of penetration, *viz.,* the transcellular and paracellular routes [137, 138]. The transcellular route involves absorption of drugs across epithelial cells whereas the paracellular route involves absorption of drugs via the interconnecting tight junctions between mucosal cells [138]. Absorption of substances via the transcellular route depends mainly on their lipophilicity and molecular size. These include active transport for amino acids and carrier-mediated transport for β-lactam antibiotics and dipeptides [137]. Passive transport, involving diffusion of a drug across the epithelial wall is known as paracellular transport and is the main mechanism by which drugs are absorbed from the rectum [121].

A suppository formulation can be a complex dosage form and many aspects of the formulation composition, in addition to physiological factors, may influence rectal absorption and consequently the systemic distribution and pharmacokinetics of a rectally administered drug [129]. These factors relate specifically to the volume and composition of the rectal fluids and the associated environment, the physicochemical properties of the drug substance in addition to the physicochemical properties of the suppository base from which the drug is to be delivered. The factors affecting rectal absorption of a drug administered in suppository formulations are summarised in Table 3.1.

Table 3.1: Factors affecting drug availability from suppositories*

Physiological Factors	Drug Substance	Formulation Parameters
Buffer capacity	Solubility	Composition
Rectal fluid volume	Surface properties	Melting behavior
Surface tension	Particle size	Rheological properties
Composition	Drug concentration	Surface tension
Motility of the rectal wall	Partition coefficient pKa and the degree of ionization	

* Adapted from references 129 and 139

3.1.2.1 Physiological factors affecting drug absorption

A major rate-limiting step in drug absorption is the rate at which diffusion of a drug takes place from a dosage form to the site from which absorption occurs [140, 141]. The diffusivity of a drug is influenced by its physicochemical nature, the physiological state of the colon and rectum, including the amount and nature of fluid and solids present [140]. In the absence of faecal matter, an administered drug will have a greater potential to make contact with the mucosal surfaces of the rectum from which absorption will take place. The membranous wall of the rectum is covered with a continuous relatively viscous mucous blanket, which acts as a mechanical barrier to the free passage of a drug through the epithelial wall [140]. The epithelial wall in the rectum has no villi or microvilli and is surrounded by a single layer of columnar or epithelium cuboidal and goblet cells with an associated approximate surface area of about 200 to 400cm^2 [129], which is 10,000 times smaller than that of the small intestine [121]. Therefore the rectum has a relatively small surface area available for drug absorption, compared to the small intestine.

When anticipating drug behavior subsequent to rectal administration, a primary element to be considered is the nature of the absorption and uptake process in the rectum [113]. The inferior, middle and superior rectal veins drain the tissues associated with the rectum. The inferior and middle rectal veins drain directly into the systemic circulation, whereas the superior rectal vein drains directly into the hepatic portal system, which flows directly to the liver [129] as shown in the schematic presentation of the venous drainage system Figure 3.1.

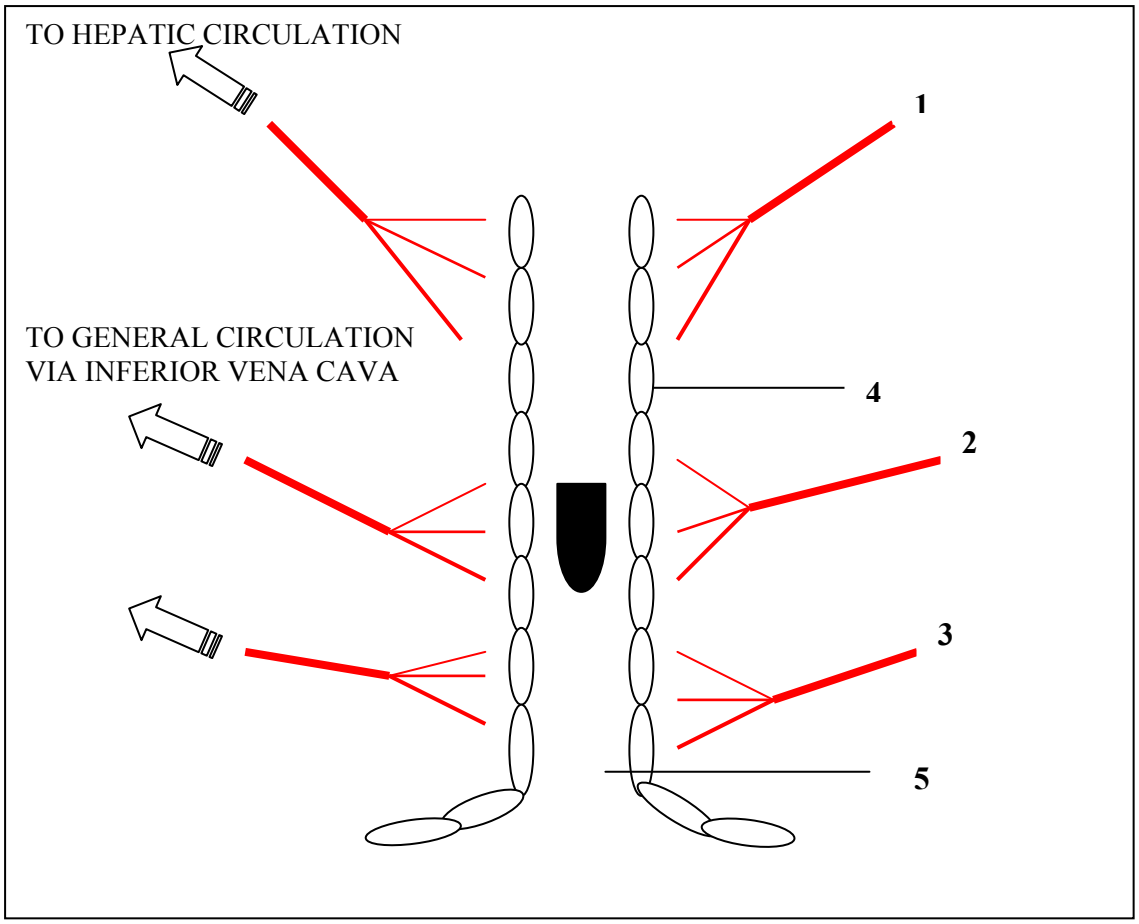


Figure 3.1: A schematic representation of the venous drainage system of the human rectum with a suppository in position, showing the superior rectal vein (1), the middle rectal vein (2), the inferior rectal vein (3), the rectal mucosa (4) and the rectal cavity (5), (adopted from reference 142).

Consequently, the positioning of a suppository in the rectum is critical in terms of the potential for exposure of a drug to liver enzymes following absorption and subsequent metabolism. A drug that is absorbed into the systemic circulation via the inferior or middle rectal veins will likely bypass the liver, resulting in a higher bioavailability than one transported by the superior vein to the liver via the hepatic portal system, prior to its entry into the systemic circulation. However, a complicating factor in this respect is the occurrence of anastomoses that defines the communication between the inferior, middle and superior rectal veins [129]. The presence of anastomoses results in the potential for the diversion of blood flow and consequently an absorbed drug may or may not pass though the liver directly and be subjected to hepatic first pass elimination to a greater or lesser extent than expected.

Consequently, it is unwise to rely solely on the positioning of a suppository in the rectal cavity when evaluating drug absorption [129].

The pH of the rectal fluids also plays a significant role in drug absorption and is often a rate-controlling step in rectal drug absorption. Rectal fluids have virtually no buffering capacity and, as a consequence, the characteristics of dissolved drugs will to a large extent determine the pH that prevails in the anorectal area following administration [141]. Coben and Lieberman [141] have demonstrated that the intra-luminal pH of the rat colon can affect the absorption of acidic and basic drugs and that the unionized form of a drug is preferentially permeable. Thus the absorption of basic drug will be more favorable from rectal fluids, since it would be largely unionized and remain unionized at rectal pH, which is approximately 7.2. Therefore, it can be suggested that ionized substances that are lipid-insoluble will be poorly absorbed through rectal tissues [141].

3.1.2.2 Physicochemical characteristics of the drug and base affecting absorption.

The relative solubility of a drug in lipid and aqueous environments in addition to the particle size of an API plays an important role in determining the rate and extent of drug absorption from the rectum. Another significant factor that must be considered is the quality and type of base used as the carrier matrix, which plays a role in regulating the rate and extent of partitioning of a drug between a suppository, the rectal fluids and ultimately the rectal mucosa.

3.1.2.2.1 Drug solubility

The solubility of an API in the vehicle to be used as the suppository base will determine whether the product that is produced is either a solution or suspension formulation and the solubility of a drug in the rectal fluid will determine the maximum attainable concentration possible, in the rectum, and consequently the driving force for the absorption process [139]. If a drug has a high oil to water partition coefficient and the base of choice is a fatty material, the API will primarily be in solution in the base. Therefore the ability or tendency of the drug to leave the vehicle will be low and the subsequent release rate into the rectal fluids will be slow [139]. On the other hand, a certain degree of lipid solubility is essential to facilitate

penetration through the rectal membranes [115]. Therefore an optimal balance between the partition coefficient of the drug and its solubility in the bases used as carriers for the API and in the rectal fluids is vital when selecting an appropriate base for suppository dosage form manufacture. The highly variable and low rectal fluid volume will affect the rate and extent to which a drug will dissolve in such fluids. The variability of fluid volume is a function of diurnal and inter-individual variability [139].

3.1.2.2.2 Partition coefficient

The lipid-water partition coefficient of a compound is one of the most important factors that should be considered during pre-formulation studies, as this parameter may assist in the prediction of drug release from a suppository base, in addition to providing insight into potential absorption and disposition challenges for the compound [143]. Drug absorption from the rectum is a consequence of the partitioning of a dissolved drug from a molten base into the rectal fluids and from the rectal fluids to the rectal mucosa, in addition to the rate of solution of the drug in the body fluids. It has been suggested that penetration of a drug through the barrier phase or epidermal mucosa of the rectum is proportional to the permeability constant of the drug, which is a complex constant taking into account factors such as transfer of drug from the base to the barrier phase and diffusion of a drug through the barrier membrane [114]. The transfer of a medicament from a base is related to the solubility of the medicaments in that base, whereas diffusion through the barrier membrane is related to the lipid/water partition coefficient of the drug between those fluids [114].

3.1.2.2.3 Particle size

If a drug with limited solubility in a suppository base is suspended in that suppository base, the particle size and size distribution of the compound may influence the rate and extent to which the active pharmaceutical is released from the dosage form, since the rate of dissolution of a drug in rectal fluids has been reported to be proportional to its particle size [114]. However, size reduction and the use of a smaller particle size does not necessarily ensure higher blood levels, as the drug release process is relatively complex and involves the melting and spreading of the base, in addition to the wetting, sedimentation and dissolution of the drug [115].

3.1.2.2.4 Surface properties

As the API contained in a suppository dosage form will be transferred from one phase to another, the surface properties of the drug particles become an important consideration in evaluation of rate of drug release [139]. If wetting of the API by the vehicle or base does not occur, powder particles may agglomerate, which in turn may affect the uniformity of dispersion of the API, due to the increased tendency for the agglomerated powder to sediment prior to the setting of the suppository. In order to reduce the surface effects of poorly wettable API's, the addition of a surfactant to a formulation will more than likely improve the wetting of the API and subsequently the facilitate dissolution of the drug in the suppository and in the rectal fluids [139].

3.1.2.2.5 Nature of the base

Suppository bases must melt, soften or dissolve in order to facilitate or promote the release of API such that it is readily available for absorption [114]. A chemical and/or physical interaction between a drug and a base may influence the stability and/or bioavailability of a drug. Potential chemical and/or physical interactions between a drug and the components of a formulation should be investigated during preformulation studies and in the early stages of formulation development. In addition, should the base irritate mucous membranes, it may initiate a colonic response and promote unwanted bowel movements resulting in the expulsion of the dosage form, thereby negating any potential drug absorption via the rectal route [140].

3.1.3 Enhancement of rectal absorption

The rate at which the drug diffuses into the rectal mucosa is influenced by the physicochemical relationship that exists between a drug, the rectal fluids, the suppository base and the membranes of the rectal cavity. Nishihata and Rytting [137] have critically reviewed the mechanisms by means of which rectal absorption can be enhanced, in particular for poorly absorbed drugs with low aqueous solubility, by modulation of the physical and/or chemical properties of the base or the drug. More specifically, drug absorption from a suppository formulation can be modulated by the incorporation of absorption or permeation

enhancers into the dosage form. Enamine derivatives of amino acids [137], surfactants [118, 144, 145], fatty acids derivatives [137], and carboxylic acid derivatives [146, 147] have been reported to act as effective absorption or penetration enhancers for rectally administered compounds.

The addition of adjuvants to a formulation can affect drug absorption by changing the rheological properties of the base at body temperature or by altering the dissolution rate of a drug in the rectal fluids [141]. The effective use of absorption promoting agents and their effect on dissolution rates have also been extensively reported [115, 117-119, 121, 122, 137, 144]. Hydrophilic polymers such as Carbopol®, in conjunction with fatty bases have recently been used to promote drug absorption [135, 136]. The safety, efficacy and compatibility of a drug and/or base with absorption enhancers must be established during preformulation studies since the addition of an absorption enhancer may either reduce or increase drug release rates, depending on the nature of the enhancer, base and drug to be incorporated into a specific formulation.

3.1.4 Suppository bases

The suppository base plays an important role in the release of an API from the dosage form and therefore has an impact on the availability of a drug for absorption and any subsequent systemic effect. The primary requirement of a suppository base is that it is solid at room temperature, but must soften, melt or dissolve readily at body temperature in order to liberate the drug incorporated into the formulation, soon after insertion of the dosage form into the rectum [140]. The melting range of the suppository should be narrow in order to ensure rapid solidification after manufacture, thereby preventing possible sedimentation of any API that is insoluble in the base or those of high density that may be prone to powder agglomeration [139].

A suppository base should have little or no irritancy potential, be nontoxic to either sensitive or inflamed tissues and must not be absorbed into or through rectal mucosal membranes. The base should be compatible with a wide variety of API's and should be easily and readily moulded into a rigid shape that will produce constant drug release rates immediately after manufacture and following long term storage.

Suppository bases are usually classified according to their physical characteristics and thus fall into one of three main classes:

1. Fatty or oleaginous bases, such as theobroma oil, or synthetic and semi-synthetic fatty bases.
2. Water-soluble or water miscible bases that may consist of glycerol, gelatin and/or polyethylene glycol.
3. Miscellaneous bases such as hydrophilic or water-dispersible compounds that may contain nonionic surfactants mixed with either vegetable oils or waxy solids [141]

Semi-synthetic fatty bases and polyethylene glycol were used for the development of a suppository formulation containing AZI following consideration of several factors as explained *vide infra* in § 3.1.4.1 and 3.1.4.2.

3.1.4.1 Synthetic and semi-synthetic fatty bases

The use of cocoa butter as a suppository base is well known [113], however, its use poses several technical challenges with respect to the manufacture of dosage forms of suitable quality. It is for this reason that research and development initiatives have focused on the search for more appropriate and suitable synthetic or semi-synthetic alternatives that retain many of the desirable characteristics yet eliminate the inappropriate ones of cocoa butter, as a suppository base.

The first semi-synthetic fat products, used as suppository bases were derived from vegetable oils such as palm kernel or arachis oils. Initially, the vegetable oils were subjected to hydrogenation and heating [141]. The hydrogenation process saturates any unsaturated glycerides and heat treatment splits some triglycerides into fatty acids and partial ester components, resulting in the formation of mono- and di-glycerides [141]. Hydrogenated palm kernel oil was first recommended as a suppository base by Caldwell in 1939 [148]. Currently, synthetic fat bases are manufactured by hydrolyzing vegetable oils and then subjecting the resultant free fatty acid product to fractional distillation, hydrogenation of the C_{12} - C_{18} fraction and then re-esterification of the fatty acids by heating with glycerol [149]. Control of the manufacturing parameters and modification of the process yields the potential to form a

wide range of hard fats with different physicochemical properties, such as, melting point, hydroxyl value and acid value [149].

Semi-synthetic fats are usually white, brittle, solid, odorless and unctuous to touch and produce suppositories that are white and have an attractive, clean, polished appearance [149]. Hard fats are available in a variety of grades with different melting ranges, hydroxyl values and other physicochemical characteristics. Examples of semi-synthetic fatty suppository bases that are available commercially include fractionated palm kernel oil B.P and hard fats such as Massa Estarium®, Massupol®, Suppocire® and Witepsol®.

The hydroxyl value is one of the physicochemical properties of a base that can be used to distinguish fatty bases in terms of their compatibility with an API and an associated extended shelf life. The hydroxyl value of a base is determined by the measurement of the proportion of mono- and di-glycerides present in the base [149]. A high hydroxyl value suggests that the base has a greater ability to absorb water relative to a base with a low hydroxyl value [139] and it has been suggested that these bases should not be used to manufacture formulations containing drugs that are readily hydrolyzed [139]. The water absorbing capacity of a suppository base could influence the formation of w/o emulsions *in situ* in the rectum, which must be avoided since drug release rates from these systems have been reported to be very slow [139]. A base with a high hydroxyl value will have a tendency to form hydrogen bonds with components of the formulation and the API, which in turn may result in relatively slow release rates of a drug from the base to the rectal mucosa [149]. Bases with a high hydroxyl value have also been reported to be irritant to the rectal mucosa [149].

The use of hard fat suppository bases is preferred over the use of cocoa butter, as they do not exhibit polymorphism and their solidification is unaffected by overheating during the manufacturing process [139, 141]. The hard fat bases have a narrow temperature interval between their melting and solidification points, which is generally between 1.5°C and 2°C and seldom over 3°C [141]. The narrow temperature range between melting and solidification aids in the manufacture of uniform suppositories, as the risk of sedimentation of an insoluble drug dispersed in the base is usually low. In addition, hard fat suppository bases contract markedly on cooling thereby reducing the need for the use of a lubricant to facilitate removal of products from moulds following manufacture.

As the presence of unsaturated fatty acids in the semi-synthetic bases is reduced, the bases are relatively resistant to oxidation when compared to cocoa butter, which contains a considerable amount of unsaturated oleic acid [139]. Semi-synthetic fatty bases have low acid and iodine values of < 2 and < 7, respectively, when compared to cocoa butter, which has an acid value of < 5 and an iodine value of 34-38. Low acid and iodine values are essential properties of suppository bases should a long shelf-life be required [139, 141]. Free acids complicate formulation work as they tend to react with other ingredients susceptible to acid hydrolysis [141]. Consequently, the possibility of decomposition by moisture, acids and oxygen, which leads to rancidity in fats, increases with high iodine values [113, 141].

3.1.4.2 Water miscible bases

Macrogol or polyethylene glycols (PEGs) are amongst the most widely used hydrophilic polymer suppository bases. PEGs are polymers of ethylene oxide and water, prepared in a variety of chain lengths, molecular weights and physical states [140]. PEGs with a molecular weight ranging between 200 and 600 exist as liquids and, as the molecular weights increase to above 1000, they exist as wax-like solids [141, 149]. In addition, as the molecular weights increase, their water solubility and hygroscopicity decreases [141]. The wide range of melting points and solubilities make possible the formulation of suppositories with various degrees of heat stability and with different dissolution rates [141]. PEGs of different molecular weight can be combined to achieve a suppository base of the desired consistency and that can achieve a specific drug release rate profile. The use of high melting point solids as suppository bases permits convenient storage of the suppositories without the need for refrigeration and without the danger of excessive softening in warm climates [141].

PEGs have inherently good solvent properties that may result in the retention of a drug in the liquefied base, in the rectum with the potential for a reduction in therapeutic activity [113]. If the drug is partly in solution and partly in suspension there is a high potential for crystal formation, which may cause the formulation to become brittle and prolong dissolution time [149]. PEG bases are hygroscopic and therefore attract water, resulting in a painful sensation for the patient [139]. It is therefore recommended that suppositories manufactured from these bases be moistened with water prior to introduction into rectum to minimize local irritation [113, 139].

PEG bases have several advantages over synthetic and semi-synthetic suppository bases, in particular with respect to stability against oxidation and other degradation processes, their inert nature and a high water absorbing capacity. Unlike glycerol-gelatin suppositories, PEG suppositories do not adhere to moulds and contract sufficiently on cooling to make mould lubrication unnecessary during manufacture. However, PEG suppositories may become brittle, unless poured at the lowest temperature possible, but this may be overcome or reduced by the addition of surfactants or plasticizing agents such as propylene glycol to the formulation [113].

3.1.5 Selection of a suitable base

The selection of a suitable suppository base depends on a number of physicochemical variables, including, but not limited to the solubility of the drug in the base and rectal fluids, in addition to the intended therapeutic goals following rectal administration. In order to ensure that the maximum amount of drug is released from a base, a principal of opposites may be applied. A water-soluble drug may be incorporated into a fatty base while a fat-soluble drug may be best incorporated into a water soluble or miscible base [115]. The selection of a suitable base cannot be made in the absence of any knowledge of the physicochemical properties and intrinsic pharmaceutical or pharmacological activity of the active ingredients to be incorporated into the suppository [141]. In addition, factors such as the oil-water partition coefficient and dissociation constant of a drug must be assessed and considered and the displacement value of the components of a formulation in a specific base must be determined.

The physical properties of a suppository base that may or may not be affected by the addition of a drug or that can influence drug release rate, as well as the stability of the final product are the melting characteristics, iodine value and the hydroxyl value [113]. These parameters are widely used in the pharmaceutical industry for a range of applications with regard to suppository base selection [113].

If a formulator selects a fatty base for the delivery of a particular drug to elicit a systemic effect, the chosen base must liquefy at or below body temperature, whereas only base softening or dispersion may be adequate for the delivery of compounds intended for local action, sustained and/or modified release of the API [113]. Suppository bases with high melting points may be useful for delivering drugs that tend to lower the melting point of a base after inclusion, or for suppositories intended for use in warm climates. The high molecular weight PEG bases, in combination with low molecular weight PEG, may, for example, be appropriate in these circumstances. If the drug to be delivered has a relatively high lipid solubility, it will dissolve in the molten base, which in turn may create difficulties if the drug deposits as crystals of different polymorphic form or with an increased particle size when the base cools. The increase in particle size may alter the rate of drug release from the formulation [149]. Low melting point bases that melt to low viscosity liquids are appropriate for the delivery of large doses of insoluble drugs [113], but there is a high risk of sedimentation during manufacture, if there is a long setting time, which is dependent on the temperature difference between the melting and solidification points of the base of choice.

The rancidification or oxidation potential of suppository bases as a result of the presence of moisture, acids and/or oxygen is increased in bases with high iodine values [141]. Furthermore, due to the sensitivity of the rectal mucosal tissues, potentially irritating antioxidants that are incorporated to prevent rancidification of the base are generally not recommended [113]. To avoid their use, bases with iodine values of < 3 and preferably less < 1 should be selected for use [113].

The hydroxyl value not only indicates the presence of mono- and diglycerides, but also the relative rate of crystallization of a base, which increases with increasing hydroxyl values [113, 141]. In general, a low hydroxyl value permits a faster rate of suppository manufacture, due to the lower melting point and solidification range [113]. A suppository base with a low hydroxyl value should be selected in cases where the API(s) to be incorporated in the delivery system is/are sensitive to the presence of the free hydroxyl radicals [1113, 139].

3.2 FORMULATION OF AZITHROMYCIN SUPPOSITORIES

3.2.1 Suppository bases

In the design of pharmaceutical dosage forms, the choice of the excipients to be used in order that the drug contained therein is released under the best absorption conditions, is one of the most important factors to be determined during preformulation studies. The suppository formulations used in these studies were prepared from either water-soluble or semisynthetic fatty bases. Polyethylene glycol was used for the manufacture of water-soluble base suppositories and two semi-synthetic fatty bases of different grades, *viz.,* Suppocire® and Witepsol® were used in these studies. Additional excipients used in the formulation studies are listed in Table 3.2.

Table 3.2: Excipients used in formulation studies

Name	Abbreviation	Composition	Manufacturer or donor
Azithromycin	AZI		Zhejiang Huayi Pharmaceutical Co., Yiwu, Zhejiang, China
Polyethylene glycol 400 1000 6000 1540	PEG PEG 400 PEG 1000 PEG 6000 PEG 1540	Linear polymers of ethylene oxide	Merck Chemicals Ltd, Wadeville, Gauteng, South Africa Aspen Pharmacare, Port Elizabeth, South Africa
Witepsol® H15 Witepsol® W35 Witepsol® E75	W-H15 W-W35 W-E75	Triglycerides of saturated vegetable fatty acids with monoglycerides	Bergen Trading C.C, Südafrika, Germany
Suppocire® NA1 25 Suppocire® NA 15 Suppocire® NA0 Suppocire® AM Suppocire® NAS 50	S-NA1 25 S-NA 15 S-NA0 S-AM S-NAS 50	Eutectic mixtures of mono-, di- and triglycerides derived from natural vegetable oils	Gattefossé Corp., Paris, France
Tween 80 Tween 20		Polysorbate 80 Polysorbate 20	Aspen Pharmacare, Port Elizabeth, South Africa
Urea	Urea		Merck Chemicals, Ltd., Wadeville, South Africa
Povidone® K-25	PVP		BASF, Ludwigshafen, Germany

Different grades of Suppocire® and Witepsol® were selected for use, based on differences in their physicochemical properties, thereby permitting evaluation of a wider selection of potentially suitable bases for the formulation of AZI suppositories for paediatric use. The characteristics of the fatty bases and water-soluble bases as specified in the certificate of analysis from the respective manufacturers and/or distributors or in a relevant Pharmacopeia [149] are summarized in Tables 3.3 and 3.4 respectively.

Table 3.3: Characteristics of the fatty bases used

Parameters	S-NA 15	S-AM	S-NAI 25	S-NAS 50	S-NA 0	W-H15	W-E75	W-W35
Acid value (mgKOH/g)	< 0.2 **(0.09)**	< 0.2 **(0.04)**	<0.3 **(0.08)**	< 0.30 **(0.14)**	≤ 0.2 **(0.07)**	≤ 0.2 **(0.1)**	≤ 1.3 **(0.96)**	≤ 0.3 **(0.26)**
Iodine value (mgl/100mg)	< 2.0 **(0.2)**	≤ 2 **(1)**	< 2 **(0.4)**	< 3.0 **(0.8)**	≤ 2 **(0.5)**	< 3 **(1.9)**	< 3 **(1.2)**	≤ 3 **(0.6)**
Melting range (°C)	-	35.0-36.5	33.5-35.5	-	35.5-37.5	33.5-35.5	37.0-39.0	33.5-35.5
Saponification No.(mgKOH/g)	230-245 **(239)**	228-252 **(242)**	225-240 **(232)**	225-235 **(230)**	230-250 **(242)**	230-245 **(236)**	220-230 **(224)**	225-235 **(228)**
3.2.1.1 Drop point	34.5-36.5 **(35.2)**	35.0-36.5 **(35.8)**	34.5-36.5 **(35.6)**	33.5-35.5 **(34.7)**	34.0-36.5 **(35.2)**	-	-	-
Solidification point (°C)	-	-	-	-	-	32.5-34.5	32.0-36.0	29-32
Hydroxyl value (mgKOH/g)	5-15 **(11.3)**	< 10 **(3.3)**	20-30 **(23.8)**	40-50 **(42.3)**	< 3 **(1.7)**	5-15 **(13.6)**	5-15 **(12.6)**	40-50 **(44.5)**

*Bold figures represents confirmed values as per the certificate of product analysis supplied by the manufacturer or product distributor, which show that they all fall within the specified pharmacopoeial range [149]

Table 3.4: Characteristics of the water-soluble bases used

PEG	Mean Molecular weight	Melting ranges (°C)	Hydroxyl value
400	400	< 10	264-300
1000	1000	33.3-33.4	107-118
1540	1450	43.1-43.3	70-86
4000	3400	57.4-57.6	30-36
6000	6750	60.7-61.0	-

3.2.2 Manufacture of AZI suppositories

The volume of a suppository from a particular mould is uniform, but the weight of the suppository may vary, due to the difference in densities between the API, adjuvants and the base with which the mould was originally calibrated [150]. Therefore, in order to prepare products accurately, allowance must be made for the differences in density of the suppository base, owing to the presence of the added API and other adjuvants. The factor used to account for these differences is termed the displacement value (D.V.), which is the amount of API by weight that displaces one part by weight of a specific base being used for the manufacture of a dosage form [150].

3.2.2.1 Determination of the D.V.

In order to produce a suppository dosage form with the requisite dose, the D.V. of AZI dihydrate was experimentally determined in the different suppository bases used in these studies [150]. Suppository moulds where calibrated with the specific unmedicated base in order to obtain an accurate weight for each unmedicated suppository for a specific mould before the D.V. of AZI was determined. Six suppositories with base only were prepared and weighed. Six suppositories containing 30% w/w of AZI were prepared by the fusion method of manufacture [151] and weighed. The D.V. was then calculated using Equation 3.1 and the results of these determinations are listed in Table 3.5. Each D.V. determination was performed in triplicate.

$$F = \frac{XB}{100(A - B) + XB} \qquad \text{Equation 3.1}$$

where,

F = the displacement value
X = the percentage of a drug used
B = the weight of the suppositories containing X% of a drug
A = the weight of unmedicated suppositories

Table 3.5: The displacement value of AZI in different suppository bases

Base	Displacement value Mean ± SD
PEG 1000/4000 (75:25) PEG 400/1540/4000/6000 (20:33:67)	1.04 ± 0.030 1.02 ± 0.005
W-H15 W-W35 W-E75	1.32 ± 0.021 1.20 ± 0.036 1.24 ± 0.025
S-NA1 25 S-AM S-NA 15 S-NA0 S-NAS 50	1.38 ± 0.315 1.22 ± 0.017 1.30 ± 0.015 1.28 ± 0.025 1.13 ± 0.015

3.2.2.2 Preparation of suppositories

The fusion or melting method was used for the manufacture of the AZI suppositories [151]. Each suppository was manufactured so as to contain an equivalent amount of 250mg of AZI in each one-gram suppository. The manufacturing process commenced with the preparation of the suppository mould. Suppository moulds were cleaned using warm water and a soap solution and were inverted to drain off excess water. Suppository moulds were not lubricated, since the bases selected for these studies contract on cooling, which permits the easy removal of the suppositories from the moulds. The moulds were left to equilibrate at room temperature prior to pouring the suppositories.

The appropriate mass of a suppository base to be used for a specific batch of product was calculated using the Equation 3.2

$$P = (N \times S) - \frac{D}{F} \qquad \text{Equation 3.2}$$

where,

P = the amount of base required

N = the number of prepared suppositories

S = the size of the mould used

D = the amount of drug that is required

F = displacement value

The fatty suppository bases were heated to 45-50°C and the PEG bases to 55-60°C in order to melt them. The bases were heated in a porcelain evaporating dish using a Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate (Laboratory Marketing Services cc. Johannesburg, South Africa).

AZI was passed through a mesh of 120 mm size to ensure an even distribution of the drug throughout the base during the mixing stage and to minimize settling in the melt when stirring ceased. Suppositories were produced manually on a small scale by mixing an amount of AZI equivalent to 262.03 mg of AZI dihydrate (equivalent to 250mg AZI) per suppository into the molten suppository base. The mixture was then poured into 1 g stainless steel moulds that had been allowed to equilibrate to room temperature and the suppositories were left to set at room temperature for 24 hours before assessment and analysis.

3.2.2.3 The addition of additives

During preformulation studies and formulation development, formulators may find that dissolution rates of an API may be suspected of being a rate-limiting step in the potential bioavailability of the API. In particular, the development of potent and more lipophilic compounds suggests that on determination of the solubility of these compounds, poor water solubility will be evident, which in turn would suggest that dissolution would invariably be a rate-limiting step in the absorption process [152].

One of the simplest approaches to enhance solubility that is used in industrial practice is the incorporation of a surfactant into a formulation to increase the dissolution rate and wettability of a drug [152]. For these studies, Tween 80 and Tween 20 were used in formulations in which a surfactant was to be incorporated, these were mixed with the molten mass prior to the addition of AZI.

A second, more complex approach that may be of value in decreasing solubility-related bioavailability difficulties involves altering the physical properties of a drug by the preparation of solid dispersions of the API with an appropriate adjuvant or carrier [153].

Solid dispersions of AZI with either urea or PVP were prepared using the solvent evaporation method described by Chiou *et al,* [153]. A concentration of 2.0% w/w of urea or PVP was used as the water-soluble carrier to form co-precipitates of AZI and the carrier. AZI and the carrier were dissolved in ethanol, and following complete dissolution, the solvent was removed using a rotary evaporator (Büchi, Optolabor (Pty) Ltd, Johannesburg, South Africa), maintained at 40°C with a water bath. The resultant residues were further dried in a dessicator for 3 days, using freshly dried silica gel in order to ensure that complete and total evaporation of the ethanol was achieved. Coevaporates were size reduced using a mortar and pestle and passed through a 120 mm mesh sieve prior to use. The size-reduced solid dispersion was then used to formulate suppositories using the procedure described in § 3.2.2.2. Physical mixtures (PM) of AZI and carrier were also manufactured using the same proportions of drug/carrier as that for the solid dispersions, for comparative purposes.

The compositions of all the formulations including PEGs and fatty bases are summarized in Tables 3.6 and 3.7 respectively.

Table 3.6: Formulation of azithromycin suppositories in PEG bases*

Batch #	**Base type (PEG) (%)**				
	400	**1000**	**1540**	**4000**	**6000**
AZI-01	-	75	-	25	-
AZI-02	20	-	33	-	47

* PEG compositions based on The Pharmaceutical Codex, 1994 [241]

Table 3.7: Formulations of azithromycin suppositories in fatty bases

Batch #	Base type (%)								Additives (%)			
	W-H15	**W-W35**	**W-E75**	**S-NA1 25**	**S-NA 15**	**S-NA0**	**S-AM**	**S-NAS 50**	**Tween 80**	**Tween 20**	**Urea**	**PVP**
AZI-03	100	-	-	-	-	-	-	-	-	-	-	-
AZI-04	-	100	-	-	-	-	-	-	-	-	-	-
AZI-05	-	-	100	-	-	-	-	-	-	-	-	-
AZI-06	-	-		100	-	-	-	-	-	-	-	-
AZI-07	-	-	-	-	100	-	-	-	-	-	-	-
AZI-08	-	-	-	-	-	100	-	-	-	-	-	-
AZI-09	-	-	-	-	-	-	100	-	-	-	-	-
AZI-10	-	-	-	-	-	-	-	100		-	-	-
AZI-11	99.5	-	-	-	-	-	-	-	0.5	-	-	-
AZI-12	99	-	-	-	-	-	-	-	1	-	-	-
AZI-13	98	-	-	-	-	-	-	-	2	-	-	-
AZI-14	96	-	-	-	-	-	-	-	4	-	-	-
AZI-15	-	99.5	-	-	-	-	-	-	0.5	-	-	-
AZI-16	-	99	-	-	-	-	-	-	1	-	-	-
AZI-17	-	98	-	-	-	-	-	-	2	-	-	-
AZI-18	-	96	-	-	-	-		-	4	-	-	-
AZI-19	-	-	-	-	-	-	99.5	-	0.5	-	-	-
AZI-20	-	-	-	-	-	-	99	-	1	-	-	-
AZI-21	-	-	-	-	-	-	98	-	2	-	-	-
AZI-22	-	-	-	-	-	-	96	-	4	-	-	-
AZI-23	-	-	-	99.5	-	-	-	-	0.5	-	-	-
AZI-24	-	-	-	99	-	-	-	-	1	-	-	-
AZI-25	-	-	-	98	-	-	-	-	2	-	-	-
AZI-26	-	-	-	96	-	-	-	-	4	-	-	-
AZI-27	-	-	-	-	-	-	-	99.5	0.5	-	-	-
AZI-28	-	-	-	-	-	-	-	99	1	-	-	-
AZI-29	-	-	-	-	-	-	-	98	2	-	-	-
AZI-30	-	-	-	-	-	-	-	96	4	-	-	-

Table 3.7: continued

Batch #	Base type								Additives			
	W-H15	**W-W35**	**W-E75**	**S-NA1 25**	**S-NA 15**	**S-NA0**	**S-AM**	**S-NAS 50**	**Tween 80**	**Tween 20**	**Urea**	**PVP**
AZI-31	-	98	-	-	-	-	-	-	-	2	-	-
AZI-32	98	-	-	-	-	-	-	-	-	2	-	-
AZI-33	-	-	-	-	-	-	98	-	-	2	-	-
AZI-34*	-	98	-	-	-	-	-	-	-	-	2	-
AZI-35**	-	98	-	-	-	-	-	-	-	-	2	-
AZI-36*	-	98	-	-	-	-	-	-				2
AZI-37**	-	98	-	-	-	-	-	-	-	-	-	2

* Batches manufactured using physical mixtures of AZI and carrier

**Batches manufactured using solid dispersions of AZI and carrier

3.3 DOSAGE FORM ANALYSIS

Pharmaceutical products must routinely undergo analytical testing throughout the product development and subsequent scale-up and manufacturing phases to ensure that quality standards are met and that the ultimate quality of a product can always be assured. Analytical testing was employed throughout studies during which dosage form design and optimization were undertaken. Following manufacture, all suppositories were routinely inspected and their appearance, after slicing lengthways, was evaluated to inspect the uniformity of distribution of API and other additives in the dosage form. Any potential solid-solid interactions between the drug and urea or PVP that were added were investigated using infrared spectroscopy, an analytical method routinely used for this purpose [120, 152, 154, 155]. Infrared spectra were determined using a Perkin-Elmer FT-IR spectrum 2000 spectrophotometer (Perkin-Elmer LTD, Beaconsfield, Bucks, England). Melting range tests was performed using a capillary tube method so as determine any potential physical and absorption characteristic changes that may have occurred in the batches manufactured in these studies.

The British Pharmacopoeia (BP) [91] has specified an apparatus that should be used for the physical assessment of suppositories should manufacturers wish to claim pharmacopoeial status for their products. In addition, the BP defines and sets limits for these tests. The specified tests include disintegration testing, softening time and resistance to rupture analysis. In order to perform these tests, specific apparatus have to be designed according to the standards specified in the BP. None of these specific apparatus were developed and adopted in our laboratory as the primary focus of these studies was to develop a proof of concept for AZI suppositories.

Content uniformity assessments were performed on all batches that were manufactured. Furthermore, residual content analyses were undertaken, following dissolution testing, to ensure that a mass balance of the amount of drug released from the suppositories and that retained by the products could be achieved. These results were important to ascertain whether the low amounts of drug released from the suppositories were not a consequence of the suppository content being less than the desired 250mg of AZI. Dissolution rate studies were performed on every batch of suppositories manufactured, in order to assess suppository

behavior *in vitro* and to evaluate the effects of changes in formulation composition on drug release rates.

3.3.1 Weight variation

Quality control tests, such as uniformity of weight, are routinely undertaken on specifically selected portions of manufactured batches of dosage forms during formulation development and large-scale batch manufacture in the pharmaceutical industry. Weight uniformity testing is used as an indicator to determine potential areas of difficulty in a manufacturing process, such as, incomplete or inefficient mixing, heterogeneous repartitioning during the melting-casting process and sedimentation, which may also affect content uniformity [139]. Twenty suppositories from each batch were individually weighed and presented as a mean with a percentage relative standard deviation (% RSD) limit of $\leq 5\%$.

3.3.2 Melting range

Various methods have been used to determine the melting characteristics of suppository bases, including the capillary melting point [156], softening point [141] and incipient melting point techniques [141]. In addition, DSC has also been used to determine the melting point of fatty bases [119, 156]. Melting range tests were only performed on the fatty base suppositories manufactured in these studies. The melting point of PEG formulations was not determined since drug release from PEG suppositories is dependent on the rate of dissolution of the PEG base rather than its melting properties. The melting characteristics of a base are expressed as a range of temperatures indicating the temperature at which the base starts melting and the temperature at which it is completely molten.

The ascending melting point method was used for the determination of the melting point of all formulations. Capillary tubes of approximately 10 cm in length were sealed at one end and were filled with the formulation to be tested to approximately 1cm height in the tube. Following filling, the tubes were placed in an automated Model SMP1 melting point test apparatus (Stuart Scientific, Laboratory and Scientific Equipment Co., Cape Town, South

Africa). Each melting point determination was performed in triplicate and the results of these tests are summarized in Table 3.8.

3.3.3 Extraction efficiency

The extraction procedure was developed and validated prior to use for the assay of AZI in suppository formulations. One of the suppository bases, W-H15 base, was selected to develop the extraction method and to demonstrate the efficiency of the extraction procedure. An amount of base equivalent to that in one suppository was accurately weighed into a beaker and melted on a magnetic stirrer fitted with a hot plate at 50°C, after which an accurately weighed amount of AZI equivalent to a 250mg dose per suppository was added to the molten base. The mixture was stirred at 510 r.p.m. for 3 minutes using the magnetic stirrer. A 15 ml aliquot of methanol, previously warmed to 50°C, was added to the melt and the mixture stirred for a further 2 minutes. The molten mix was then allowed to cool and was left to set in a refrigerator for 10 minutes.

Following solidification of the lipophilic phase, the solvent phase was decanted and filtered through ashless filter paper (Schleicher and Schüll GmbH, Dassel, Germany) and quantitatively transferred into a 50ml A-grade volumetric flask. The filter paper was returned to a beaker following filtration and the process was repeated a further two times using the same conditions, using a new piece of filter paper on each occasion. At the end of the extraction process, the filter papers were washed with a predetermined amount of warm methanol (5 ml), cooled and the resultant solution filtered with a new piece of filter paper and then added to the volumetric flask to make the solution up to volume. In order to ascertain whether any AZI was retained by the filter paper, the same extraction procedure was followed, without the addition of the suppository base. In all cases, the internal standard was added to the volumetric flask after the completion of the extraction, prior to making the solution up to volume. Aliquots of the extracted solution were filtered though a Millipore Millex-GV Hydrophilic PVDF 0.22μm filters (Millipore Co., Billerica, USA) before HPLC analysis, using the method described in Chapter 2. All determinations were performed in triplicate.

3.3.4 Content uniformity

The determination of content uniformity is critical when a semi-automated manufacturing procedure is used to produce large-scale batches. Of particular concern in large-scale manufacture is the potential for incomplete mixing or irregular base melting and mould filling, characteristics that may lead to a high degree of batch-to-batch inconsistency. An assay in which the API is quantitatively measured in a sample of the large batch can be used to infer that the individual dosage units conform to the specified labeled content.

Conventional methods for the extraction of API from suppository type formulations involves several steps, such as melting, dispersion of the matrix in an appropriate hot organic solvent, cooling, centrifugation and/or filtration [122, 127, 156, 158, 159]. In addition, the solvent selected for the extraction procedure should solubilize the analyte of interest without significantly extracting matrix components or other materials that may interfere with the analytical procedure. This can be a challenge when attempting to quantitate non-polar analytes dissolved in fatty matrices. The use of supercritical CO_2 for the isolation of acetaminophen from non-polar suppositories has been described as one of the non-conventional methods for the treatment of suppositories, prior to the determination of the API by HPLC method [160].

The conventional extraction methods that have been used for the determination of API in suppository bases are time consuming and use fairly large quantities of organic solvents, which is of concern from an environmental perspective. Recently, Labbozzetta *et al,* [161] described the use of focused microwave-assisted extraction of an API from suppository dosage forms that was reported to have several advantages over conventional extraction methods, such as the reduction of extraction time and organic solvent consumption. A conventional extraction method was developed and used for the determination of AZI in these studies, since microwave-assisted extraction requires the availability of specialized equipment, which was not available in our laboratory [161].

In order to assess the content uniformity of suppositories manufactured in these studies, eight suppositories were accurately weighed and placed into a 100 ml Erlenmeyer conical flask and melted on a magnetic stirrer hot plate, maintained at 50°C. The melt was mixed continuously at 510 r.p.m., using a magnetic stirrer for 3 minutes prior to placement in a refrigerator to solidify. A portion (n = 3) of the solidified mass equivalent of the weight of one suppository was then harvested by chipping and quantitatively transferred to a beaker and once again heated to 50°C. The extraction procedure described in § 3.3.3 was used to extract AZI from the molten base. Content uniformity was determined and expressed as the mean percentage drug recovered for the eight suppositories used for the assay.

3.3.5 Residual content analysis

In order to determine whether any AZI was retained in the fatty base suppositories following the 8 hour dissolution studies, residual content analyses were performed on the dosage forms at the end of the dissolution testing. The same extraction procedure as described in § 3.3.3 was used to analyse each of the resultant suppository cores (n = 3) to determine the percentage of drug retained in the dosage form, for mass balance purposes.

3.3.6 Results and Discussion

3.3.6.1 Weight variation

Suppositories for adults generally weigh approximately 2 g and are about 2.54 cm to 3.80 cm in length, whereas the weight of paediatric suppositories is approximately one half that of adult rectal suppositories [114]. For these studies, a target weight of one gram per suppository was desired, as the objective of these studies was to develop AZI suppositories for paediatric use. All batches of suppositories that were manufactured were assessed for weight uniformity and were found to comply with the requirements for weight uniformity of suppositories, as described in the BP [91], which recommends a maximum percentage deviation of 5%. The results of these studies for selected batches are listed in Table 3.8 and the results for batches AZI-11 – AZI-37 are included in Appendix II together with the batch summary records for these batches.

Table 3.8: Mean suppository weight and melting range of selected fatty base formulations

Batch #	Excipients	Target Weight (mg)	Actual Weight (mg)	Melting Range (°C)	
				Base	Base and Drug
AZ-01	PEGs[a]	1.1880	1.1800 ± 0.48	-	-
AZ-02	PEGs[b]	1.3105	1.2926 ± 3.43	-	-
AZ-03	W-H15	1.1109	1.0159 ± 1.32	32.5-34.5	33.5-35.0
AZ-04	W-W35	1.1019	1.1174 ± 1.22	34.0-35.5	34.5-35.5
AZ-05	W-E75	1.0507	1.0339 ± 1.25	36.5-39.0	37.5-40.0
AZ-06	S-NA1 25	1.0771	1.0416 ± 3.17	34.5-35.0	34.5-36.0
AZ-07	S-NA 15	1.0486	1.0344 ± 1.72	34.0-35.0	34.5-35.5
AZ-08	S-NA 0	1.0827	1.0405 ± 1.62	35.0-36.5	35.5-36.5
AZ-09	S-AM	1.1017	1.1008 ± 1.46	35.0-36.0	35.5-37.0
AZ-10	S-NAS 50	1.0888	1.1109 ± 0.91	36.0-37.5	35.5-37.5

[a]: PEG 1000: PEG4000 (75:25), [b]: PEG400:PEG1500:PEG6000 (20:33:47)

3.3.6.2 Melting range

The addition of AZI to the suppository bases did not significantly change the melting behavior of the bases and the melting point determinations revealed considerable variability between the fatty bases tested as can be seen from the data listed in Table 3.8. Suppositories made with W-E75 and S-NAS 50 showed a higher and wider melting range, with and without the addition of AZI, than suppositories manufactured with other bases. The melting range has a direct impact on the rate of drug release from suppository formulations, since the rate of drug release from fatty bases depends on the melting rate of the base. The consequences of these effects are discussed further in § 4.3.1 in Chapter 4. The effect of various formulation additives on the melting characteristics of the bases is also discussed in § 4.3.1 in Chapter 4. The melting range of the fatty bases without the addition of AZI corresponds to the reported melting ranges for the bases [149]. The melting range for batches AZI-11 – AZI-37 are included in Appendix II together with the batch summary records for these batches.

3.3.6.3 Extraction efficiency

The mean recovery value obtained for AZI from the mixture of the suppository base and drug used for extraction efficiency studies was found to be 96.63 ± 2.45 % and that from a mixture of AZI in methanol was found to be 99.93 ± 1.63%. The difference of 3.4% may be due to the failure of AZI to partition out of the fatty base completely into the methanol layer, thus remaining in the fatty layer during the decantation of the methanolic phase into the beaker. The results reveal that the extraction procedure was reliable and valid and that it was suitable for the assay of AZI in fatty base suppository formulations, since the method extracted in excess of 97% of the original AZI content which is within the BP limits [91] and the %RSD for these determinations was less than 5%, which was deemed acceptable. The extraction of AZI from the suppository base was clean and the peak of interest was easily quantitated without interference from the base excipient. The resultant chromatograms of AZI following extraction are depicted in Figure 3.2.

Table 3.9: Extraction efficiency data after extraction of AZI from both methanol and fatty base (W-H15), using the method described in § 3.3.3

Procedure	Actual concentration extracted (µg/ml) ± %RSD	Percentage recovery
Extraction from mixture of AZI and methanol	249.95 ± 1.63	99.93
Extraction from mixture of fatty base and AZI	241.58 ± 2.45	96.63

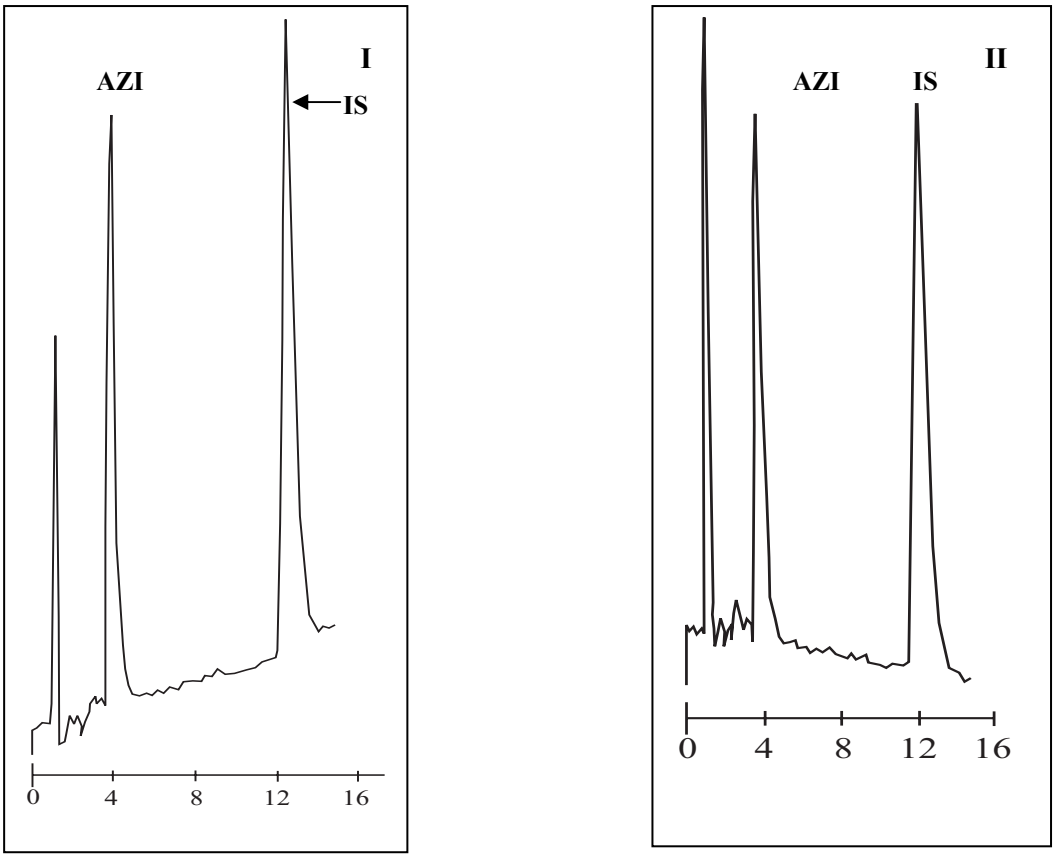


Figure 3.2: HPLC chromatograms of (I) extraction of AZI from methanol and (II) extraction of AZI from a suppository base W-H15, with internal standard IS (100 µg/ml)

3.3.6.4 Content uniformity

The results of the content uniformity studies are summarized in Table 3.10. The content uniformity of all batches of suppositories in which content uniformity was investigated, was found to be within the limits prescribed in the first step of the official content uniformity specifications for suppositories listed in the BP [91]. Although some of the dosage forms showed a relatively low recovery, *viz.,* batches AZ-06 and AZ-04, which may be attributed to the failure of AZI to partition into the methanol layer, thus remaining in the fatty layer during the extraction procedure. The content uniformity results for batches AZI-11 – AZI-37 are included in Appendix II together with the batch summary records for these batches.

Table 3.10: Content, residual content and percentage drug released at the end of the dissolution test for selected batches

Batch #	Content (%)	Final % released	Residual Content	Final % released	Mass balance
AZI-01	98.87 ± 1.72	99.01 ± 4.13	0	99.01 ± 4.13	0
AZI-02	98.19 ± 0.76	98.05 ± 4.08	0	98.05 ± 4.08	0
AZI-03	98.08 ± 2.71	39.59 ± 4.89	56.07 ± 4.45	39.59 ± 4.89	95.66
AZI-04	98.31 ± 1.90	30.02 ± 1.89	62.26 ± 4.86	30.02 ± 1.89	92.28
AZI-05	93.20 ± 6.37	**	95.15 ± 2.83	**	95.15
AZI-06	97.24 ± 2.75	23.70 ± 1.29	75.19 ± 2.44	23.70 ± 1.29	98.89
AZI-07	96.06 ± 2.54	20.74 ± 0.52	75.65 ± 4.52	20.74 ± 0.52	96.39
AZI-08	100.05 ± 4.20	16.82 ± 0.78	82.43 ± 3.18	16.82 ± 0.78	99.25
AZI-09	94.81 ± 1.39	12.28 ± 1.89	73.86 ± 3.20	12.28 ± 1.89	86.14
AZI-10	95.52 ± 2.25	12.06 ± 1.52	80.19 ± 3.59	12.06 ± 1.52	92.25

** AZI was not released after the test period of 8 hours.

3.3.6.5 Residual content

The results of the residual content analyses of selected batches are summarized in Table 3.10 and it can be seen that batches AZ-04, AZ-06, AZ-09 and AZ-10 retained AZI to a greater extent than the other formulations listed in the table. This result is more than likely a result of their melting range temperatures and the physicochemical composition of the suppository base. The results obtained correspond favorably with the total percentage of drug released at the end of an 8 hour dissolution test period, as seen in the dissolution rate profiles obtained throughout this study, which are discussed further in § 4.3.1 and 4.3.2 in Chapter 4.

3.3.7 Characterization of solid dispersion formulations

Fourier transform infrared spectroscopy (FTIR) is one of the standard methods used for the characterization of the solid-state properties of solid dispersion co-precipitates [120, 155, 162]. FTIR analysis can form part of preliminary studies in which potential interactions between a drug and a carrier can be determined by the study of chemical bond shifts in the IR spectrum. Other methods that can be used for this purpose include X-ray crystallographic analysis, differential scanning calorimetry (DSC), calorimetric analysis of a solution and microscopic methods, including polarization microscopy and scanning electron microscopy [163]. FTIR spectrometry was used in these studies using a KBr disc, to assess whether any

changes in the IR absorption spectrum of AZI occurred as a result of mixing AZI with urea and PVP in solid dispersions and as physical mixtures.

3.3.7.1 FTIR Methodology

The FTIR spectra of physical mixtures of AZI and urea or PVP, in addition to co-evaporates, were obtained using a Perkin-Elmer FT-IR spectrum 2000 spectrophotometer (Perkin-Elmer Ltd, Beaconsfield, England). Approximately 2 mg samples of the powders in 200 mg KBr were prepared for analysis as KBr disks. Spectral data were collected over the IR spectrum region of 400-4000cm^{-1}. The resultant chemical bond shifts were used to analyse the spectra for any possible drug-excipient interactions.

3.3.7.2 Results and discussion

The term solid dispersion describes a dispersion of one or more active ingredients in an inert carrier matrix at solid state [162]. They can be prepared by a number of methods, including melt-fusion and solvent evaporation. In order to prepare a solid dispersion, both a given drug and a carrier should be completely dissolved in an organic solvent if the solvent method of preparation is used, or fused by heating, if the melting method is used for their preparation [153]. For these studies, the solvent method of preparation was selected for use, with ethanol selected as the organic solvent. Ethanol is classified as a Class 3 solvent according to the ICH Harmonized Tripartite guidelines [164]. Ethanol is less toxic than many other organic solvents [10] and is therefore an ideal solvent for the preparation of the coprecipitates for pharmaceutical use and more specifically from a patient perspective. The term coprecipitate has been used to refer to dispersions obtained by using the solvent method of manufacture [153].

Leurner *et al,* [163] have described various methods that can be used to characterize solid dispersions, of which the most important ones are thermoanalytical, X-ray diffraction, infrared spectroscopy and measurements of the release rate of drugs from the dispersions. For these studies, infrared spectroscopy and measurements of the release rate of AZI were investigated.

Structural changes and the lack of a crystal structure that may affect the physicochemical properties of the drug, can lead to changes in bonding between functional groups, which can be detected by using the IR method of investigation [163]. Therefore FTIR studies were undertaken to investigate the potential interaction between AZI and urea or PVP and assessment release rates are discussed further in § 4.3.2.2, Chapter 4.

The IR spectrum of AZI shown in Figure 1.5, § 1.5.6 in Chapter 1, reveals characteristic shoulders in the AZI IR spectrum that occur at 3433 cm^{-1} for the O-H stretch 2968 and 2935 cm^{-1} for the C-H stretch and 1723 cm^{-1} for the C=O stretch of the ester form. Peaks that occur at 1458 and 1376 cm^{-1} represent bond vibration of the CH_3O and CH_2O functionalities, respectively and those at 1167 and 1050 cm^{-1} represent the asymmetrical and symmetrical aliphatic ether respectively [25]. These bands were also observed for the physical mixture of urea and AZI with the same absorbance as shown in Figure 3.3. From these results, it can be confirmed that there is no interaction between AZI and urea in the physical mixture. The band due to the C=O stretching vibration of AZI that appears at 1723 cm^{-1} was shifted when AZI was co-evaporated with urea and a less intense band that was shifted to 1720 cm^{-1} was observed and is clearly evident in Figure 3.4. In addition, the O-H stretch also shifted to 3435 cm^{-1}, while other stretching vibrations of AZI were not affected.

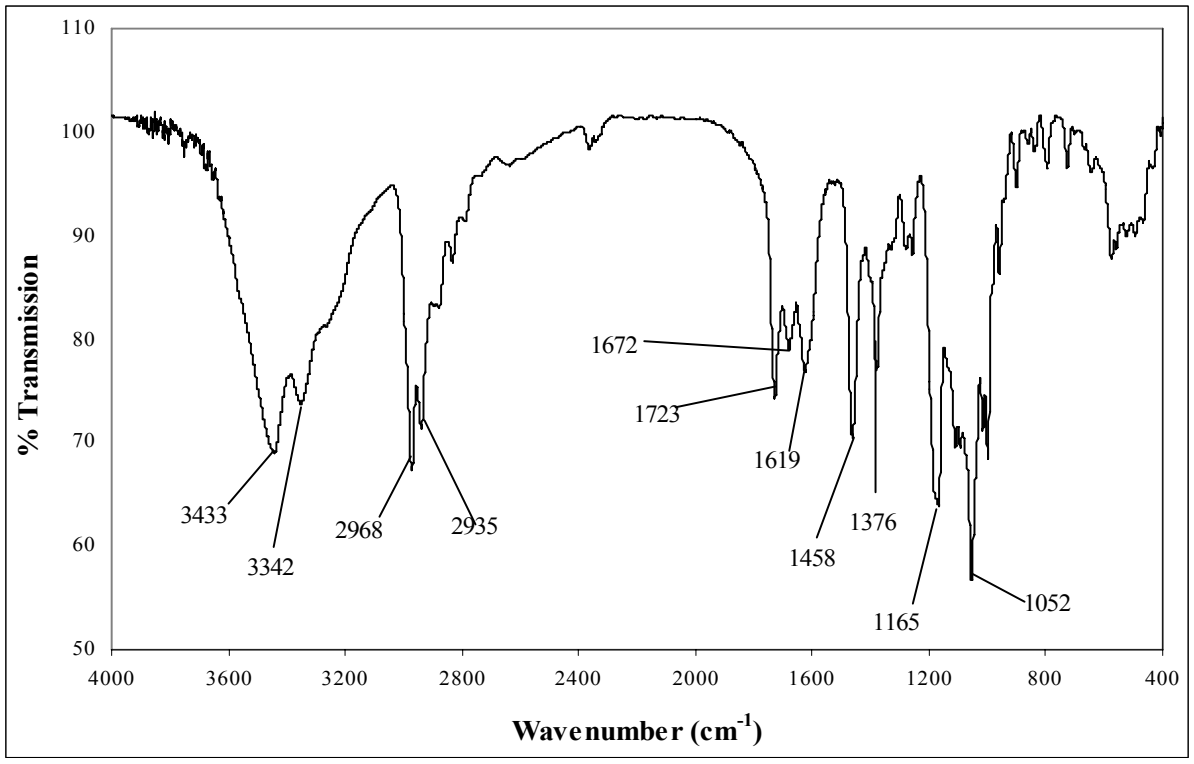


Figure 3.3: Infrared spectrum of a physical mixture of AZI and Urea (2% w/w)

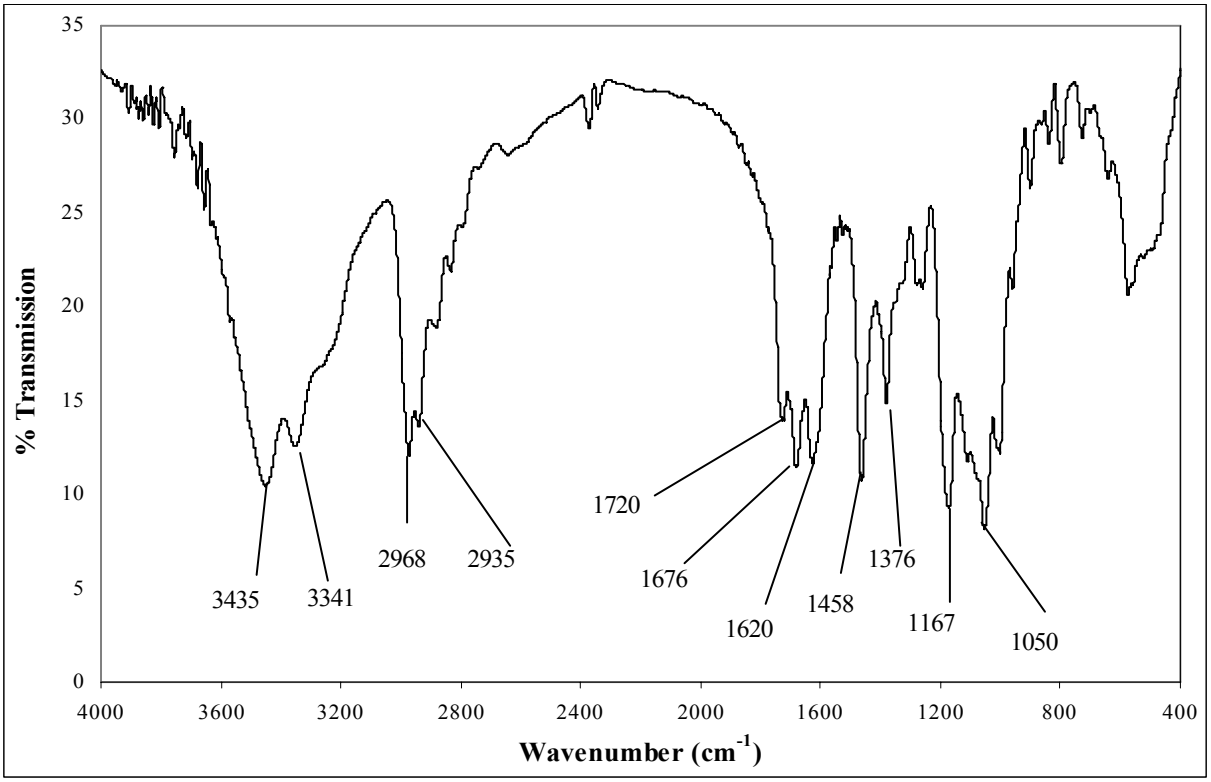


Figure 3.4: Infrared spectrum of AZI and Urea (2% w/w) prepared as a solid dispersion

The vibration bands at 3433 cm^{-1} and 3342 cm^{-1} observed in Figure 3.3 and 3435 cm^{-1} and 3341 cm^{-1} in Figure 3.4, represent the coupling of the N-H and O-H stretch of the combination of urea and AZI [25]. The characteristic shoulders observed at 1672 cm^{-1} and 1619 cm^{-1} in Figure 3.3 and at 1676 cm^{-1} and 1620 cm^{-1} in Figure 3.4 represent the carbonyl stretch of the amide band present in urea [25].

The characteristic shoulders of a physical mixture of PVP and AZI, as seen in Figure 3.5, revealed a shift of the carbonyl shoulder to 1726 cm^{-1}. Other stretch vibrations of AZI where not affected. The intensity of the carbonyl stretch band was not reduced as compared to that of the IR spectrum of AZI and PVP prepared by solid dispersion, as shown in Figure 3.6. In this spectrum, the carbonyl stretch band was reduced in intensity and was shifted to 1719 cm^{-1}. In addition, the O-H band of the PVP solid dispersion coprecipitates was broader and was shifted to 3422 cm^{-1}.

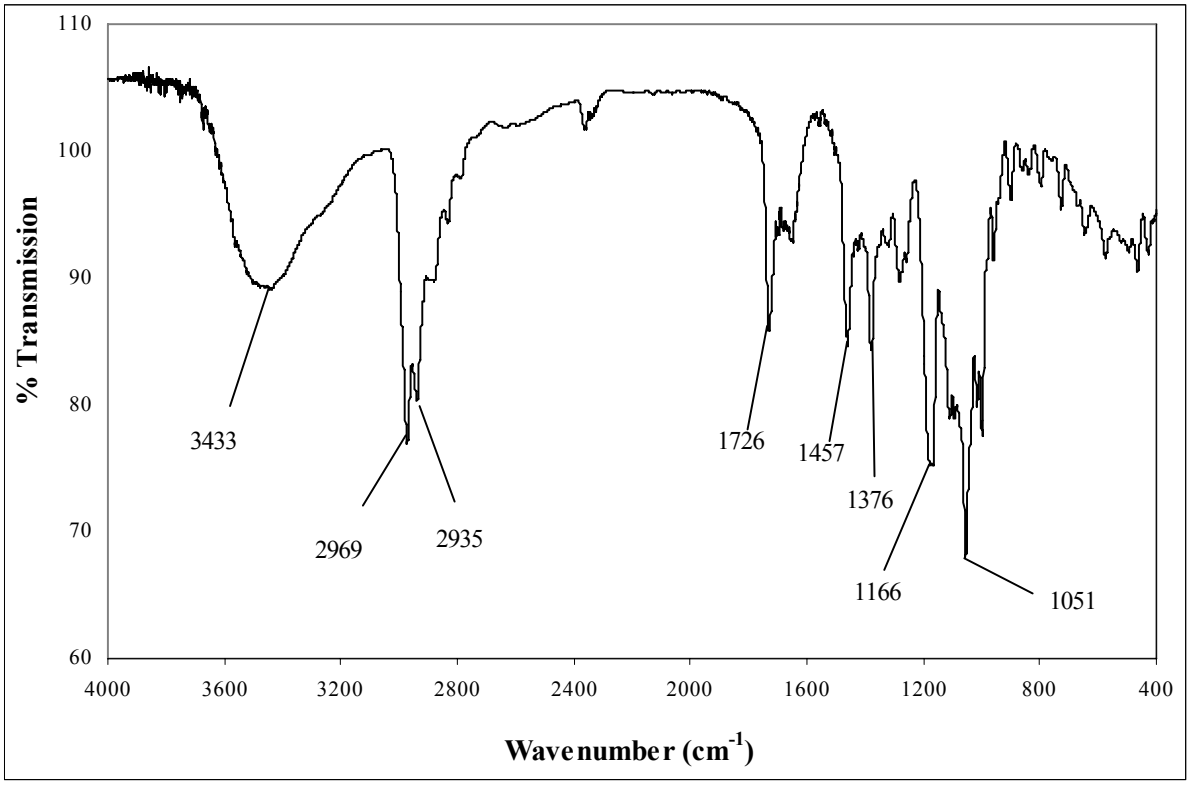


Figure 3.5: Infrared spectrum of a physical mixture of AZI and PVP (2% w/w)

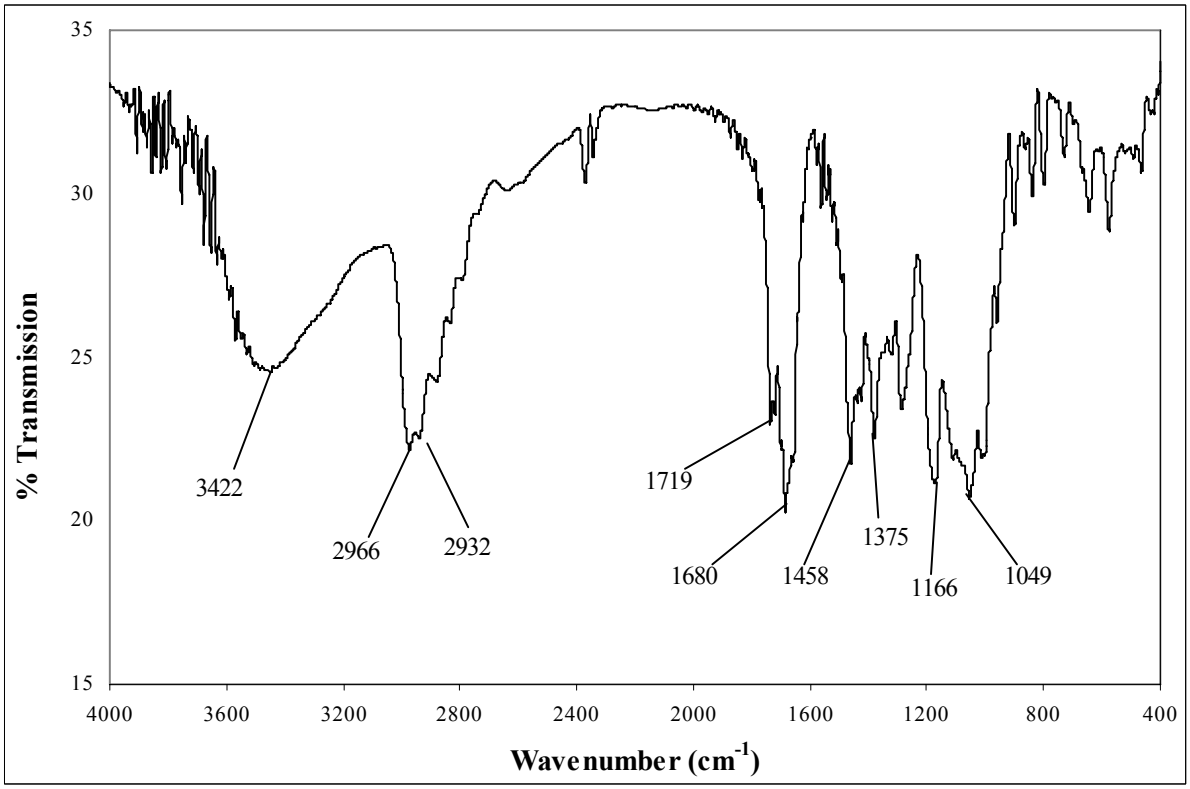


Figure 3.6: Infrared spectrum of AZI and PVP (2% w/w) prepared as a solid dispersion

The shifts observed in the characteristic bands of AZI suggest that a chemical interaction may occur between AZI and any of the carrier substances tested [120, 152, 155, 165]. These results also suggest that the carbonyl and hydroxyl functional groups of AZI can interact with various functional groups of the carrier compounds at a molecular level [152, 165] when prepared as a solid dispersions and when a physical mixture technique is used to mix AZI with PVP. It is well established that both intra- and inter-molecular hydrogen bonding in alcohols results in a downward frequency shift in absorption peaks [152]. In addition, intra-molecular hydrogen bonding yields a greater downward frequency shift than inter-molecular hydrogen bonding [25, 152]. Therefore, the results may suggest that, in the presence of AZI/PVP co-precipitates prepared by solid dispersion, the O-H groups of AZI may interact with the C=O group of PVP, since there was a less pronounced downward shift of the O-H vibration to 3422 cm^{-1} from 3433 cm^{-1}.

3.4 CONCLUSIONS

Although rectal dosage forms are not commonly used because of the cultural and psychological biases, continued research on rectal drug administration in humans [152, 166, 167] has shown its advantages over the oral and parental route, particularly in elderly and paediatric patients. Suppositories are complex dosage forms and many pharmaceutical and physiological factors can influence drug availability for absorption. The composition and performance of pharmaceutical formulations may play a major role in the control of rectal absorption and consequently the systemic distribution and pharmacokinetics of a drug administered in a suppository dosage form.

Dosage form analysis is an important assessment tool for the assurance of product quality. All suppositories manufactured in these studies complied with the BP specifications for weight and content uniformity and therefore may be considered to be of satisfactory quality, using these parameters. The use of appropriate analytical tools allows the formulator to evaluate the effect of changing formulation variables over drug release rates. The results obtained from dosage form analyses were used to characterise the dosage forms that were developed and manufactured and to explain the behavior of AZI release rates *in vitro* from suppository formulations.

Although potential interactions were detected using FTIR, they did not seem to warrant the exclusion of either PVP or urea as carriers for AZI in these formulations. Similar experiments have yielded comparable results and yet achieved the goal of improving the dissolution rate of poorly water soluble drugs [120, 152, 153, 155, 165, 168]. However, long term stability studies would be essential to exclude any possibility of degradation and/or complexation of AZI with the components of a formulation in these rectal delivery systems.

CHAPTER FOUR

4. DRUG RELEASE FROM SUPPOSITORY DOSAGE FORMS

4.1 INTRODUCTION

The absorption of drugs following administration in a specific suppository base usually entails the release of that drug from that base and subsequent diffusion from the base through the rectal fluids and mucosa to the site of action. The mechanism by which a drug is made available for absorption from suppositories manufactured using hydrophilic bases is quite different to that from suppositories manufactured using lipophilic bases. Drug release from hydrophilic bases such as PEG is a result of the progressive dissolution of the base and associated excipients in the intrarectal fluids [169, 170]. By contrast, drug release from lipophilic suppository bases is the result of a series of successive steps that involve the melting of the base at or below body temperature (37°C), migration of the drug particles to the interface between the melted excipients and the rectal secretions, diffusion of drug molecules from the molten base to the rectal barrier membranes and subsequent absorption of the drug into general circulation [171].

Drug release mechanisms from fatty bases must account for the contact area between the molten base and the membranes, in addition to the rate of drug diffusion per unit area [172]. Furthermore, the hydrophilic or lipophilic nature of the drug incorporated into a suppository formulation must be accounted for when describing the release characteristics of the drug from such dosage forms. A schematic diagram summarizing the aforementioned release processes of a drug from a lipophilic suppository formulation is depicted in Figure 4.1.

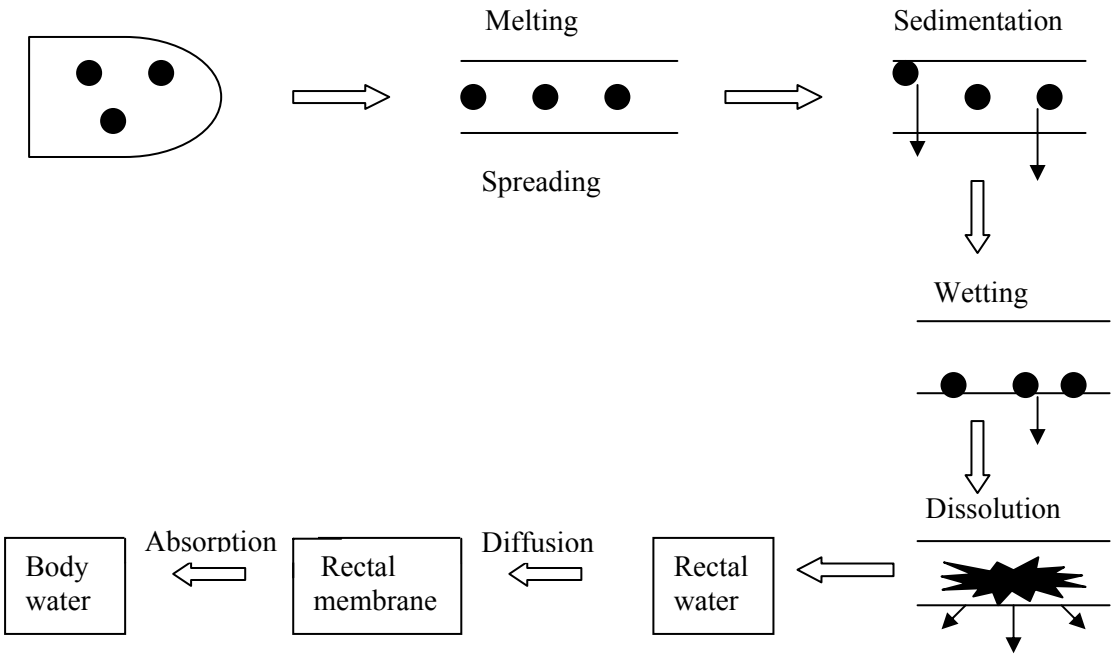


Figure 4.1: Schematic representation of a drug release process dispersed in a lipophilic suppository base

The rate of diffusion of a drug is determined by the drug concentration in the small volume of intrarectal fluids in the rectal cavity, which must be high enough to support a concentration gradient of the drug between the rectal and plasma fluids to ensure that absorption by passive diffusion can occur [169]. The concentration gradient is in turn conditioned by the solubility and dissolution rate of the drug that has been released from the suppository base, which is also influenced by the limited rectal fluid volume of approximately 3-5 ml [139].

In the presence of lipophilic excipients, drug availability can be correlated to the solubility of the drug in water, under rectal conditions [169]. The presence of hydrophilic excipients, such as PEG, in a suppository formulation may influence the *in vitro* availability of a drug considerably, such that it can no longer be correlated with drug solubility in the rectal fluids [169]. The osmotic effect of PEG draws water into the rectal compartment, resulting in an increase in volume of the intrarectal fluids that not only contributes to increased drug solubility, as a result of increased fluid volume, but also to the rate of diffusion of the drug [169, 170].

The determination of *in vitro* drug release rates through dissolution testing and batch uniformity testing in the pharmaceutical industry are valuable tools for the validation of dosage form performance. In particular, drug availability, batch homogeneity and batch conformity are of paramount importance [173]. Suppository dosage forms have not been investigated to as great an extent as oral dosage forms with respect to establishing a correlation between the *in vitro* dissolution rate and *in vivo* absorption potential of compounds [158]. Despite studies showing satisfactory *in vitro-in vivo* correlations for specific drugs [120, 123, 125, 174-176], *in vitro* dissolution testing for suppositories are not applicable for the assessment and confirmation of bioavailability following rectal administration [158]. However, *in vitro* dissolution testing can be used as a tool during initial dosage form development and routine control of batch quality during production. In addition, dissolution testing can be used to provide valuable information regarding the physical stability of drugs in suppositories, in addition to the assessment of the reliability of dosage form performance [158].

A number of techniques have been used for the study of *in vitro* drug release from suppository dosage forms. The techniques that are in use differ mainly in the extent to which they are able to mimic *in vivo* physiological conditions. Two basic techniques have been employed, *viz.,* those that use membranes in the assessment of drug release and those that do not. Animal studies have also been used in conjunction with *in vitro* dissolution studies, in an attempt to correlate *in vitro-in vivo* drug availability [144, 174, 175, 177-179].

4.1.1 *In vitro* dissolution methods without membranes

The most frequently used techniques for the measurement of *in vitro* drug release from suppository dosage forms appear to be those used for the assessment of drug release from solid oral dosage forms as described in the USP [10]. The apparatus that has been used includes the USP Apparatus I or basket apparatus, USP Apparatus II or paddle apparatus and USP Apparatus IV or flow-though cell apparatus, or modifications thereof [10]. Comparative studies of rectal formulations have been performed using all three of the aforementioned techniques [180, 181], in an attempt to determine whether or not the methods described for the assessment of oral dosage forms are applicable to the assessment of drug release from rectal dosage forms, in addition to determining whether they are reproducible [182].

An unmodified version of the USP apparatus I has been used to study the *in vitro* release of drugs from both hydrophilic and lipophilic suppository formulations [118, 144, 156, 180, 181, 183] using basket rotation speeds of either 50 or 100 rpm and a basket of mesh size 40. Dash *et al,* [184] used a modified rotating basket apparatus to characterize drug availability of nicotine from fatty base suppository formulations. In these studies, conventional USP stainless steel baskets were replaced with polyurethane baskets of the same external dimensions as the official USP basket, but with 12 linear slots of 0.25 mm allowing for a porosity of 52%. The increase in porosity more than likely increases contact of the dissolution medium with the suppository, hence facilitating hydrodynamic flow of the dissolution media through the melted suppository.

The use of USP apparatus II for the assessment of drug release from suppository dosage forms often requires some modification of the method in order to prevent the suppositories being tested from floating on the surface of the dissolution medium after dropping the dosage form into the beaker. Modifications that have been made have included the use of a stainless steel screen of mesh size ranging between 1 and 2 mm, placed between the paddle and the suppositories at the base of the dissolution vessel, or the use of a metal helix wound around the suppository to prevent the dosage form from floating [127, 178, 181]. Rotational speeds of the paddles in these assessments are normally 50 rpm.

The British Pharmacopoeia [91] has recently introduced a suggested dissolution apparatus for the testing of *in vitro* drug release rates of API from suppository dosage forms. The suggested apparatus makes use of a flow-through cell approach for the assessment of drug release from the suppository. However, slight changes in the cell design of the USP flow-through dissolution test system, *viz.,* Apparatus IV [10] can also be used for testing drug release from suppository dosage forms [185].

The USP Apparatus IV consists of a single chamber in which the dosage form is placed, and the system can be operated as an open system with fresh solvent from the reservoir continuously passing through the cell in which the dosage form is initially accommodated, or as a closed system by recycling a fixed volume of liquid through the cell [185]. The BP flow-through cell for suppository dosage form testing consists of two adjacent chambers, in one of which a suppository is placed and the system is operated continuously as an open system, so that the suppository is continually exposed to the flow of a fresh dissolution medium.

A schematic representation of the BP flow-through cell is depicted in Figure 4.2. The system consists of two adjacent chambers, in one of which *viz.* the chamber labeled A, a suppository is placed. As the heated dearated dissolution medium flows past the suppository, the dosage form melts or dissolves depending on the primary constituents of the base. The upward stream of dearated dissolution medium flows past the molten suppository base into a second chamber, *viz.* chamber B and subsequently flows through a filter into a collecting vessel. Molten suppository bases that are of lower density than the hydrophilic dissolution fluid are trapped in a niche area, *viz*. C, located at the top of chamber A.

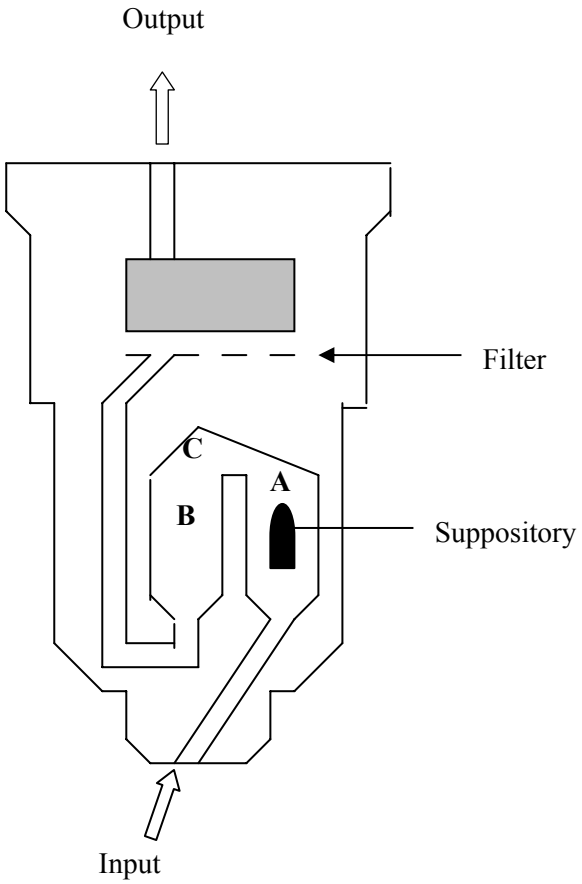


Figure 4.2: A schematic representation of the flow-through dissolution cell described in the BP in which A represents a dissolution chamber, B an adjacent chamber and C, a niche for the retention of molten lipids. The arrows indicate the direction of flow of the dissolution medium in this system [91].

The flow-through cell method or a modified version thereof has been used extensively for the assessment of a variety of different suppository formulations [117, 176, 180, 186-188]. Drug exchange at the lipid/water interface becomes the rate-limiting step of drug release from suppository formulations apart from the apparent solubility of the drug contained in the formulation [186]. In a study in which the *in vitro* release rates and bioavailability of

paracetamol and codeine were evaluated, it was found that the flow-through cell can be used to create test conditions that generate results that are consistent with *in vivo* conditions, provided that the flow rate that is used is indicative of the change in agitation and the amount of medium available for dissolution in the rectum, and is kept constant at a low flow rate of 8 ml/min [176].

Comparative studies [180-182] in which the *in vitro* drug release characteristics from lipophilic and hydrophilic suppositories were assessed using various USP Apparatus, revealed that drug release from hydrophilic suppositories appeared to be less sensitive to the type of dissolution method used when compared to lipophilic base suppositories. Furthermore, consistent release rate results were obtained for suppositories that dissolve than for those that must first melt prior to releasing the active ingredient. Therefore for the *in vitro* characterisation of hydrophilic suppositories that release API as a consequence of the base dissolving in rectal fluids, the basket, paddle, or flow-through cell apparatus can be considered equivalent.

In vitro release studies of drugs from lipophilic suppositories have always posed challenges, as a result of differences in melting rates and the deformation and dispersion of the molten base in a dissolution medium. As different dissolution techniques will result in different degrees of spreadibility, high variability of *in vitro* dissolution patterns may result, which may ultimately impact on drug release rates. The use of the basket method for the assessment of *in vitro* release rates from lipophilic suppositories may from time to time result in the molten mass leaving the basket through gaps between the basket and the basket holder, which in turn may result in higher drug release rates and an overestimation of that rate of release, as a consequence of greater surface area exposure for diffusion in the dissolution medium [181]. In addition, the different spreading conditions of a molten mass observed using the flow-through apparatus suggest that this technique may result in rapid drug dissolution rates compared to those obtained with other methods [181].

The continuous flow of fresh dissolution medium through the molten suppository mass as compared to the constant exposure that prevails with USP Apparatus I and II as a result of a constant volume of dissolution medium may also facilitate rapid drug release rates [181]. A flow-through cell technique in which the suppositories are enclosed in a bed of glass beads will allow direct contact of the suppository with the dissolution medium, whilst controlling

the interface exposed for dissolution of the drug in the suppository [180]. The latter method has been compared to the rotating basket technique for the analysis of in *vitro* release rates of benzocaine-containing fatty base formulations and the results were found to be reproducible using both methods [180].

4.1.2 *In vitro* dissolution methods with membranes

In reality, the physiological environment in which drug release from a suppository is achieved can only occur in the presence of a small volume of rectal fluid or secretions of approximately 3-5 ml. Subsequently, drug that is released, is transferred through a highly viscous mucous barrier to the rectal membranes and following absorption, into the systemic circulation to exert a therapeutic effect [171, 189]. An ideal model for the evaluation of *in vitro* drug availability should therefore consist of at least two aqueous phases, viz. one phase of small volume in which the suppository is in direct contact with the dissolution fluids, thereby simulating the intrarectal lumen, and a second phase of larger volume simulating the systemic circulation. For the purposes of mimicking *in vivo* conditions, the two aqueous phases should be separated by a membrane that represents the rectal barrier membranes, through which the drug must diffuse [171, 189].

The use of the membrane method was initially proposed by Muranish *et al,* [190] in an attempt to minimize variation of drug release rates and to reflect drug release under physiological conditions, when testing the release rate of ampicillin from various types of suppository bases. The Muranish method [190] made use of a filter membrane to achieve drug diffusion phenomena in these systems. Semi-synthetic dialysis membranes [120, 147, 157, 171, 186, 191, 192] in addition to natural membranes [177, 193] have also been used in an attempt to simulate such *in vivo* conditions.

Realdon *et al,* [189] have recently proposed a new alternative method for the determination of drug availability from the suppositories formulation, as a substitute for the use of biological tissues [193] in *in vitro* testing. An artificial membrane consisting of a cellulose ester polymer, coupled with filter paper was used to separate the chambers. The membrane was soaked in *n*-octanol and the filter paper was soaked in a phosphate buffer so as to provide an integrated hydro-lipophilic simulation of the biological membrane [189]. The proposed

simulated model provided a pattern of drug availability that was closer to that of the *in vitro* simulated rat rectum model [193].

The use of a membrane method for the assessment of drug release, potentially avoids surface variation effects that may occur between the suppository and a receptor phase, which is one of the major causes of poor reproducibility of the methods that do not use membranes to assess drug release rates from suppositories. In addition, membrane methods facilitate sampling and analysis since a clear filtered solution is sampled for analysis, rather than a complex mixture of dissolution medium and suppository base [186]. These models also take into consideration factors such as type of excipients used, viscosity of molten bases and water solubility of the drug, which might influence the availability of drugs for dissolution and subsequent absorption *in vivo*, in particular when a drug is administered in combination with lipophilic excipients in the form of suppositories [171].

Several reports in which satisfactory *in vitro-in vivo* correlations have been established using membrane systems have been published [116, 147, 179, 193]. However, the implementation and use of such systems has drawbacks [139]. Since drug release is measured in the outer compartment, monitoring of the actual release process, taking place in the inner compartment, is not achieved and consequently drug release rates may be underestimated [139]. The use of an isolated rat rectum to assess the release of paracetamol from suppositories and establish an *in vitro-in vivo* correlation between *in vitro* pharmaceutical availability and bioavailability has been reported [177]. However, a poor *in vitro-in vivo* correlation was obtained in these studies, suggesting that factors other than the physicochemical properties of the excipient and drug may influence drug availability when the formulation is administered *in vivo*. Therefore, the use of membrane systems for the initial characterisation of rectal formulations is not recommended [139, 194].

Comparative studies on drug availability in which methods that use membranes and those that do not have been reported [182, 186, 195] and their results reveal that the techniques are not comparable. Aoyagi *et al,* [182] investigated inter-laboratory reproducibility of drug release rate tests using the paddle method, the Muranish method [190] and a dialysis tubing technique with two types of suppository, *viz.* fatty and water miscible base formulations. In these studies, a modified Muranish and dialysis tubing method was used to assess drug release from a fatty base, whereas a modified Muranish and paddle method was used to

determine drug release rates from water-soluble bases. The Muranish method [190] was modified so that no membrane was used. From these studies it was concluded that both paddle and modified Muranish methods may be used for the assessment and quality control of suppositories manufactured using water soluble bases and that neither the Muranish nor the dialysis tubing methods can be reliably used for the assessment and quality control of suppositories manufactured using fatty bases, as a consequence of their poor reproducibility. The lack of reproducibility may be attributed to the difficulty in achieving an exact position for the Muranish cell or due to complete removal of residual test fluids in the dialysis tubes during testing, thereby changing the dynamics of the test system [182].

Two different apparatus have been designed and compared for the assessment of *in vitro* dissolution kinetics of indomethacin from either hydrophilic or lipophilic base suppositories, *viz.* a flow through cell and a rotating dialysis cell [186]. The rotating dialysis membrane method generated faster release rates for the API from lipophilic suppositories than the flow-through method, whereas the flow-through method resulted in faster release rates for the API from hydrophilic suppositories than the dialysis membrane method.

Ermis and Taimci [195] reported *in vitro* release and diffusion rate data for the release of ketoprofen from PEG bases using USP Apparatus I and the modified Muranish method [190]. The results revealed a significant difference between the release profiles obtained using the basket or the modified Muranish methods. It is evident that ketoprofen diffused across a membrane of constant surface area to the aqueous dissolution medium in the latter method, whereas ketoprofen was released directly into the aqueous dissolution medium from the surface of the suppository using the former method.

It is therefore apparent that no single test method is suitable for the assessment and evaluation of drug release for all types of suppository formulations. However, it should be possible to select an appropriate *in vitro* dissolution test method from those reported in the literature, based on factors such as the physicochemical properties of the drug, the base of choice and changes in rectal dosage forms that may occur following insertion into the rectum. Since drug release patterns are influenced by both suppository composition and the dissolution test method selected for use, factors such as dissolution medium flow rate, temperature, composition and contact area, must be controlled to ensure reproducibility of batch-to-batch assessments [139]. It has been recommended, when starting development of an *in vitro*

dissolution or release test, that the official USP Apparatus specified for solid oral dosage forms or modifications thereof be used [194]. However, the true validation of *in vitro* release testing remains the subsequent *in vivo* performance of developed dosage forms [139], as in many cases, the results of *in vitro* dissolution testing do not correlate with *in vivo* blood levels [177, 186].

4.2 *IN VITRO* RELEASE STUDIES OF AZITHROMYCIN FROM SUPPOSITORY BASES

4.2.1 Procedures

The *in vitro* release of AZI from the different formulations listed in Tables 3.6 and 3.7 were assessed using a fully automated Model SR 8 PLUS dissolution apparatus (Hanson Research Corporation, Chartsworth, CA, USA) fitted with an Autoplus™ Multifill™ and a Maximizer Syringe Fraction Collector (Hanson Corporation, Chartsworth, CA, USA). The release studies were conducted at 37°C ± 0.5°C using USP Apparatus 1 [10] with USP baskets of mesh size 40 rotated at 100 rpm, in replicates of six. Phosphate buffer (900 ml, 0.1 M, pH 7.2) was used as the dissolution medium, following degassing. The dissolution medium was degassed by filtration through a 0.45 µm Durapore® HVLP membrane filter (Millipore Corporation, Bellerica, MA, USA) using an Eyela Vacuum pump (Tokyo Rikakikai Co., Tokyo, Japan). Samples (3ml) were withdrawn at the pre-determined times listed in Table 4.1 and automatically filtered through 0.40 µm filters (Cannula Filters, Lab House, Cramerview, South Africa) prior to analysis. Samples were analysed using the validated HPLC method described in § 2.3, Chapter 2. An equivalent amount (3 ml) of previously warmed phosphate buffer was immediately replaced in the dissolution vessels to compensate for the volume of the sample removed for analysis. A summary of the dissolution test conditions for these studies is listed in Table 4.1.

Table 4.1: Summary of *in vitro* dissolution conditions used in these studies

Parameter	
Dissolution apparatus	USP Apparatus I
Dissolution medium	0.1M Phosphate buffer pH 7.4
Temperature	37 ± 0.5°C
Initial volume	900 ml
Basket speed	100 rpm
Basket screen size	40
Filter size	0.40 µm
Volume withdrawn	3 ml
Volume replaced	3 ml
Sampling times	
Water miscible bases	0, 10, 20, 30, 60, 120 minutes
Fatty bases	0, 30, 60, 120, 180, 240, 360, and 480 minutes

The suppositories were manufactured as described in § 3.2.2.2, Chapter 3, and the dissolution profiles characterized 24 hours after completion of the manufacturing process and again following storage of the suppositories for one month at room temperature (22°C) and in the refrigerator (4°C), to determine whether storage had any effect on the amount of AZI released from selected formulations. A mass balance analysis was conducted at the completion of each dissolution test, as described in § 3.3.5, in order to account for any drug that had not been released from a suppository base during the 480 minute dissolution test period. The content uniformity, weight variation and melting range, as described in § 3.3, Chapter 3, were assessed for all formulations studied.

4.2.2 Statistical interpretation of the data

The comparison of the dissolution data profiles based on statistical models and mathematical relationships were performed, so as to provide valuable information during the product development process. The difference and similarity factors f_1 and f_2 defined by Moore and Flanner [196] and endorsed by several regulatory agencies [197-199], in addition to a relatively new similarity factor, S_d [200], were used to compare the dissolution profiles of formulations that had been modified to a reference formulation, *viz.* suppositories manufactured with AZI in suppository base only. In addition, the application of mathematical models such as the Korsmeyer-Peppas, Zero order, First order and Higuchi models to dissolution results was undertaken to elucidate the mechanism by means of which AZI was released from the suppositories manufactured in these studies. A detailed explanation and

description of the use of statistical and mathematical models is presented in Chapter 5 *vide infra*.

Suppository formulations manufactured using water-soluble and fatty bases and that released the highest amount of AZI during dissolution testing were selected for stability studies. The batches selected for these studies were batches AZI-01, AZI-02 and AZI-18. Individual suppositories from these batches were wrapped in foil and stored at room temperature (22°C ± 0.5°C) and at 4°C for one month. Suppositories that were stored at 4°C were left to equilibrate to room temperature for 24 hours prior to analysis. The statistical procedure described by Timm *et al,* [112] and reported in § 2.3.8, Chapter 2, was adapted in order to determine whether storage had any effect on the drug release characteristics of AZI from the suppository formulations selected for stability assessment.

4.3 RESULTS AND DISCUSSION

4.3.1 The effect of base type on the rate and extent of AZI release

The dissolution profiles of AZI from suppositories manufactured using different compositions of PEG, *viz.* batches AZI-01 and AZI-02, are illustrated in Figure 4.3. It is clearly evident that more than 95% of the AZI contained in the suppositories from both batches tested was released in less than 60 minutes. Polyethylene glycols are water soluble polymers that often melt at temperatures higher than the rectal or physiological temperature of 37°C. Consequently, when used in rectal formulations, the drug is released gradually, as a result of the progressive dissolution of the PEG excipients in an aqueous dissolution medium [169].

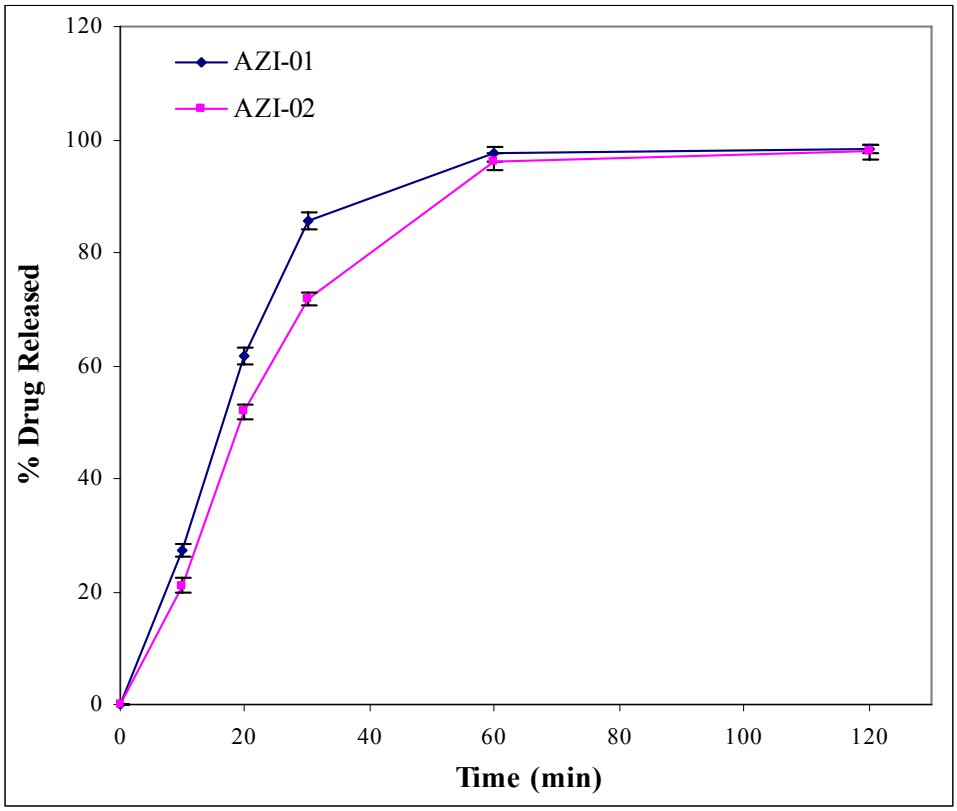


Figure 4.3: Dissolution profiles of AZI from batches AZI-01 and AZI-02, PEG base formulations

High molecular weight PEGs have high melting points, as seen in Table 3.4, and consequently have slower dissolution rates, compared to PEGs of intermediate molecular weight. Batch AZI-02 released AZI at a slower rate than Batch AZI-01, since batch AZI-02 contained PEG 1540 and 6000, whereas batch AZI-01 contained PEG 1000 and 4000. PEG bases are also known to have a solubilizing effect [169], which may in part explain the higher drug release rates from these suppositories when compared to suppositories in which fatty bases are used, as can be seen from the AZI dissolution profiles depicted in Figures 4.4 and 4.5.

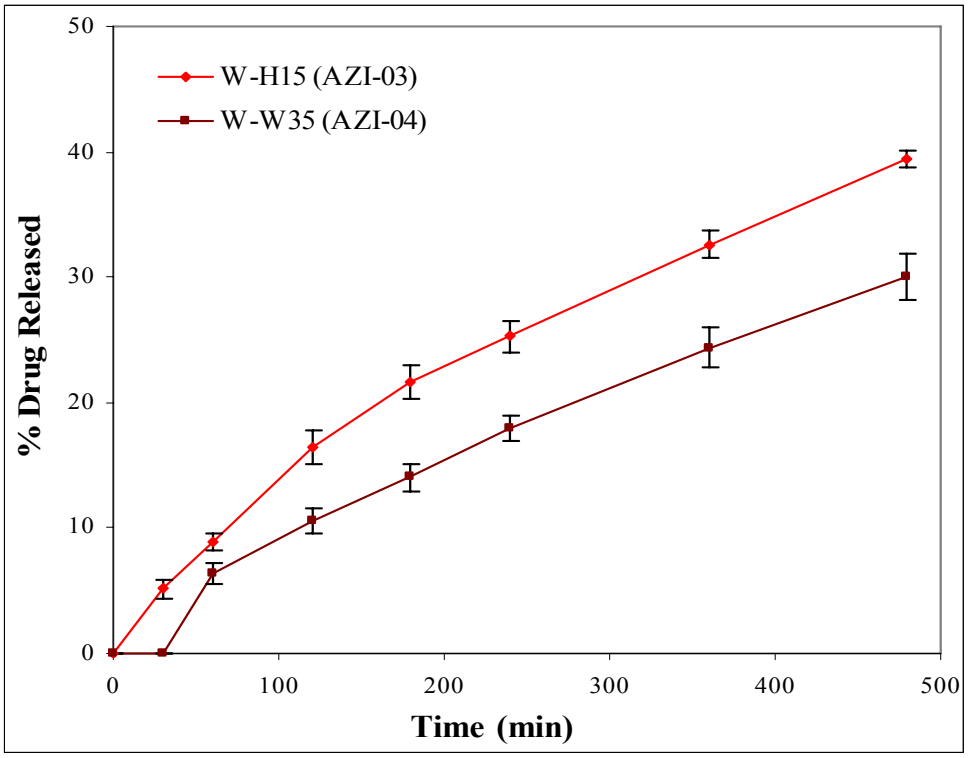


Figure 4.4: Release profiles of AZI from Witepsol® W-H15 (AZI-03) and W-W35 (AZI-04) formulations

Although the PEG formulations released AZI to a greater extent than from fatty base suppositories, the research conducted focused on improving the rate and extent of release of AZI from fatty bases, since PEG bases have been reported to be irritant to mucosal tissues [178, 201]. The interaction of a base and a base/drug complex with the rectal mucosa can significantly alter the physiological barrier in drug absorption [178, 201]. Research conducted by Young *et al,* [1178], Van Hoogdalem *et al,* [202] and Reid *et al,* [201] revealed that the interaction of a number of fatty bases, namely W-H12, W-H15 and S-AP with the rectal mucosa, was reversible within 24 hours, whereas that of the PEG bases appeared to aggravate the mucosal membrane tissue irritation for a longer period .

The release of AZI from suppositories manufactured using different types of fatty bases, *viz.* Witepsol® and Suppocire®, showed distinctive release profiles. The results of dissolution testing of batches AZI-03 and AZI-04 are depicted in Figure 4.4 and reveal that drug release was more complete than from batches AZI-06 – AZI-10, as depicted in Figure 4.5. Drug release rates from suppositories are known to be dependent upon several excipient properties, such as melting and fusion rates, viscosity and hydro-lipophilic characteristics [203]. The fatty bases used in these studies differ from each other mainly in their content of mono-, di-,

and triglycerides of saturated fatty acids, which confers specific characteristics on the base that are listed in Table 3.3.

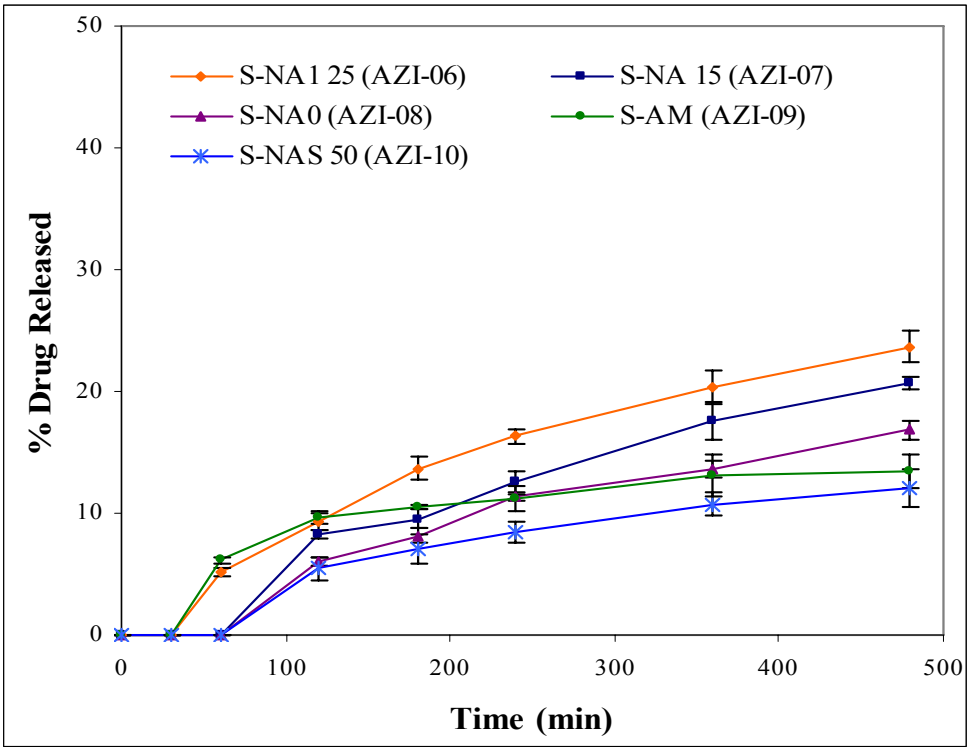


Figure 4.5: Release profiles of AZI from Suppocire® S-NA1 25 (AZI-06), S-NA 15 (AZI-07), S-NA0 (AZI-08), S-AM (AZI-09) and S-NAS 50 (AZI-10) formulations

When considering the mechanisms of drug release from lipophilic suppositories, a correlation can be established between the different physicochemical properties of the bases and their resultant *in vitro* release profiles [171]. However, no clear conclusions could be drawn following the examination of the dissolution profiles generated in these studies. Nevertheless, two factors were found to be of some significance, *viz.* the hydroxyl value and the melting range of the specific suppository bases.

Semi-synthetic suppository bases are mixtures of fatty acids and esters with certain amounts of glycerides. The hydroxyl values reported for specific bases represent the proportions of free mono- and diglycerides and therefore, the availability of free hydroxyl functional groups that are available for interaction [139]. A high hydroxyl value is an indication of the potential for a base to adsorb water, where a higher value is indicative of the ease with which water adsorption can occur. The presence of a high hydroxyl value in fatty bases imparts a hydro-dispersable character to the base [188].

Drug release from formulations manufactured with W-H15, batch AZI-03, which has a low hydroxyl value of 13.6, was faster and more complete than that from formulations manufactured using W-W35, batch AZI-04, which has a high hydroxyl value of 44.5. The dissolution profiles depicting AZI release from batches AZI-03 and AZI-04 are depicted in Figure 4.4. Similar results were also observed by Webster *et al,* [157] for amoxycillin release from Witepsol® suppositories and Othman *et al,* [204] for indomethacin release from Witepsol® suppositories. A maximum of 39.41 ± 0.73% and 30.02 ± 1.89% of AZI was released from batches AZI-03 and AZI-04 during dissolution testing of freshly manufactured suppositories, respectively. Furthermore, suppositories manufactured using W-E75, which has a hydroxyl value of 12.6 (batch AZI-05), did not release any AZI for the duration of the 480 minute test period.

By contrast, the hydroxyl value appears to be of some significance for suppositories manufactured using various grades of Suppocire®. The highest rate and extent of AZI release was observed for batch AZI-06, which was prepared using S-NA1 25, which has a relatively high hydroxyl value of 23.8, compared to those of 11.3, 1.7, and 3.3 of Suppocire® grades S-NA 15, S-NA0 and S-AM, that were used to manufacture batches AZI-07, AZI-08 and AZI-09, respectively. The resultant dissolution profiles following testing of these batches are shown in Figure 4.5. A maximum of 23.70 ± 1.30% AZI was released from batch AZI-06, whereas batches AZI-07, AZI–08 and AZI-09 released 20.74 ± 0.52%, 16.82 ± 0.80% and 13.43 ± 1.41% respectively.

These results can not be accounted for by hydroxyl value alone, since, when batch AZI-10 prepared using S-NAS 50 base was tested, a relatively slow release rate was observed, despite the base having a high hydroxyl value of 42.3 when compared to the other grades of Suppocire® used. The resultant dissolution profile is also depicted in Figure 4.5. A maximum of 12.06 ± 1.52% AZI was released from batch AZI-10 over the 480 minutes test period.

The results observed in these studies may be accounted for on the basis of simple partitioning of AZI between the aqueous dissolution medium and the lipoidal suppository base phase [188, 204]. The partitioning of a drug between bases with high hydroxyl values, such as W-W35 and S-NAS 50, appears to favor the lipid phase [183]. Therefore, further research must be conducted on the basis of drug partitioning in the presence of particular suppository base-

rectal fluid systems, to further elucidate and/or predict the process of drug release [143]. In studies in which the partition coefficient of a drug between lipophilic bases and aqueous phases was studied, it was found that the partition coefficient varied depending on the nature and type of fatty base used to deliver the drug [177].

An examination of the physicochemical properties of the bases tested in relation to AZI release, revealed that the melting ranges of the fatty bases, as reported in § 3.3.6.2, Chapter 3, appeared to play, at least in part, a greater role in release rates than that contributed by the hydroxyl values alone. The melting of a base is a prerequisite for drug liberation, in addition to contributing to a dissolution lag time. Dissolution lag time has been defined as the time in which less than 5% of the drug has been dissolved [205]. Melting of a fatty base is followed by several successive mechanistic steps, such as spreading of the molten mass, particle sedimentation, passage across the interface and finally dissolution of the drug in the hydrophilic rectal fluids or dissolution test media [173]. It is therefore clearly evident that the complete melting of a suppository in a dissolution vessel is required for AZI to have the potential to be released completely during *in vitro* testing. If melting is not complete or does not occur, drug release is likely to be extremely slow, as was observed during the assessment of batches AZI-05, AZI-08, AZI-09 and AZI-10.

A similar conclusion was drawn by Janick *et al,* [158] and Bergren *et al,* [206] who studied the dissolution rates of paracetamol from different fatty bases and the release of meteneprost potassium from vaginal suppositories using pharmacopoeial test conditions and the USP basket apparatus, respectively. The effect of hydroxyl value and melting range, of the fatty bases used in these studies, on the rate and extent of AZI release are summarized in Table 4.2.

Table 4.2: The effects of hydroxyl value and melting range on the rate and extent of AZI release

Batch #	Base	Hydroxyl value	Melting range °C	Lag time min	% released t = 480 min
AZI-03	W-H15	13.6	32.5-34.5	*	39.41
AZI-04	W-W35	44.5	34.0-35.5	30	30.02
AZI-05	W-E75	12.6	36.5-39.0	**	0.00**
AZI-06	S-NA1 25	23.8	34.5-35.0	30	23.70
AZI-07	S-NA 15	11.3	34.0-35.0	60	20.74
AZI-08	S-NA 0	1.7	35.0-36.5	60	16.82
AZI-09	S-AM	3.3	35.0-36.0	30	13.43
AZI-10	S-NAS 50	42.3	36.0-37.5	60	12.06

*Dissolution lag time was less than 30 minute, **AZI was not released after 480 minute test run

AZI release from batch AZI-03 exhibited a lag time of < 30 minutes, whereas that from batches AZI-04, AZI-06 and AZI-09 exhibited lag times of > 30 minutes and that from batches AZI-07, AZI-08 and AZI-10 exhibited lag times of up to 60 minutes. The presence of a lag time indicates that a period of time is required prior to the commencement of base melting. Batches of suppositories manufactured with bases with a lower melting range had shorter lag times when compared to batches manufactured with bases with higher melting ranges.

The drug release rate from batches AZI-06 and batch AZI-07 was relatively slower than that from batch AZI-04, despite having been manufactured using bases with a similar melting range. The difference in release rates from bases with similar melting ranges may, in part, be accounted for on the basis of simple partitioning rates of AZI between the lipoidal base and aqueous dissolution medium, in addition to the decreasing viscosity of the molten base, thus influencing the rate of drug migration from within the suppository to the surface of the molten dosage form [177, 204].

Suppositories from batch AZI-05 melted at a temperature of approximately 39°C and therefore dissolution of AZI from these products would be delayed until the base had completely melted. Since all dissolution studies were conducted at 37°C ± 0.5°C, AZI release from suppositories was not observed during the 480 minute test period as the base had not melted and therefore migration of AZI to the interface, prior to partitioning from the fatty base into the aqueous dissolution medium, was not possible. Such observations have raised the question as to the definition of a suitable temperature for use for *in vitro* dissolution testing of suppository formulations.

It is evident that the development of a successful *in vitro* dissolution test requires an adequate knowledge of the melting range of the base and final product being tested. However, when developing such a procedure, the physiological conditions within the rectum must be considered if an acceptable *in vitro-in vivo* correlation is to be established. Therefore, as the rectal temperature has been reported to be typically in the region of 36°C to 37°C [194], bases with high melting ranges, such as that used for the manufacture of batch AZI-05, were not considered as appropriate for rectal formulation development and were therefore not considered further, despite the fact that additives are known to reduce melting points [158].

4.3.2 The effect of additives on AZI release

In order to improve the rate and extent of AZI release from suppositories manufactured using fatty bases, two different manufacturing and formulation approaches were investigated. The first of these two approaches involved the addition of a surfactant to the formulation composition. The polysorbates or Tween® surfactants have been shown to increase the dissolution rate of a number of drugs by micellar solubilization and improved wetting [116, 118, 207, 208]. Tween® 20 and 80 were used as the surfactants of choice for these investigations. The second, more complex, approach involved the alteration of the physical properties of AZI via the formation of solid dispersions of AZI with an 'inert' water soluble carrier [153]. The use of this technique has been reported to increase the dissolution rates of poorly water-soluble drugs intended for rectal use [120, 191, 209-212]. Urea and PVP-K25 were used as potential water-soluble carriers for the preparation of solid dispersions of AZI for these studies.

4.3.2.1 The effect of surfactant content on AZI release

To evaluate the effects of incorporating surfactants into the formulation on AZI dissolution rates, the dissolution behavior of formulations containing different proportions of Tween® 80, which has a hydrophilic-lipophilic balance (HLB) of 14.9 and Tween® 20, which has an HLB value of 16.7, were compared to that of drug release from formulations that contained AZI and that specific base only. The surfactants were incorporated in concentrations of 0.5% w/w, 1.0% w/w, 2.0% w/w, 4.0% w/w for Tween® 80 and 2.0% w/w for Tween® 20 into batches manufactured using W-H15, W-W35, S-NA1 25, S-AM and S-NAS 50 fatty bases. The different bases were selected in order to assess the impact of hydroxyl value on drug release in the presence of surfactants. The rationale for using the surfactants at a concentration of up to 4.0% w/w was to ensure that the amount of the surfactant incorporated reflected the intermediate amount of surfactant concentration that has been shown to be safe following rectal administration to humans [117, 123].

Surfactant addition to the suppository bases resulted in a reduction in the temperature at which melting occurred and thus, in effect, the melting range was reduced when compared to suppositories manufactured with AZI in a suppository base alone. The melting points of all formulations with surfactant added ranged between 32.0°C and 35.5°C and the results of the melting point determinations are listed in Table 4.3.

Table 4.3: The effect of surfactant on the melting range of selected fatty bases

Batch #	Batch Composition	Melting range (°C)	% released t= 480 min
AZI-03	W-H15	32.5-34.5	39.41
AZI-11	W-H15/ 0.5%Tw80	33.5-35.5	36.83
AZI-12	W-H15/ 1.0%Tw80	32.5-34.0	33.42
AZI-13	W-H15/ 2.0%Tw80	32.5-33.5	38.30
AZI-14	W-H15/ 4.0%Tw80	32.0-33.5	32.94
AZI-32	W-H15/ 2.0%Tw20	32.0-33.5	36.20
AZI-04	W-W35	34.0-35.5	30.02
AZI-15	W-W35/ 0.5%Tw80	32.5-33.5	48.43
AZI-16	W-W35/ 1.0%Tw80	33.0-35.0	57.08
AZI-17	W-W35/ 2.0%Tw80	32.5-34.5	63.64
AZI-18	W-W35/ 4.0%Tw80	32.0-33.5	57.10
AZI-31	W-W35/ 2.0%Tw20	32.5-34.0	53.61
AZI-09	S-AM	35.0-36.0	13.43
AZI-19	S-AM/ 0.5%Tw80	35.0-35.5	9.20
AZI-20	S-AM/ 1.0%Tw80	33.0-35.0	11.41
AZI-21	S-AM/ 2.0%Tw80	32.0-34.5	12.12
AZI-22	S-AM/ 4.0%Tw80	32.0-34.0	11.37
AZI-33	S-AM/ 0.2%Tw20	32.5-34.0	10.05
AZI-06	S-NA1 25	34.5-35.0	23.70
AZI-23	S-NA1 25/ 0.5%Tw80	34.0-35.0	38.66
AZI-24	S-NA1 25/ 1.0%Tw80	33.5-34.5	41.71
AZI-25	S-NA1 25/ 2.0%Tw80	32.5-34.5	41.85
AZI-26	S-NA1 25/ 4.0%Tw80	32.0-34.0	41.95
AZI-10	S-NAS 50	36.0-37.5	12.06
AZI-27	S-NAS 50/ 0.5%Tw80	32.5-35.5	42.65
AZI-28	S-NAS 50/ 1.0%Tw80	32.5-34.0	45.75
AZI-29	S-NAS 50/ 2.0%Tw80	32.0-34.5	44.06
AZI-30	S-NAS 50/ 4.0%Tw80	32.0-34.5	42.15

However, the reduction in the melting points of the formulations did not have a significant impact on the rate and extent of release of AZI from these formulations and the resultant dissolution profiles are shown in Figures 4.6 and 4.8. It is apparent that, in these formulations, the presence of hydroxyl values together with the addition of surfactants appears to have greater impact on release rates than the reduction in the melting range of the base.

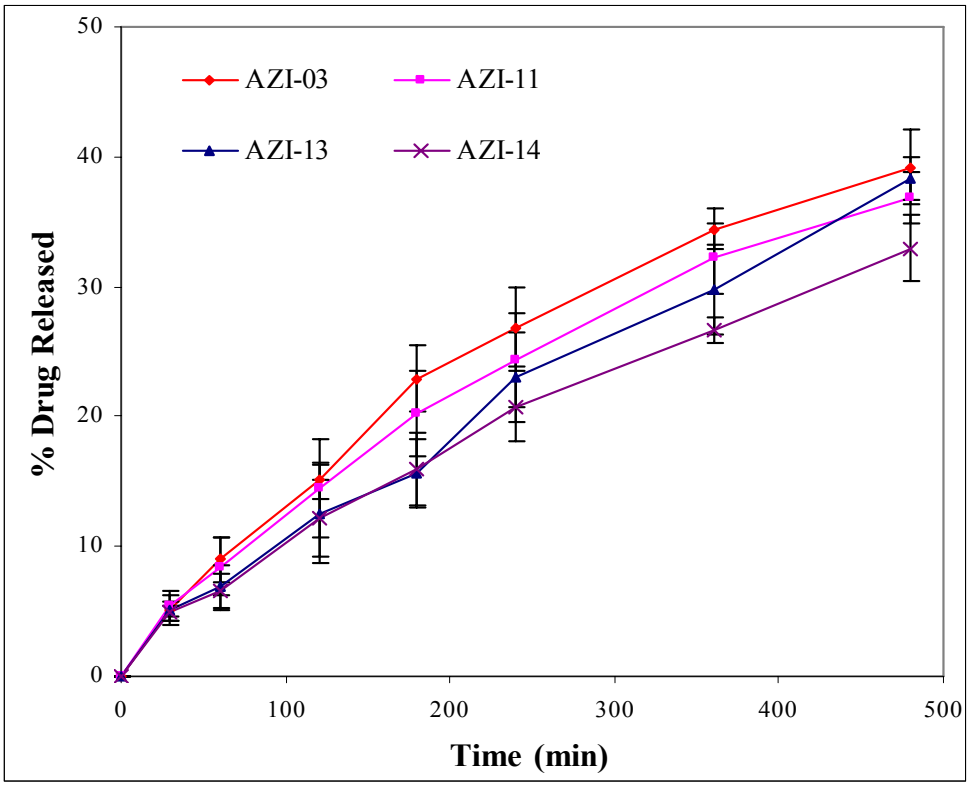


Figure 4.6: The effect of addition of Tween® 80 on the release of AZI from W-H15 base formulations containing 0% w/w (AZI-03), 0.5% w/w (AZI-11), 2.0% w/w (AZI-13) and 4.0% w/w (AZI-14)

The addition of Tween® 80 to suppositories manufactured using W-H15 in concentrations of 0.5% w/w, 1.0% w/w and 2.0% w/w, *viz.* batches AZI-11, AZI-12 and AZI-13 respectively, did not result in a significant increase in the rate and extent of AZI release, in comparison to that from batch AZI-03 and, in fact, the release rate of AZI from these products was slightly reduced. The decrease in drug release could be a consequence of the added surfactant, the formation of a stable complex between AZI and the base and/or the dissociation of the complex was influenced unfavorably by the use of the additive [116]. The dissolution profile for Batch AZI-12 is included in Appendix II. Furthermore, the addition of Tween® 80 at a concentration of 4.0% w/w to W-H15 to produce batch AZI-14 did not increase the rate and extent of release of AZI.

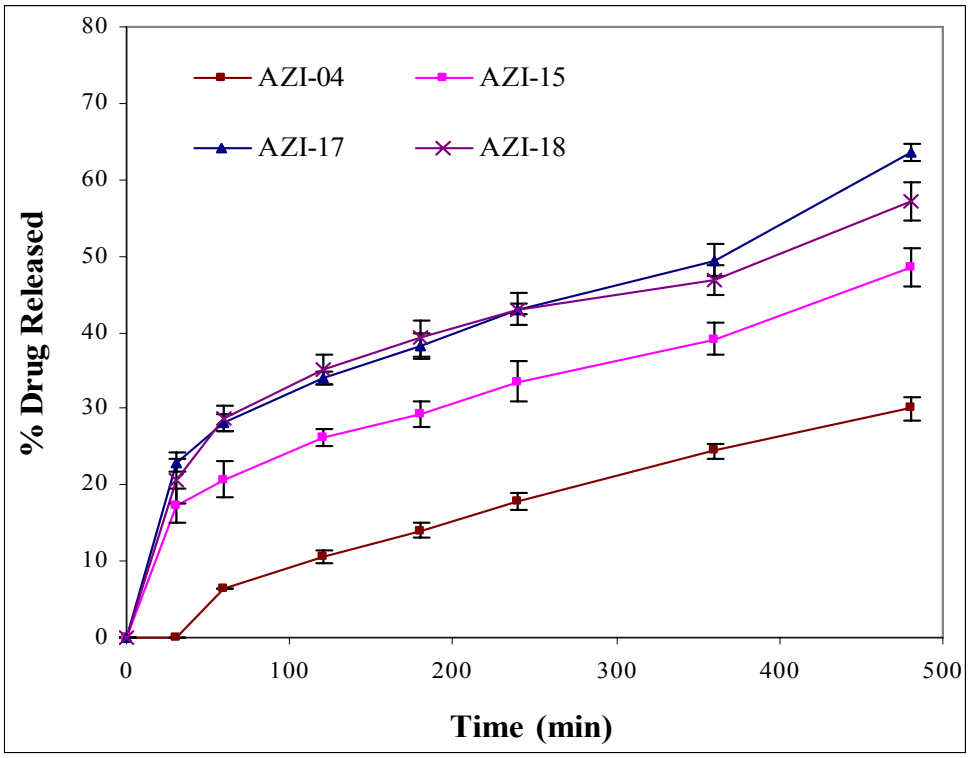


Figure 4.7: The effect of addition of Tween® 80 on the release of AZI from W-W-35 base formulations containing 0% w/w (AZI-04), 0.5% w/w (AZI-15), 2.0% w/w (AZI-17) and 4.0% w/w (AZI-18)

The rate and extent of release of AZI from W-W35 suppository base formulations, following the addition of Tween® 80, was significantly greater than that from batch AZI-04, in which AZI was formulated with base only. The resultant release profiles are depicted in Figure 4.7. It is clearly evident that the rate and extent of AZI release increased as the surfactant concentration was increased from 0.5% w/w up to 4.0% w/w for batches AZI-15 – AZI -18. The dissolution profile for batch AZI-16 has been included in Appendix II for reference purposes.

It is evident from these studies that the addition of a surfactant to a fatty suppository base appears to have a significant effect on the release of AZI from bases with high hydroxyl values. The mechanism by which added surfactant may influence release rates is complex and is not fully understood, due to the large variety of effects surfactants may produce [139]. It has been reported that, in the presence of a surfactant, drug release from suppositories is favored by various mechanisms, *viz.* increasing the surface area of the suppository mass, as a result of their moistening effects, shortening disintegration times of lipophilic suppositories as a result of changing their lipophilic characteristics to a lipo-hydrophilic nature [116, 118],

decreasing the interfacial tension between the suppository base and the dissolution medium [118] or by enhanced wetting of the drug [118].

Furthermore, bases with high hydroxyl values have higher monogylyceride contents compared to bases with intermediate or low hydroxyl value [177] and as monoglycerides are known to interact with polar groups of surrounding dissolution media, thereby disordering the hydrophobic region of the lipophilic suppository bases, drug release may be enhanced [177]. The combined effect of surfactant addition and the presence of high hydroxyl values may be synergistic and could have contributed to the higher rate and extent of release of AZI from these formulations.

Further addition of Tween® 80 up to a concentration of 4.0% w/w in batch AZI-18 did not significantly increase the rate and extent of release of AZI compared to that observed for batch AZI-17, which was manufactured using a 2.0% w/w content of Tween® 80. This result could be a consequence of micellar solubilization of the AZI by the surfactant or of exceeding the critical micellar concentration (CMC). The presence of surfactants in formulations at concentrations higher than their CMC may retard drug release, as a result of micellar entrapment of the drug [139].

A similar trend was observed when AZI release from formulations manufactured using Suppocire® bases with high hydroxyl value and added surfactant was examined. As can be seen in the dissolution profiles depicted in Figure 4.8, the increased rate and extent of release of AZI was not significant from formulations manufactured using S-AM base, which has a low hydroxyl value of 3.3 and surfactant concentration of 0.5% w/w in batch AZI-19, 1.0% w/w in batch AZI-20 and 2.0% w/w for batch AZI-21.

However, the effects were significant for formulations prepared with Suppocire® bases S-NA1 25, that has an intermediate hydroxyl value of 23.8, *viz*. batches AZI-23 – AZI-25, and S-NAS 50, that has a high hydroxyl value of 42.3, *viz.* batches AZI-27 – AZI-29, as can be seen from the dissolution profiles shown in Figures 4.9 and 4.10 respectively. The dissolution profiles for batches AZI-20 (S-AM and 1.0% w/w Tween® 80), AZI-24 (S-NA1 25 and 1.0% w/w Tween® 80) and AZI-28 (S-NAS 50 and 1.0% w/w Tween® 80) are included in Appendix II for reference purposes.

Similarly, the addition of Tween® 80 to concentrations of 4% w/w did not appear to make a difference to the rate and extent of AZI release as can be seen from the release rate profiles of batches AZI-22, AZI-26 and AZI-30 formulated with S-AM, S-NA1 25 and S-NAS 50 bases respectively. The dissolution profiles for batches AZI-22, AZI-26 and AZI-30 are included in Figures 4.8, 4.9 and 4.10 respectively.

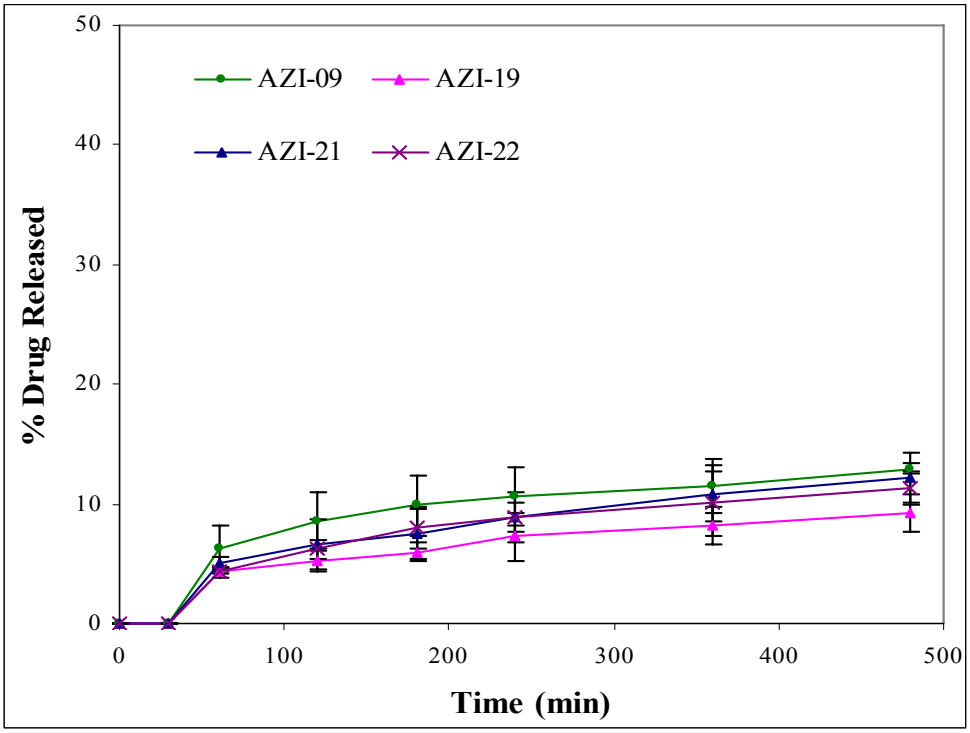


Figure 4.8: The effect of addition of Tween® 80 on the release of AZI from S-AM base formulations containing 0% w/w (AZI-09), 0.5% w/w (AZI-19), 2.0% w/w (AZI-21) and 4.0% w/w (AZI-22)

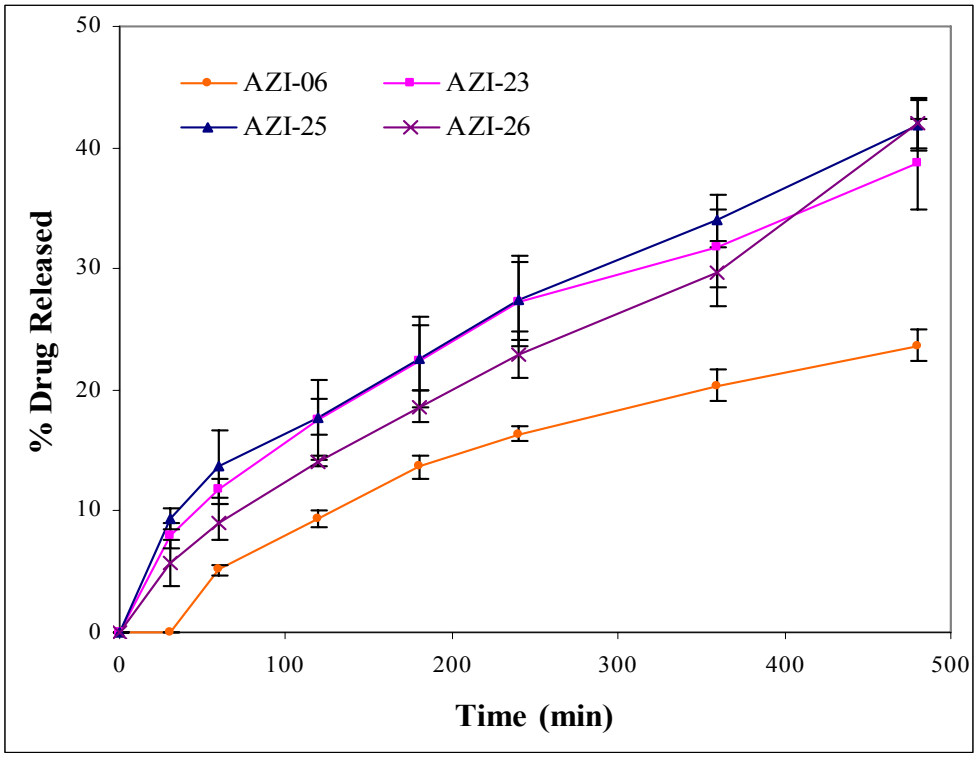


Figure 4.9: The effect of addition of Tween® 80 on the release of AZI from S-NA1 25 base formulations containing 0% w/w (AZI-06), 0.5% w/w (AZI-23), 2.0% w/w (AZI-25) and 4.0% w/w (AZI-26)

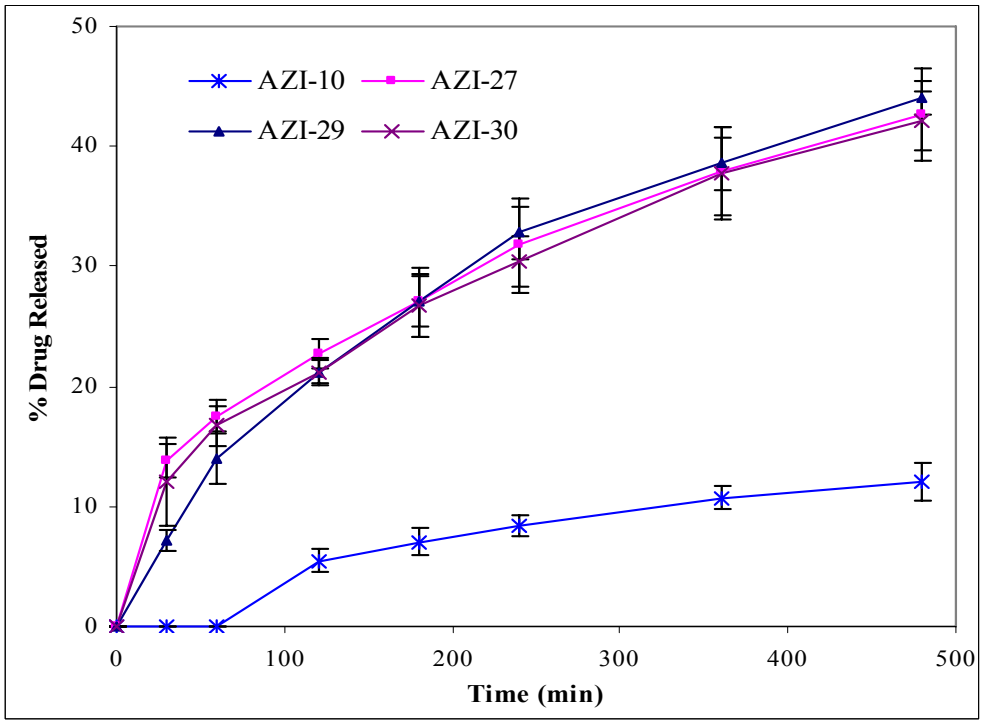


Figure 4.10: The effect of addition of Tween® 80 on the release of AZI from S-NAS 50 base formulations containing 0% w/w (batch AZI-10), 0.5% w/w (AZI-27), 2.0% w/w (AZI-29) and 4.0% w/w (AZI-30)

The effect of the addition of Tween® 80 on the rate and extent of release of AZI from fatty bases was also analysed statistically, using the GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA) computer program. For statistical evaluations, data were analysed by ANOVA with the Newman-Keuls test. It can be concluded that drug release from fatty bases of high hydroxyl value, *viz.* W-W35 ($^{***}P<0.001$), S-NA1 25 ($^{***}P<0.001$) and S-NAS 50 ($^{***}P<0.001$), was significantly increased when surfactant was added at concentrations of 0.5% w/w, 1.0% w/w, 2.0% w/w and 4.0% w/w, whereas the rate and extent of AZI release was significantly reduced when Tween® 80 was incorporated at concentrations of 0.5% w/w, 1.0% w/w and 4.0% w/w into formulations with fatty bases of intermediate hydroxyl value, W-H15 ($^{***}P<0.001$), while at surfactant concentration 2.0%w/w the release was approximately the same ($^{ns}P>0.05$) as from the formulation in which AZI and base only were used. The release of AZI from the fatty base S-AM, which has a low hydroxyl value, was not significantly different ($^{ns}P>0.05$) when Tween® 80 was incorporated at concentrations of 1.0% w/w, 2.0% w/w and 4.0% w/w, when compared to the formulation manufactured with base only. The overall effect of the addition of Tween® 80 in concentrations of 0.5% w/w, 1.0% w/w, 2.0% w/w and 4.0% w/w on the release of AZI from different fatty suppository bases is summarized in Figure 4.11.

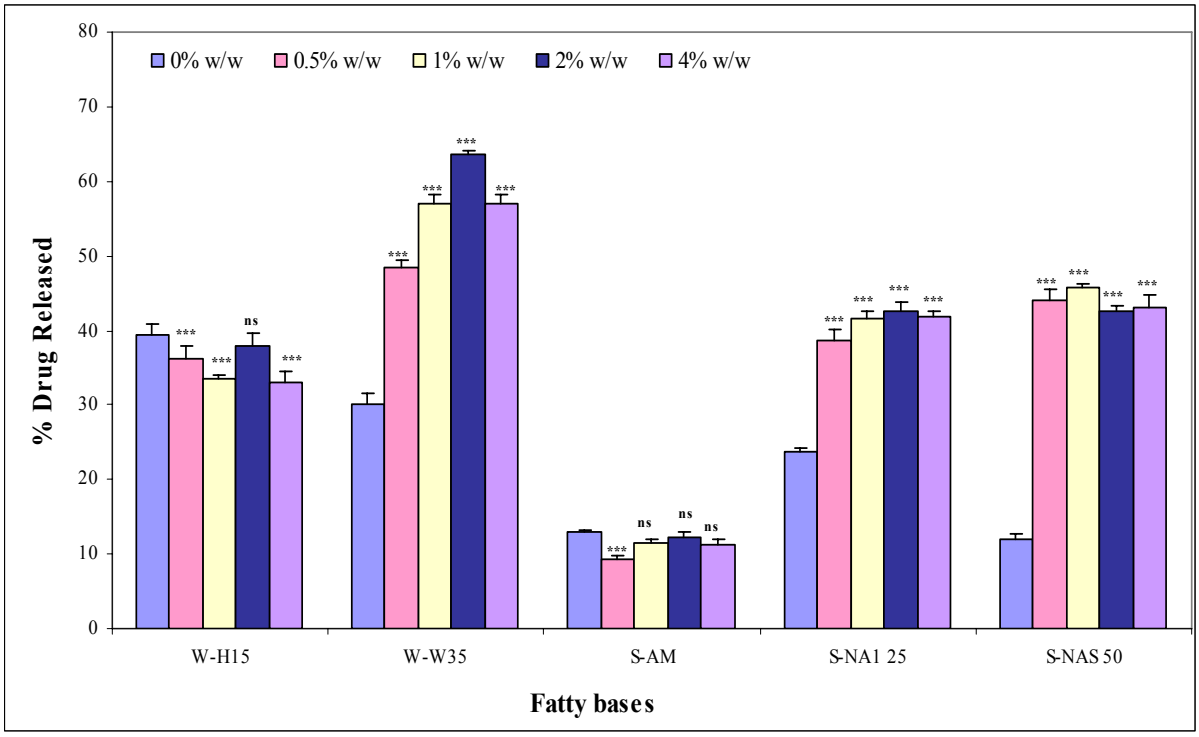


Figure 4.11: A summary of the effects of Tween® 80 on the rate and extent of release of AZI from different suppository bases after 480 minutes ($^{ns}P> 0.05$, $^{***}P < 0.001$ versus control (± SD), analysis of variance using Newman-Keuls test).

The effects of a surfactant with a high HLB value, such as Tween® 20, on AZI release rates from selected fatty suppository bases was also examined. Fatty bases W-W35, W-H15 and S-AM, with high, intermediate or low hydroxyl values respectively, were used to assess the impact of Tween® 20 on AZI release from these suppositories. The incorporation of Tween® 80 into suppositories manufactured using W-H15 and S-AM bases did not yield significant effects on AZI release during *in vitro* testing and therefore the use of Tween® 20 was expected to enhance drug liberation from these bases more than that observed when using Tween 80 as an additive.

The HLB value of a surfactant provides an indication of the hydrophilic-lipophilic balance of the compound and the higher the HLB value, the more hydrophilic the compound [103]. A surfactant with a high HLB value should therefore enhance the wetting of lipid excipients by aqueous dissolution media. In addition, an increase in the hydrophilic character of a fatty base may reduce the affinity of a base for lipophilic substances, thereby promoting drug release [118]. Initially, a concentration of 2.0% w/w of Tween® 20 was selected for these studies, since the same concentration of Tween® 80 appeared to facilitate the release of AZI to a greater extent from the fatty base products than the other concentrations tested. However, the results from these studies revealed that surfactants with low HLB values are more likely to promote AZI release from these suppositories than a surfactant with a high HLB value. These findings are consistent with those in which the effects of surfactants such as Tween® 20 and 80 on carbamazepine release from lipophilic suppositories were investigated [208].

The incorporation of Tween® 20 into formulations manufactured using the W-W35 suppository base, which has a high hydroxyl value, significantly increased the release of AZI when compared to that for batch AZI-04, manufactured with base only. However, AZI release from batch AZI-17, in which 2.0% w/w Tween® 80 had been incorporated, was greater than that from batch AZI-31, to which 2.0% w/w Tween® 20 had been added. The resultant dissolution profiles for these dissolution tests are depicted in Figure 4.12.

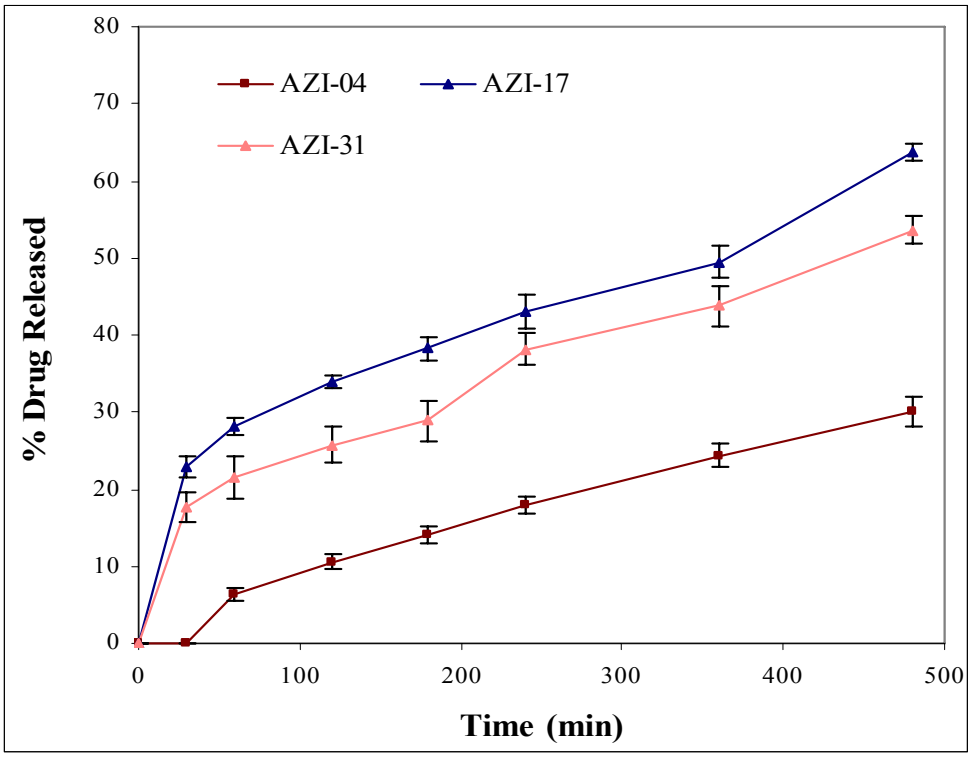


Figure 4.12: The effect of addition of Tween® 20 in comparison to Tween® 80 on the release of AZI from W-W35 base formulations containing 0% w/w (AZI-04), 2.0% w/w Tween® 80 (AZI-17) and 2.0% w/w Tween® 20 (AZI-31)

The addition of Tween® 20 to the formulations manufactured using bases with intermediate and low hydroxyl values did not significantly increase the rate and extent of release of AZI, as shown in Figures 4.13 and 4.14 respectively. It is clearly evident that AZI release from batches AZI-13 and AZI-21, to which Tween® 80 was added, was not significantly different from batches AZI-32 and AZI-33, in which Tween® 20 had been used.

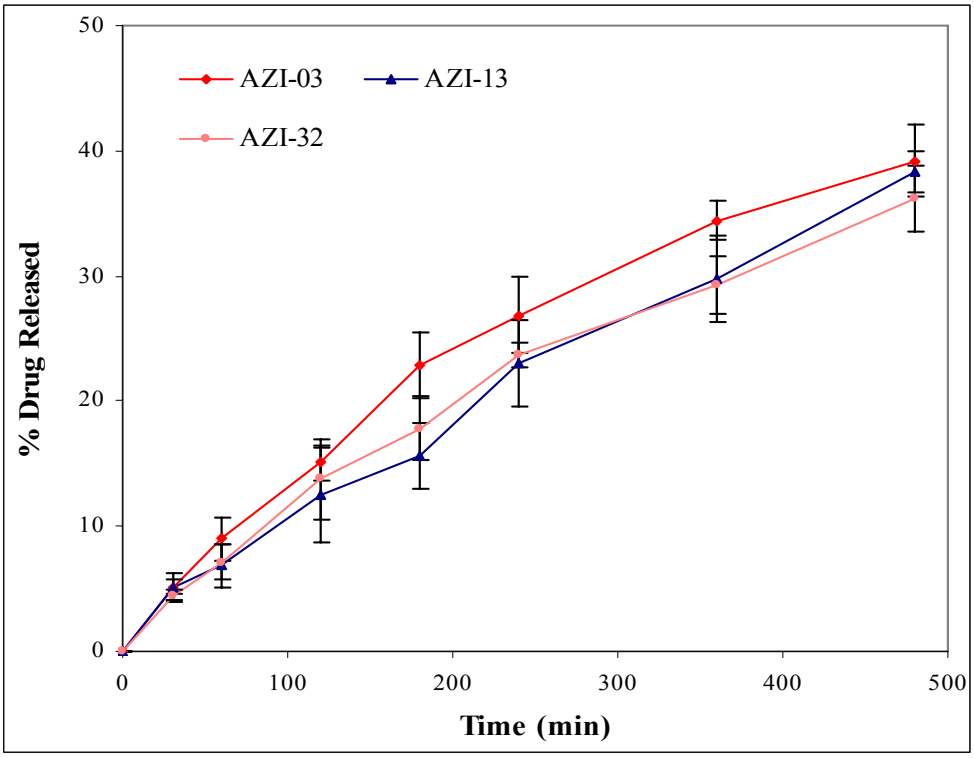


Figure 4.13: The effect of addition of Tween® 20 in comparison to Tween® 80 on the release of AZI from W-H15 base formulations containing 0% w/w (AZI-03), 2.0% w/w Tween® 80 (AZI-13) and 2.0% w/w Tween® 20 (AZI-32)

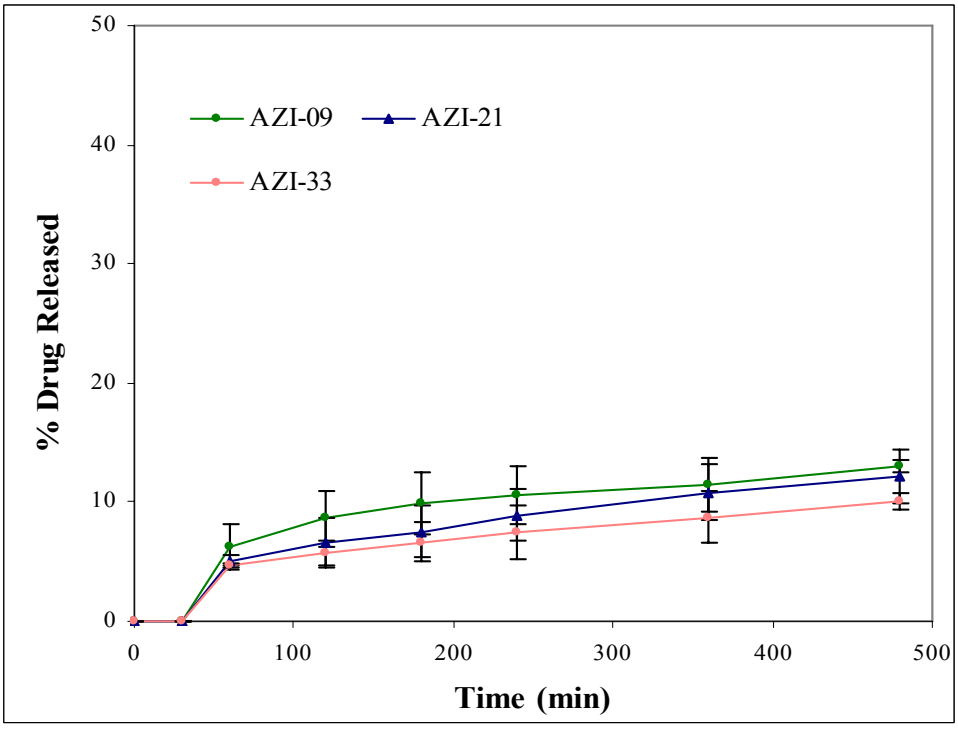


Figure 4.14: The effect of addition of Tween® 20 in comparison to Tween® 80 on the release of AZI from S-AM base formulations containing 0% w/w (AZI-09), 2.0% w/w Tween® 20 (AZI-21) and 2.0% w/w Tween® 80 (AZI-33)

The surfactants Tween® 20 and 80 consist of long chain hydrophilic polyoxyethylene derivatives and differ due to the composition of their lipophilic polymeric composition, which imparts a difference in their ability to effect drug release [208]. The size and number of micellar aggregates of the surfactant increases with increasing hydrocarbon chain length [208]. Tween® 80 is an oleic acid ester with an 18-unit hydrocarbon chain (C_{18}), whereas Tween® 20 is a lauric acid ester with a 12-unit hydrocarbon chain (C_{12}). Therefore, micelles assembled from Tween® 80 may have a better incorporation capacity for AZI than those assembled from Tween® 20, as a result of their longer lipid chains, which may explain the higher observed AZI release from batch AZI-17 compared to that from batch AZI-31. Furthermore, micellar solubilization could explain the reduced rate and extent of release of AZI, if the critical micelle concentration for each of the surfactants had been exceeded in these systems.

4.3.2.2 The effect of urea and polyvinylpyrrolidone (PVP) on AZI release

The inclusion of urea or PVP in suppositories manufactured from base W-W35 in the form of drug-carrier coprecipitates was evaluated in order to determine their effects on drug release rates. Physical mixtures of urea or PVP with AZI were also studied for comparative purposes. The W-W35 base was selected for initial studies since it has a high hydroxyl value and a low melting range and these factors appeared to play a significant role when evaluating AZI release from suppositories manufactured in earlier studies. Urea was selected as a potential release enhancer due to its osmotic potential and its compatibility with body fluids, as it is a normal component of biological fluids [120]. The impact of urea on the rate and extent of AZI release from W-W-35 suppositories is shown in Figure 4.15.

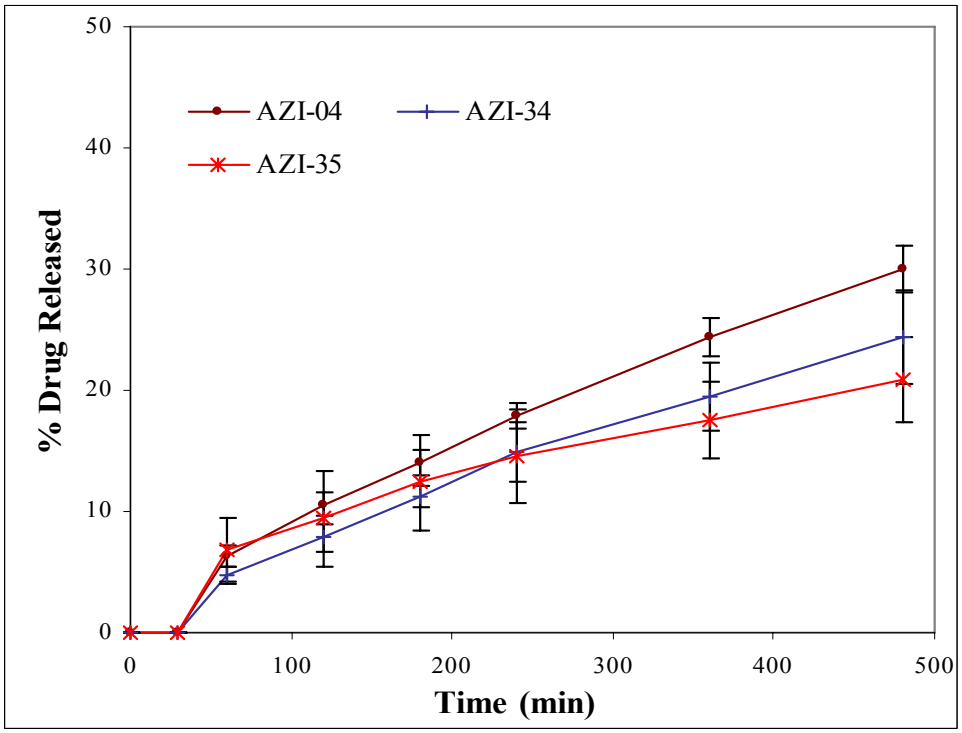


Figure 4.15: The effect of addition of urea on the release of AZI from W-W35 base formulations containing 0% w/w urea (AZI-04), 2.0% w/w urea in a physical mixture (AZI-034) and solid dispersion coprecipitates (AZI-35)

The addition of urea to W-W35 formulations revealed that AZI release was decreased when compared to that from suppositories manufactured using the base only, *viz*. batch AZI-04. The method of incorporation of urea, either as a physical mixture or as a solid dispersion, as described in § 3.2.2.3, did not appear to influence the rate and extent of AZI release from these dosage forms. The maximum amount of AZI released from batch AZI-34, manufactured from a physical mixture of urea, and from batch AZI-35, manufactured from a solid dispersion of AZI and urea, were found to be 24.11% ± 4.45% and 20.88 ± 3.59% respectively.

PVP derivatives are particularly suitable for the preparation of solid dispersions by solvent evaporation methods as a result of their excellent solubility characteristics in a wide variety of organic solvents. In addition, PVP derivatives display excellent aqueous solubility and can improve the wettability of dispersed compounds [213]. To investigate the effects of PVP on AZI release from W-W-35 suppositories, PVP-K25 was selected and the results of these investigations are shown in Figure 4.16.

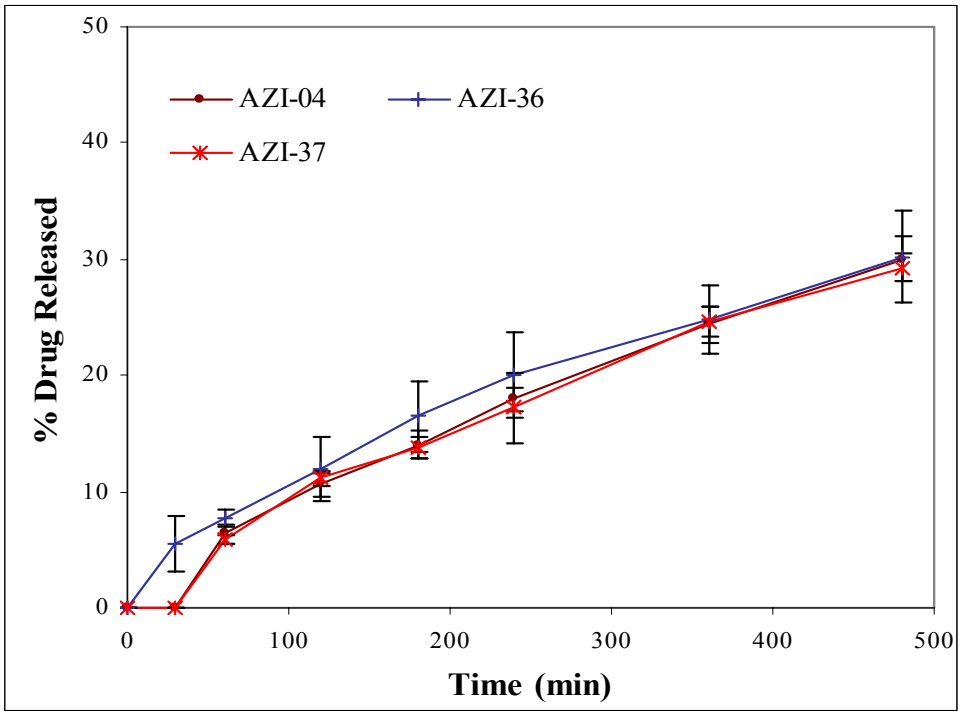


Figure 4.16: The effect of addition of PVP on the release of AZI from W-W35 base formulations containing 0% w/w PVP (batch AZI-04), 2.0% w/w PVP in a physical mixture (AZI-36) and solid dispersion coprecipitates (AZI-37)

The incorporation of PVP into W-W35 suppository formulations did not have a significant effect on the rate and extent of AZI release from these products. The maximum AZI released from batch AZI-36, manufactured from a physical mixture of PVP and AZI, and from batch AZI-37, manufactured from a solid dispersion of AZI and PVP, were found to be 29.30 ± 1.24% and 30.18 ± 3.97% respectively.

Several mechanisms have been proposed to explain drug release from solid dispersions, but the dissolution behavior of individual components of a solid dispersion, *i.e.* the drug and the carrier, are fundamental to understanding the performance of the solid dispersion. It has been proposed that solid dispersion systems may exhibit carrier-controlled release [162, 213, 214] in which the rate of drug release is determined by the rate of dissolution of the inert carrier and that such release is independent of the drug loading in a dosage form or system. Several systems have demonstrated that drug release is drug dissolution rate-controlled, as opposed to carrier-controlled dissolution [162, 213, 214]. In order to determine whether or not the mechanism of dissolution from a solid dispersion coprecipitate is carrier-controlled, a high carrier/drug ratio must be used [55, 213]. These effects were investigated since the dose of AZI administered for paediatric patients is usually 200-400 mg [37]. Therefore, the amount

of dispersion required to administer the usual dose of the drug may be too high to produce suitable one-gram suppositories, which are most appropriate for rectal administration to pediatric patients The maximum quantity of additives and API that can be incorporated into a suppository formulation should comprise approximately 30% of the blank weight of the suppository, so as to avoid the challenges posed by increased viscosity, sedimentation and dispersion of the active ingredient during manufacture, in addition to the possible formation of suppositories that are too brittle for handling [151].

It has been suggested that if drug release from a dosage form is dissolution-controlled then, the incorporation of a proportion of low molecular weight material, such as polyethylene glycol 400, or the addition of surfactants, may have a beneficial effect, thereby promoting drug release [214]. The use of surfactants in solid dispersion formulations has been reported to increase the dissolution rate of poorly water soluble drugs, when used at a low concentration of approximately 0.5% w/w [152, 209]. Mixtures of polyethylene glycol and polysorbates such as Tween® 80 have been reported to have potential for use as surface-active carriers in solid dispersions, to improve the solubility of drugs that are poorly water-soluble and poorly wetted [215].

Therefore the decrease in the rate and extent of AZI release observed for batch AZI-35 and the slight and insignificant increase observed for batch AZI-37 may be a consequence of the dissolution properties of the solid dispersion coprecipitate that requires further investigation. The decrease in AZI release may be explained by a theory proposed by Frijilink *et al,* [211, 212] who suggest that, for lipophilic compounds, following partial dissociation of the coprecipitate complex, free drug that has been liberated from the complex may undergo back-diffusion into the lipid phase, from which it has been liberated. Similar observations were reported for studies in which the effect of urea on allopurinol release from hydrophilic and lipophilic suppository bases were investigated [141]. Consequently, the low amount of AZI released from these suppositories may be a consequence of back diffusion of the drug into the lipophilic suppository bases.

4.3.3 Stability studies of selected water soluble and fatty base formulations

Dosage forms and active ingredients must be chemically and physically stable for extended periods, in order to provide an adequate shelf-life for products. The USP [10] description of stability considerations for suppositories in long term storage includes observations for excessive softening and evidence of oil stains on packaging materials. Excessive softening is the major indication of instability in suppository products [10]. As a general rule, the USP [10] recommends storage in a refrigerator, unless otherwise indicated. Rectal delivery systems must be stable under refrigerated (4°C ± 1°C) and ambient (25°C ± 3°C) storage conditions for at least two years [141]. In order to determine whether or not AZI suppositories manufactured in our laboratories were potentially stable, preliminary stability studies were conducted for a period of one month at 4°C and room temperature (RMT), which was 22°C.

The bioavailability of chemically stable rectally administered APIs is known to be influenced by the physical stability of suppositories on long-term storage [115]. Suppositories are known to undergo hardening in storage, which may result in an increase in the melting time of the suppository, with a corresponding decrease in dissolution rates of drugs from the suppositories [115]. The hardening effect in storage may explain the increased dissolution time and slower release rates of AZI from suppositories manufactured using PEG bases, following storage for one month. The rate of AZI release from formulations stored for the period of one month was reduced but the extent of release was similar since approximately 95% and 96% of AZI was released from batch AZI-01 and AZI-02 respectively stored at both temperatures (at 4ºC and at 22ºC) for the period of one month compared to 98% and 97% released from freshly prepared batch AZI-01 and AZI-02 respectively. From the chemical perspective, the experiments indicate that AZI is stable in PEG formulations tested under the specified conditions and that the release rate is affected by the hardening effect of the PEG base. The rate and extent of AZI release from batch AZI-01 stored at 4°C was similar to that stored at room temperature (f_1 = 6.8, f_2 = 73.6); similar results were also observed for batch AZI-02 (f_1 = 15.0, f_2 = 63.5). The resultant dissolution profiles, following stability testing, are shown in Figures 4.17 and 4.18 for batches AZI-01 and AZI-02 respectively.

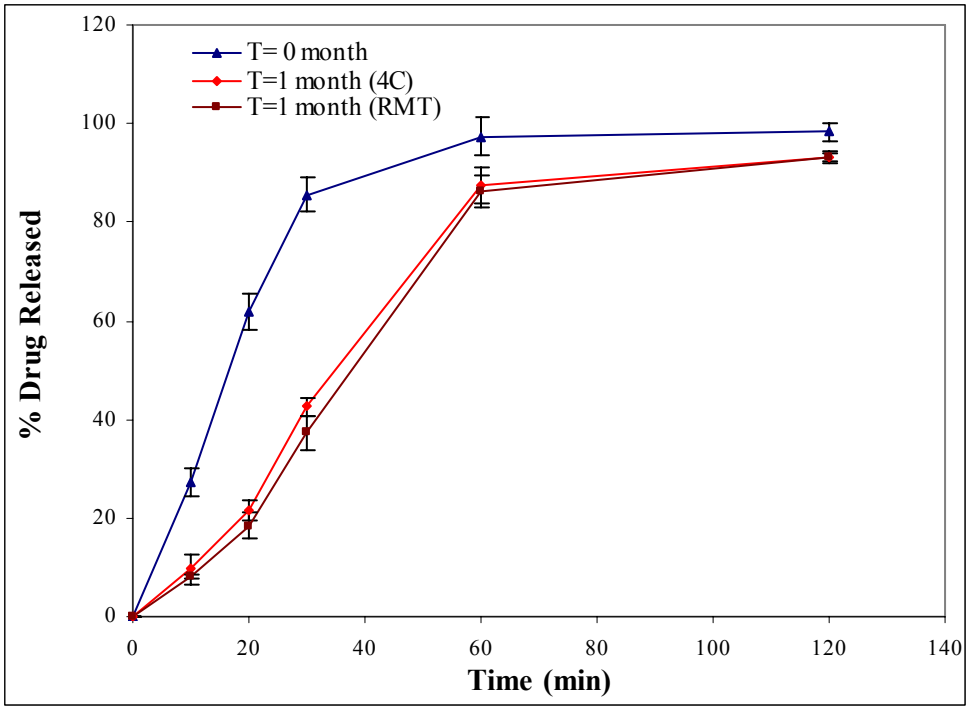


Figure 4.17: Dissolution profiles of AZI from suppositories formulations, batch AZI-01 immediately after manufacture (T=0) and following one month (T=1) of storage at 4°C and room temperature (22ºC)

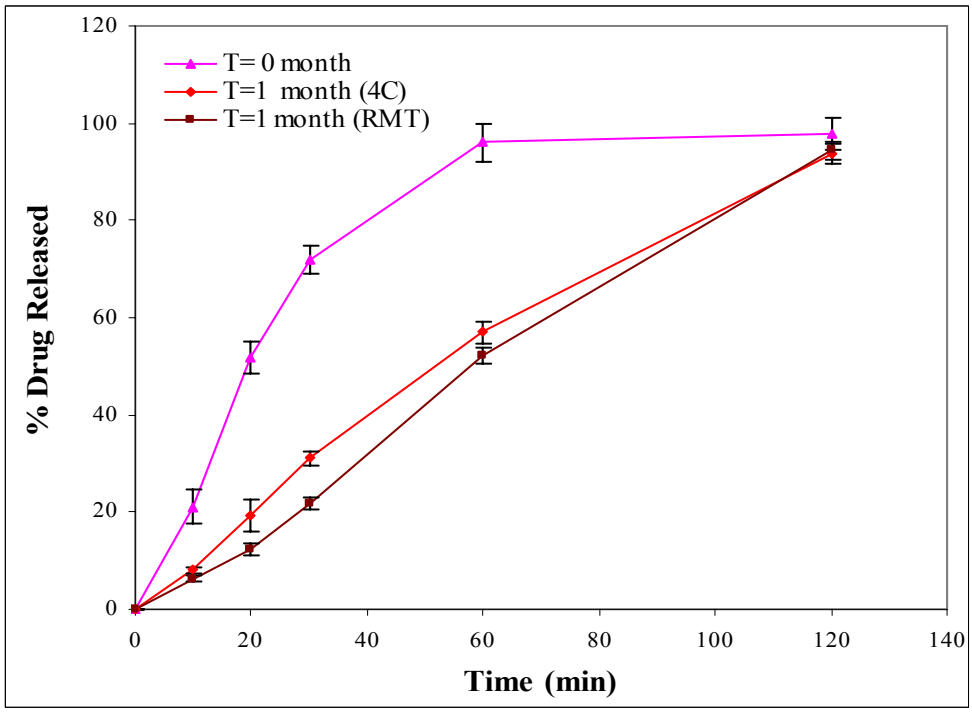


Figure 4.18: Dissolution profiles of AZI from suppositories formulations, batch AZI-02 immediately after manufacture (T=0) and following one month (T=1) of storage at 4°C and room temperature (22ºC)

On prolonged storage, semisynthetic fatty suppository bases have also been shown to be subject to hardening, with a corresponding increase in melting times [115]. The melting characteristics, hardness and drug release profiles tend to change after storage for a few weeks and melting points may rise by as much as 0.5°C, following storage for several months [149]. Any hardening effect, resulting in little or no suppository melting, can cause local irritation, a defaecatory reflex or bowel obstruction and therefore are important considerations during formulation development [216]. Due to the complexity of the fatty bases, elucidation of the mechanisms that induce these changes with ageing have been difficult to isolate [149]. However, is has been postulated that hardening of suppositories in storage may be a consequence of polymorphic phase transitions, increased crystallinity and/or increased transesterification of the bases [119, 216, 217]. Therefore, long term storage of suppositories manufactured using semisynthetic fatty suppository bases may result in a marked reduction in drug release from these dosage forms [183].

The consequences of hardening, polymorphism formation or increased crystallinity [217] may explain, in part, the lower AZI release from batch AZI-17, manufactured using W-W35 with 2.0% w/w Tween® 80, following storage for one month at 4°C and 22°C, as seen in Figure 4.19. AZI dispersed in a freshly prepared mixture of fatty base W-W35 and Tween 80 was mainly in suspension and in the presence of aqueous dissolution media the release rate was enhanced, due to the synergistic effect of surfactant and presence of high hydroxyl values. During prolonged storage, it is possible that AZI dissolved in the interior of the excipient and that, during dissolution testing, the partitioning of the drug appears to favour the lipid phase, which is indicated by the lower rate of release observed for the formulations stored for the period of one month as shown in Figure 4.19. AZI release from batch AZI-17 stored at 4ºC was similar to that of AZI-17 stored at room temperature ($f_1 = 12.2, f_2 = 74.8$).

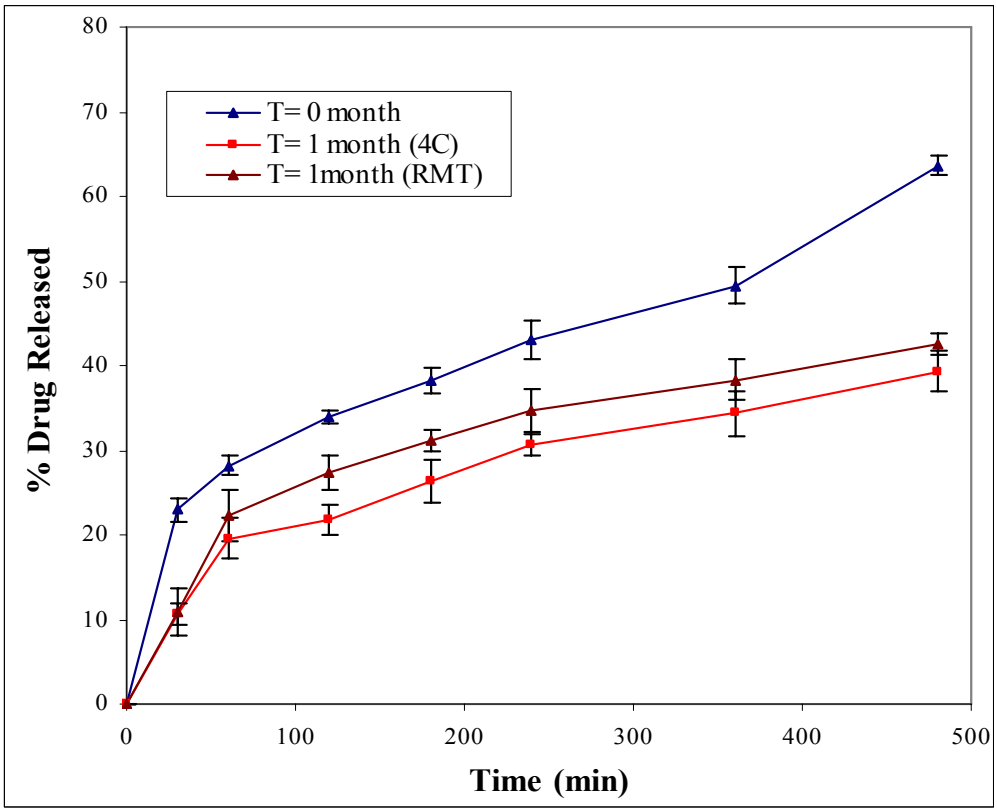


Figure 4.19: Dissolution profiles of AZI from suppositories formulations, batch AZI-17 immediately after manufacture (T=0) and following one month (T=1) of storage at 4°C and room temperature (22ºC)

The interpretation of stability results was undertaken using the method described by Timm *et al,* [112]. The method is used to assess whether or not differences in the amount of AZI released from the suppositories placed in storage, from that obtained at the time of manufacture (T=0 month) may be considered relevant and/or significant. The interpretation of stability results previously described in § 2.3.8 were applied to the data for these experiments and the resultant confidence intervals calculated for these determinations are depicted in Figure 4.20 and are reported as the percentage change from the initial amount of AZI released at T=0 month.

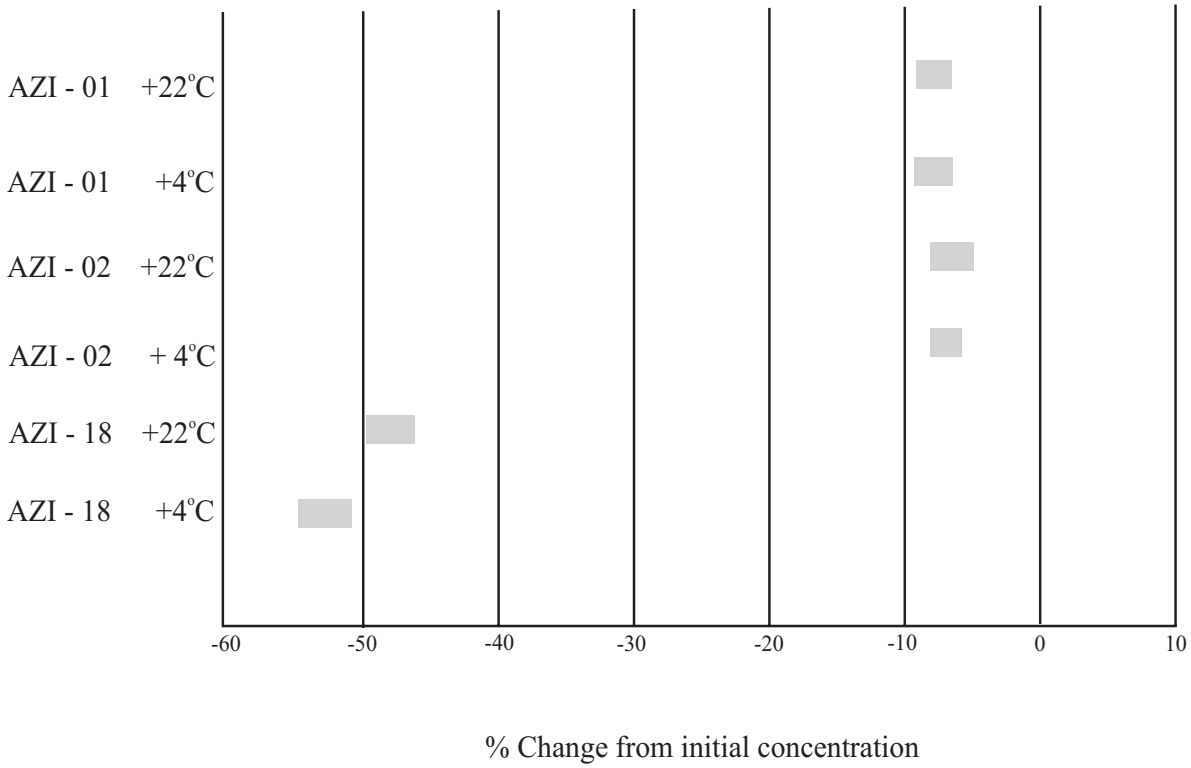


Figure 4.20: Stability of AZI in water soluble base (AZI-01 and AZI-02) and fatty soluble (AZI-18) base formulations stored at +4°C and +22°C for one month

The rate and extent of AZI release from batches AZI-01 and AZI-02 stored at 4°C and 22°C showed a significant decrease, which may not be relevant. Stability studies of a longer duration would provide data to ascertain whether the response observed following one month of storage is significant and relevant. From the results of statistical analyses, it is clear that after 4 weeks of storage at 4°C and 22°C, batch AZI-18 showed a significant and relevant decrease in AZI release, indicating that studies should be conducted to establish the effect of the base and/or additive on the stability of AZI, which may have led to a reduced rate and extent of release.

4.4 CONCLUSIONS

The objective of this study was to develop and assess a suppository formulation containing an equivalent of 250 mg AZI and to determine the impact of different types of formulation approaches on drug release rates. The USP basket apparatus was successfully employed to assess the release behavior of AZI from water-soluble PEG and semi-synthetic fatty base suppositories. The results of these studies serve as a guide for the selection of an optimal formulation for AZI paediatric suppositories, in particular with respect to the type of base and concentration of potential absorption enhancers required for optimal *in vitro* performance. The results of *in vitro* release studies revealed that AZI suppositories manufactured using PEG bases released the API faster and to a greater extent than those manufactured using semisynthetic fatty bases.

In addition, different approaches were used in the manufacture of these suppositories with the primary aim of improving AZI release from the fatty base suppository dosage forms. These approaches included the incorporation of a surfactant, the use of additives as a physical mixture and alteration of the physical properties of the API via a solid dispersion technique. Fatty suppository bases with intermediate and lower hydroxyl values did not yield a significant increase in the rate and extent of AZI release when a surfactant was added to the formulation. However, a significant increase in the rate and extent of release was observed from formulations in which bases with a high hydroxyl value were used, in combination with a surfactant. These results suggest that the nature and physicochemical properties of a base play an important role in determining the rate and extent of drug release from lipophilic suppository formulations.

The addition of urea or PVP as a physical mixture or as a solid dispersion with AZI did not significantly increase drug release rates. These studies serve as a preliminary investigation into the use of solid dispersion techniques to improve the aqueous solubility of AZI. Further investigations should focus on a full understanding of the mechanism of drug release from solid dispersion coprecipitates and/or complexes. In addition, investigations as to whether the solid dispersion remains physically stable during manufacture and in storage must also form part of future studies.

The results of the preliminary stability study indicate that additional long-term studies must be conducted to characterise the rate and extent of AZI release and to elucidate any potential interactions between AZI and the specific suppository base used. These long-term investigations are crucial, in particular for antibiotics, to ensure that effective antimicrobial activity is retained in such products during long-term storage.

Although it appears that PEG suppository formulations may be more appropriate when considering the results of *in vitro* dissolution rate testing of AZI formulations, fatty bases are better from a patient comfort perspective, since the PEG bases are known to cause rectal mucosa irritation, due to their osmotic effects. Therefore, further research should focus on understanding the physicochemical properties of AZI in relation to those of fatty suppository bases in order to improve *in vitro* release rates of AZI.

CHAPTER FIVE

5. STATISTICAL COMPARISONS AND MATHEMATICAL MODELING OF DISSOLUTION RATE PROFILES

5.1 INTRODUCTION

In dosage form development, *in vitro* dissolution results are used as a guide for formulation optimization and, where appropriate, they are used to compare the impact of different formulations on resultant drug release rates [218]. Dissolution specifications are often used for quality control purposes, to demonstrate consistency of product performance following large scale manufacture and long-term storage of dosage forms during stability testing [218].

A number of qualitative data interpretations and statistical comparisons of dissolution rate profiles have been reported [188, 218-221]. Although qualitative interpretations and statistical comparisons of dissolution profiles can provide some information about drug release profiles, mathematical modeling of dissolution rate data is recommended for the analysis of release kinetics, which can then be tested statistically [173]. The shape of a dissolution profile is largely affected by the properties of a drug, vehicle and manufacturing methods used to produce a dosage form [173]. Therefore finding a mathematical function that is valid for all kinetic variations and that is independent of the properties of a drug, the vehicle and the test apparatus used, can be problematic [173]. Hence, in order to describe drug release from different dosage forms, there is a need to identify or develop an adequate mathematical model or theory for specific types of drug delivery system [222].

There are a number of different methods that have been reported in the literature for the comparison of dissolution rate profiles and these can be classified into three main groups, *viz*. methods that are based on exploratory data analysis [220], model-independent methods [196, 200, 218, 223, 224] and model-dependent methods [223, 225, 226].

5.1.1 Exploratory data analysis methods

Exploratory data analysis methods have been used for the statistical comparison of the dissolution profiles [220]. Although exploratory data analysis methods are not currently endorsed by the FDA, this method of data evaluation can be used to compare dissolution profile curves in a graphical and numerical manner [220]. Graphical evaluation of dissolution profiles, is achieved by plotting the mean dissolution profile data for each formulation tested with error bars that extend to twice the standard error (SE) at each dissolution time point. The dissolution profiles for two formulations, *viz.* the test and reference products, may be considered to be significantly different to each other, if the error bars at each dissolution time point do not overlap [220]. The error bars at each dissolution time point are considered to be an approximate 95% confidence interval, therefore, if the confidence interval for the two formulations at a given time point does not overlap, then the mean dissolution profiles at that particular time point may be considered to be significantly different from each other [220].

The data may also be summarized numerically, to complement the graphical illustration method, by presenting the mean and standard deviation (SD) of the dissolution data at each dissolution time point for the test and reference formulations [220]. The dissolution data presented in a numerical manner may be considered to differ significantly from each other if the 95% confidence interval for the mean difference at a specific dissolution time point does not contain zero. Differences that exist at a specific dissolution time point are considered to be significant at a 5% level. The exploratory data analysis method was applied for the comparison of dissolution rate profiles in these studies and the results of both the graphical and numerical methods are presented *vide infra* in § 5.2.1.

5.1.2 Model-independent methods

Model-independent data analysis techniques can be further divided into pair-wise or ratio test comparative methods. A simple model-independent approach that uses a difference factor (f_1) and a similarity factor (f_2) for the comparison of dissolution profiles has been described by Moore and Flanner [196]. These factors are also known as fit factors and they are calculated using Equations 5.1 and 5.2 respectively.

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100\% \qquad \text{Equation 5.1}$$

$$f_2 = 50 \log_{10} \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} w_t (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \qquad \text{Equation 5.2}$$

where

n = the number of dissolution time points

R_t = percent of the reference product dissolved at time t

T_t = percent of the test product dissolved at time t and

w_t = an optional weight factor, usually set at 1

The difference factor, f_1, measures the percent error between two dissolution curves and the percent error is equal to zero when the dissolution profiles of a test and a reference product are identical. The value of f_1 increases proportionally as the two curves being compared become dissimilar [196]. The similarity factor, f_2, is a logarithmic transformation of the sum of the squared error of differences between a test and a reference product and fits the results between 0 and 100. The f_2 value is 100 when the test and reference dissolution profiles are identical and approaches zero as they become dissimilar [196].

Both factors have been endorsed by the FDA and are recommended for use in several FDA Guidance documents, including the Guidance on Immediate Release Solid Oral Dosage forms [197] and that for Scale-up and Postapproval change [198]. Resultant f_1 values of < 15 and f_2 values > 50 < 100 are indicative of equivalence between two dissolution profiles [196]. Shah *et al,* [221] has proposed that the number of sample points be limited to not more than one, once any of the products has released more than 85% of its label claim, due to the reduced sensitivity of these factors to be able to discriminate between measurements, after 85% dissolution has occurred.

The main advantages of the use of the f_1 and f_2 difference and similarity factors are that they are easy to compute and they provide a single number for the determination of the closeness between two resultant dissolution profiles [220]. However, there are limitations to the use of

this method that could affect the outcome of data analysis and both the f_1 and f_2 values are sensitive to the number of dissolution time points used. The FDA has recommended that model-independent methods are most suitable for dissolution profile comparison when three to four or more dissolution time points, excluding zero, are available for computing the factors [197]. Measurement of the f_1 or f_2 factors for a pair of dissolution profiles are often based on combining the difference between the curves at all time points into one measurement, and such measurements are estimated by substituting sample means for the actual means [221].

Dissolution data that have been correlated at specific sample time points are in fact estimates, which are complicated in that the variation of the estimates are difficult to calculate and the estimates themselves may be biased [220, 221]. The f_2 factor is insensitive to the shape of dissolution curves and does not take into account information about, for example, unequal spacing between sample time points [224]. It is therefore impossible to determine the frequency and prevalence of false positive or false negative rates of decision for approval of drug products based on these factors [224, 225].

Gohel and Panchal [200] have proposed a new model-independent mathematical approach for dissolution profile comparison, using a similarity factor, S_d that is calculated using Equation 5.3.

$$S_d = \frac{\sum_{t=1}^{n-1} \left| \log\left(\frac{AUC_{Rt}}{AUC_{Tt}} \right) \right|}{n-1} \qquad \text{Equation 5.3}$$

where

n = the number of data points collected during an *in vitro* dissolution test

AUC_{Rt} = area under the dissolution curve of the reference product at time = t

AUC_{Tt} = area under the dissolution curve of the test product at time = t

The primary advantage of the similarity factor S_d, over the similarity factor, f_2 is the simplicity and flexibility of determination of S_d. The data to be used for the determination of the S_d factor can be expressed as either an amount of drug dissolved or as a percentage drug

dissolved [200, 227]. For a test and reference formulation to be considered similar, the S_d value should be close to or equal to zero [200, 227].

Ratio tests determine the relationship between parameters obtained from the assessment of drug release from a reference and test formulation at a particular time point. The analysis can be extended from a simple ratio of percentage drug dissolved ($t_{x\%}$), to a ratio of area under the dissolution curve, which is also known as dissolution efficiency (DE) or even to a ratio of mean dissolution times (MDT) [218, 225, 228, 229].

The $t_{x\%}$ parameter corresponds to the time taken for the release of a pre-determined percentage of drug (X%) at a sample time (t), which corresponds to the amount of drug dissolved at that time [225]. The official pharmacopoeias often use this parameter as an acceptance limit for dissolution testing, *e.g*. the acceptance limit for *in vitro* dissolution testing of azithromycin capsules requires that at t_{45min}, ≥ 75% of the labeled amount in a capsule must have dissolved [10].

The MDT parameter is calculated using Equation 5.4 and is the sum of the individual times during which a specific fraction of the total dose is released [229]. It has been reported that the application of MDT provides a more accurate representation of drug release rates compared to the $t_{x\%}$ approach [196].

$$MDT = \sum_{i=1}^{n} \hat{t}i \frac{M_t}{M_\infty} \qquad \text{Equation 5.4}$$

where

n = the number of dissolution samples

i = the sample number

$\hat{t}i$ = the time at the midpoint between t_i and $t_{i\text{-}1}$ and is calculated with the expression $(t_i + t_{i\text{-}1})/ 2)$

M_t = the fraction of dose released at time t_i

M_∞ = corresponds to the loading dose in the dosage form

The concept of dissolution efficiency (DE) of a pharmaceutical dosage form has been proposed by Khan [228] and is defined as the area under the dissolution curve up to a certain time, t. The DE is expressed as a percentage of the area of a rectangle described by 100% dissolution in the same time, and can be calculated using Equation 5.5.

$$DE = \frac{\int_0^t y.dt}{y_{100} \cdot t} \times 100\% \qquad \text{Equation 5.5}$$

where

y = the percentage drug dissolved at time t

The ratio test model-independent methods, in particular MDT and DE, have been used for the study of drug release profiles from lipophilic and hydrophilic suppositories [118, 181, 187, 188].

For these studies, however, the similarity factor, f_2, was calculated for the comparison of dissolution profiles, as it is recommended by the FDA [197] and other regulatory agencies [198, 199] and the difference factor, f_1, was used as a supplementary parameter. In addition, the S_d factor was also calculated for each comparison and compared to the f_2 factor to determine whether or not a relationship between these two parameters can be established to describe dissolution curve similarity. The S_d values were calculated using AUC_{Rt} and AUC_{Tt} values that had been determined by use of the trapezoidal rule [218].

5.1.3 Model-dependent methods

Model-dependent approaches for data analysis require that model specifications, characterised by suitable mathematical functions, in order to describe the dissolution data, are established [223, 226]. Once a mathematical function is selected, the dissolution profiles are evaluated in terms of the resultant model parameters [226]. Dissolution profiles are generated using a number of time points, until either 80% of the drug has been released or the dissolution profile reaches an asymptote [220]. The use of model-dependent approaches has been recommended for the evaluation of dissolution rate profiles, consisting of at least four or more dissolution data points and excluding zero [226]. The model-dependent approach could

therefore not be used to evaluate dissolution data from PEG formulations due to the fact that 80% of the drug from these products was released in less than 30minutes resulting in too few data points being available to perform data modeling.

Mathematical models have been used extensively for the parametric representation of drug release kinetics from suppository formulations. Models that have been used include, the Zero order [117, 127, 195, 209, 230], First order [117, 127, 178, 195, 209, 203], Higuchi [117, 157, 195, 209, 230], Korsmeyer-Peppas [192] and Weibull models [127, 173]. Mathematical models that have been reported in the literature for the characterisation of drug release from suppository formulations, *viz*. Zero order, First order, Korsmeyer-Peppas and Higuchi model, were used in these studies to evaluate the release kinetics of AZI from fatty base suppository formulations.

5.1.3.1 Zero order model

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the same amount of drug per unit of time in order to archive a prolonged pharmacological action, can be represented by a zero order release kinetic model [225]. The mathematical expression of this model is shown in Equation 5.6.

$$Q_1 = Q_0 + K_0 t \qquad \text{Equation 5.6}$$

where

Q_t = the amount of drug dissolved in time = t

Q_o = the initial amount of drug in solution

K_o = zero order release rate constant

Ermiş *et al,* [195] have reported that the zero order release kinetic process from systems such as water-soluble suppository formulations containing polyethylene glycol, in which the drug is released in a controlled manner, is independent of drug concentration. This model was applied to the evaluation of ketoprofen release from controlled release systems containing hydroxypropyl methylcellulose phthalate in PEG suppository bases. A similar phenomenon was observed by Assasy [230] and Samy [209], when evaluating the release kinetics of flurbiprofen and tenoxicam respectively, in systems containing PEG suppository bases. It was

observed that as the dissolution medium comes into contact with a suppository containing PEG as a component, the dissolution medium dissolves the base and a nearly equal amount of drug simultaneously and the same amount of drug is released per unit time [209, 230] and drug release is said to be zero-order.

5.1.3.2 First order model

The application of a first order kinetic model to dissolution test results in which the experiments were performed under sink conditions has been reported [231]. The first order release model can be represented mathematically using Equation 5.7.

$$\ln Q_t = \ln Q_0 + K_1 t \qquad \text{Equation 5.7}$$

where

Q_t = the amount of drug released in time = t

Q_0 = the initial amount of drug in solution

K_1 = the first order release rate constant

Pharmaceutical dosage forms, from which drug release is adequately described by this model, include those that have been manufactured using water-soluble drugs in porous matrices so that drug is released in a manner that is proportional to the amount of drug remaining in the matrix and the amount of drug released per unit time diminishes as dissolution continues [225]. In general, the model describes drug release under ideal or sink conditions from systems in which the release rate is concentration dependent [231]. A study done by Assasy *et al,* [230] revealed that the release of flurbiprofen, a non-steroidal antinflammatory agent, from lipophilic suppository bases tends to follow first order kinetics.

5.1.3.3 The Higuchi model

Higuchi [232, 233] described the rate of release of both poorly water soluble and water-soluble drugs from semisolid and/or solid matrices as a square root of time-dependent process that was based on Fickian diffusion. The Higuchi model can be simplified to the

mathematical expression shown in Equation 5.8 and this relationship is generally known as 'the modified Higuchi model' [225].

$$Q_t = K_H t^{0.5} \qquad \text{Equation 5.8}$$

where

Q_t = the amount of drug released in time = t

K_H = Higuchi dissolution rate constant

This modified Higuchi relationship has been used to describe drug release from various types of modified release pharmaceutical dosage forms [225] and for lipophilic suppository formulations containing the sparingly soluble drug acetaminophen [157] and hydrophilic suppository formulations containing the water-soluble drug chloroquine phosphate [117].

5.1.3.4 The Korsmeyer-Peppas model

Korsmeyer *et al,* [234] developed a simple, semi-empirical model that can be used to analyse dissolution data from controlled release devices that have been manufactured as polymeric based drug delivery systems. The model is able to predict the fractional release of a drug and reveals that drug release is related to time in an exponential manner. The model is described by Equation 5.9 and is depicted in a log-transformed relationship in Equation 5.10.

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{Equation 5.9}$$

$$\log\left(\frac{M_t}{M_\infty}\right) = \log k + n\log(t) \qquad \text{Equation 5.10}$$

where

M_t/M_∞ = the fraction of drug released at time = t

k = a kinetic constant incorporating the structural and geometric characteristics of the dosage form

n = the release exponent and is indicative of the mechanism of drug release

The numerical value of the release exponent, *n,* is characteristic of the mechanism of diffusional release [234, 235] from delivery system, for which drug release data have been fitted to the model using the relationship defined in Equation 5.9. Peppas [235] used the *n* value to characterise different release mechanisms from non-eroding polymeric slabs and the data are summarised in Table 5.1.

Table 5.1: Interpretation of diffusion release mechanisms from non-eroding polymeric dosage forms using the value of the release exponent, *n*

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 (non-Fickian) transport	t^{n-1}
1.0	Case-II transport	Zero order release
$n > 1.0$	Super-Case II transport	t^{n-1}

The release exponent, *n,* for delivery systems in which drug release is primarily controlled by Fickian diffusion is 0.5 and for systems in which non-Fickian transport occurs, *n* will lie between 0.5 and 1.0. Furthermore, drug release from polymeric slabs that is independent of time and approaches zero order release is known as Case II transport. Drug release from devices of different shape, such as a cylinder or sphere, reveal Case II transport as *n* approaches 1.0 and in such cases, the release exponent $n = 1.0$ is indicative of non-Fickian transport [235, 236]. Moreover, in cases in which drug transport is best described by a value $n > 1.0$, transport mechanisms are referred to as super Case II transport. Clearly, the value of *n* can and does change with changes in the geometric shape of a matrix. In the case of a cylinder, Fickian diffusion is predominant when $n = 0.45$ instead of 0.5, and for Case II transport when $n = 0.89$, instead of 1.0 [235].

A modified form of Equation 5.10 [237, 238] was developed to accommodate a lag time (*l*) at the beginning of drug release studies from pharmaceutical dosage forms. The modified form of this relationship is depicted in a linear form as Equation 5.11 and in a log-transformed form as Equation 5.12.

$$\frac{M_{(t-l)}}{M_{\infty}} = k(t-l)^{n} \qquad \text{Equation 5.11}$$

$$\log\left(\frac{M_{(t-l)}}{M_{\infty}}\right) = \log k + n\log(t-l) \qquad \text{Equation 5.12}$$

where

M_t/M_∞ = the fraction of drug released at time = t

k = a kinetic constant incorporating the structural and geometric characteristics of the dosage form

n = the release exponent and is indicative of the mechanism of drug release

l = lag time

The mathematical relationship depicted in Equations 5.11 and 5.12 can be used to characterise only the initial stages of a drug release profile where $M_t/M_\infty < 0.6$ [225, 234, 235]. The Korsmeyer-Peppas model, also known as the "Power Law", has been used to characterise diclofenac sodium release from poloxomer based solid suppositories and the dissolution rate of the API was found to be independent of the time, *viz.* the exponent *n* approached 1.0 [192].

5.1.3.5 The Weibull model

A general empirically derived mathematical model that can be applied to all common types of dissolution profiles was reported by Langenbucher [239]. When this model is applied to drug dissolution or release from pharmaceutical dosage forms, the Weibull equation expresses the accumulated fraction, (Q_t/Q_∞), of the material in solution at time, t, as shown in Equation 5.13 [239].

$$\frac{Q_t}{Q_\infty} = 1 - \exp\left[-(t-T_i)^{b/a}\right] \qquad \text{Equation 5.13}$$

Rearrangement of Equation 5.13 into a logarithmic form results in Equation 5.14

$$\log\left[-\ln\left(1-\frac{Q_t}{Q_\infty}\right)\right] = b\log(t-T_i) - \log a \qquad \text{Equation 5.14}$$

where

Q_t = the amount of drug remaining in the pharmaceutical dosage form at time = t

Q_∞ = the maximal amount of drug that can be released at infinite time

a = the time scale of the process

T_i = the lag time before the actual onset of the dissolution process

b = the shape parameter that characterises the curve shape and is obtained from the slope of the line

It can be seen from Equation 5.14 that a linear relationship can be obtained from a log-log plot of –ln (1-Qt/Q∞) versus time t, and the shape parameter, *b*, can be obtained from the slope of the line and the scale parameter, *a*, is estimated from the ordinate value (1/*a*) at time t = 1 [225, 239]. The scale parameter '*a*' can be replaced by a more informative time, T_d, that represents the time interval necessary for 63.2% of the drug present in a dosage form to be dissolved or to be released [173, 239] and this implies that the model will be valid for dissolution profiles from which more than 63.2% of the drug has been released.

Drug release from lipophilic suppository formulations is often accompanied by a more or less long-lasting lag phase, that occurs as a result of the need for the base to melt prior to drug release and therefore the melting rate of the base is a factor that contributes to the lag time. Loth *et al,* [173] suggested that in order to use the Weibull model for mathematical modeling of the dissolution rate profiles of API from lipophilic suppository formulations, a necessary correction of the model functions is crucial to accommodate the experimental time that incorporates the lag time effects. The Weibull function for curve fitting was therefore modified such that t_{lag}, t', and n, were obtained from data fitting. The modified Weibull model was used to study the release kinetics of caffeine and theobromine from lipophilic suppositories manufactured using a variety of different grades of Witepsol® bases [173]. The modified Weibull model that was used for that analysis is depicted in Equation 5.15.

$$r_p = 100 - 100\exp\left\{-\left[\left(t \ldots t_{lag}\right)/t'\right]^n\right\}$$
$$r_p = 100\left\{1 - \exp\left[-\left(t_{corr}/t'\right)^n\right]\right\} \qquad \text{Equation 5.15}$$
$$r_p = 100\left\{1 - \exp\left[-\left(f_{lib} \cdot t_{corr}\right)^n\right]\right\}$$

where

r_p = release as the portion of the used amount of drug (%) at time = t

t_{lag} = lag time

t' = time at which 63.2% of the drug has been liberated

$t_{corr} = t - t_{lag}$, corrected experimental time

$f_{lib} = 1/t'$, liberation or release rate coefficient

n = curve parameter

The authors were able to correlate the effect of particle size distribution of an API and the base from which the suppository was manufactured, on drug release rates [173]. By use of the modified Weibull function, it was concluded that the vehicle influence on drug release rates is a function of interactions of components of hard fat bases with the surfaces of drug particles, with the result that there is competition for wetting by the hydrophilic dissolution phase. Furthermore, the structure of the sediment and displacement of the base from inter-particulate spaces, by the dissolution medium, depends on the rheological properties of the vehicle melt, such that the particle size distribution of the drug exerts an influence on the vehicle, with a resultant effect on release rates [173]. The release rates of caffeine and theobromine were therefore found to be time dependent.

As the Weibull function is an empirical model that has not been deducted from any kinetic foundation, it has some deficiencies that have been the subject of criticism such as that there is no single parameter related to the intrinsic dissolution rate of the drug and that it is of limited use for establishing *in vitro/in vivo* correlations [225]. This model was not applied to the assessment of AZI dissolution profiles in these studies since all of the fatty base suppositories, except for batch AZI-17, released less than 63.2% of the total amount of the drug per 1-gram suppository over the 480-minute dissolution test period.

Model-independent methods make no assumptions regarding the shape of dissolution curves, whereas model-dependent methods involve the use of defined mathematical equations in which parameters defining the shape of a profile are optimised [218, 226]. It has been recommended that, where the shape of the dissolution profile is important, it is necessary to describe the profile by at least two parameters, whereby one or more of these describes the shape of the profile and the other is indicative of the rate of dissolution [218].

5.1.4 Selection of the best-fit mathematical model

The correlation coefficient (R) [117], coefficient of determination (R^2) [223, 240], sum of squared of residuals (SSR) [223, 240], the F-ratio probability test [227] and the adjusted coefficient of determination ($R^2_{adjusted}$) [225] are commonly used as drug-release best fitting model selection criteria. It has been recommended that, when comparing models with different numbers of parameters, that the $R^2_{adjusted}$ is a more meaningful criterion for the selection the best fit, compared to the coefficient of determination R^2 [225]. The R^2 value increases or remains constant when adding new model parameters, whereas $R^2_{adjusted}$ may decrease, thus giving an indication whether or not the new parameter improves the model or may lead to over-fitting of data [225].

In order to determine the best fit model for the evaluation of AZI release from suppositories manufactured in these studies, the $R^2_{adjusted}$ parameter was adopted as the selection criterion of choice. Models with the highest $R^2_{adjusted}$ value were considered the best fit for the data evaluated. A value for $R^2_{adjusted} > 0.950$ was considered acceptable for the purposes of comparison of modeling dissolution profiles generated in these studies.

The $R^2_{adjusted}$ value was calculated following the fitting of AZI dissolution rate data to different models using Equation 5.16.

$$R^2_{adjusted} = 1 - \frac{(n-1)}{(n-p)}\left(1 - R^2\right) \qquad \text{Equation 5.16}$$

where

n = number of dissolution data points

p = number of parameters in the model

R^2 = coefficient of determination

5.2 RESULTS AND DISCUSSION

5.2.1 Exploratory data analysis

The exploratory data method of analysis was used for the comparison of drug release profiles from formulations in which PEG had been used as the base, *viz.* batches AZI-01 and AZI-02. Batch AZI-01 was used as the reference product and batch AZI-02 as the test product for the purposes of this evaluation. Statistical differences were assessed at a 95% confidence level. The same dissolution rate data were used for both the graphical and numerical data presentation, shown in Figure 5.1 and Table 5.2 respectively.

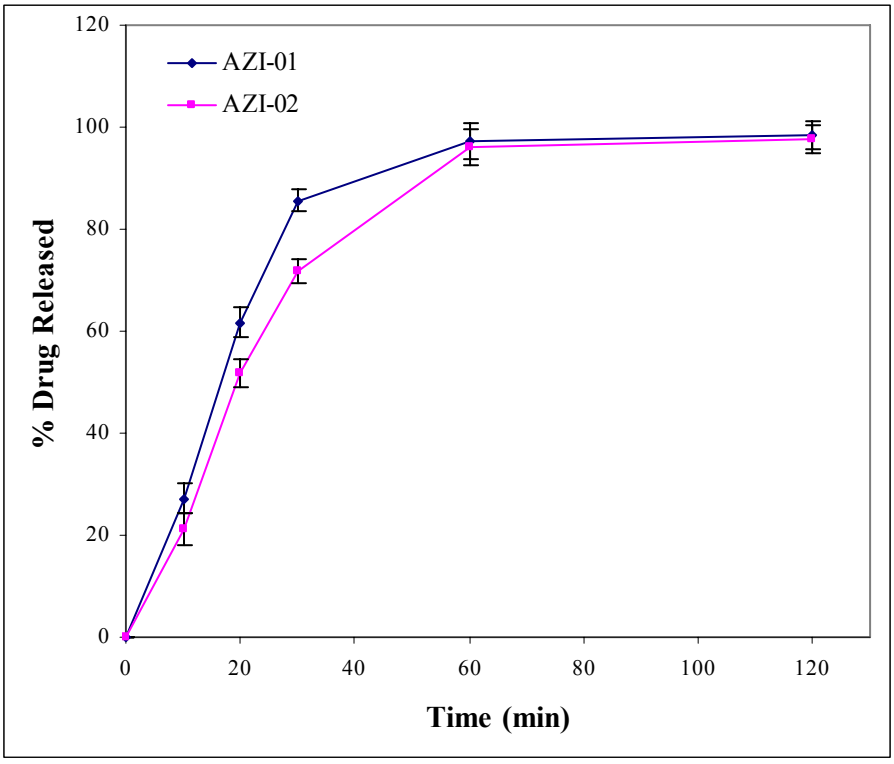


Figure 5.1: Statistical comparisons of the mean dissolution profiles (n=6) of the reference (AZI-01) and test (AZI-02) AZI formulations. Note: Error bars are extended to twice the standard errors on either side of the mean at each dissolution time point for comparative purposes.

Table 5.2: Summary statistics for percentage drug released for the Test (AZI-02) and Reference (AZI-01) formulations

Time (min)	Reference % dissolved mean ± (SD) (n=6)	Test mean % dissolved mean ± (SD) (n=6)	Difference (Reference-Test)	95% Confidence interval for difference LL, UL
0	0 (0)	0 (0)	0	-3.15, 3.154
10	27.25 (1.16)	21.10 (1.36)	-6.15	-9.306, -3.00
20	61.70 (1.47)	51.81 (1.27)	-9.90	-13.05,-6.74
30	85.62 (1.43)	71.76 (1.16)	-13.86	-17.01, -10.70
60	97.39 (1.48)	95.98 (1.58)	-1.40	-4.56, 1.75
120	98.33 (0.70)	97.78 (1.36)	-0.55	-3.70, 2.61

The results presented in Figure 5.1 reveal that there are significant differences between the mean amount released from the reference and test formulations at specific time points, which are in agreement with those results presented in the numerical summary listed in Table 5.2. The mean amount dissolved from batch AZI-02 at times 10, 20, and 30 minutes, were significantly lower when compared to those observed for Batch AZI-01 at the same times. As discussed in § 4.3.1, the decrease in AZI release was more than likely due to the incorporation of a PEG of a high molecular weight, which has a high melting range that will slow the dissolution rate of AZI from batch AZI-02 as compared to batch AZI-01, resulting in slower release of AZI from this product. The error bars, as seen in Figure 5.1, at dissolution times of 60 and 120 minutes clearly overlapped for both products and as is evident in the 95% confidence interval for the differences between the profiles at the same times, as shown in Table 5.2, zero is included in the confidence interval, indicating that there was no difference at a 95% level between these two curves. Therefore the dissolution data for both PEG batches may be considered similar at these times.

Despite the simplicity and ease of use of this method of data analysis, it is not considered as comprehensive due to several limitations [220], for example that it is difficult to conclude definitively that the dissolution profiles for the two formulations being compared are significantly different, as the error bars overlap only at some times and not others, as shown in Figure 5.1. The same challenges are evident when evaluating the results of numerical data analysis for the 95% confidence interval for the difference between the reference and test formulations. These data reveal that zero is included in the confidence interval at some time points and not at others, as seen in the data presented in Table 5.2 making it difficult to draw

comprehensive conclusions about the entire dissolution rate profiles. Nevertheless, the application of this method may be useful as a first step in obtaining an improved understanding of dissolution data generated in testing, when specific time points in dissolution profiles are to be compared and therefore its use can be recommended.

5.2.2 Difference and Similarity Factors (f_1 and f_2 fit factors)

The *in vitro* dissolution profiles of AZI from all fatty base suppositories with the addition of additives were compared to formulations manufactured with AZI and base only, by evaluating the f_1 and f_2 difference and similarity factors. Formulations that contained additives were treated as test formulations, whereas those manufactured with AZI and base only were used as the reference formulations. For PEG formulations, the dissolution profile of batch AZI-01 was considered the reference product that was compared to that of batch AZI-02 that was considered the test product. In addition, the Gohel and Panchal [200] similarity factor S_d was calculated for each of the comparisons undertaken with the fit factor, using AUC_{Rt} and AUC_{Tt} values that had been determined by the trapezoidal rule. The calculated fit factors f_1 and f_2 and the similarity factor S_d for all comparisons are summarised in Table 5.3.

Table 5.3: Statistical comparisons of formulations manufactured with AZI and base only (reference product) and formulations manufactured with the addition of AZI, base and an additive (test product) for all suppositories

Batches compared	Comparison	f_1	f_2	S_d
AZI-01 vs AZI-02	PEG formulations	11.5	52.0	0.0074
AZI-03 vs AZI-11	W-H15 vs W-H15/ 0.5%Tw80	8.1	81.8	0.0047
AZI-03 vs AZI-12	W-H15 vs W-H15/ 1.0%Tw80	15.9	70.6	0.0121
AZI-03 vs AZI-13	W-H15 vs W-H15/ 2.0%Tw80	13.7	72.7	0.0094
AZI-03 vs AZI-14	W-H15 vs W-H15/ 4.0%Tw80	11.4	77.4	0.0153
AZI-11 vs AZI-13	W-H15/ 0.5%Tw80 vs W-H15/ 2.0%Tw80	9.8	79.9	0.0047
AZI-13 vs AZI-14	W-H15/2.0%Tw80 vs W-H15/ 4.0%Tw80	9.3	78.3	0.0060
AZI-13 vs AZI-32	W-H15/ 2.0%Tw80 vs W-H15/ 2.0%Tw20	5.5	89.5	0.0004
AZI-04 vs AZI-15	W-W35 vs W-W35/ 0.5%Tw80	91.1	41.7	0.0398
AZI-04 vs AZI-16	W-W35 vs W-W35/ 1.0%Tw80	>100	36.1	0.0474
AZI-04 vs AZI-17	W-W35 vs W-W35/ 2.0%Tw80	>100	31.2	0.0557
AZI-04 vs AZI-18	W-W35 vs W-W35/ 4.0%Tw80	>100	32.2	0.0537
AZI-15 vs AZI-17	W-W35/ 0.5%Tw80 vs W-W35/ 2.0%Tw80	29.9	51.2	0.0153
AZI-17 vs AZI-18	W-W35/ 2.0%Tw80 vs W-W35/ 4.0%Tw80	4.6	76.8	0.0020
AZI-17 vs AZI-31	W-W35/ 2.0%Tw80 vs W-W35/ 2.0%Tw20	17.5	57.1	0.0110
AZI-09 vs AZI-19	S-AM vs S-AM/ 0.5%Tw80	31.8	75.3	0.0140
AZI-09 vs AZI-20	S-AM vs S-AM/ 1.0%Tw80	21.9	81.4	0.0116
AZI-09 vs AZI-21	S-AM vs S-AM/ 2.0%Tw80	13.5	87.5	0.0088
AZI-09 vs AZI-22	S-AM vs S-AM/ 4.0%Tw80	17.1	86.1	0.0115
AZI-19 vs AZI-21	S-AM/ 0.5%Tw80 vs S-AM/ 2.0%Tw80	26.9	84.1	0.0152
AZI-21 vs AZI-22	S-AM/ 2.0%Tw80 vs S-AM/ 4.0%Tw80	6.30	97.1	0.0027
AZI-21 vs AZI-33	S-AM/ 2.0%Tw80 vs S-AM/ 2.0%Tw20	15.0	88.8	0.0110

Table 5.3: Continued

Batches compared	Comparison	f_1	f_2	S_d
AZI-06 vs AZI-23	S-NA1 25 vs S-NA1 25/ 0.5%Tw80	68.5	50.0	0.0326
AZI-06 vs AZI-24	S-NA1 25 vs S-NA1 25/ 1.0%Tw80	65.8	49.0	0.0288
AZI-06 vs AZI-25	S-NA1 25 vs S-NA1 25/ 2.0%Tw80	54.0	52.0	0.0358
AZI-06 vs AZI-26	S-NA1 25 vs S-NA1 25/ 4.0%Tw80	76.6	47.0	0.0267
AZI-23 vs AZI-25	S-NA1 25/ 0.5%Tw80 vs S-NA1 25/ 2.0%Tw80	5.3	85.8	0.0032
AZI-25 vs AZI-26	S-NA1 25/ 2.0%Tw80 vs S-NA1 25/ 4.0%Tw80	13.4	71.6	0.0091
AZI-10 vs AZI-27	S-NAS 50 vs S-NAS 50/ 0.5%Tw80	>100	33.4	0.0863
AZI-10 vs AZI-28	S-NAS 50 vs S-NAS 50/ 1.0%Tw80	>100	31.5	0.0893
AZI-10 vs AZI-29	S-NAS 50 vs S-NAS 50/ 2.0%Tw80	>100	33.3	0.0855
AZI-10 vs AZI-30	S-NAS 50 vs S-NAS 50/ 4.0%Tw80	>100	34.0	0.0847
AZI-27 vs AZI-29	S-NAS 50/ 0.5%Tw80 vs S-NAS 50/ 2.0%Tw80	4.6	86.4	0.0008
AZI-29 vs AZI-30	S-NAS 50/ 2.0%Tw80 vs S-NAS 50/ 4.0%Tw80	4.0	85.4	0.0008
AZI-04 vs AZI-34*	W-W35 vs W-W35/ 2.0%Urea	20.5	71.8	0.0140
AZI-04 vs AZI-35**	W-W35 vs W-W35/ 2.0%Urea	22.3	66.1	0.0163
AZI-04 vs AZI-36*	W-W35 vs W-W35/ 2.0%PVP	7.4	88.3	0.0051
AZI-04 vs AZI-37**	W-W35 vs W-W35/ 2.0%PVP	2.9	97.5	0.0006

*Physical mixture formulations, **Solid dispersion formulations

Shaded areas represent values that fall outside the specification

Due to the sensitivity of the f_1 and f_2 fit factors to measurements that exceed 85% dissolution [221], the comparison of PEG formulations were calculated for data generated for only 60 minutes following the commencement of dissolution. The PEG formulations, batches AZI-01 and AZI-02, were found to be comparable up to 60 minutes, with a resultant f_1 value of 11.5 and f_2 value of 52.0. Although the exploratory data analysis method described in § 5.2.1 revealed that these profiles are similar only at dissolution times of 60 and 120min. This is because the measurements of f_1 or f_2 of the profiles are often based on combining the differences, at all time points, into one measurement [221] and it is clear that the dissolution profile of the test product (AZI-02) was closer to that of the reference product (AZI-01) at the early time points and again at later time points, as shown in Figure 5.1. Therefore overall similarity between the two curves can be declared.

Suppositories in which a surfactant was incorporated into a fatty base of intermediate hydroxyl value, *viz*. W-H15, and low hydroxyl value, *viz.* S-AM, were found to be comparable and the resultant f_2 values ranged between 70.6-81.8 and 75.3-86.1 for these formulations respectively, when compared to formulations in which AZI was included in suppositories manufactured using W-H15 and S-AM bases without additional additives. Whilst the f_2 values for the bases with high hydroxyl values, *viz.* W-W35 and S-NAS 50, ranged between 31.2-41.7 and 31.5-34.0 respectively, suggesting that differences between the bases with additives and without additives do exist, as seen from the data in Table 5.3.

The f_2 values for the comparison of batches manufactured with S-NA1 25 with the addition of different concentrations of surfactant to that of the base only revealed similarity in some cases, *viz.* batches AZI-23 and AZI-25, despite the base having a high hydroxyl value. However, the trends in the drug release characteristics are clearly evident, such that there is a difference between the profiles, as shown in Figure 4.9, § 4.3.2.1. In addition, when considering the f_1 values determined for the same base type, S-NA1 25, there is a difference as all the f_1 values were greater than 15. However, the FDA [198] and Human Medicine Evaluation Unit of The European Agency for the Evaluation of Medicinal Products (EMEA) [199] recommend the use of only f_2 for the statistical comparison of dissolution profiles. Therefore, according to f_2 values, dissolution profiles of batches manufactured with S-NA1 25 base with the addition of 1.0% and 4.0% w/w Tween 80, *viz*. AZI-24 and AZI-26 respectively, may be considered to be similar to that of batch AZI-06 containing the base only. Whilst dissolution profiles of batches AZI-23 and AZI-25, containing 0.5% and

2.0%w/w Tween® 80 respectively, where considered to be dissimilar to that of batch AZI-06 containing the base only. However, for the purposes of these studies, both factors must comply with the specifications in order for dissolution profiles to be declared similar or different.

An increase in the Tween® 80 content to 2% and 4% w/w in formulations containing base type W-H15, W-35, S-NA1 25, S-AM and S-NAS 50, did not improve the rate and extent of AZI release, compared to the formulations that had been produced using 0.5% w/w Tween® 80, as all f_2 values were > 50, as listed in Table 5.3. Formulations of base type W-H15, W-W35 and S-AM in which Tween 20 was incorporated at a 2% w/w level were also found to be comparable to formulations of the same base type in which Tween 80 had been incorporated at a 2% w/w level, as listed in Table 5.3.

The incorporation of urea or PVP into formulations in which W-W35 was used as the base, as a physical mixture or as a solid dispersion with AZI, did not appear to change the dissolution characteristics of AZI, compared to the formulation in which AZI and the base only had been used, and the resultant f_2 values ranged between 66.1 and 97.5 for these comparisons.

It can be clearly seen from the data listed in Table 5.3 that there seems to be a relationship between the f_2 values and the Gohel and Panchal similarity factor S_d [200, 227]. Batches that showed values of f_2 greater than 50 resulted in S_d values of ≤ 0.015, whereas for those comparisons in which the curves being compared were shown to be different on the basis of f_2 values, S_d values of ≥ 0.03 were observed. However, suppositories manufactured with S-NA1 25 base and Tween® 80, *viz*. batches AZI-23 – AZI-26, showed S_d values of ≥ 0.03, when compared to a formulation with the base only, despite some of the formulations showing f_2 values of ≥ 50. Therefore, from a practical perspective, the selection of a limit to determine similarity based on S_d may not provide convincing results due to the variability of this parameter as observed in this study.

Gohel and Panchal [227] studied the release of diltiazem and observed that when the S_d value was close to zero, the dissolution profiles showed similarity and when the value approached unity, the dissolution profiles were dissimilar. For this study, it was observed that the S_d values approached 0.10 as the dissimilarity between the two dissolution profiles increased

and approached 0.0004 as the similarity between the dissolution profiles increased, when using the f_1 and f_2 factors for comparative purposes.

5.2.3 Mathematical modeling

In order to determine the mechanism by which AZI is released from suppositories manufactured using fatty bases, dissolution data were fitted to selected mathematical models previously described in § 5.1.3. The Korsmeyer-Peppas model was used to characterise drug release behavior from each of the products produced with fatty bases, in the absence and presence of additives. In order to assess drug release from these formulations, Equation 5.10 was used to model drug release data from dosage forms in which an experimental lag time was not observed. In addition, the modified Korsmeyer-Peppas model [237, 238], as described in Equations 5.10 and 5.12, was used to obtain a linear plot of the log of M_t/M_∞ *vs.* log (t), and the log of ($M_{(t-l)}/M_\infty$ *vs*. log of (t-l) respectively. The data were also fitted to the Higuchi, Zero and First order mathematical models to determine which model best described the release kinetics of AZI from these dosage forms.

5.2.4 Application of the Korsmeyer-Peppas model

The mechanism of drug liberation from suppositories manufactured using hard fats as the base is complex, since the physicochemical properties of the drug, drug-vehicle interactions and the vehicle properties can and often do affect the release process. An analysis of the fitting of experimental data to the Korsmeyer-Peppas model, as described in Equations 5.10 and 5.12, in addition to the interpretation of the corresponding release exponent values (n) were used to characterise and understand the mechanism by which AZI was released from these products.

Dissolution rate data generated by monitoring AZI release from formulations containing drug and base only were initially fitted to the Korsmeyer-Peppas model to assess the impact of base type on the resultant release exponent, kinetic constants and ultimately the mechanism of drug release from these products. The best-fit model parameters obtained following fitting of experimental data obtained from these formulations are listed in Table 5.4.

Table 5.4: Summary of Korsmeyer-Peppas best-fit parameters for batches AZI-03 – AZI-10

Batch #	Base type	M_t/M_∞ $t_{480\ min}$	n	k %/min^n	R^2
AZI-03	W-H15	0.39	0.6065	0.8698	0.9882
AZI-04	W-W35	0.30	0.5478	0.9736	0.9972
AZI-06	S-NA1 25	0.24	0.5235	0.9543	0.9973
AZI-07	S-NA15	0.21	0.4961	0.9993	0.9958
AZI-08	S-NA0	0.17	0.4173	1.1628	0.9688
AZI-09	S-AM	0.13	0.3552	1.0762	0.9851
AZI-10	S-NAS 50	0.12	0.4134	0.9998	0.9997

The release exponent *n* was found to be 0.6065 and 0.5478 for batches AZI-03 and AZI-04, indicating that the release mechanism from these dosage forms was controlled by non-Fickian diffusion, whereas the *n* value determined for batches AZI-06 and AZI-07 were found to be 0.5235 and 0.4961 respectively, suggesting that the release mechanism of AZI from these batches was predominantly controlled by Fickian diffusion as $n \approx 0.50$. The release mechanism elucidated for batches AZI-08, AZI-09 and AZI-10 was not able to be explained by the Korsmeyer-Peppas model [235], since the resultant *n* values ($n<0.5$) did not fall within the specified range. The inability to ascribe the mechanism of release to these batches of suppositories may in part be explained by the change in geometry of these dosage forms on melting, since *n* is affected by the change in shape of the matrix undergoing investigation.

The melting rate of a fatty suppository base is a precondition for drug liberation and is responsible for the lag time(s) observed. In addition, the change of the original shape of the suppositories as melting commences, is a contributing factor that can affect drug release. It was observed that as the suppository melted, it acquired the shape of the base of the basket in which it was placed during dissolution testing. These suppositories changed from a cone-like shape, in the solid state, to a flattened circular-disk shape after melting. Each of the fatty bases used in these studies had a different melting range with the fatty bases S-NA0, S-AM and S-NAS 50 having the highest range of between 35.0°C and 37.5°C, compared to the reported melting ranges for the W-H15, W-W35, S-NA1 25 and S-NA15 bases, which melt between 32.5°C and 35.0°C, as summarised in Table 3.3, § 3.3. Therefore the rate at which the base changes shape from the solid state to attain that of the basket, *viz.* the circular-disk shape, varies depending on the melting rate of the base. The *n* value is affected by the change in matrix geometry [235, 238] and therefore, the change in shape of a suppository mass could in part explain the variable results obtained for *n,* following fitting of the dissolution data to the Korsmeyer-Peppas model.

An *n* value of between 0.5 and 1.0 is usually indicative of more than one type of release phenomenon facilitating drug release and is termed anomalous transport kinetics. It is evident that some of the dissolution data could be evaluated and explained as having complex profiles with associated anomalous transport mechanisms controlling drug release [225]. Anomalous transport processes will undoubtedly play a significant role in describing drug release from suppository formulations, due to the complexity of the drug release process from suppositories, which involves a series of steps such as melting, drug partitioning and diffusion through molten base to the hydrophilic dissolution medium in which the tests are conducted [171, 173].

The kinetic constants (k) calculated from a linear plot of the log of M_t/M_∞ *vs*. log t, using Equation 5.10, or from a plot of log of $M_{(t-l)}/M_\infty$ *vs*. log (t-*l*), using Equation 5.12, are summarised in Table 5.4. Since the Korsmeyer-Peppas kinetic constant incorporates the structural and geometric characteristics of dosage forms, the change in matrix geometry, as implicated by the change in *n* value, affected the k value directly. It is clear that there is no direct relationship between the total percentage AZI released and the kinetic rate constant, since batches AZI-07 – AZI-10 had a high k and yet had a lower percentage AZI released compared to batches AZI-04 – AZI-06. This could be explained by the change in geometric characteristics of the dosage form as it melts. This implies that the melting rate of the formulations, which are affected by the melting rate of the particular base, affects the structural and geometric characteristics of the suppository formulations, which in turn affects the resultant *n* and k values.

The dissolution rate data for suppositories in which the formulation had been modified to include a surfactant were also fitted to the Korsmeyer-Peppas model in order to assess the impact of the addition of surfactant at different concentrations on the release mechanism, as well as the kinetic constant for these dosage forms. A plot of the release exponent derived from modeling of the experimental data versus surfactant concentration is depicted in Figure 5.2 and the best fit model parameters for these studies are summarised in Table 5.5.

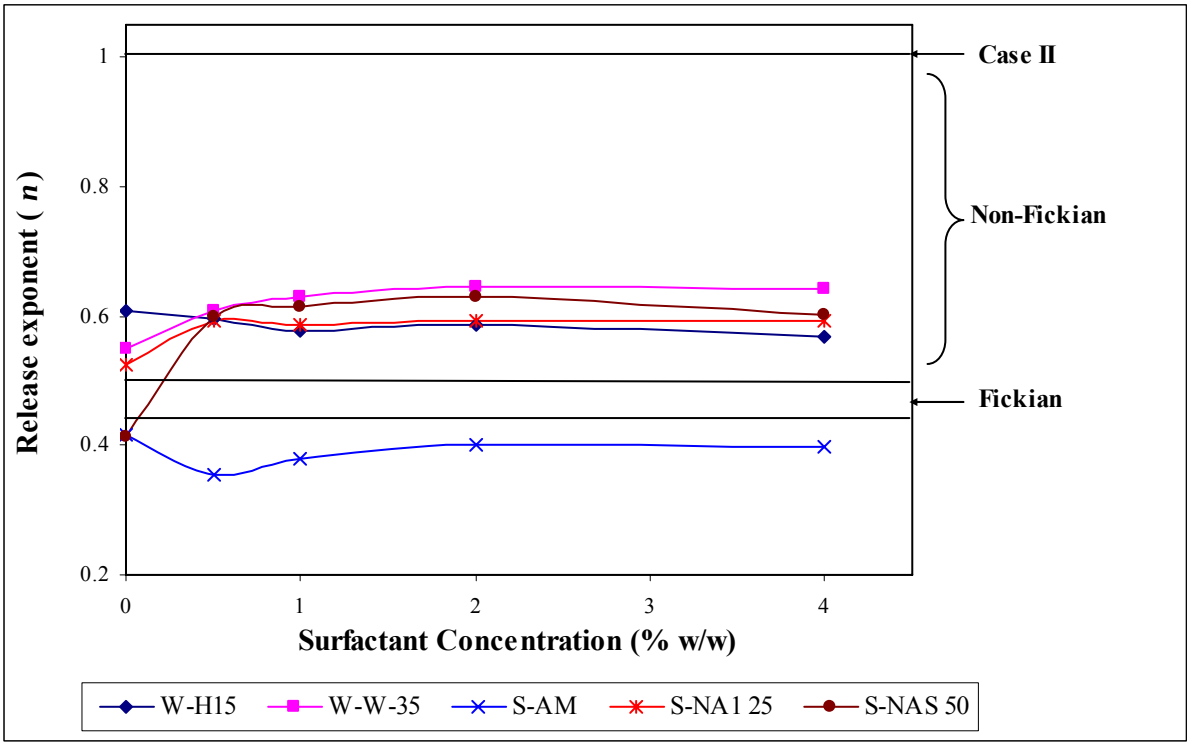


Figure 5.2: Effect of surfactant concentration on the release exponent of formulations in which W-H15, W-W35, S-AM, S-NA1 25 and S-NAS 50 were used as bases.

Table 5.5: Summary of the best-fit parameters for Batches AZI-03 - AZI-30 generated by fitting dissolution data to the Korsmeyer-Peppas model

Batch #	Batch composition	M_t/M_∞ $t_{480\ min}$	*n*	k %/min^n	R^2
AZI-03	W-H15	0.39	0.6065	0.8698	0.9882
AZI-11	W-H15/ 0.5%Tween 80	0.37	0.5961	0.8723	0.9890
AZI-12	W-H15/ 1.0%Tween 80	0.33	0.5777	0.8504	0.9820
AZI-13	W-H15/ 2.0%Tween 80	0.38	0.5869	0.8236	0.9746
AZI-14	W-H15/ 4.0%Tween 80	0.33	0.5685	0.8451	0.9806
AZI-32	W-H15/ 2.0%Tween 20	0.32	0.5926	0.8125	0.9751
AZI-04	W-W35	0.30	0.5478	0.9736	0.9972
AZI-15	W-W35/ 0.5%Tween 80	0.48	0.6069	1.3388	0.9553
AZI-16	W-W35/ 1.0%Tween 80	0.57	0.6305	1.3274	0.9601
AZI-17	W-W35/ 2.0%Tween 80	0.64	0.6454	1.4275	0.9422
AZI-18	W-W35/ 4.0%Tween 80	0.57	0.6407	1.4227	0.9434
AZI-31	W-W35/ 2.0%Tween 20	0.54	0.6228	1.3080	0.9613
AZI-09	S-AM	0.13	0.4173	1.1628	0.9688
AZI-19	S-AM/ 0.5%Tween 80	0.09	0.3552	1.0762	0.9851
AZI-20	S-AM/ 1.0%Tween 80	0.10	0.3778	1.0935	0.9771
AZI-21	S-AM/ 2.0%Tween 80	0.12	0.4007	1.0752	0.9888
AZI-22	S-AM/ 4.0%Tween 80	0.11	0.3992	1.0382	0.9970
AZI-33	S-AM/ 0.2%Tween 20	0.10	0.3672	1.0879	0.9840
AZI-06	S-NA1 25	0.24	0.5235	0.9543	0.9973
AZI-23	S-NA1 25/ 0.5%Tween 80	0.39	0.5918	1.0266	0.9993
AZI-24	S-NA1 25/ 1.0%Tween 80	0.42	0.5859	0.9752	0.9961
AZI-25	S-NA1 25/ 2.0%Tween 80	0.42	0.5940	1.0804	0.9956
ZI-26	S-NA1 25/ 4.0%Tween 80	0.42	0.5923	0.8870	0.9905
AZI-10	S-NAS 50	0.12	0.4127	0.9980	0.9997
AZI-27	S-NAS 50/ 0.5%Tween 80	0.43	0.6001	1.2440	0.9744
AZI-28	S-NAS 50/ 1.0%Tween 80	0.46	0.6140	1.1943	0.9836
AZI-29	S-NAS 50/ 2.0%Tween 80	0.44	0.6286	0.9851	0.9957
AZI-30	S-NAS 50/ 4.0%Tween 80	0.42	0.6012	1.1896	0.9836

It is clearly evident that the addition of surfactant had no effect on the mechanism of AZI release from formulations in which W-H15, *viz.* batches AZI-11 – AZI-14, and S-AM, *viz*. batches AZI-19 – AZI-22, were used as the bases of choice. A slight increase in the *n* value was observed for formulations in which W-W35, *viz*. batches AZI-15 – AZI-18, and S-NA1 25, *viz*. batches AZI-23 – AZI-26, were used, however, the release mechanism was unaltered and was considered to be non-Fickian diffusion controlled. The observed shift of the release exponent from $n < 0.5$, *i.e.* release is neither Fickian nor non-Fickian diffusion controlled, to $n > 0.50$, *viz*. non-Fickian release mechanisms predominate, was observed for the formulations manufactured using S-NAS 50 with added surfactant. However, as the surfactant content increased from 0.5 to 4% w/w, the calculated *n* value remained constant, indicating that the presence of surfactant did not in any way affect the mechanism of AZI release from these dosage forms.

Observations during dissolution testing of the suppositories containing surfactant revealed that as the suppositories melted, the molten fat escaped from the basket through the mesh and from between the basket and the basket holder, resulting in a change in shape of the molten base to small spherical globules. The change in shape, absence of lag time and increased release rate of AZI may have contributed to a shift of the release exponent for batches AZI-27 – AZI-30, although this was not the case for formulations in which W-W35, *viz.* batches AZI-15 – AZI-18 and batch AZI-32, and S-NA1 25, *viz*. batches AZI-23 – AZI-26, were used as the bases of choice. The change in shape was clearly observed for these batches of suppositories and there was a corresponding increase in the release rate of AZI from these dosage forms, yet the calculated *n* value remained constant.

The value of the kinetic constant increased when a surfactant was added to formulations in which W-W35 and S-NAS 50 were used as the carrier base, whereas the value of the kinetic constant for formulations in which W-H15, S-AM, and S-NA1 25 were used remained similar and these data are graphically represented in Figure 5.3. It was expected that the value for the kinetic constant would remain constant on addition of surfactant to the formulations with bases of intermediate hydroxyl value, *viz.* W-H15, and low hydroxyl value, *viz.* S-AM, since the percentage drug released was similar to that from bases without additives and the mechanism of release defined by the *n* value remained unchanged.

However, formulations in which S-NA1 25 was used as the base, *viz.* batches AZI-23 – AZI-26 revealed that there was an increase in the percentage AZI released, but the value of the kinetic constant was relatively stable. This further explains the influence of the change in geometric characteristics of dosage forms following the addition of surfactant, on the resultant values for k. Furthermore, an increase in the value of *n* from n = 0.52 for the formulation without the addition of surfactant (batch AZI-06) to n ≈ 0.60 for the formulations in which surfactant was included (batches AZI-23 – AZI-26) was observed, indicating that a change in release mechanism had been effected by use of surfactants in the formulation.

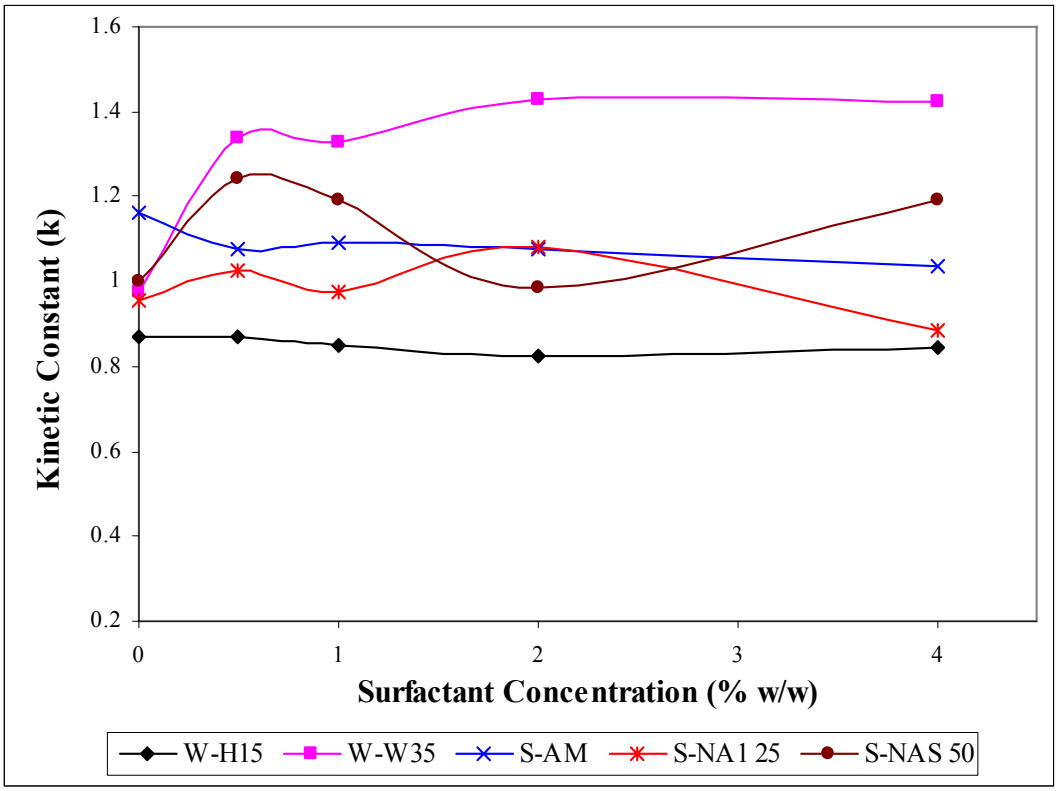


Figure 5.3: Effect of surfactant concentration on the kinetic constant (k) of formulations in which W-H15, W-W35, S-AM, S-NA1 25 and S-NAS 50 were used as bases.

The addition of urea to suppository bases as a physical mixture, *viz.* batch AZI-34, or as a solid dispersion with AZI, *viz.* batch AZI-35, resulted in values for *n* values of approximately 0.5, indicating that Fickian diffusion was the predominant mechanism controlling drug release from the modified formulations, when compared to the control formulation, batch AZI-04, which exhibited drug release that was controlled by non-Fickian release mechanisms. However, values for *n* that were estimated by modeling drug release data for formulations in which PVP had been added as a physical mixture or solid dispersion with AZI, *viz.* batches AZI-36 and AZI-37 respectively, were relatively constant, as shown in

Table 5.6. The kinetic constant, k was not glaringly different from those that were calculated when urea or PVP had been added to the bases, as compared to the control formulation, as shown in Table 5.6.

Table 5.6: Summary of Korsmeyer-Peppas best-fit parameters for batches AZI-04, AZI-34 and AZI- 37

Batch #	Batch composition	M_t/M_∞ $t_{480\ min}$	*n*	k %/min^n	R^2
AZI-04	W-W35	0.30	0.5478	0.9736	0.9972
AZI-34*	W-W35/ 2%Urea	0.24	0.5154	0.9072	0.9889
AZI-35**	W-W35/ 2%Urea	0.21	0.4887	1.0718	0.9934
AZI-36*	W-W35/ 2%PVP	0.30	0.5527	0.9140	0.9938
AZI-37**	W-W35/ 2%PVP	0.29	0.5479	0.9590	0.9972

*Physical mixture formulations, **Solid dispersion formulations

The complexity of the formulations manufactured and tested, in addition to the components that were used to modulate drug release, indicate that drug release is controlled by more than one type of release process, thus the effect of formulation composition and test methods on the rate and extent of drug release must be thoroughly investigated during formulation development studies of these types of dosage forms.

The interpretation of the results following the use of the Korsmeyer-Peppas model for the analysis of the mechanism of drug release from complex systems such as suppositories, has to be conducted with caution, since the assumptions under which the model was developed were based on a concentration independent drug diffusion coefficient [235]. The Korsmeyer-Peppas model has also been used for modeling drug release from specific types of different geometric matrix shapes, such as slabs, spheres and cylinders. Any violation of the assumptions inherent in the model, may lead to the reporting of the values of a release exponent that are not readily explained by this model. Such reports were observed for formulations AZI-08 – AZI-10, as the reported *n* values were less than 0.45. Peppas [235] reported that an *n* value of less than 0.5 may be the consequence of challenges and difficulties in the statistical analysis of dissolution data. Therefore, the results from fitting suppository dissolution data to the Korsmeyer-Peppas model should be interpreted with caution.

5.2.5 Application of other mathematical models

Mathematical models have been used extensively for the parametric representation of dissolution data from a variety of dosage forms [117, 127, 157, 173, 178, 209, 230]. To establish the kinetics of drug release more comprehensively, dissolution data generated during these studies were fitted to various drug release kinetic models, including the Higuchi, Zero and First order models. The shape of a dissolution curve, especially that describing drug release from lipophilic suppositories, is greatly affected by phases of the liberation process because the properties of the drug, vehicle and preparations under investigation affect the process differently [173]. One of the parameters that affects the liberation process of a drug from fatty suppositories is the melting rate of the lipophilic suppository bases, which can result in the observation of an experimental lag phase. Most of the dissolution curves showed a relatively long-lag phase and, since time is one of the parameters that are incorporated in the computation of dissolution data when using selected mathematical models, it may be appropriate to correct the experimental times to incorporate a lag time. Modified Higuchi, Zero and First order models in which the lag time can be taken into account have yet to be elucidated and are beyond the scope of this dissertation. Therefore, dissolution data were fitted to the aforementioned models with the inclusion and exclusion of the lag time in order to determine the extent of the effect of the experimental lag time on the resultant dissolution rate profiles.

The selection criterion for the best-fit model was based on the adjusted coefficient of determination, $R^2_{adjusted}$. The $R^2_{adjusted}$ value was used to compare the results of fitting data to kinetic models with different numbers of parameters, since it takes into account the inclusion in a model of a variable number of parameters that may lead to an inaccurate conclusion being drawn, as a result of over-fitting of experimental data if unadjusted values of R^2 were used to a determine which model best fitted the experimental data [191]. The results of fitting the dissolution data to selected mathematical models are summarized in Table 5.7.

Table 5.7: Resultant model parameters and descriptive statistics obtained following fitting of AZI dissolution data for batches AZI-03 – AZI-37 to selected mathematical models

Batch #	Zero order model				First order model				Higuchi model			
	With lag time		Without lag time		With lag time		Without lag time		With lag time		Without lag time	
	R^2	K_0	R^2	K_0	R^2	K_1	R^2	K_1	R^2	K_H	R^2	K_H
AZI-03	-	-	0.9458	0.0817	-	-	0.6133	0.0060	-	-	0.9745	1.8274
AZI-11	-	-	0.9572	0.0758	-	-	0.6171	0.0058	-	-	0.9774	1.7818
AZI-12	-	-	0.9597	0.0682	-	-	0.6417	0.0057	-	-	0.9733	1.5991
AZI-13	-	-	0.9475	0.0775	-	-	0.6891	0.0060	-	-	0.9480	1.7695
AZI-14	-	-	0.9658	0.0664	-	-	0.6614	0.0057	-	-	0.9669	1.5419
AZI-32	-	-	0.9604	0.0740	-	-	0.6586	0.0060	-	-	0.9670	1.7216
AZI-04	0.9675	0.0623	0.9605	0.0638	0.6658	0.0068	0.5873	0.0057	0.9474	1.4696	0.9925	1.7490
AZI-15	-	-	0.8389	0.0811	-	-	0.4325	0.0048	-	-	0.9752	2.0138
AZI-16	-	-	0.8729	0.0970	-	-	0.0048	0.0051	-	-	0.9772	2.366
AZI-17	-	-	0.8273	0.1040	-	-	0.4066	0.0050	-	-	0.9631	2.582
AZI-18	-	-	0.7578	0.0931	-	-	0.4000	0.0049	-	-	0.9535	2.387
AZI-31	-	-	0.8799	0.0936	-	-	0.4577	0.0051	-	-	0.9760	2.280
AZI-07	0.9409	0.0472	0.9026	0.0451	0.7670	0.0071	0.6266	0.0056	0.8948	1.0768	0.9632	1.3933
AZI-08	0.9352	0.0383	0.8924	0.0364	0.7833	0.0066	0.6532	0.0053	0.8961	0.8756	0.9652	1.1322
AZI-09	0.6982	0.0256	0.6290	0.0141	0.5602	0.0048	0.4576	0.0037	0.8658	0.6526	0.7842	0.6646
AZI-19	0.7707	0.0184	0.7241	0.0163	0.6323	0.0042	0.5516	0.0034	0.9001	0.4577	0.8483	0.4799
AZI-20	0.8067	0.0219	0.7695	0.0197	0.6331	0.0045	0.5529	0.0037	0.9045	0.536	0.8620	0.5703
AZI-21	0.8238	0.0248	0.7907	0.0224	0.6406	0.0048	0.5625	0.0039	0.9250	0.6058	0.8991	0.6528
AZI-22	0.8020	0.0235	0.7602	0.0212	0.6472	0.0048	0.5700	0.0039	0.9207	0.5812	0.8931	0.6268
AZI-33	0.7798	0.0199	0.7364	0.0176	0.6263	0.0044	0.5439	0.0035	0.9037	0.4936	0.8548	0.5185

- Absence of lag time, Shaded area represents the selected model with the highest $R^2_{adjusted}$ value

Table 5.4: Continued

Batch, #	Zero order model				First order model				Higuchi model			
	With lag time		Without lag time		With lag time		Without lag time		With lag time		Without lag time	
	R^2	K_0	R^2	K_0	R^2	K_1	R^2	K_1	R^2	K_H	R^2	K_H
AZI-06	0.9262	0.0515	0.9093	0.0494	0.6540	0.0064	0.6416	0.0054	0.9502	1.212	0.9861	1.4157
AZI-23	-	-	0.9240	0.0739	-	-	0.5805	0.0053	-	-	0.9946	1.7763
AZI-24	-	-	0.9757	0.0788	-	-	0.6430	0.0056	-	-	0.9563	1.8211
AZI-25	-	-	0.9402	0.0783	-	-	0.5648	0.0053	-	-	0.9908	1.8664
AZI-26	-	-	0.9821	0.0804	-	-	0.6888	0.0058	-	-	0.9500	1.8417
AZI-10	0.8878	0.0280	0.8126	0.2530	0.7594	0.0058	0.6139	0.0046	0.8916	0.6531	0.9148	0.7988
AZI-27	-	-	0.8582	0.0769	-	-	0.4695	0.0049	-	-	0.9934	1.9049
AZI-28	-	-	0.8877	0.0850	-	-	0.4959	0.0052	-	-	0.9977	2.0806
AZI-29	-	-	0.9030	0.0876	-	-	0.5781	0.0057	-	-	0.9899	2.1216
AZI-30	-	-	0.8792	0.8792	-	-	0.4952	0.0050	-	-	0.9969	1.9139
AZI-04	0.9675	0.0623	0.9605	0.0638	0.6658	0.0068	0.8967	0.0035	0.9414	1.4696	0.9925	1.7490
AZI-34	0.9706	0.0518	0.9635	0.0508	0.7537	0.0065	0.7061	0.0056	0.9379	1.1883	0.9950	1.4257
AZI-35	0.8886	0.0430	0.8682	0.0401	0.6479	0.0059	0.5717	0.0048	0.9465	1.0291	0.9544	1.1546
AZI-36	-	-	0.9560	0.0595	-	-	0.6643	0.0053	-	-	0.9843	1.4030
AZI-37	0.9638	0.0629	0.9556	0.0613	0.7157	0.0068	0.6583	0.0057	0.9408	1.4498	0.9916	1.7253

- Absence of lag time, Shaded area represents the selected model with the highest $R^2_{adjusted}$ value

When comparing the results of model fitting using the $R^2_{adjusted}$ selection criteria, the Higuchi model was defined as the model that best fitted the dissolution data for AZI release from suppository formulations. When model fitting was conducted without a lag time as indicated in Table 5.4, $R^2_{adjusted}$ values for these studies ranged between 0.9500 and 0.9960, indicating that diffusion is the predominant factor that controls drug release from these dosage forms.

Since the dissolution rate profiles are evaluated depending on model parameters that are derived from the model and one of these is the experimental time [223], it was therefore decided to establish the extent to which the experimental time parameter affected model fitting, by fitting drug release data with the inclusion or exclusion of the lag time parameter. Variable results were observed when fitting dissolution data to the selected mathematical models for formulations that showed a lag phase, as can be seen from the data highlighted in Table 5.4.

The highest $R^2_{adjusted}$ values obtained following model fitting were observed when AZI release data from batches AZI-04, AZI-34 and AZI-37 were fitted to a zero order model with the inclusion of the lag time and for batches AZI-06 and AZI-35 when fitted to the Higuchi model with the inclusion of lag time. The dissolution profiles for batches AZI-07 – AZI-10, AZI-19 – AZI-22 and AZI-33 could not be fitted to any of the selected models when a lag time was included and the resultant $R^2_{adjusted}$ values were below the limit of 0.9500 set as a lower limit for this criterion, when determining the suitability of a model to describe drug release data.

However, when fitting AZI release data that had an observed lag time to mathematical models without the inclusion of lag time, different results where obtained and these data are also summarised in Table 5.4. In these studies, the highest $R^2_{adjusted}$ values where observed for the fitting of AZI release data, without a lag time, to the Higuchi model for suppositories from batches AZI-04, AZI-06 – AZI-08, AZI-34 and AZI-35. Dissolution data from batches AZI-09, AZI-10, AZI-19 – AZI-22, and AZI-33 could not be fitted to any of the selected models, despite the exclusion of a lag time. These results coincide with the results obtained from fitting dissolution data to the Korsmeyer-Peppas model, which for the same batches, *viz.* AZI-09, AZI-10, AZI-19 – AZI-22 and AZI-33, resulted in the prediction of *n* values of less than 0.5 that are not easily explained by that model.

These results indicate that model parameters are an important aspect to be considered when evaluating drug release mechanisms from dosage forms. Furthermore, model parameters must be comprehensively defined before fitting experimental data to any models, as experimental parameters may affect the shape of the dissolution profiles and consequently skew the results obtained from modeling. Loth *et al,* [173] have suggested that, when fitting dissolution data from lipophilic suppository formulations to mathematical models, it is necessary to correct the experimental lag time parameter for particular models as the use of square and cubic root equations have been proven to be inappropriate for describing drug release in the presence of a lag phase.

The fitting of drug release data to the Higuchi model revealed that the K_H or rate constant values increased for batches formulated with fatty bases with a high hydroxyl value, *viz.* W-W35, S-NA1 25, S-NA 50 bases with the addition of Tween® 80 for batches AZI-15 – AZI-18 and AZI-31 or for batches AZI-23 – AZI-26 or for batches AZI-27 – AZI-30 respectively, as listed in Table 5.4. The results of fitting drug release data to the Higuchi model, for batches formulated with a base of intermediate hydroxyl value, such as W-H15, *viz.* batches AZI-11 – AZI-14 and AZI-32, revealed that the value for K_H remained constant on addition of a surfactant. The effect of surfactants on the release rate constant, K_H, of AZI from fatty bases of high and intermediate hydroxyl value is shown in Figure 5.4. The rate constant, K_H, also remained relatively constant when either urea or PVP were added to the formulations in which W-W35 was used as the base of choice to manufacture batches AZI-34 – AZI-37 and these data are also summarised in Table 5.4.

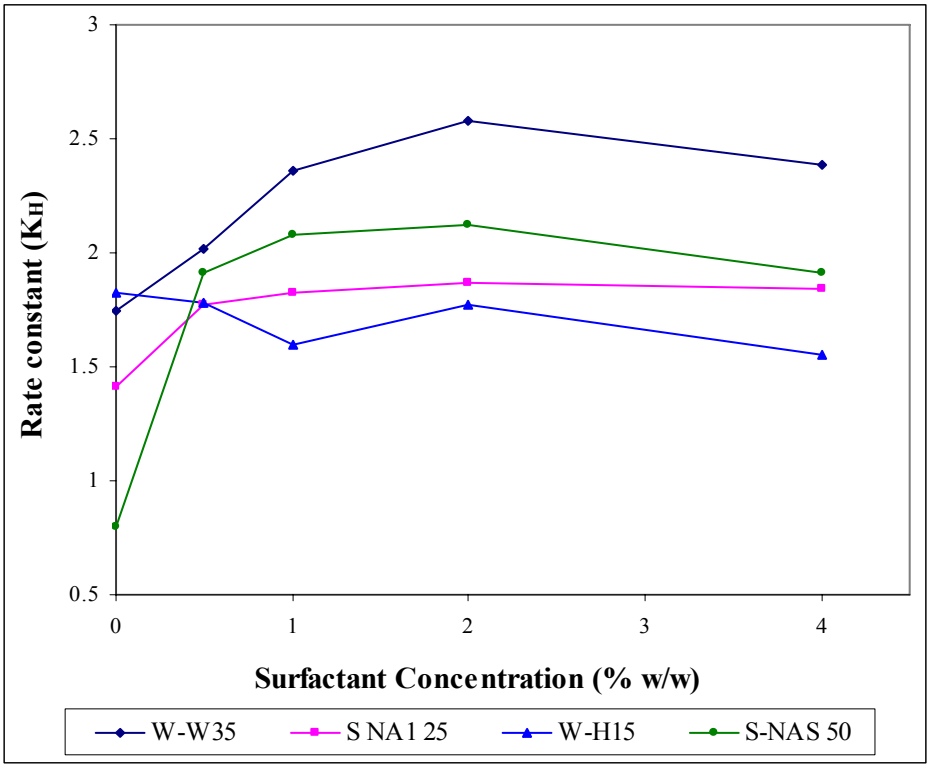


Figure 5.4: Effect of surfactant on the Higuchi kinetic constant (K_H) for formulations manufactured using W-H15, W-W35, S-NA1 25 and S-NAS 50 suppository bases.

The addition of surfactant or PVP did not change the order of drug release kinetics of AZI from formulations manufactured using W-H15, W-W35, S-SNA1 25 bases, as reported in Table 5.4. However, the release kinetics of AZI from suppositories manufactured using S-NAS 50 as the base, changed to a diffusion controlled mechanism on addition of a surfactant to the formulations (Table 5.4).

5.3 CONCLUSIONS

The pharmaceutical suppository dosage forms manufactured in these studies have been critically assessed using both model dependent and independent analytical methods. The release characteristics of AZI from these dosage forms were found to be affected by factors such as the type of vehicle used, the physicochemical properties of the drug and test parameters, such as experimental time or lag times.

The exploratory data analysis method was used as an initial approach for the statistical comparison of AZI dissolution profiles from PEG suppositories and the results that were presented in both a graphical and numerical manner reveal that there is some merit to this approach for to the early evaluation of drug release data. Although the method appears to be useful, definitive conclusions as to the similarity or difference between two dissolution profiles, other than at specific time points, could not be drawn.

The difference, f_1, and similarity, f_2, factors were used to characterise the *in vitro* dissolution profiles of AZI from suppositories manufactured using different bases, with and without the addition of additives. AZI release from suppositories in which surfactants were added to a formulation in which a base with a low hydroxyl value S-AM, was used, or with an intermediate hydroxyl value, such as W-H15, was found to be comparable to those manufactured with the base only. The dissolution rate profiles of AZI release from formulations manufactured using bases with a high hydroxyl value, *viz*. W-W35 and S-NAS 50, with the addition of surfactant, were found to be different to the formulations containing base only. However, when the fit factors were used for the comparison of dissolution profiles of formulations manufactured using S-NA1 25 base with the addition of surfactant to that of the base only, definitive conclusions as to whether the two dissolution profiles were similar or dissimilar could not be drawn. This is because in same cases the f_2 values where greater than 50, an indication of similarity, while the f_1 values where greater than 15, indicating that dissolution profiles where dissimilar. This raises the question as to why the regulatory agencies, such as the FDA and EMEA, only endorse the use of f_2 and the sensitivity of the fit factors in comparing dissolution profiles.

The Gohel and Panchal similarity factor, S_d [190, 194], was also used in combination with the existing f_1 and f_2 methods and was found to be of value when comparing *in vitro* dissolution data. The S_d approach is similar to the f_1 and f_2 methods of comparison, in that it characterises the entire dissolution curve as a single measurement, which is a better approach than a single-point technique used in exploratory data analysis or $t_{x\%}$ methods, when attempting to conclude whether drug release profiles from a reference and a test product are equivalent.

The selection of an appropriate model is vital to provide a better understanding of the drug release mechanisms from dosage forms. The mechanism of AZI release from each type of fatty base formulation was analysed using the Korsmeyer-Peppas model, in the first instance. The modified Korsmeyer-Peppas model [237, 238] was also used to evaluate the mechanism of release for formulations that showed an experimental lag time. The Korsmeyer-Peppas model could not be used to assess the release mechanism of AZI from batches AZI-09, AZI-10, AZI-19 – AZI-22 and AZI-33, since the resultant release exponent value, *n,* fell below 0.50. This result points to the importance of selecting an appropriate model based on the stated assumptions for a particular model and its apparent applicability to the fitting of drug release from a particular pharmaceutical dosage form. However, for the rest of the batches tested, this model appeared to describe the release kinetics of AZI adequately and the reported release exponent, *n*, was between 0.50 and 1.00, indicating that AZI release was a function of anomalous transport kinetics.

The release kinetics of AZI from each of the fatty base formulations were also fitted to the Higuchi, Zero and First order models and the model with the highest $R^2_{adjusted}$ value was selected as the best fit model. It is apparent that the experimental time parameter plays a major role in data interpretation after fitting dissolution data to a particular model. It is important that mathematical relationships used to describe drug release from suppository formulations incorporate a time factor. This was evident from the variable results obtained for fitting drug release data with and without the inclusion of lag times during data analysis. The same observations were also made by Loth *et al,* [173] who suggested that the use of the Weibull function when analysing drug release from lipophilic suppositories be preferred, since its takes into account the presence of lag time. This model could not be applied for the characterisation of the AZI dissolution profiles, since the fatty bases formulated released less than 63.2%, the lowest limit required for the computation of this model. However, for most of the formulations tested, the data were best fitted to the Higuchi model, which complements

the results obtained when fitting data to the Korsmeyer-Peppas model, suggesting that the release mechanism from lipophilic suppositories involves more than one process, such as diffusion, and should consider the combined effects of melting and drug partitioning in addition to diffusion.

It is evident that there are a number of challenges associated with the mathematical evaluation of dissolution profiles of API from solid suppository dosage forms, when using a model-dependent approach. Therefore, it is important that model selection is performed with caution and that the model assumptions are continually checked in order to incorporate an appropriate change in parameters that may affect the end result of data processing [223].

Based on the results obtained in these studies, it is apparent that further work and the development of an appropriate mathematical model(s) may be necessary for the evaluation of drug release from suppository dosage forms. The models that are developed should take into account the shape of the curves that are affected by the various stages of the drug liberation process, which in turn result in a variable experimental time that is an important parameter that needs to be defined when using mathematical models to characterise dissolution profiles of drugs that are lipophilic from lipophilic suppository formulations.

CHAPTER SIX
6. CONCLUSIONS

AZI is a broad spectrum, semi-synthetic antibiotic that belongs to a new class of macrolide antibiotics, the azalide group. AZI can be used for the treatment of a wide range of paediatric infections including those of the upper and lower respiratory tract and the skin and soft tissues. AZI has also been widely used for the treatment of community-acquired respiratory tract and cutaneous infections in children. Administration of AZI in a suppository dosage form may be a useful alternative for the treatment of the aforementioned diseases when patients, especially children, are unwilling or unable to take oral medications.

An HPLC method was developed and validated according to the ICH guidelines for the quantitation of AZI in a pharmaceutical dosage form, *viz.* a suppository formulation. The method was found to be linear over the concentration range of 25 μg/ml to 500 μg/ml and the intra-day and inter-day precision of the method was determined. The resultant % RSD values were found to be ≤ 2.5% RSD for all concentrations studies, which were within the limits set in our laboratory of ≤ 5%. The method was also found to be accurate with %RSD values of ≤ 2% for accuracy standards within the tolerance limit set in our laboratory. In addition the method was considered selective for the detection and quantitation of AZI in the presence of formulation excipients and degradation products, thus the method can also be considered as stability indicating.

AZI was formulated in two main types of suppository bases, *viz*, water soluble and fatty bases, in an attempt to manufacture an antibiotic suppository containing 250 mg AZI for paediatric use. PEG bases were used as the water-soluble base and different grades of Witepsol® and Suppocire® fatty bases were selected for use, based on their physicochemical properties. Suppositories were manufactured by the fusion method of manufacture and were assessed in terms of their weight and content uniformity and were found to comply with the BP pharmacopoeial specifications for these parameters. In addition the melting range of the products and residual content analysis was undertaken at the end of dissolution testing.

In vitro dissolution testing was performed on all suppositories using USP apparatus I and the discriminatory dissolution method was used to differentiate between dissolution rate profiles of AZI from the resultant formulations. The greatest amount of AZI was released from PEG suppositories and more than 95% of the 250 mg dose of AZI was released in less than 60 minutes from these dosage forms. The amount of AZI released from fatty bases suppositories was substantially lower with 39%, 30%, 25%, 20%, 16%, 13% and 12% of AZI being released from Witepsol® W-H15, W-W35 and Suppocire®, S-NA1 25, S-NA15, S-NA0, S-AM and S-NAS 50 suppository formulations respectively. The release of AZI from fatty bases was significantly influenced by the physicochemical properties, such as the melting point and hydroxyl values of the bases, in addition to the partitioning of AZI from the lipid phase to the aqueous dissolution medium. Although PEG base formulations released AZI to a greater extent than fatty suppositories, the investigations in this dissertation focused on improving the rate and extent of release of AZI from fatty suppositories, since PEG bases have been reported to irritate mucosal tissues, unlike fatty bases.

Two different manufacturing and formulation approaches were investigated, *viz*. the addition of surfactants, Polysorbates (Tween® 20 and 80) and the alteration of the physical properties of AZI via the preparation of solid dispersions of AZI with 'inert' water soluble carriers, either urea or PVP-K25. The addition of surfactants significantly increased the release rate of AZI from formulations manufactured using fatty bases with high hydroxyl values, *viz*. Witepsol® W-W35, Suppocire® S-NA1 25 and S-NAS 50. The surfactant wetting effects together with the presence of a high hydroxyl value may be synergistic and could have contributed to the increased rate and extent of AZI release from these formulations.

Solid-state characterisation of solid dispersion coprecipitates of AZI and urea or PVP were performed using FTIR methods with the aim of identifying any potential interaction between the carrier and the drug at molecular level. The results of these studies reveal that a potential interaction may occur between AZI and urea or PVP, when formulated together as a solid dispersion mixture. To further support these findings, further studies with the use of DSC, X-ray diffraction and microscopic methods such as scanning electron microscopy (SEM), need to be conducted to determine whether such interactions are real or artefacts of the manufacturing methods used to prepare these suppository dosage forms.

The inclusion of urea or PVP in a solid dispersion did not significantly increase the release of AZI from the formulations manufactured using the fatty base, W-W35. In fact a decrease in the release rate of AZI was observed when urea was used to formulate solid dispersion coprecipitates with AZI, which could be explained by the theory proposed by Frijilink *et al,* [211, 212] that suggest that following partial dissociation of a coprecipitate complex, a lipophilic drug may undergo back-diffusion into the lipid suppository phase, from which it has been liberated. Furthermore, the insignificant increase in release rate of AZI observed may be a consequence of the dissolution properties of the solid dispersion coprecipitates, which requires further studies in order to establish whether or not drug release is carrier dependent or independent to enable appropriate selection of a carrier for future studies.

The results from preliminary stability studies for selected formulations that released the greatest amount of AZI, *viz*. PEG and W-W35 formulations with the addition of 2.0% Tween® 80, revealed that there was a change in the rate and extent of drug release from these dosage forms following storage. Therefore, further studies should be conducted to elucidate any potential interactions between AZI and the specific bases used. Analytical methods, such as DSC, can be used to investigate drug/excipient incompatibility and would be of value when undertaking these investigations. Long-term stability studies are crucial to ensure that effective antimicrobial activity is retained in such products when stored under specified storage conditions.

Mathematical analysis of AZI dissolution data was undertaken using model independent and dependent methods. The purpose of these studies was to further characterise AZI release rate profiles from fatty suppository base formulations. Model independent statistical parameters, such as the difference, f_1, and similarity, f_2, factors suggested that AZI release from formulations containing fatty bases of intermediate hydroxyl value, *viz.* W-H15, and low hydroxyl value, *viz.* S-AM, with the addition of surfactant, were comparable to those in which the base only were used and values of $f_1 < 15$ and $f_2 > 50$ were obtained for these comparisons. In contrast, the f_1 and f_2 values for formulations containing fatty bases with high hydroxyl values, *viz.* W-W35 and S-NAS 50, were found to be > 15 and < 50 respectively, suggesting that differences exist between these products. The Gohel and Panchal similarity factor, S_d [200, 226], was tested along with the existing f_1 and f_2 methods and was found to be applicable for the comparison of dissolution data in these studies.

The release data obtained from *in vitro* release studies were also fitted to various mathematical models, such as the Zero order, First order and Higuchi models. In addition, the mechanism of AZI release from fatty suppositories was evaluated using the Korsmeyer-Peppas model. The drug release mechanism can be considered to occur primarily by means of anomalous transport kinetics, which is an indication of the presence of more than one type of release phenomenon. These findings were not entirely unexpected, due to the complexity of the drug release process from suppository the formulation that involves a series of consecutive steps, such as melting, drug partitioning and diffusion through the molten base to the hydrophilic dissolution medium. The fitting of the dissolution data to the aforementioned mathematical models revealed that the experimental time parameter plays a major role in data interpretation and that it is vital to correct mathematical models to incorporate this time factor. For most of the formulations tested, the data were best fitted to the Higuchi model, indicating that diffusion is one of the primary mechanisms governing drug release from the lipophilic suppository formulations tested.

Despite the apparent complexity of suppository formulations, these studies have shown the applicability of using fatty bases of different physicochemical properties for the formulation of AZI suppository dosage forms for paediatric use. It has been observed that the use of surfactant in combination with fatty bases of high hydroxyl value can improve the release of AZI from such suppositories. The formulations developed and assessed in these studies have defined a starting point for further studies in which the impact of the nature and physicochemical properties of each base in relation to the physicochemical properties of the drug can be assessed. Further studies must be conducted on the basis of determining drug partitioning in the presence of particular suppository base-rectal fluid systems, to further elucidate and/or predict the process of drug release. Analytical methods, such a DSC and SEM, can be used to further investigate the impact of drug-excipient and excipients-excipient interactions on drug release. The actual true validation of *in vitro* release testing of the suppository dosage forms remains the evaluation of *in vivo* performance of these dosage forms [139]. Therefore, it would be necessary to determine the *in vivo* bioavailability of AZI, of the suppository dosage forms prior to determining whether an *in vitro-in vivo* correlation exists for AZI following administration from a rectal suppository formulation.

APPENDIX I
BATCH PRODUCTION RECORDS

Only a sample of one production record for the PEG and the fatty base formulations with the addition of surfactant and AZI as a solid dispersion coprecipitate with urea, are included. The production and summary records for the other formulations manufactured and assessed in these studies are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-01 **Batch size:** 30 g

MANUFACTURING APPROVALS

Batch record issued by: _____ **Date:**_____

Master record issued by: _____ **Date:**_____

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-01 **Batch size:** 30 g

MASTER FORMULA AND BATCH FORMULA						
Component	Quantity	RM#	Amount /Batch	Amount Dispensed	Dispensed by	Checked by
AZI	262.03 mg	RM000157				
PEG 1000	75% w/w	RM000167				
PEG 4000	25% w/w	RM000170				

RHODES UNIVERSITY, Faculty of Pharmacy, Pharmaceutics Department, Grahamstown SOUTH AFFRICA

BACTH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-01 **Batch size:** 30 g

EQUIPMENT VERIFICATION			
Description	**Type**	**Verified By**	**Confirmed By**
Sieves	120 mm mesh		
Scale	Melter Model AE 163		
Evaporating dish	Porcelain		
Magnetic stirrer/Hot plate	Labcon™ Model MSH10		
One Gram Mould	Stainless steel		

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-01 **Batch size:** 30 g

Date:

MANUFACTURING PROCEDURE				
Step	**procedure**	**Time**	**Done By**	**Checked By**
1	Clean the mould with warm water, invert to drain off excess water, leave the mould to equilibrate at room temperature Room temperature:			
2	Work out the amount of base needed as follow: No. of suppositories required × calibrated mould size: Amount of AZI needed/Displacement value: Amount of the base needed:			
3	Weigh the amount of PEG base needed as follow: PEG 1000 (75% w/w): PEG 4000 (25% w/w):			
4	Melt the calculated amount of the base Temperature:			

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-01 **Batch size: 30 g**

Date:

MANUFACTURING PROCEDURE				
Step	**procedure**	**Time**	**Done By**	**Checked By**
5	Screen AZI through 120 mm mesh screen			
6	Place AZI in 2 and mix for 3 min at 510 r.p.m, setting speed of 3 Weight of AZI:			
7	Poor the mixture 4 into 1 and let it set at room temperature for 24 hours before analysis			

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-01 **Batch size:** 30 g

SIGNATURE AND INITIAL REFERENCE			
Full Name (Print)	**Signature**	**Initials**	**Date**

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-11 **Batch size:** 30 g

MANUFACTURING APPROVALS

Batch record issued by: _____ **Date:**_____

Master record issued by: _____ **Date:**_____

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-11 **Batch size:** 30 g

MASTER FORMULA AND BATCH FORMULA						
Component	Quantity	RM#	Amount /Batch	Amount Dispensed	Dispensed by	Checked by
AZI	262.03 mg	RM000157				
Witepsol® H15	99.5% w/w	RM000143				
Tween® 80	0.5% w/w	RM000173				

RHODES UNIVERSITY, Faculty of Pharmacy, Pharmaceutics Department, Grahamstown SOUTH AFFRICA

BACTH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-11 **Batch size:** 30 g

EQUIPMENT VERIFICATION			
Description	**Type**	**Verified By**	**Confirmed By**
Sieves	120 mm mesh		
Scale	Melter Model AE 163		
Evaporating dish	Porcelain		
Magnetic stirrer/Hot plate	Labcon™ Model MSH10		
One Gram Mould	Stainless steel		

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-11 **Batch size:** 30 g

Date:

MANUFACTURING PROCEDURE				
Step	**procedure**	**Time**	**Done By**	**Checked By**
1	Clean the mould with warm water, invert to drain off excess water, leave the mould to equilibrate at room temperature Room temperature:			
2	Work out the amount of base needed as follow: No. of suppositories required × calibrated mould size: Amount of AZI /Displacement value: Amount of Tween® 80: Amount of the base needed:			
3	Mix Tween® 80 with the melted amount of the base Temperature:			

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-11 **Batch size:** 30 g

Date:

MANUFACTURING PROCEDURE				
Step	**procedure**	**Time**	**Done By**	**Checked By**
4	Screen AZI through 120 mm mesh screen			
5	Place AZI in 3 and mix for 3 min at 510 r.p.m, setting speed of 3 Weight of AZI:			
6	Poor the mixture 4 into 1 and let it set at room temperature for 24 hours before analysis			

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-11 **Batch size:** 30 g

SIGNATURE AND INITIAL REFERENCE			
Full Name (Print)	**Signature**	**Initials**	**Date**

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-35 **Batch size:** 30 g

MANUFACTURING APPROVALS

Batch record issued by: _____ **Date:**_____

Master record issued by: _____ **Date:**_____

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-35 **Batch size:** 30 g

MASTER FORMULA AND BATCH FORMULA						
Component	Quantity	RM#	Amount /Batch	Amount Dispensed	Dispensed by	Checked by
AZI	262.03 mg	RM000157				
Witepsol® W35	98% w/w	RM000166				
Urea	2% w/w	RM000164				

RHODES UNIVERSITY, Faculty of Pharmacy, Pharmaceutics Department, Grahamstown SOUTH AFFRICA

BACTH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-35 **Batch size:** 30 g

EQUIPMENT VERIFICATION			
Description	**Type**	**Verified By**	**Confirmed By**
Sieves	120 mm mesh		
Scale	Melter Model AE 163		
Rotary evaporator	Rotavapor-R		
Mortar and Pestle	Porcelain		
Sonicator	Ultrasonic bath Model B-12		
Dessiccator	Packed with dried silica gel		
Evaporating dish	Porcelain		
Magnetic stirrer/Hot plate	Labcon™ Model MSH10		
One Gram Mould	Stainless steel		

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-35 **Batch size:** 30 g

Date:

MANUFACTURING PROCEDURE				
Step	**procedure**	**Time**	**Done By**	**Checked By**
1	Prepare the solid dispersion of urea and AZI as follow: Weigh the AZI in excess: Size reduce urea and sieve though 120 mm mesh screen Weigh urea in excess (2% w/w): Dissolve AZI in ethanol 50 ml followed by urea with the aid of sonication for 5 min, Evaporate the solvent using rotary evaporator at 40ºC, Place the resultant residue in a dessicator containing dried silica gel for 3 day			

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-35 **Batch size:** 30 g

Date:

MANUFACTURE PROCEDURE				
Step	**Procedure**	**Time**	**Done By**	**Checked By**
2	Size reduce the coprecipitates using mortar and pestle			
3	Clean the mould with warm water, invert to drain off excess water, leave the mould to equilibrate at room temperature Room temperature:			
4	Work out the amount of base needed as follow: No. of suppositories required × calibrated mould size: Amount of AZI/Displacement value: Amount of Urea (2% w/w): Amount of the base needed:			
5	Melt the calculated amount of the base Temperature:			
6	Screen the coprecipitates through 120 mm mesh screen			
7	Place coprecipitates in 5 and mix for 5 min at 510 r.p.m, setting speed of 3 Weight of coprecipitates:			
8	Poor the mixture 7 into 3 and let it set at room temperature for 24 hours before analysis			

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name : Azithromycin Dihydrate **Page 1 of**

Batch # : AZI-35 **Batch size:** 30 g

SIGNATURE AND INITIAL REFERENCE			
Full Name (Print)	**Signature**	**Initials**	**Date**

APPENDIX II
BATCH RECORD REPORT

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-01 **Batch Size** : 30 g

Melting temperature : 55ºC **Mixing time :** 3 minutes

Manufactured Date : 31/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	9.24 g	RM000157
PEG 1000	75% w/w	19.80 g	RM000167
PEG 4000	25% w/w	6.60 g	RM000170

Target Weight : 1.1880 g

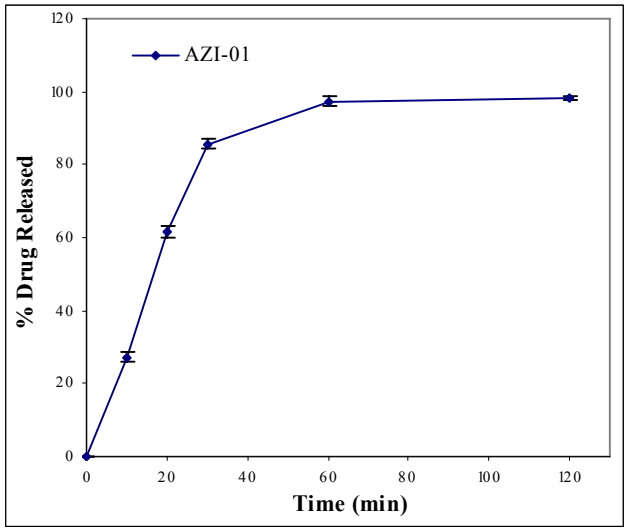
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± SD	% RSD
Weight (g)	1.1800 ± 0.006	0.48
Content uniformity (%)	98.87 ± 0.021	1.72
Melting range (ºC)	Not performed	Not performed

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced
- no surface abrasion during removal of the suppository from the mould
- good surface finish was observed
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content : not performed

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-02 **Batch Size** : 30 g

Melting temperature : 57ºC **Mixing time :** 3 minutes

Manufactured Date : 31/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	10.25 g	RM000157
PEG 400	20% w/w	5.80 g	RM000169
PEG 1500	33% w/w	9.60 g	RM000171
PEG 6000	47% w/w	13.66 g	RM000168

Target Weight : 1.3105 g

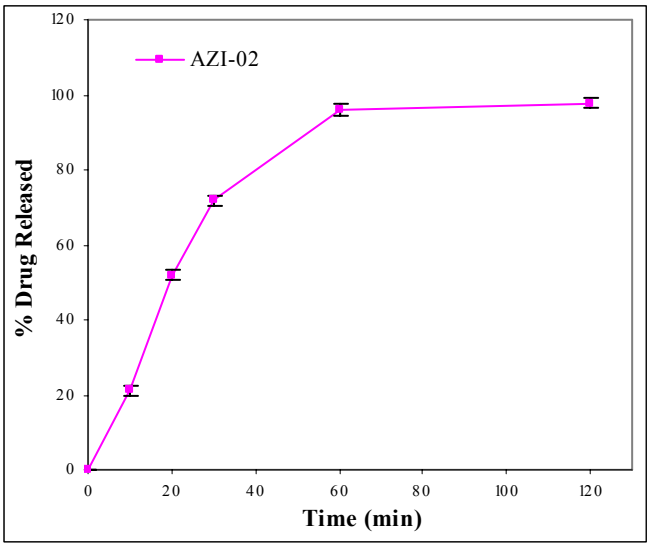
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± SD	% RSD
Weight (g)	1.2926 ± 0.044	3.42
Content uniformity (%)	98.19 ± 0.009	0.77
Melting range (ºC)	Not performed	Not performed

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced, suppositories were slightly harder, good surface finish was observed
- no surface abrasion during removal of the suppository from the mould
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content : not performed

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-03 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 18/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.27 g	RM000157
Witepsol® H15	75% w/w	25.30 g	RM000167

Target Weight : 1.1109 g

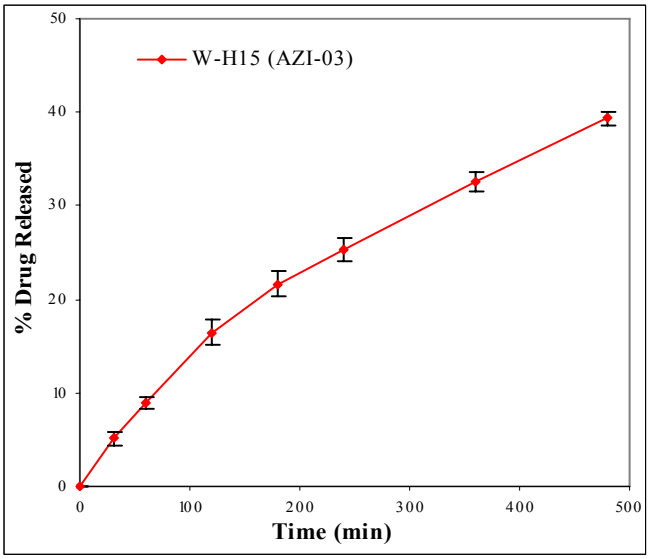
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0159 ± 1.32
Content uniformity (%)	98.08 ± 2.71
Melting range (ºC)	33.5 – 35.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 56.07 ± 4.45% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-04 **Batch Size** : 30 g

Melting temperature : 48.5ºC **Mixing time :** 3 minutes

Manufactured Date : 21/06/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Witepsol® W35	100% w/w	24.70 g	RM000166

Target Weight : 1.0982 g

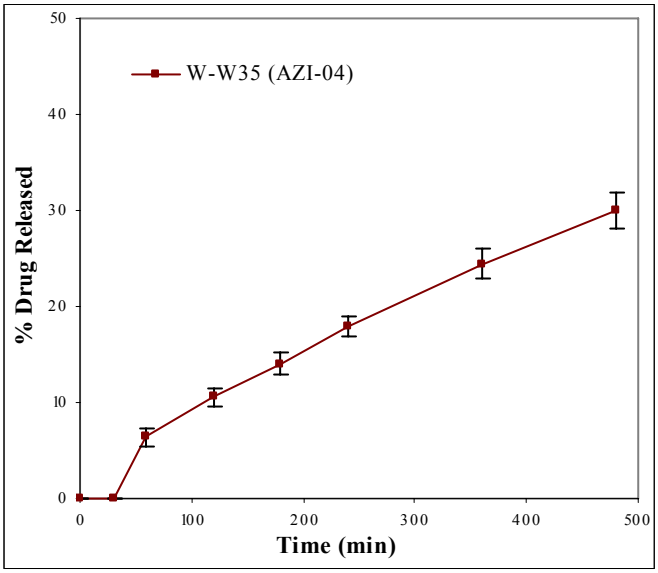
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1174 ± 1.22
Content uniformity (%)	98.31 ± 1.90
Melting range (ºC)	34.5 – 35.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Suppositories had a good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 30.02 ± 1.89% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-05 **Batch Size :** 30 g

Melting temperature : 45ºC **Mixing time :** 3 minutes

Manufactured Date : 18/06/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.86 g	RM000157
Witepsol® E75	100% w/w	23.66 g	RM000144

Target Weight : 1.0507 g

Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0339 ± 1.25
Content uniformity (%)	93.20 ± 6.37
Melting range (ºC)	37.5 – 40.0

Dissolution

- AZI was not released after the dissolution test period of 8 hours

Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Suppositories had a smooth surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 95.15 ± 2.83% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-06 **Batch Size** : 30 g

Melting temperature : 47.5ºC **Mixing time :** 3 minutes

Manufactured Date : 28/06/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.90 g	RM000157
Suppocire® NA1 25	100% w/w	24.43 g	RM000148

Target Weight : 1.0771 g

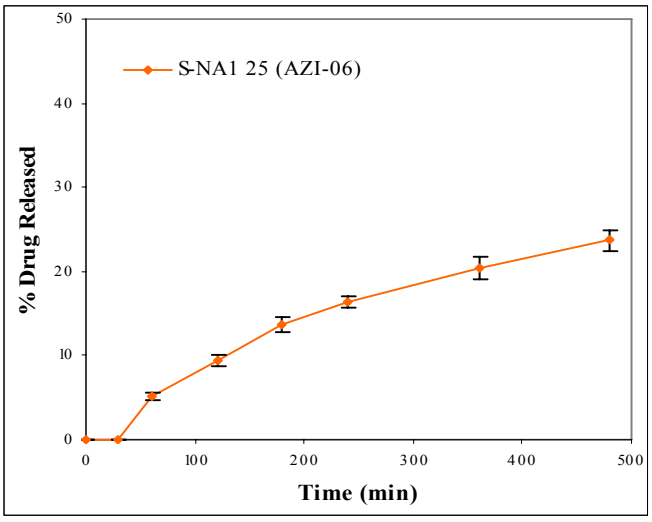
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0416 ± 3.17
Content uniformity (%)	97.24 ± 2.75
Melting range (ºC)	34.5 – 36.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 75.19 ± 2.44% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-07 **Batch Size :** 30 g

Melting temperature : 49ºC **Mixing time :** 3 minutes

Manufactured Date : 27/06/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.89 g	RM000157
Suppocire® NA15	100% w/w	23.57 g	RM000147

Target Weight : 1.0486 g

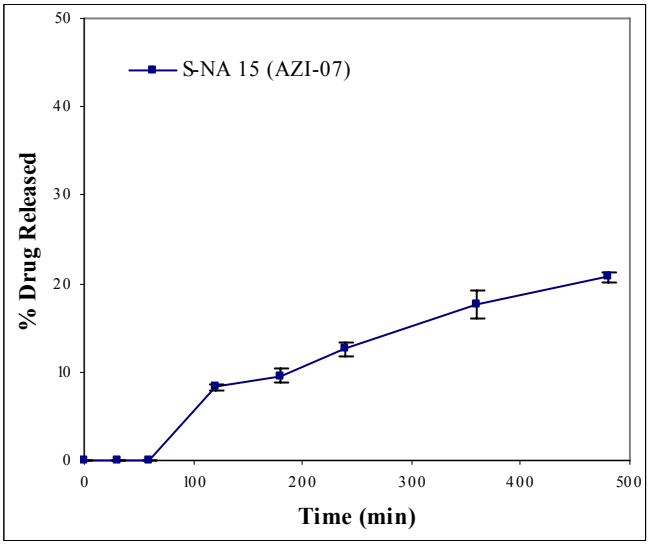
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0344 ± 1.72
Content uniformity (%)	96.06 ± 2.54
Melting range (ºC)	33.5 – 35.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Uniform distribution of the powder was observed
- Suppository weight and content uniformity complied with the BP specification
- Residual content (%) : 75.65 ± 4.52% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-08 **Batch Size :** 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 28/06/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.95 g	RM000157
Suppocire® NA 0	100% w/w	24.46 g	RM000146

Target Weight : 1.0827 g

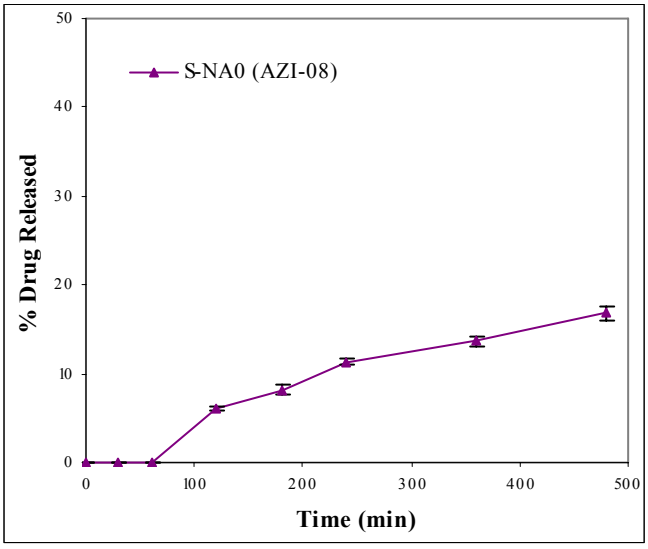
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0405 ± 1.65
Content uniformity (%)	100.05 ± 4.20
Melting range (ºC)	35.5 – 36.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Suppositories with a smooth surface were produced
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 82.43 ± 3.18% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-09 **Batch Size :** 30 g

Melting temperature : 49.5ºC **Mixing time :** 3 minutes

Manufactured Date : 27/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.27 g	RM000157
Suppocire® AM	100% w/w	24.78 g	RM000145

Target Weight : 1.1017 g

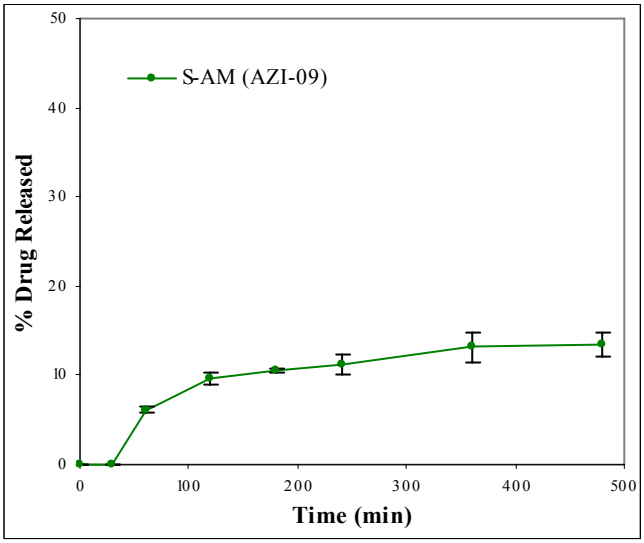
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.008 ± 1.46
Content uniformity (%)	94.81 ± 1.39
Melting range (ºC)	35.5 – 37.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with a smooth surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 73.86 ± 3.20% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-10 **Batch Size** : 30 g

Melting temperature : 49.5ºC **Mixing time :** 3 minutes

Manufactured Date : 12/09/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Suppocire® NAS 50	100% w/w	24.35 g	RM000162

Target Weight : 1.0888 g

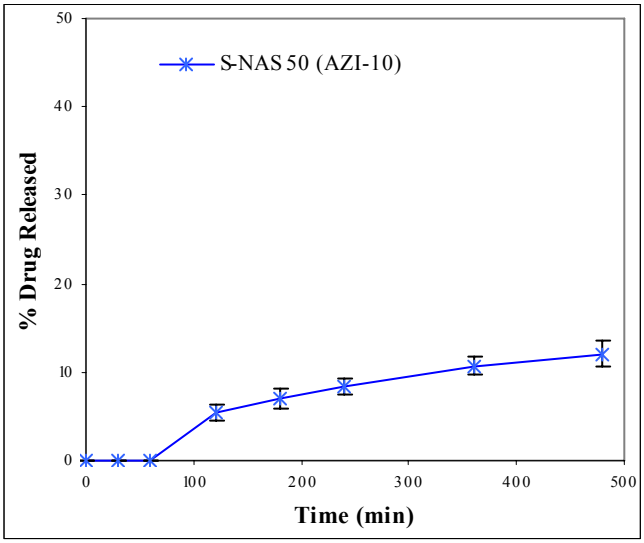
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1109 ± 0.91
Content uniformity (%)	95.52 ± 2.25
Melting range (ºC)	35.5 – 37.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 80.19 ± 3.59% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-11 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 18/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.43 g	RM000157
Witepsol® H15	99.5% w/w	22.58 g	RM000167
Tween® 80	0.5% w/w	0.1420 g	RM000173

Target Weight : 1.0050 g

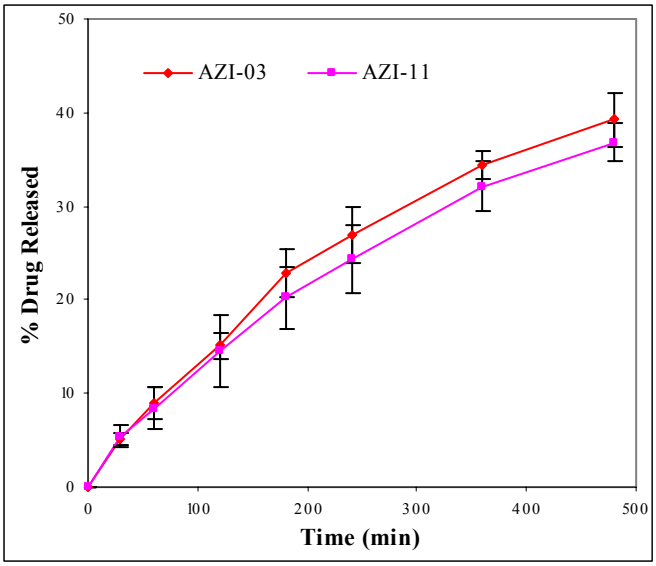
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0137 ± 1.7368
Content uniformity (%)	97.34 ± 1.44
Melting range (ºC)	33.5 – 35.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was off white with smooth surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Some of the suppository molten mass managed to escape through the basket mesh and therefore residual content was not performed

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-12 **Batch Size** : 30 g

Melting temperature : 45ºC **Mixing time :** 3 minutes

Manufactured Date : 19/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.28 g	RM000157
Witepsol® H15	99% w/w	24.98 g	RM000167
Tween® 80	1.0% w/w	0.3157 g	RM000173

Target Weight : 1.1190 g

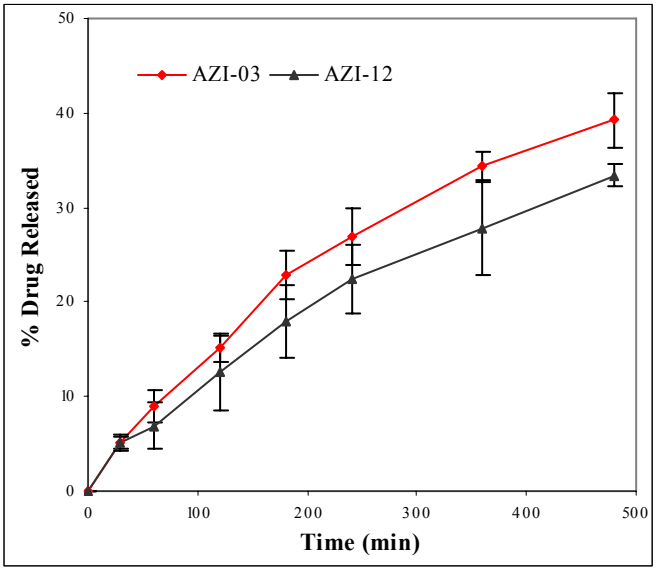
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1033 ± 2.69
Content uniformity (%)	99.40 ± 1.67
Melting range (ºC)	32.5 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed, same observations as batch AZI-11

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-13 **Batch Size** : 30 g

Melting temperature : 47ºC **Mixing time :** 3 minutes

Manufactured Date : 20/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.14 g	RM000157
Witepsol® H15	98% w/w	24.27 g	RM000167
Tween® 80	2.0% w/w	0.6210 g	RM000173

Target Weight : 1.1008 g

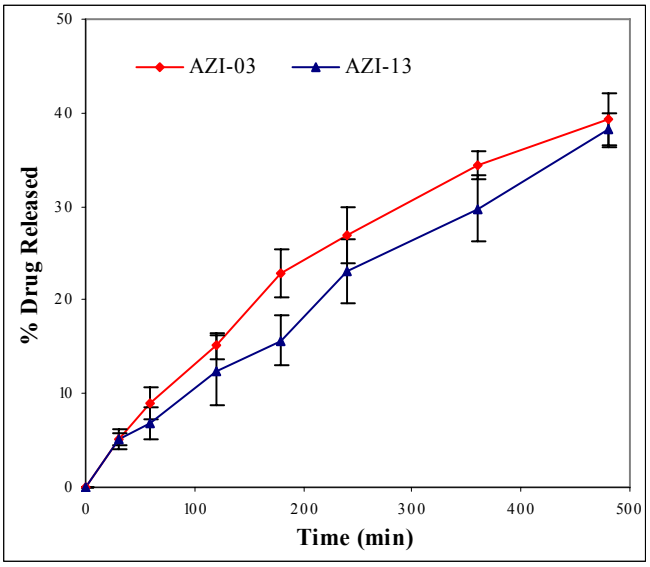
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1093 ± 2.48
Content uniformity (%)	97.50 ± 1.01
Melting range (ºC)	32.5 – 33.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was off white
- no surface abrasion during removal of the suppository from the mould
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed, same observations as batch AZI-11

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-14 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 21/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.14 g	RM000157
Witepsol® H15	96% w/w	23.65 g	RM000167
Tween® 80	4.0% w/w	1.2421 g	RM000173

Target Weight : 1.1008 g

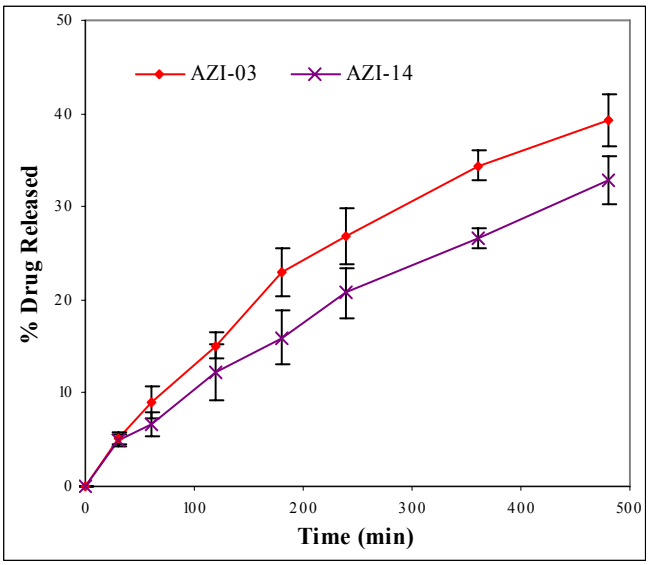
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1118 ± 1.17
Content uniformity (%)	98.25 ± 0.74
Melting range (ºC)	32.0 – 33.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed, same observations as batch AZI-11

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-15 **Batch Size** : 30 g

Melting temperature : 45ºC **Mixing time :** 3 minutes

Manufactured Date : 02/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.86 g	RM000157
Witepsol® W35	99.5% w/w	23.30 g	RM000166
Tween® 80	0.5% w/w	0.1500 g	RM000173

Target Weight : 1.0437 g

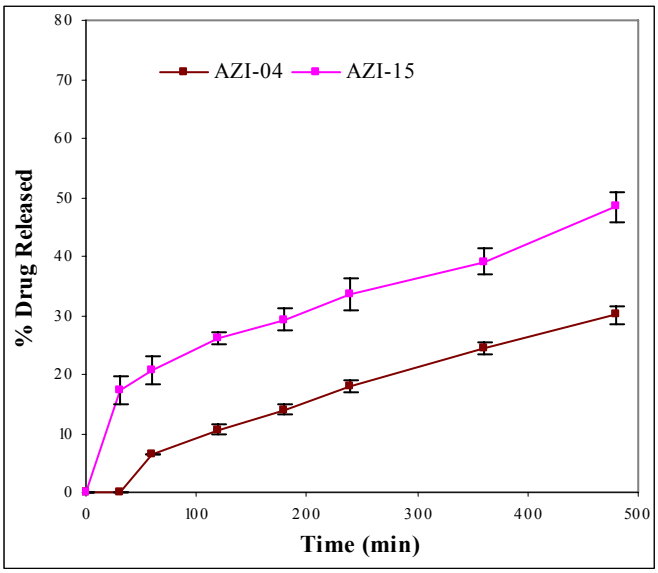
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0345 ± 3.60
Content uniformity (%)	97.01 ± 1.82
Melting range (ºC)	32.5 – 33.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour off white
- no surface abrasion during removal of the suppository from
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Some molten mass escaped the basket mesh and therefore the residual content was not performed

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-16 **Batch Size** : 30 g

Melting temperature : 48ºC **Mixing time :** 3 minutes

Manufactured Date : 06/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.87 g	RM000157
Witepsol® W35	99% w/w	23.15 g	RM000166
Tween® 80	1.0% w/w	0.3000 g	RM000173

Target Weight : 1.0437 g

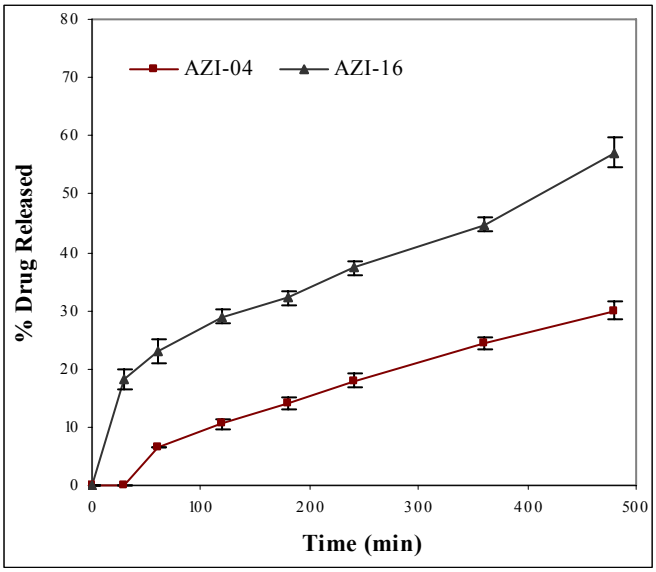
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0115 ± 3.02
Content uniformity (%)	95.68 ± 2.72
Melting range (ºC)	33.0 – 35.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed same observation as batch AZI-15

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-17 **Batch Size** : 30 g

Melting temperature : 48ºC **Mixing time :** 3 minutes

Manufactured Date : 06/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.86 g	RM000157
Witepsol® W35	98% w/w	22.85 g	RM000166
Tween® 80	2.0% w/w	0.6011 g	RM000173

Target Weight : 1.0437 g

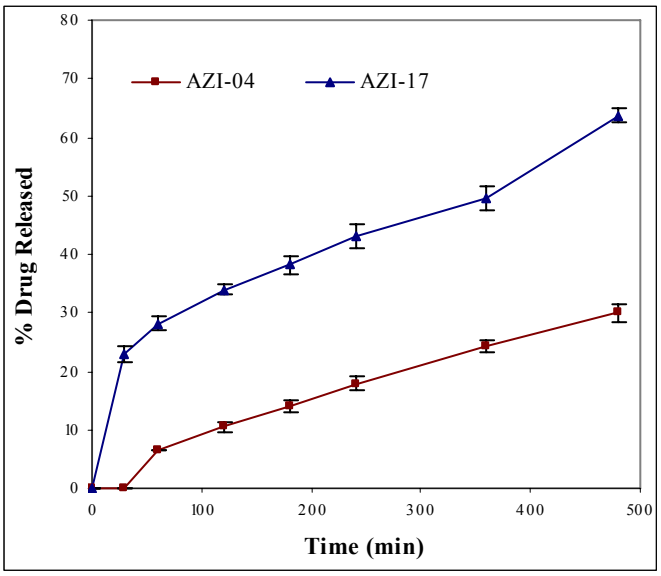
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0192 ± 2.20
Content uniformity (%)	96.71 ± 2.13
Melting range (ºC)	32.5 – 34.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed same observation as batch AZI-15

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-18 **Batch Size** : 30 g

Melting temperature : 45ºC **Mixing time :** 3 minutes

Manufactured Date : 06/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.88 g	RM000157
Witepsol® W35	96% w/w	22.25 g	RM000166
Tween® 80	4.0% w/w	1.2020 g	RM000173

Target Weight : g

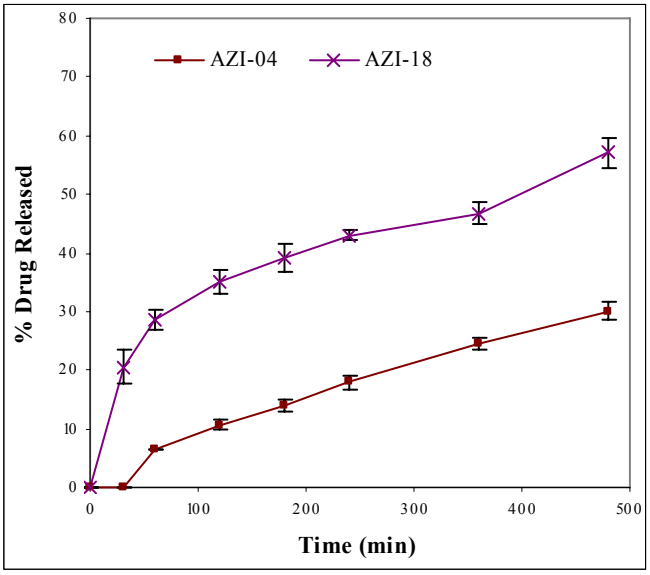
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0328 ± 1.21
Content uniformity (%)	96.83 ± 1.92
Melting range (ºC)	32.0 – 33.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed same observation as batch AZI-15

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-19 **Batch Size** : 30 g

Melting temperature: 47.5ºC **Mixing time :** 3 minutes

Manufactured Date :22/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03mg	8.30 g	RM000157
Suppocire® AM	99.5% w/w	24.63 g	RM000145
Tween® 80	0.5% w/w	0.1570 g	RM000173

Target Weight : 1.1019 g

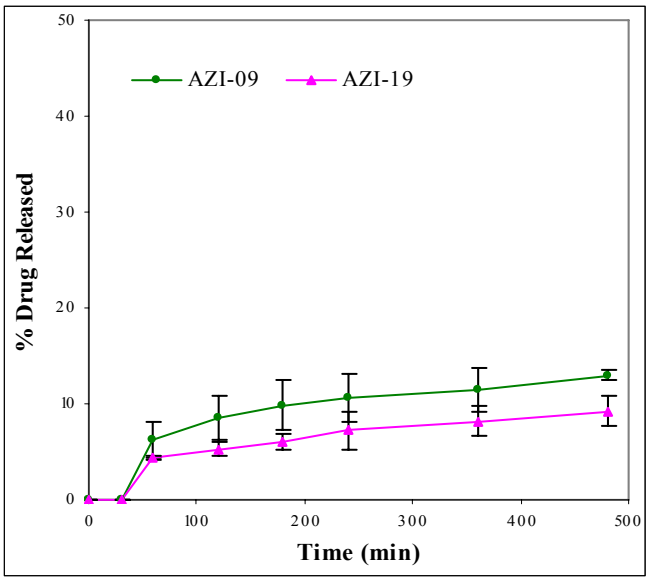
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1076 ± 0.71
Content uniformity (%)	97 ± 1.34
Melting range (ºC)	35.0 – 36.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-20 **Batch Size :** 30 g

Melting temperature : 45ºC **Mixing time :** 3 minutes

Manufactured Date : 23/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.28 g	RM000157
Suppocire® AM	99% w/w	24.48 g	RM000145
Tween® 80	1.0% w/w	0.3150 g	RM000173

Target Weight : 1.1019 g

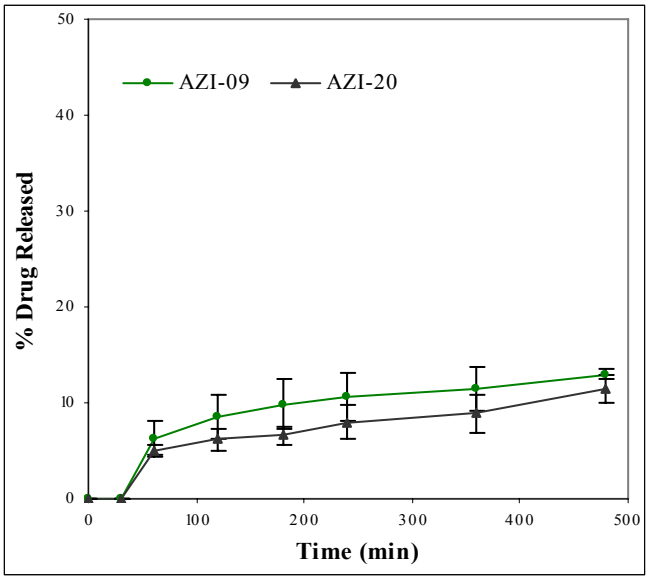
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0837 ± 3.53
Content uniformity (%)	96.05 ± 1.44
Melting range (ºC)	33.0 – 35.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-21 **Batch Size** : 30 g

Melting temperature : 50ºC **Mixing time :** 3 minutes

Manufactured Date : 26/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Suppocire® AM	98% w/w	24.15 g	RM000145
Tween® 80	2.0% w/w	0.6320 g	RM000173

Target Weight : 1.1019 g

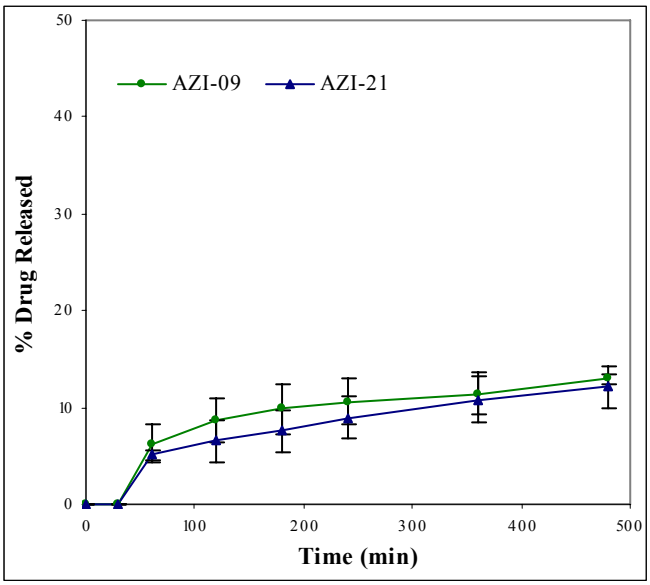
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0943 ± 2.61
Content uniformity (%)	96.15 ± 2.19
Melting range (ºC)	33.5 – 35.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-22 **Batch Size** : 30 g

Melting temperature : 47ºC **Mixing time :** 3 minutes

Manufactured Date : 27/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.27 g	RM000157
Suppocire® AM	96% w/w	22.03 g	RM000145
Tween® 80	4.0% w/w	1.2630 g	RM000173

Target Weight :1.1019 g

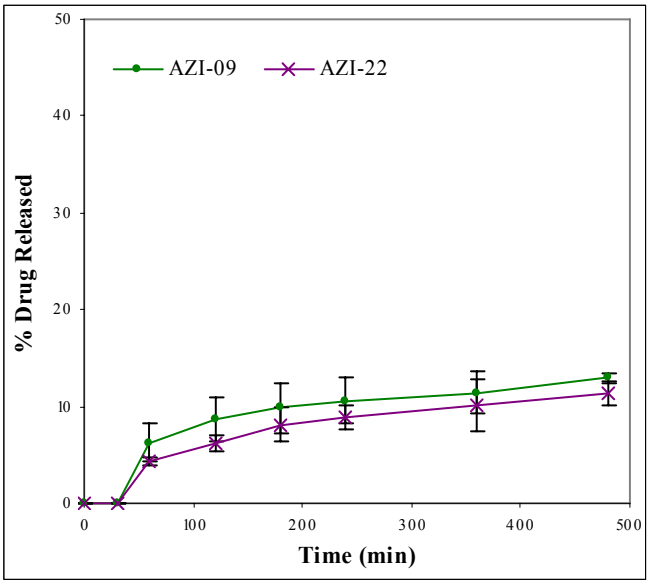
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0956 ± 2.48
Content uniformity (%)	95.81 ± 1.37
Melting range (ºC)	32.0 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-23 **Batch Size** : 30 g

Melting temperature : 48.5ºC **Mixing time :** 3 minutes

Manufactured Date : 09/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.43 g	RM000157
Suppocire® NA1 25	99.5% w/w	22.83 g	RM000148
Tween® 80	0.5% w/w	0.1418 g	RM000173

Target Weight : 1.0132 g **Room Temperature**:

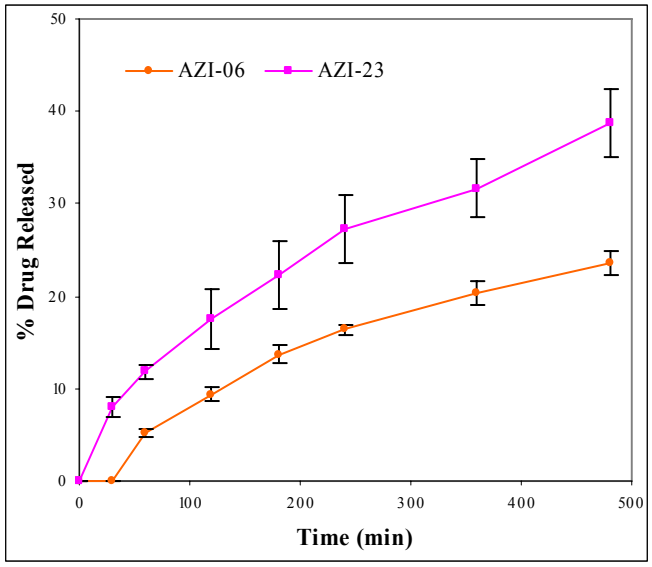
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0121 ± 1.01
Content uniformity (%)	96.48 ± 2.52
Melting range (ºC)	33.5 – 34.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-24 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 09/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.27 g	RM000157
Suppocire® NA1 25	99% w/w	25.20 g	RM000148
Tween® 80	1.0% w/w	0.3150 g	RM000173

Target Weight : 1.1266 g

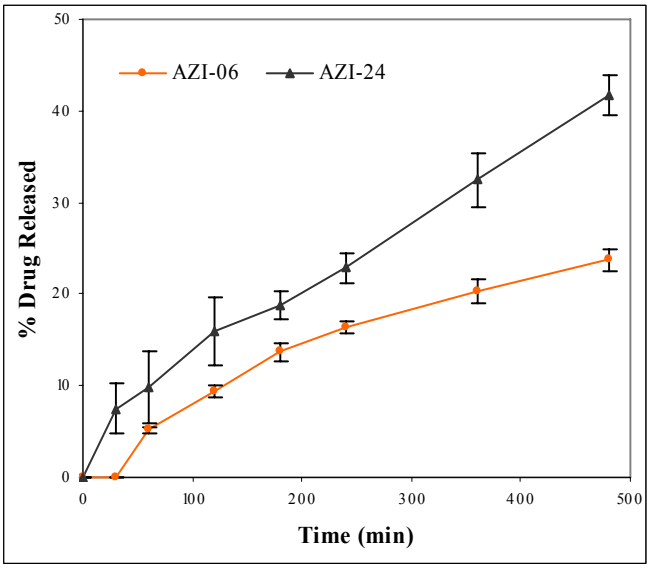
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1251 ± 0.66
Content uniformity (%)	96.35 ± 2.44
Melting range (ºC)	33.5 – 34.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-25 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 10/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.42 g	RM000157
Suppocire® NA1 25	98% w/w	22.40 g	RM000148
Tween® 80	2.0% w/w	0.5672 g	RM000173

Target Weight : 1.0132 g

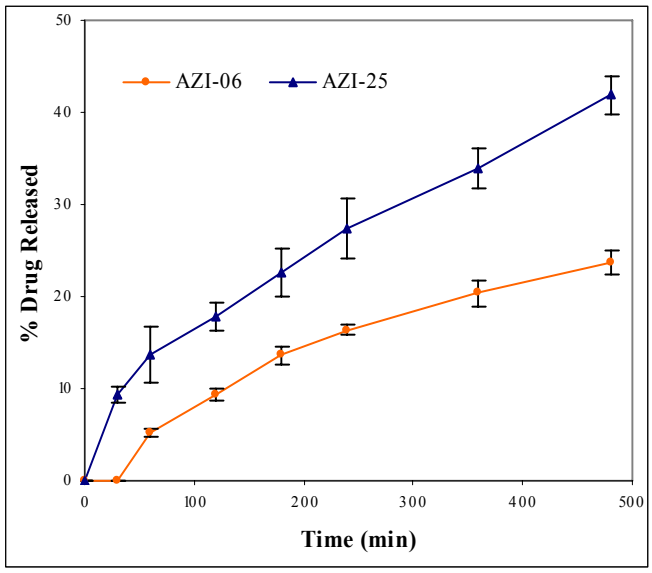
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0181 ± 1.34
Content uniformity (%)	95.62 ± 1.50
Melting range (ºC)	32.5 – 34.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-26 **Batch Size** : 30 g

Melting temperature : 48ºC **Mixing time :** 3 minutes

Manufactured Date : 10/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.31 g	RM000157
Suppocire® NA1 25	96% w/w	24.27 g	RM000148
Tween® 80	4.0% w/w	1.2610 g	RM000173

Target Weight : 1.1266 g

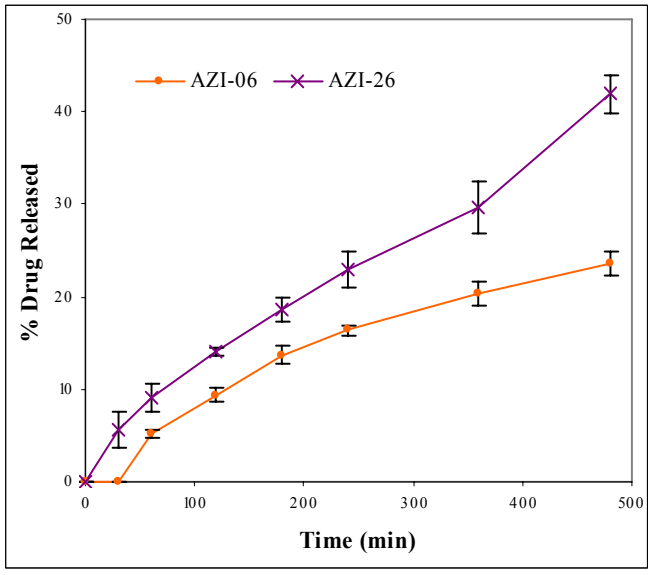
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1286 ± 0.91
Content uniformity (%)	96.70 ± 2.39
Melting range (ºC)	32.0 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-27 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 13/09/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Suppocire® NAS 50	99.5% w/w	24.10 g	RM000162
Tween® 80	0.5% w/w	0.1580 g	RM000173

Target Weight : 1.0837 g

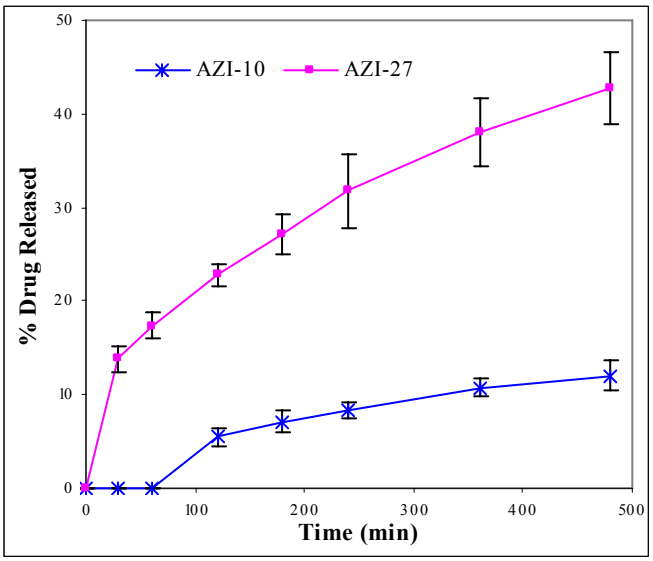
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0975 ± 0.47
Content uniformity (%)	95.44 ± .2.24
Melting range (ºC)	32.5 – 35.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-28 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 13/09/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.28 g	RM000157
Suppocire® NAS 50	99% w/w	23.93 g	RM000162
Tween® 80	1.0% w/w	0.3160 g	RM000173

Target Weight : 1.0837 g

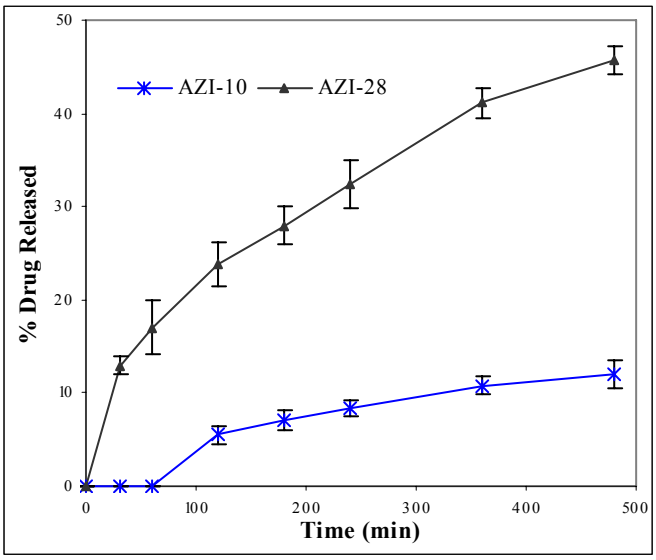
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0284 ± 2.62
Content uniformity (%)	94.61 ± 2.27
Melting range (ºC)	32.5 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-29 **Batch Size** : 30 g

Melting temperature : 45ºC **Mixing time :** 3 minutes

Manufactured Date : 14/09/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Suppocire® NAS 50	98% w/w	23.60 g	RM000162
Tween® 80	2.0% w/w	0.6310 g	RM000173

Target Weight : 1.0837 g

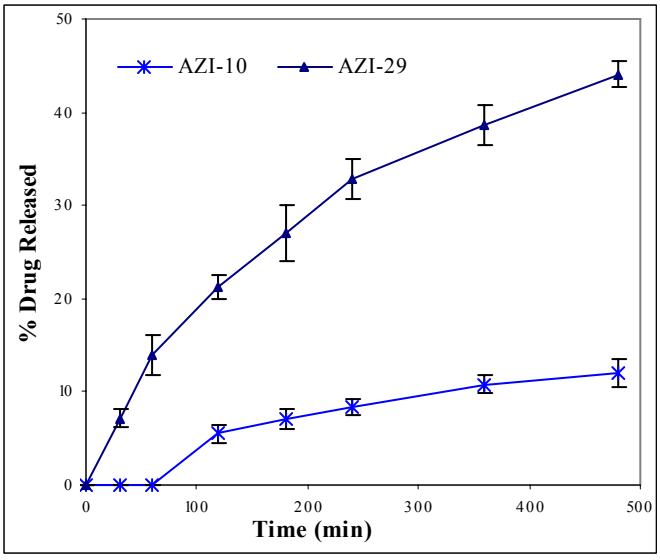
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0889 ± 1.32
Content uniformity (%)	95.60 ± 1.85
Melting range (ºC)	32.0 – 34.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-30 **Batch Size** : 30 g

Melting temperature : 48.5ºC **Mixing time :** 3 minutes

Manufactured Date : 14/07/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Suppocire® NAS 50	96% w/w	22.98 g	RM000162
Tween® 80	4.0% w/w	1.2620 g	RM000173

Target Weight : 1.0837 g

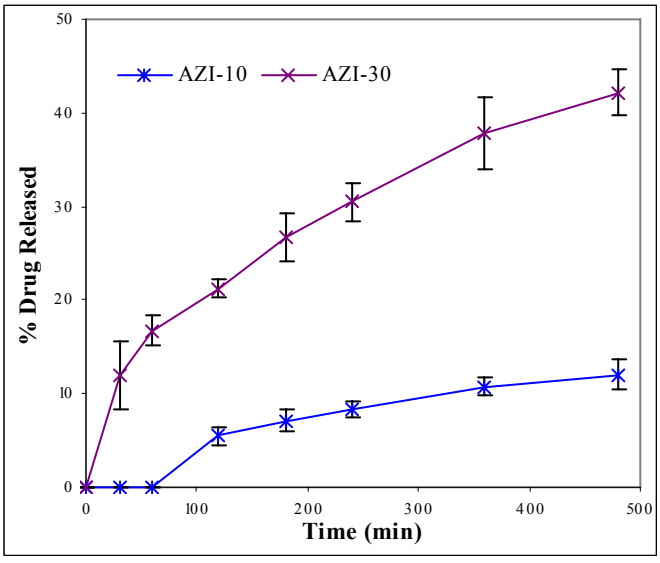
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1026 ± 0.87
Content uniformity (%)	95.17 ± 1.73
Melting range (ºC)	32.0 – 34.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-31 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 02/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.88 g	RM000157
Witepsol® W35	98% w/w	22.86 g	RM000166
Tween® 20	2.0% w/w	0.6023 g	RM000172

Target Weight : 1.0437 g

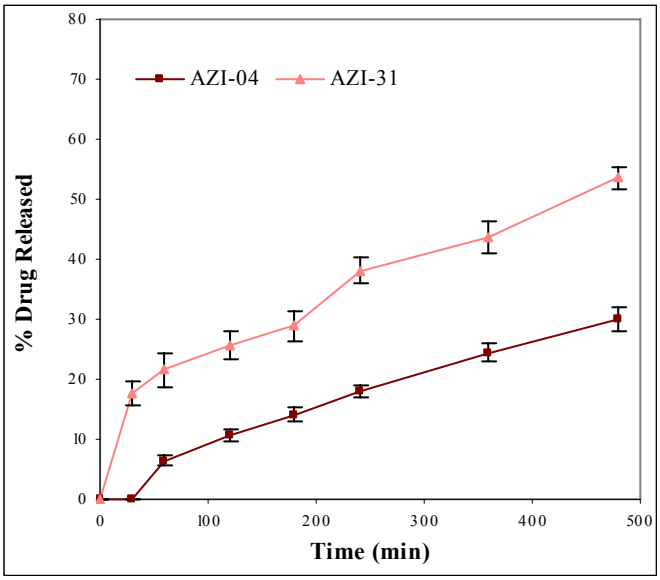
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0335 ± 2.06
Content uniformity (%)	94.63 ± 2.16
Melting range (ºC)	32.5 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-32 **Batch Size** : 30 g

Melting temperature : 46ºC **Mixing time :** 3 minutes

Manufactured Date : 01/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.12 g	RM000157
Witepsol® H15	98% w/w	24.27 g	RM000167
Tween® 20	2.0% w/w	0.6201 g	RM000172

Target Weight : 1.1008 g

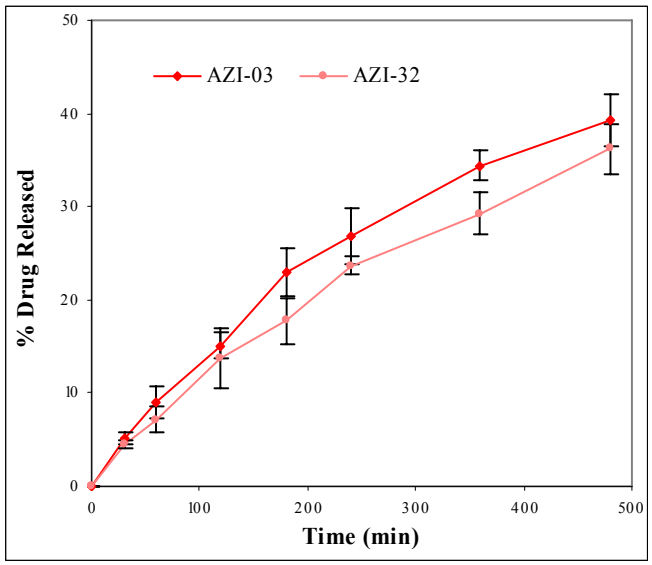
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1088 ± 1.20
Content uniformity (%)	95.94 ± 1.21
Melting range (ºC)	32.0 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content was not performed same observations as batch AZI-11

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-33 **Batch Size** : 30 g

Melting temperature : 50ºC **Mixing time :** 3 minutes

Manufactured Date : 1/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.27 g	RM000157
Suppocire® AM	98% w/w	24.16 g	RM000145
Tween® 20	2% w/w	0.6303 g	RM000172

Target Weight : 1.1019 g

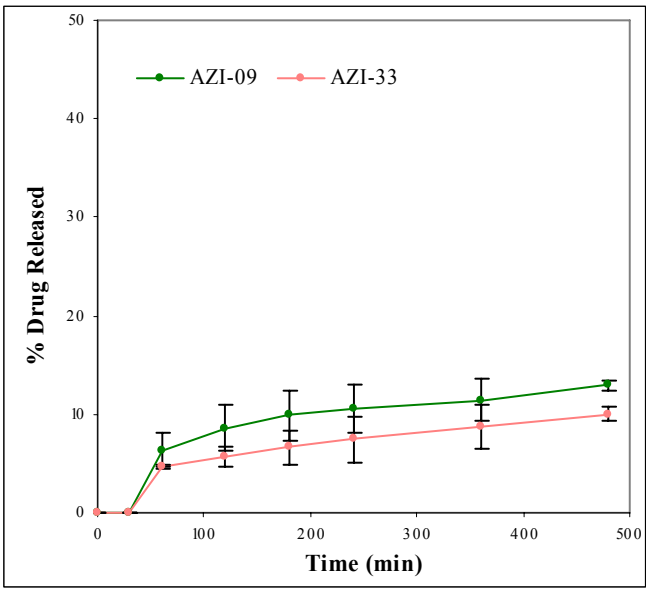
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1022 ± 0.10
Content uniformity (%)	95.21 ± 1.04
Melting range (ºC)	32.5 – 34.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-34 (Physical Mixture) **Batch Size** : 30 g

Melting temperature : 50.5ºC **Mixing time :** 5 minutes

Manufactured Date : 15/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	7.43 g	RM000157
Witepsol® W35	98% w/w	21.60 g	RM000166
Urea	2% w/w	0.5672 g	RM000164

Target Weight : 0.9900 g

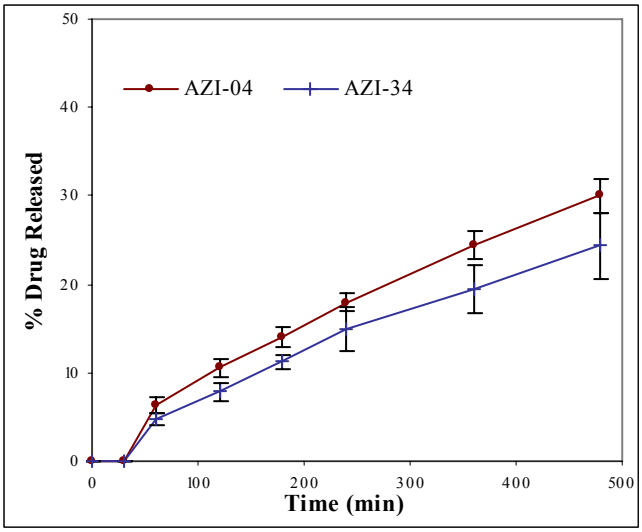
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scentific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	0.9968 ± 0.50
Content uniformity (%)	97.18 ± 2.11
Melting range (ºC)	34.5 – 35.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 71.09 ± 3.45% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-35 (solid dispersion) **Batch Size** : 30 g

Melting temperature : 50.5ºC **Mixing time :** 5 minutes

Manufactured Date : 22/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03 mg	8.30 g	RM000157
Witepsol® W35	98% w/w	24.04 g	RM000166
Urea	2% w/w	0.6310 g	RM000164

Target Weight : 1.0979 g

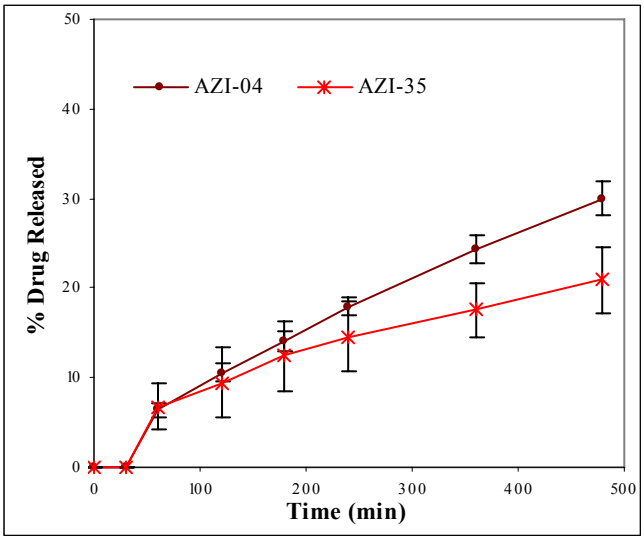
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.0811 ± 2.00
Content uniformity (%)	95.81 ± 3.25
Melting range (ºC)	34.5 – 36.0

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 72.67 ± 3.42% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-36 **Batch Size** : 30 g

Melting temperature : 49ºC **Mixing time :** 5 minutes

Manufactured Date : 15/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03	8.30 g	RM000157
Witepsol® W35	98% w/w	24.05 g	RM000166
PVP-K25	2% w/w	0.6310 g	RM000165

Target Weight : 1.0979 g

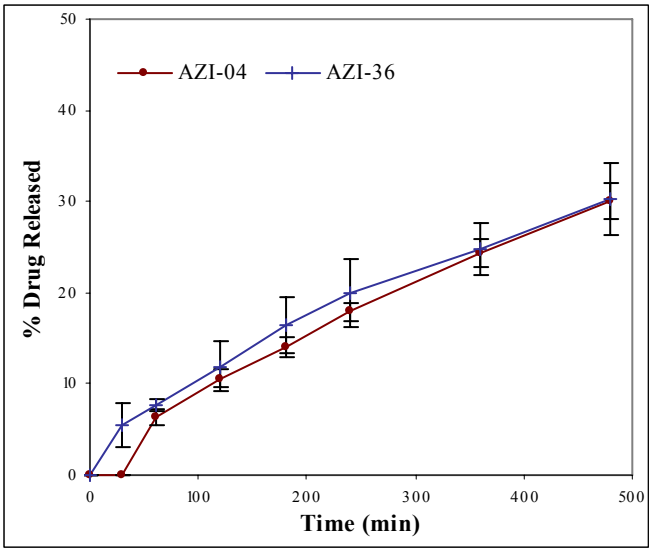
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1043 ± 1.16
Content uniformity (%)	97.19 ± 2.10
Melting range (ºC)	35.0 – 36.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 65.96 ± 4.73% RSD

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics, Grahamstown, SOUTH AFRICA

BATCH RECORD REPORT

Formulator : Happiness Mollel

Product : Azithromycin dihydrate

Batch # : AZI-37 **Batch Size** : 30 g

Melting temperature : 50ºC **Mixing time :** 5 minutes

Manufactured Date : 22/08/2005

Formula

Material	Quantity	Added amount	Rhodes #
AZI	262.03	8.30 g	RM000157
Witepsol® W35	98% w/w	24.00 g	RM000166
PVP-K25	2% w/w	0.6310 g	RM000165

Target Weight : 1.0979 g

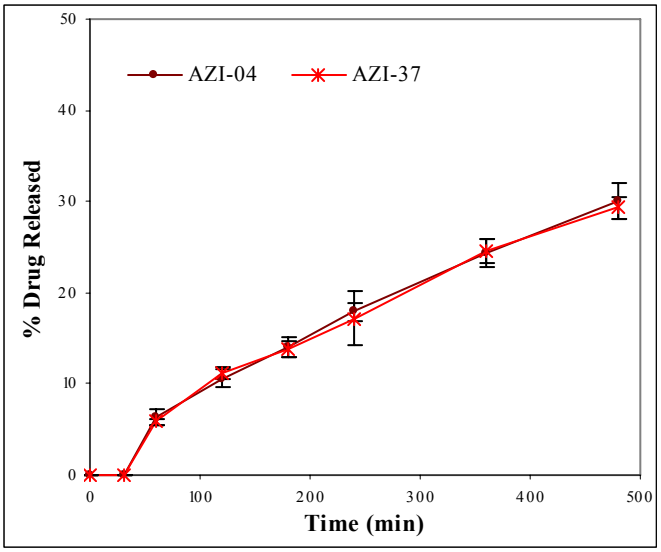
Production equipment used: Labcon™ Model MSH10 Magnetic Stirrer fitted with a hot plate

Melting range test equipment used: Stuart Scientific Model SMP1 melting point test apparatus

Tests performed:

	Mean ± % RSD
Weight (g)	1.1024 ± 1.54
Content uniformity (%)	96.68 ± 2.32
Melting range (ºC)	34.5 – 35.5

Dissolution



Comments / Observations

- Torpedo-shaped suppositories were produced , the product colour was white to off white
- no surface abrasion during removal of the suppository from the mould with good surface finish
- Uniform distribution of the powder was observed
- Suppository weight did not vary and content uniformity complied with the BP specification
- Residual content (%) : 67.44 ± 4.06% RSD

REFERENCES

1. L. Pacifico, C. Chiesa. Azithromycin in children: A critical review of the evidence. *Current Therapeutic Research.* (2002) 63, 54-76.
2. G. Treadway, A. Reisman. Tolerability of 3-day, once daily Azithromycin suspension versus standard treatments for community-acquired paediatric infectious diseases. *International Journal of Antimicrobial Agents.* (2001) 18, 427-431.
3. Drew R.H., Gallis H.A. Azithromycin-spectrum of activity, pharmacokinetics and clinical applications. *Pharmacotherapy.* (1992) 12 (3), 161-173.
4. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry 11th Edition. J.H. Block, J.M. Beale (Eds). Lippincot Williams and Wilkins. London. 2004, pp 352.
5. N. Kujundžič, G. Kobrehel, Z. Banič, Ž. Kelnerić, B. Koić-Prodić. Azalides: Synthesis and antibacterial activity of novel 9a-N-(N'-Substituted Carbamoyl and Thiocarbamoyl) derivatives of 9-deoxo-9a-aza-9a-homoerythromycin A. *European Journal of Medicinal Chemistry.* (1995) 30, 455-462.
6. History Highlights. Pliva, Pharmaceutical Industry. http://www.pliva.hr. Retrieved 20/11/2005.
7. J.C. Kremer. Azithromycin-A new macrolide. *Antibiotic review, Prim Care Update Ob/Gyns.* (2002) 9, 174-175.
8. M.I. Hoepelman, E.M.M. Schneider. Azithromycin: The first of the tissue-selective azalides. *International Journal of Antimicrobial agents.* (1995) 5, 145-167.
9. C. Harold. Clinical microbiological of azithromycin. *The American journal of Medicine.* (1991) 91 (3, S_1), S_{12}-S_{18}.
10. United States Pharmacopoeia 28th Edition. United States Pharmacopeial Convention, Twinbrook Parkway, Rockville, MD, USA. 2005, pp 208-210, 2748-2751, 2748-2751.
11. Martindale. The complete drug reference 33rd Edition. Pharmaceutical Press (PhP), London. 2002, pp 153.
12. W. Yong-Jin. Highlights of semi-synthetic developments from erythromycin A. *Current Pharmaceutical Design.* (2000) 6, 181-223.
13. J.R. Brennan, J. Barber. Full assignments of the ^{13}C and ^{1}H NMR spectra of azithromycin in buffered D_2O and DMSO-d_6. *Magnetic resonance in chemistry.* (1992) 30, 327-333.
14. J. Retsema, Fu. Wench. Macrolides: Structure and microbial targets. *International Journal of Antimicrobial Agents.* (2001) 18, S_3-S_{10}.
15. G. Kobrehel, G. Lazarevski, S. Dokic, L. Kolacny-Babic, N. Kucisec-Iepes, M. Cvrlje. Synthesis and antimicrobial activity of *O*-methylazithromycin derivatives. *Journal of Antibiotics.* (1992) 45 (4), 527-534.
16. E.F Fiese, S.H. Steffen. Comparison of the acid stability of azithromycin and erythromycin A. *Journal of Antimicrobial Chemotherapy.* (1990) 25 (Suppl. A), 39-47.

17. R. Gardhi, O. Pillai, R. Thilagavathi, B. Gopalakrishnan, C.L. Kaul, R. Panchagnula. Characterization of azithromycin hydrates. *European Journal of Pharmaceutical Science.* (2002) 16, 175-184.
18. British Pharmacopoeia, The stationary Office, London, Volume I. pp 129-194.
19. J.W. McFarland, C.M. Berger, S.A. Froshauer, S.F. Hayashi, S.J. Hecker, B.H. Jaynes, R.M. Jefson, J.B. Kamicker, C.A. Lipinski, K.M. Lundy, C.P. Rees, Vu. B.Chi. Quantitative structure-activity relationships among macrolide antibacterial agents: In vitro and in vivo potency against *Pasteurella multocida. Journal of Medicinal Chemistry.* (1997) 40, 1340-1346.
20. C.R. Goldman, W.S. Fesik, C.C. Doran. Role of protonated and neutral forms of macrolides in binding to ribosomes from gram positive and gram negative bacteria. *Antimicrobial Agents Chemotherapy.* (1990) 34, 426-431.
21. Z. Mandić, Z. Weitner, M. Ilijas. Electrochemical oxidation of azithromycin and its derivatives. *Journal of Pharmaceutical and Biomedical Analysis.* (2003) 33, 647-654.
22. H. Chanteux, I. Paternotte, M.P. Mingeot-Leclercq, R. Brasseur, E. Sonveaux, P.M. Tulkens. Cell handling membrane-binding properties, and membrane penetration modeling approaches of Pivampicillin. Two basic esters of Ampicillin in comparison with Chloroquine and Azithromycin. *Pharmaceutical Research.* (2003) 20 (4), 624-631.
23. S.K. Poole, C.F. Poole. Separation methods for estimating octanol-water partition coefficients. *Journal of Chromatography B.* (2003) 797, 3-9.
24. S.F. Donovan, M.C. Pescatore. Method for measuring the logarithm of the octanl-water partition coefficient by usinging short octadecyl-poly (vinly alcohol) high-performance liquid chromatography columns. *Journal of Chromatography A.* (2002) 953 (1-2), 47-61.
25. M.R. Silverstein, G.C. Blassler, Morrill T.C. Spectrometric identification of organic compounds 4th Edition. John Wiley and Sons, New York. 1981, pp 95-137.
26. K.B. Mafatlal, G.S. Krishnamurthy. Manufacturing of clear liquid pharmaceutical composition of azithromycin. *PCT International Application. No. 2002007736,* 2002.
27. M. Pesachovich, S. Issacs, C. Singer, E. Schwartz, E. Berger. Degradation products of azithromycin and methods for their identification. *United State Patent Application No. 20040266997, A1. December, 30, 2004.*
28. M. Pesachovich, S. Issacs, C. Singer, E. Schwartz, E. Berger. Methods of stabilizing azithromycin during storage by packaging in a gas impermeable container. *United State Patent Application No. 20040226852, A1. Nov, 11, 2004.*
29. R. Jenengauzer, J. Schwarz, J. Hrakovsky, T. Lessen, L. Khondo, M. Mathivanan, C. Singer, M. Pesachovich. Stabilized azithromycin composition with antioxidants. *United State Patent Application No. 20030176369, A1. August, 18, 2003*.
30. H.R. Drew, A.H. Gallis. Azithromycin-spectrum of activity, pharmacokinetics and clinical applications. *Pharmacotherapy.* (1992) 12(3), 161-173.

31. A.J. Retsema. Susceptibility and resistance emergence studies with macrolides. *International Journal of Antimicrobial Agents.* (1999) 11 (Suppl. 1), S_{15}-$S_{21.}$
32. G. Foulds, R.M. Shepard, R.B. Johnson. The pharmacokinetics of azithromycin in human serum and tissue. *Journal of Antimicrobial Chemotherapy.* (1990) 25 (Suppl. A) 73-82.
33. G.W. Amsden. Erythromycin, Clarithromycin and Azithromycin: Are the difference real? *Clinical Therapeutics.* (1996) 18, 56-71.
34. A service of the U.S. Department of Helath and Human Services. *AIDSinfo*. http://aidsinfo.nih.gov. Retrieved 30/06/2004.
35. L.S. Young, L. Wiviott, M. Wu, P. Kolonosiki, R. Bolan, C.B. Inderlied. Azithromycin for treatment of mycobacterium avium-intracellular complex infection in patients with AIDS. *The Lancet.* (1991) 338, 1107-1109.
36. C.L. Pacifico, C. Chiesa. Azithromycin in children: A critical review of the evidence. *Current Therapeutic Research.* (2002) 63, 54-76.
37. South Africa Medicine Formulary 6th Edition. C.J. Gibbon. (Ed). South Africa medical association, Health and Medical Publishing Group, South Africa. 2001, pp 269, 271, 464.
38. J.C. Pechĕre. A new perspective on macrolide antibiotics. *International Journal of Antimicrobial Agents.* (2001) 18, S_{93}-S_{97}.
39. D.E. Koch, A. Bhandari, L. Close, R.P. Hunter. Azithromycin extraction from municipal wastewater and quantitation using liquid chromatography/mass spectrometry. *Journal of Chromatography A*. (2005) 1074, 17-22.
40. F.V. Bambeke, P.M Tulkens. Macrolides: Pharmacokinetics and pharmacodynamics. *International Journal of Antimicrobial Agents.* (2001) 18, S_{17}-S_{23}.
41. G.W. Amsden. Pharmacological consideration in the emergence of resistance. *International Journal of Antimicrobial Agents.* (1999) 11 (suppl.1) S_7-S_{14}.
42. M.B. Kays, G.A. Denys. In vitro activity and pharmacodynamics of Azithromycin and Clarithromycin against *Streptococcus pneumoniae*. Based on serum and intrapulmonary pharmacokinetics. *Clinical Therapeutics.* (2001) 23(3), 413-423.
43. G.E. Stein, S. Schooley. Comparative serum bactericidal activity of Clarithromycin and Azithromycin against macrolide-sensitive and resistant strains of streptococcus pneumoniae. Antimicrobial susceptibility studies. *Diagnostic Microbiology and Infectious Diseases.* (2001) 39, 181-185.
44. D.H. Peters, H.A. Friedel, D. McTavish Azithromycin: A review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. *Drugs.* (1992) 44(5), 750-799.
45. T. Mazzei, C. Surrenti, A. Novelli. Pharmacokinetics of azithromycin in patients with impaired hepatic function. *Journal of Antimicrobial Chemotherapy.* (1993) 31 (suppl.E), 57-63.

46. P. Coates, R. Daniel, C.A. Huston. An open study to compare the pharmacokinetics, safety and tolerability of a multiple-dose regimen of Azithromycin in young and elderly volunteers. *European Journal of Clinical Microbial Infectioncious Diseases.* (1991) 10, 850-862.
47. D.E. Amacher, S.J. Schomaker, J.A. Retsema. Comparison of the effects of the new azalides antibiotic Azithromycin and Erythromycin Estolate on rat liver cytochrome p-450. *Antimicrobial Agents and Chemotherapy.* (1991) 35, 1186-1190.
48. P.J. Chave, A. Munofo, Y.J. Chatton. One-a-week Azithromycin in AIDS patients: Tolerability, kinetics and effects on Zidovudine disposition. *Antimicrobial Agents Chemotherapy.* (1991) 36, 1013-1018.
49. K.L. McCall, A.H. Glenn, A.D. Jones. Determination of the lack of a drug interaction between Azithromycin and Warfarin. *Pharmacotherapy.* (2004) 24(2), 188-194.
50. S. Hopkin. Clinical safety and toleration of Azithromycin in children. *Journal of Antimicrobial Chemotherapy.* (1993) 31 (suppl. E), 111-118.
51. Daily Drug Use revised edition: A Guide for the Health Professional. Turner L. (Ed). The Tincture Press, South Africa. 2001, pp 290-291.
52. D.C Rothermel. Single dose azithromycin for acute otitis media: A pharmacokinetic/pharmacodynamic rationale. *Current Therapeutic Research.* (2003) 64 (suppl. A), A_4-A_{15}.
53. C.J. Dunn, B.L. Barradell. Azithromycin: A review of its pharmacological properties and use as 3-day therapy in respiratory tract infectious. *Drugs*. (1996) 51(3), 483-505.
54. G. Treadway, A. Reisman. Tolerability of 3-day, once daily Azithromycin suspension versus standard treatments for community-acquired paediatric infectious diseases. *International Journal of Antimicrobial Agents.* (2001) 18, 427-431.
55. K.W. Garey, W.G. Amsden. Intravenous Azithromycin. *Annals Pharmacotherapy.* (1999) 33, 218-228.
56. Package Insert. Zithromax®: azithromycin for injection (for IV infusion only). Pfzer Laboratories. New York, USA. 2003.
57. R.P. Rapp. Pharmacokinetics and pharmacodynamics of Intravenous and oral Azithromycin: Enhanced tissue activity and minimal drug interactions. *The Annals of Pharmacotherapy.* (1998) 32, 785-793.
58. Zithromax product information. Pfizer Laboratories, New York. 2003. http://www.pfizer.com./pfizer/download/uspi_zithromax600mg.pdf. Retrieved 30/11/2005.
59. A.D. Paolo, C. Barbara, A. Chella, C.A. Angeletti, M.D. Tacca. Pharmacokinetics of Azithromycin in lung tissue, bronchial washing, and plasma in patients given multiple oral doses of 500 and 1000 mg daily. *Pharmacological Research.* (2002) 46 (6), 545-550.
60. W.G. Amsden. Advanced–generation macrolides: Tissue-directed antibiotics. *International Journal of Antimicrobial Agents.* (2001) 18, S_{11}-S_{15}.

61. N.J. Lalak, D.L. Morris. Azithromycin clinical pharmacokinetics. *Clinical Pharmacokinetics.* (1993) 25 (5), 370-374.
62. L.D. Morris, A. De Souza, A.J Jones, E.W. Morgan. High and prolonged pulmonary tissue concentrations of azithromycin following a single oral dose. *European Journal of Clinical Microbiology and Infectious Diseases.* (1991) 10, 859-861.
63. D.A. Raines, A. Yusuf, M.H. Jabak, W.S. Ahmed, Z.A Karcioglu. Simultaneous high-performance liquid chromatography analysis of azithromycin and two of its metabolites in human tears and plasma. *Therapeutic Drug Monitoring.* (1998) 20, 680-684.
64. R.P. Hunter, D.E. Koch, R.L. Coke, M.A. Goatley, R. Isaza. Azithromycin metabolie identification in plasma, bile and tissues of the ball python (*Python regius*). *Journal of Veternary Pharmacology and Therapeutics*. (2003) 26, 117-121.
65. E. Bergone-Bérézin, A. Bryskier. The suppository form of antibiotic administration: pharmacokinetics and clinical application. *Journal of Antimicrobial Chemotherapy*. (1999) 43, 177-185.
66. L.S. Stratchunsky, A.D. Nazarov, A.A. Firsov, N.A. Petrachenkova. Age dependence of erythromycin rectal bioavailability in children. European *Journal of Drug Metabolism and Pharmacokinetics*. (1991) Spec. No. 3, 321-323.
67. E.J. van Hoogdalem, A.G. de Boer, D.D. Breimer. Pharmacokinetics of rectal drug administration, part II. Clinical applications of peripherally acting drugs and conclusions. *Clinical Pharmacokinetics*. (1991) 21 (2), 110-128.
68. E. Pozzi, A. Ferrara, A. Sardi, M.A. Berti, G. Coppi. Bio-availability studies on erythromycin administered by rectal and oral routes. Current Therapeutic Research. (1982) 31 (4).
69. B. Lingerfelt, W.S. Champney. Macrolide and ketolide antibiotic separation by reversed phase high performance liquid chromatography. *Journal of Pharmaceutical and Biomedical analysis.* (1999) 20, 459-469.
70. T. Turčinov, S. Pepeljnjak. Azithromycin potency determination: Optimal conditions for microbiological diffusion method assay. *Journal of Pharmaceutical and Biomedical Analysis.* (1998) 17, 903-910.
71. R.A Breier, C.V. Garcia, P.T. Oppe, M. Steppe, E.E.S. Schapoval. Microbiological assay for azithromycin in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis.* (2002) 29, 957-961.
72. O.A.E. Farghaly, N.A.L. Mohamed. Voltammetric determination of azithromycin at the carbon paste electrode. *Talanta.* (2004) 62, 531-538.
73. B. Nigović, B. Šimunić. Voltammetric assay of azithromycin in pharmaceutical dosage forms. *Journal of Pharmaceutical and Biomedical Analysis.* (2003) 32, 197-202.

74. K.L Šebojka, B. Nigović. Identification of 5-aminosalicylic acid, ciprofloxacin and azithromycin by abrasive stripping voltammetry. *Journal of Pharmaceutical and Biomedical Analysis.* (2004) 36, 81-89.
75. B. Nigović. Adsorptive stripping voltammetric determination of azithromycin at a glassy carbon electrode modified by electrochemical oxidation. *Analytical Sciences*. (2004) 20, 639-643.
76. P.Y. Khashaba. Spectrofluorimetric analysis of certain macrolide antibiotics in bulk and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis.* (2002) 27, 923-932.
77. A. Khedr, M. Sheha. Quantitative TLC method of analysis of azithromycin in pure and capsule forms. *Journal of Chromatographic Science.* (2003) 41, 10-16.
78. P. Zubata, R. Ceresole, M.A. Rosasco, M.T. Pizzorno. A new HPLC method for azithromycin quantitation. *Journal of Pharmaceutical and Biomedical Analysis.* (2002) 27, 833-836.
79. R. Gandhi, L.C. Kaul, R. Panchagnula. Validated LC method for in-vitro analysis of azithromycin using electrochemical detection. *Journal of Pharmaceutical and Biomedical Analysis.* (2000) 23, 1073-1079.
80. F.N. Kamau, H.K. Chepkwony, K.J. Ngugi, D. Debremaeker, E. Roets, J. Hoogmartens. Isocratic liquid chromatographic method for the analysis of azithromycin and its structurally related substances in bulk samples. *Journal of Chromatographic Science.* (2002) 40, 529-533.
81. L. Miguel, C. Barbas. LC determination of impurities in azithromycin tablets. *Journal of Biomedical and Biomedical Analysis.* (2003) 33, 211-217.
82. E. Wilms, H. Trumpie, W. Veenendaal, D. Touw. Quantitative determination of azithromycin in plasma, blood and isolated netrophils by liquid chromatography using pre-column derivatization with 9-fluorescence detection. *Journal of Chromatography B.* (2005) 814, 37-42.
83. I. Kanfer, M.F. Skinner, R.B. Walker. Analysis of macrolide antibiotics. *Journal of Chromatography A*. (1998) 812, 255-286.
84. R.M. Sherpard, G.S. Duthu, R.A. Ferraina, M.A. Mullins. High-performance liquid chromatographic assay with electrochemical detection for azithromycin in serum and tissues. *Journal of Chromatography: Biomedical Applications.* (1991) 565 (1-2), 321-337.
85. C. Taninaka, H. Ohtani, E. Hanada, H. Kotaki, H. Sato, T. Iga. Determination of erythromycin, clarithromycin, roxithromycin and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. *Journal of Chromatography B.* (2000) 738, 405-411.
86. F. Kees, S. Spangler, M. Wellenhofer. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection. *Journal of chromatography A.* (1998) 812, 287-293.

87. D.A. Raines, A. Yusuf, M.H. Jabak, W.S. Ahmed, Z.A. Karcioglu. Simultaneous high-performance liquid chromatography analysis of azithromycin and two of its metabolites in human tears and plasma. *Therapeutic Drug Monitoring.* (1998) 20, 680-684.

88. Y-H. Kim, J.V. Pothuluri, C.E. Cerniglia. Voltametric investigation of macrolides by an HPLC-coulometric assay. *Journal of Pharmaceutical and Biomedical Analysis* (2005) 38, 390-396.

89. J.S. Toraño, J.H. Guchelaar. Quantitative determination of the macrolide antibiotics erythromycin, roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbnyl chloride and fluorescence detection. *Journal of Chromatography B.* (1998) 720, 89-97.

90. Z. Song, C. Wang. Ultrasensitive assay of azithromycin in medicine and bio-fluids based on its enhanced luminal-H_2O_2 chemiluminescence reaction using flow injection technique. *Bioorganic Medicinal Chemistry.* (2003)11, 5375-5380.

91. British Pharmacopoeia, The stationery Office, London, Volume VI, 2005, pp A273-275, A378-381, A294-296, A480-481.

92. L.R. Snyder, J.J. Kirkland. Liquid-solid chromatography. Introduction to modern liquid chromatography 2nd edition. John Wiley and sons Inc. New York. 1979, pp 349-409.

93. R.J. Hamilton, P.A. Sewell. Introduction to high performance liquid chromatography 2nd Edition. Chapman and Hall, London. 1982, pp 127-140.

94. Liquid chromatography in pharmaceutical development: An introduction. I.W. Wainer (Ed). Ater publishing Corporation, USA. 1985, pp 149-179.

95. Practical HPLC method development 2nd Edition. L.R. Snyder, J.J. Kirkland, J.L. Glajch. (Eds). John Wiley and Sons Inc, New York. 1997, pp 59-99, 174-231, 293-349, 643-684.

96. H.G. Fouda, R.P. Schneider. Quantitative determination of the antibiotic azithromycin in human serum by high-performance liquid chromatography (HPLC)-atmospheric pressure chemical ionization mass spectrometry: Correlation with a standard PHLC-electrochemical method. *Therapeutic Drug monitoring.* (1995) 17, 179-183.

97. D. Debremaeker, D. Visk, H.K. Chepkwony, A.V. Schepdael, E. Roets, J. Hoogmertens. Analysis of unknown compounds in azithromycin bulk samples with LC coupled to ion trap mass spectrometry. *Rapid communication in mass spectrometry.* (2003) 17, 342-350.

98. J. Jiao, A.J. Carella, G.S. Steeno. Optimization of triple quadrupole mass spectrometry for quantitation of trace degradants of pharmaceutical compounds. *International Journal of Mass Spectrometry.* (2002) 216, 209-218.

99. R.J.M. Vervoort, E. Ruyter, A.J.J. Debets, H.A. Claessens, C.A. Cramers, G.J. Dejong. Characterization of reversed-phase liquid chromatography stationary phases for the analysis of basic pharmaceuticals: eluent properties and comparison of empirical test methods. *Journal of Chromatography A.* (2001) 931, 67-79.

100. D.C. Harries. Quantitative chemical analysis 5th Edition. W.H. Freeman and Company, New York. 1999, pp 713-753.
101. B. Law, S.J. Houghton, P. Ballard. An approach to the evaluation and comparison of reversed-phase high-performance liquid chromatography stationary phases. *Journal of Pharmaceutical and Biomedical Analysis*. (1998) 17, 443-453.
102. K. Hammarstand. Internal standard in gas chromatography. *Varian Instrument Applications*. (1976) 10 (1), 10-11.
103. A. Martin. Physical pharmacy 4th Edition. Lea and Febiger, Philadelphia, London, 1993, pp 169-189, 370-372.
104. J.J. Kirkland, J.D. Martosella, J.W. Henderson, C.H. Dilks, J.B. Adams. HPLC of basic compound at high pH with a silica-based dibentate-C18 bonded-phase column. *American Laborator. (22/11/1999)*, 23-27.
105. I.E. Davidson. Development and validation of analytical methods, volume 3. Riley M.C., Rosank W.T., (Eds). Perganon, UK. 1996, pp 75-99.
106. M.E. Swartz, I.S. Krull. Analytical method development and validation. Marcel Dekker Inc, New York, 1997, pp 20-82.
107. H. Rosing, W.Y. Man, E. Doyle, A. Bult, H.J. Beijnen. Bioanalytical liquid chromatographic method validation. A review of current practices and procedures. *Journal of liquid chromatography and related technologies*. (2000) 23 (3), 329-354.
108. The European Agency for the Evaluation of Medicinal Products. Human Medicines Evaluation Unit.ICH topic Q2B, validation of analytical procedures: methodology. Note for guidance on validation of analytical procedures: methodology. (CPMP/ICH/281/95). November 1996. http://www.emea.eu.int/pdfs/human/ich/028195en.pdf. Retrieved 4/12/2005.
109. M. Thompson, S.L.R. Ellison, R. Wood. Hormonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical report). *Pure Applied Chemistry*. (2002) 74 (5), 835-855.
110. G.S. Clarke. The validation of analytical methods for drug substances and drug products in UK pharmaceutical laboratories. *Journal of Pharmaceutical and Biomedical Analysis*. (1994) 12 (5), pp 643-652.
111. T.C. Paino, A.D. Moore. Determination of the LOD and LOQ of an HPLC method using four different techniques. *Pharmaceutical Technology*. (1999), 86-92.
112. U. Timm, Wall M., Dell D. A new approach for dealing with the stability of drugs in biological fluids. *Journal of Pharmaceutical Sciences*. (185) 74 (9), 972-977.
113. Pharmaceutical dosage forms. Disperse systems, Volume 2. H.A. Lieberman, M.M. Riger, G.S. Banker (Eds). Mercel Dekker Inc. New York, 1998, pp 533-566.
114. Remington's Pharmaceutical Science 7th Edition. R.A. Gennore (Ed). Marck Publishing Co. Easton, Pennysylvania. 1985, pp 1580-1584.

115. T.W. Hermann. Recent research on bioavailability of drugs from suppositories. *International Journal of Pharmaceutics.* (1995) 123, 1-11.
116. B. Szilvia, R. Géza, D. Estzter, F. George, E. István. In vitro and in vivo study in rats of rectal suppositories containing furosemide. *European Journal of Pharmaceutics and Biopharmaceutics.* (2002) 53, 311-315.
117. C.O. Onyeji, A.S. Adebayo, C.P. Babalola. Effects of absorption enhancers in chloroquine suppository formulations: I In vitro release characteristics. *European Journal of Pharmaceutical Science*. (1999) 9, 131-136.
118. J. Hanace, Y. Javadzadeh, S. Taftachi, D. Farid, A. Nokhodchi. The role of various surfactants on the release of salbutamol from suppositories. *IL Farmaco.* (2004) 59, 903-906.
119. M.M. Victoria, C.J. David. Thermal and rheological study of lipophilic ethosuximide suppositories. *European Journal of Pharmaceutical Science.* (2003) 19, 123-128.
120. E.S. Samy, M.A. Hassan, S.S. Tous, C.T. Rhodes. Improvement of availability of allupurinol from pharmaceutical dosage forms I- suppositories. *European Journal of Pharmaceutics and Biopharmaceutics.* (2000) 49, 119-127.
121. E. Bergogne-Bérézin, A. Bryskier. The suppository form of antibiotic administration: pharmacokinetics and clinical application. *Journal of Pharmaceutics and Biomedical Analysis.* (2000) 23, 955-964.
122. C.W. Blissitt, R.B. Tinker, W.J. Husa. Comparison of the antibacterial activity of erythromycin in various suppusitory bases. *Journal of Pharmaceutical Sciences*. (1991) 50 (1), 56-58.
123. E.J. van Hoogdalem, A.G. de Boer, D.D. Breimer. Pharmacokinetics of rectal drug administration, Part II Clinical application of peripherally acting drugs and conclusions. *Clinical Pharmacokinetics.* (1991) 21 (2), 110-128.
124. F. Moolenaar, J.W. Meijler, W.M. Frijilink, J. Visser, H.J. Proost. Clinical efficacy, saety and pharmacokinetics of a newly developed controlled released morphine sulphate suppositories in patients with cancer pain. *European Journal of Clinical Pharmacology.* (2000) 56, 219-223.
125. A. Schneeweis, C.C. Muller-Goymann. Controlled release of solid-reversed-micellar-solution (SRMS) suppositories containing metoclopramide-HCl. *International Journal of Pharmaceutics.* (2000) 196, 193-196.
126. Y. Azechi, K. Ishikawa, N. Mizuno, K. Takahashi. Sustained release of diclofenac from polymer-containing suppository and the mechanism involved. *Drug Development and Industrial Pharmacy.* (2000) 25, 1177-1183.
127. U. Gülçin, B. Nazan. In vitro drug liberation and kinetics of sustained release indomethacin suppository. *IL Farmaco.* (2003) 58, 509-512.
128. J.L Bolognia, L. Freije, L. Amici, J. Dellostritto, F.P. Gasparro. Rectal suppositories of 8-methoxsalen produce fewer gastrointestinal side effects than theoral formulation. *Journal of the American Academy of Dermatology*. (1996) 35 (3), 424-427.

129. G.A. DeBoer, F. Moolenaar, L.G.J. DeLeede, D.D. Breimer. Rectal drug administration: Clinical pharmacokinetic considerations. *Clinical Pharmacokinetics*. (1982) 7, 285-311.
130. C. Han-Gon, L. Mi-Kyung, K. Moon-Hee, K. Chong-Kook. Effect of additives on the physical properties of liquid suppository bases. *International Journal of Pharmaceutics.* (1999) 190, 13-19.
131. Y. Mi-Ok, C. Han-Gon, J. Jae-Hee, K. Chong-Kook. Development of a thermo-reversible insulin liquid suppository with bioavailability enhancement. *International Journal of Pharmaceutics.* (1999) 189, 137-145.
132. Y.S. Chul, Y. Ha. Chae, R. Jong-Dal, L. Beom-Jin, K. Dong-Chool, K. Dae-Duk, K. Chong-Kook, C. Jun-Shik, C. Han-Gon. Enhanced rectal bioavailability of ibuprofen in rats by poloxamer 188 and menthol. *International Journal of Pharmaceutics.* (2004) 269, 169-176.
133. S.S. Abd Elhady, N.D. Mortada, G.A.S. Awad, N.M. Zaki, R.A.Taha. Development of in situ gelling and mucoadhesive mebeverine hydrochloride solution for rectal administration. *Saudi Pharmaceutical Journal.* (2003) 11 (4), 159-171.
134. K. Chong-Kook, L. Sa-Won, C. Han-Gon, L. Mi-Kyung, G. Zhong-Gao. Trials of in situ gelling and mucoadhesive acetaminophen liquid suppository in human subjects. *International Journal of Pharmaceutics.* (1998) 174, 201-207.
135. R. Yahagi, O. Hiraku, M. Yoshiharu. Preparation and evaluation of double-phased mucoadhesive suppositories of lidocaine utilizing Carbopol® and white beeswax. *Journal of Control Release.* (1999) 61, 1-8.
136. R. Yahagi, Y. Machida, H. Onishi. Mucoadhesive suppositories of ramosetron HCl utilizing carbopol®. *International Journal of pharmaceutics.* (2000) 193, 205-212.
137. N. Toshiaki, J.H. Rytting. Absorption-promoting adjuvants: enhancing action on rectal absorption. *Advance drug delivery reviews.* (1997) 205-228.
138. W.J. van Hoogdalem, A.G. de Boer, D.D. Breimer. Pharmacokinetics of rectal drug administration part I. General considerations and clinical application of centrally acting drugs. *Clinical Pharmacokinetics*. (1991) 21 (1), 11-26.
139. Rectal and vaginal drug delivery. Pharmaceutics: The science of dosage form design 2nd Edition. Aulton E.M (Ed). Churchill, Livingston. 2002, pp 534-543.
140. Suppositories and other rectal vaginal and urethral preparations. Introduction to Pharmaceutical dosage forms. Ansel C.H (Ed). Lea and Febiger, Philadelphia. 1981, pp 328-344.
141. L.J. Coben, H.A. Lieberman. The theory and practice of industrial pharmacy. Lea and Febiger, Philadelphia. 1986, pp 564-588.
142. J.A. Webster, R. Dowse, R.B. Walker. Suppositories: an underutilized dosage form. *The Journal of Morden Pharmacy*. (1996) 3(2), 26-30.
143. B. Dimitrova, I. Doytchinova, M. Zlatkova. Reversed-phase high-performance liquid chromatography for evaluating the distribution of pharmaceutical substances in suppository

base-phosphate buffer system. *Journal of Pharmaceutical and Biomedical Analysis*. (2000) 23, 955-964.

144. M. Masateru, K. Naoki, O. Yoshikazu, M. Tadashi, M. Takanori, T. Hajime, O. Masaaki, O. Ken-ichi, H. Kazutaka, K. Toshikiro. Development of Suppository formulation safety improving rectal absorption of rebamipide, a poorly absorbable drug, by utilizing sodium laurate and taurine. *Journal of controlled release.* (2004) 99, 63-71.
145. K. Nakanishi, M. Masada, T. Nadai. Effect of pharmaceutical adjuvants on the rectal permeability of drugs II. Effect of tween-type surfactants on the permeability of drugs in the rat rectum. *Chemical and Pharmaceutical Bulletin*. (1983) 31 (9), 3255-3263.
146. T. Lindmark, J. D. Söderholm, G. Olaison, G. Alván, G. Ocklind, P. Arturson. Mechanism of absorption enhancement in human after rectal administration on ampicillin in suppositories containing sodium caprate. *Pharmaceutical Research*. (1997) 14 (7), 930-935.
147. K.I. Nishimura. Studies on the promoting effects of carboxylic acid derivatives on the rectal absorption of β-lactam antibiotics in rats. *Chemical and Pharmaceutical Bulletin*. (1985) 33 (1), 282-291.
148. A.F. Caldwell. Hydrogenated fats in suppository bases for tropical courtiers. *Quarterly Journal of Pharmacy Pharmacology.* (1939) 12, 689-698.
149. Handbook of pharmaceutical excipients 4th Edition. R.C. Rowe, P.J. Sheskey, P.J. Weller (Eds). Pharmaceutical Press (PhP), London. 2003, pp 454-459, 636-643.
150. N.J. Vidras, V.E. Reid, N.R. Bohidar, P.M. Plakogiannis. Medicament release from suppository bases I: physicochemical characteristics and bioavailability of indomethacin in rabbits. *Journal of Pharmaceutical Sciences*. (1982) 71(8), 945-949.
151. L.V. Allen. Compounding Suppositories Part I. Current and Practical Compounding Information for the Pharmacist. *Secundum Artem* Volume 3, Number 3.
152. C-Y. Perng, A.S. Kearney, K. Patel, N.R. Palepu, G. Zuber. Investigation of formulation approaches to improve the dissolution of SB-210661, a poorly water soluble 5-lipoxygenase inhibitor. *International Journal of Pharmaceutics*. (1998) 176, 31-38.
153. W-L. Chiou, R. Sidney. Pharmaceutical applications of solid dispersion systems. *Journal of Pharmaceutical Science.* (1971) 60 (9), 1281-1302.
154. O.I. Corrigan, T. Stanley. Mechanism of drug dissolution rate enhancement from β-cyclodextrin-drug systems. *Journal of Pharmacy and Pharmacology*. (1982) 34, 621-626.
155. M. Franco. Dissolution properties and anticonvulsant activity of phenytoin – polyethylene glycol 6000 and –polyvinylpyrrolidone K-30 solid dispersion. *International Journal of Pharmaceutics*. (2001) 225, 63-73.
156. Y.E. Hammouda, N.A. Kasim, A.H. Nada. Formulation and *in vitro* evaluation of verapamil HCl suppositories. *International Journal of Pharmaceutics*. (1993) 89, 111-118.

157. T. Takatori, N. Shimono, K. Higaki, T. Kimura. Evaluation of sustained release suppositories prepared with fatty bases including solid fats with high melting points. *International Journal of Pharmaceutics*. (2004) 278, 275-282.

158. S. Janicki, M. Sznitowska, W. Zebrowska, H. Gabiga, M. Kupiec. Evaluation of paracetamol suppositories by a pharmacopoeial dissolution test, comments on methodology. *European Journal of Pharmaceutics and Biopharmaceutics*. (2001) 52, 249-254.

159. A. Mohammadi, I. Kanfer, R.B. Walker. A capillary zone electrophoresis (CZE) method for the determination of cyclizine hydrochloride in tablets and suppositories. *Journal of Pharmaceutical and Biomedical Analysis*. (2004) 35, 233-239.

160. R.A. Almodóvar, R.A. Rodríguez, O. Rosario. Inverse supercritical extraction of acetaminophen from suppositories. *Journal of Pharmaceutical and Biomedical Analysis*. (1998) 17, 89-93.

161. S. Labbozzetta, L. Valvo, P. Bertocchi, L. Manna. Focused microwave-assisted extraction and LC determination of the active ingredient in naproxen-based suppositories. Journal of *Pharmaceutical and Biomedical Analysis*. (2005) 39, 463-468.

162. S. Esnaashari, Y. Javadzadeh, H.K. Batchelor, B.R. Conway. The use of microviscometry to study polymer dissolution from solid dispersion drug delivery systems. *International Journal of Pharmaceutics*. (2005) 292, 227-230.

163. C. Leuner, J. Dressman. Improving drug solubility for oral delivery using solid dispersions. *European Journal of Pharmaceutics and Biopharmaceutics.* (2000) 50, 47-60.

164. European Medicines Agency Inspections. Commitee for medicinal products for human use (CHMP) and Commitee for medicinal products for veterinary use (CVMP), February 2005. Annexes to: CMP/ICH/283/95 Impurities: Guidance for residual solvents and CVMP/VICH/502/99 Guidance on impurities: residual solvents. http://www.emea.eu.int/pdfs/human/qwp/045003en.pdf. Retrieved 20/11/2005.

165. K. Yamashita. Establishment of new preparation method for solid dispersion formulation of tacrolimus. *International Journal of Pharmaceutics*. (2003) 267, 79-91.

166. H. Lennernäs, K. Gjellan, R. Hällgren, C. Graffner. The influence of caprate on rectal absorption of phenoxymethylpenicillin: experience from an *in vivo* perfusion in humans. *Journal of Pharmacy and Pharmacology*. (2002) 54, 499-508.

167. H. Kokki, M. Karvinen, P. Suhonen. Pharmacokinetics of intravenous and rectal ketoprofen in young children. *Clinical Pharmacokinetics*. (2003) 42, 373-379.

168. V. Tantishaiyakul. N. Kaewnopparat, S. Ingkatawornwong. Properties of solid dispersions of piroxicam in polyvinylpyrrolidone. *International Journal of Pharmaceutics*. (1999) 181, 143-151.

169. N.Realdon, EUG.Ragazzi, ENR.Ragazzi. Effect of drug solubility on *in vitro* availability rate from suppositories with polyethylene glycol excipients. *Pharmazie* (2001) 56, 163-167.

170. I.W. Kellaway, C. Marriott. Correlations between physical and drug release characteristics of polyethylene glycol suppositories. *Journal of Pharmaceutical Sciences*. (1975) 67 (7), 1162-1166.
171. N.Realdon, EUG.Ragazzi, ENR.Ragazzi. Effect of drug solubility on the *in vitro* availability rate from suppositories with lipophilic excipients. *Pharmazie* (2000) 55 (5), 372-377.
172. A.J.M. Schoonen, F.Moolenaar, T. Huzinga. Release of drugs from fatty suppository bases I. The release mechanism. *International Journal of Pharmaceutics*. (1979), 4, 141-152.
173. H. Loth, P. Bosche. Kinetics, mechanism and *in vitro* measurement of drug liberation from suppositories. *Pharmazie*. (1996) 51 (8), 571-577.
174. A. Babar, T. Bellete, F.M. Plakogiannis. Ketoprofen suppository dosage forms: *in vitro* release and *in vivo* absorption studies in rabbits. *Drug Development and Industrial Pharmacy*. (1999) 25 (2), 241-245.
175. D.J. Morgan, Y. McCormick, W. Cosolo, L. Roller, J. Zalcberg. Prolonged release of morphine alkaloid from a lipophilic suppository base *in vitro* and *in vivo*. *International Journal of Clinical Pharmacology, Therapy and Toxicology*. (1992) 30 (12), 576-581.
176. K. Gellan, C. Graffner, H. Quiding. Influence of amount of hard fat in suppositories on the in vitro release rate and bioavailability of paracetamol and codeine. I. A comparison of three suppository compositions *in vivo*. *International Journal of Pharmaceutics*. (1994) 102, 71-80.
177. D. Chicco, I. Grabnar, A. Škerjanec, D. Vojnovic, V. Maurich, N. Realdon, E. Ragazzi, A. Belič, R. Karba, A. Mrhar.. Correlation of *in vitro* and *in vivo* paracetamol availability from layered excipient suppositories. *International Journal of Pharmaceutics*. (1999) 189, 147-160.
178. C. Young, K.J. Palin, A.S. Reid, N.W. Thomas, P.L. Gould. Formulation of Fenbufen suppositories. II. Selection of a suppository base using dissolution studies and histological studies in rats. *International Journal of Pharmaceutics.* (1987) 40, 187-191.
179. T.Oribe, M.Yamada, K.Takeuchi, S.Tsunemi, K.Imasaka, O.Shirakura et al. Formulation and *in vivo-in vitro* correlation of the dissolution property of lemildipine solid dispersions-incorporated suppositories. *International Journal of Pharmaceutics* (1995) 124**,** 27-35.
180. J.C.McElnay, A.C.Nicol. The comparison of a novel continuous-flow dissolution apparatus for suppositories with the rotating basket technique. *International Journal of Pharmaceutics* (1984) 19**,** 89-96.
181. K.Gjellan, C.Graffner. Comparative dissolution studies of rectal formulations using the basket, the paddle and the flow-through methods: II. Ibuprofen in suppositories of both hydrophilic and lipophilic types. *International Journal of Pharmaceutics* (1994) 112, 233-240.
182. N.Aoyagi, N.Kaniwa, M.Uchiyama. Inter-laboratory reproducibility of release tests for suppositories. *Drug Development and Industrial Pharmacy* (1995) 21 175-183.
183. J.A.Webster, R.Dowse, R.B.Walker. *In vitro* release of amoxicillin from lipophilic suppositories. *Drug Development and Industrial Pharmacy.* (1998) 24**,** 395-399.

184. A.K.Dash, Z.Gong, D.W.Miller, H.Huai-Yan, J-P.Laforet. Development of a rectal nicotine delivery system for the treatment of ulcerative colitis. *International Journal of Pharmaceutics* (1999) 190**,** 21-34.

185. N. Fotaki, C. Reppas. The flow-through cell methodology in the evaluation of intralumenal drug release characteristics. *Dissolution Technologies*. May, 2005, 17-21.

186. G.Lootvoet, E.Beyassac, G.K.Shiu, J.M.Aiache, W.A.Ritschel. Study on the release of indomethacine from suppositories: *in vitro-in vivo* correlation. *International Journal of Pharmaceutics* (1992) 85, 113-120.

187. K.Gjellan, C.Graffner. Influence of amount of hard fat in suppositories on the *in vitro* release rate and bioavailability of paracetamol II. A comparison between three compositions and a rectal solution. *International Journal of Pharmaceutics* (1994) 104**,** 215-226.

188. J.Lauroba, I.Diez, M.Rius, C.Peraire, J.Domenech. Study of the release process of drugs: suppositories of paracetamol. *International Journal of Clinical Pharmacology,Therapy and Toxicology* (1990) 28**,** 118-122.

189. N. Realdon, EUG. Ragazzi, M. Morpurgo, ENR. Ragazzi. *In vitro* methods for the evaluation of drug availability from suppositories: Comparison between biological and artificial membranes. *Pharmazie*. (2005) 60 (10), 756-760.

190. S. Muranish, Y. Okuba, H. Sezaki. Manufacture and examination of apparatus for drug release from suppositories. *Yakazaigaku*. (1979) 39, 1-7.

191. A.H.Abd El-Gawad, E.Zin El-Din, H.Abd El-Alim. Effect of surfactant incorporation techniques on sulphamethoxazole suppository formulations. *Pharmazie* (1988) 43**,** 624-627.

192. C.S.Yong, Y-K Oh, Y-I.Kim, J.O.Kim, B-K.Yoo, J-D.Rhee, K.C.Lee, D-D.Kim, Y-J.Park, C-K.Kim, H-G.Choi. Physicochemical characterization and *in vivo* evaluation of poloxamer-based solid suppository containing diclofenac sodium in rats. *International Journal of Pharmaceutics* (2005) 301**,** 54-61.

193. E.Ízgü, Ü.Güngör. The use of natural membranes for *in vivo* determination of absorption rates of drugs from suppository bases. *International Journal of Pharmaceutics* (1981) 9, 107-120.

194. M. Siewert, J. Dressman, C.K. Brown, V.P. Shah. FIP/AAPS Guidelines to dissolution/in vitro release testing of novel/special dosage forms. *AAPS PharmSci Tech*. (2003) 4(1) Article 7.

195. D. Ermis, N. Tarimci. Ketoprofen sustained-release suppositories containing hydrxypropylmethycellulose phthalate in PEG bases. *International journal of Pharmaceutics.* (1995) 113, 65-71.

196. J.W. Moore, H. Flanner. Mathematical comparison of dissolution profiles. *Pharmaceutical Technology*. (1996) 6, 64-74.

197. US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research CDER. Guidance for industry: Dissolution testing of immediate-

release solid oral dosage forms. August 1997. http://www.fda.gov/cder/guidance/1713bpl.pdf. Retrieved 20-11-2005.

198. US Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research CDER November 1995. Guidance for industry: Immediate release solid oral dosage forms. Scale-up and post-approval changes. Chemistry, manufacturing and controls. *In vitro* dissolution testing and *in vivo* bioequivalence documentation. http://www.fda.gov/cder/guidance/cmc5.pdf. Retrieved 24-11-2005.
199. European Agency for the Evaluation of Medicial Products. Human Medicines Evaluation Unit, Jully 1999. Note for guidance on quality of medified release products. A. Oral dosage forms; B. Transdermal dosage forms; Section I (Quality), CPMP/QWP/604/96. http://www.eu.int/pdfs/human/qwp/060496en.pdf. Retrieved 24- 11-2005.
200. M.C.Gohel, M.K.Panchal. Comparison of *in vitro* dissolution profiles using a novel, model-independent approach. *Pharmaceutical Technology* (2002) 92-102.
201. A.S. Reid, N.W. Thomas, K.J. Palin, P.L. Gould. Formulation of fenbufen suppositories. I. Quantitative histological assessment of the rectal mucosa of rats following treatment with suppository bases. *International Journal of Pharmaceutics*. (1987) 40, 181-185.
202. E.J. van Hoogdalem, C. Vermeij-Kerrs, A.G. DeBoer. Topical effects of absorption ehancing agents on the rectal mucosa of rats *in vivo*. *Journal of Pharmaceutical Sciences*. (1990) 79, (10), 866-870.
203. N. Realdon, E. Ragazzi, M. DalZotto, G.D. Fini. Layered excipients suppositories: the possibility of modulating drug availability. *International Journal of Pharmaceutics*. (1997) 148, 155-163.
204. S.Othman, H.Muti. The effect of bases and formulation on the release of indomethacin from suppositories. *Drug Development and Industrial Pharmacy* (1986) 12**,** 1813-1831.
205. Guidlines for bioequivalence studies of generic products, December, 1997. http://www.nihs.go.jp/drug/be-guide(e)/form/form-change.pdf. Retrieved 30-11-2005.
206. M.S.Bergren, M.M.Battle, G.W.Halstead, D.L.Theis. Investigation of the relationship between melting-related parameters and *in vitro* drug release from vaginal suppositories. *Journal of Pharmaceutical and Biomedical Analysis* (1989) 7, 549-561.
207. E.A.Hosny, S.S.Abdel-hady, K.E.H.El-Tahir. Formulation, *in vitro* release and *ex-vivo* spasmolytic effects of mebeverine hydrochloride suppositories containing polycarbophil or polysorbate 80. *International Journal of Pharmaceutics* (1996) 142**,** 163-168.
208. J.E.Fontan, R.Arnaud, J.C.Chaumeil. Enhancing properties of surfactants on the release of carbamazepine from suppositories. *International Journal of Pharmaceutics* (1991) 73**,** 17-21.
209. E.M. Samy. Formulation and evaluation of tenoxicam suppositories. *Bulletin of the Faculty of Pharmacy Cairo University*. (2001) 39 (1), 331-344.

210. T.Oribe, M.Yamada, K.Takeuchi, S.Tsunemi, K.Imasaka, O.Shirakura et al. Formulation and *in vivo-in vitro* correlation of the dissolution property of lemildipine solid dispersions-incorporated suppositories. *International Journal of Pharmaceutics* (1995) 124, 27-35.
211. H.W. Frijlink, A.C. Eissens, A.J.M. Schoonen, C.F. Lerk. The effects of cyclodextrins on drug release from fatty suppository bases. I. In vitro observations. *European Journal of Pharmaceutics and Biopharmaceutics. (*1991) 37 (3), 178-182.
212. H.W. Frijlink, A.C. Eissens, A.J.M. Schoonen, C.F. Lerk. The effects of cyclodextrins on drug release from fatty suppository bases. II. In vivo observations. *European Journal of Pharmaceutics and Biopharmaceutics.* (1991) 37 (3), 183-187.
213. C. Leurner, J. Dressman. Improving drug solubility for oral delivery using solid dispersions. *European Journal of Drug Metabolism and Pharmacokinetics* (2000) 50, 47-60.
214. D.Q.M.Craig. The mechanisms of drug release from solid dispersions in water-soluble polymers. *International Journal of Pharmaceutics* (2002) 231**,** 131-144.
215. H.N. Joshi, R.W.Tejwani, M.Davidovich, V.P.Sahasrabudhe, M.Jamel, M.S.Bathala, S.A.Varia, A.T.M.Serajuddin. Bioavailability enhancement of a poorly water-soluble drug by solid dispersion in polyethylene glycol-polysorbate 80 mixture. *International Journal of Pharmaceutics* (2004) 269**,** 251-258.
216. L.J. Coben, N.G. Lordi. Physical stability of semi-synthetic suppository bases. *Journal of Pharmceutical Sciencs*. (1980) 69(8), 955-960.
217. E.M. Samy. Formulation and evaluation of tenoxicam suppositories. *Bulletin of the Faculty of Pharmacy of Cairo University*. (2001) 39 (1), 331-344.
218. N.H.Anderson, M.Bauer, N.Boussac R.Khan-Malek, P.Munden, M.Sardaro. AN evaluation of fit factors and dissolution efficiency for the comparison of *in vitro* dissolution profiles. *Journal of Pharmaceutical and Biomedical Analysis* (1998) 17**,** 811-822.
219. Y.Tsong, T.Hammerstrom, P.Sathe, V.P.Shah. Statistical assessment of mean differences between two dissolution data sets. *Drug Information Journal* (1996) 30**,** 1105-1112.
220. T.O´Hara, A.Dunne, J.Butlet, J.Devane. A review of methods used to compare dissolution profile data. *Plasma Sources, Science and Technology* (1998) 1**,** 214-223.
221. V.P.Shah, Y.Tsong, P.Sathe, J-P.Liu. *In vitro* dissolution profile comparison-statistics and analysis of the similarity factor, f_2. *Pharmaceutical Research* (1998) 15**,** 889-896.
222. AJ. Siepmann. Mathematical modeling of controlled drug delivery. *Advanced Drug Delivery Reviews* (2001) 48**,** 137-138.
223. N.Yuksel, A.E.Kanik, T.Baykara. Comparison of *in vitro* dissolution profiles by ANOVA-based, model-dependent and -independent methods. *International Journal of Pharmaceutics* (2000) 209**,** 57-67.
224. P.Costa. An alternative method to the evaluation of similarity factor in dissolution testing. *International Journal of Pharmaceutics* (2001) 220, 77-83.

225. P.Costa, J.M.S.Lobo. Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences* (2001) 13**,** 123-133.

226. P.M.Sathe, Y.Tsong, V.P.Shah. In-vitro dissolution profile comparison: statistics and analysis, model dependent approach. *Pharmaceutical Research* (1996) 13**,** 1799-1803.

227. M.C. Gohel, M.K. Panchal. Novel use of similarity factors f_2 and S_d for the development of diltiazem HCl modified-release tablets using a 3^2 factorial design. *Drug Development and Industrial Pharmacy.* (2002) 28(1), 77-87.

228. K.A.Khan. The concept of dissolution efficiency. *Journal of Pharmacy and Pharmacology* (1975) 27**,** 48-49.

229. V.Pillay, R.Fassihi. Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method. *Journal of Controlled Release* (1998) 55**,** 45-55.

230. A.H.ElAssays, N.H.Foda, S.S.Badawi, R.T.Abd.ElRehim. Formulation of flurbiprofen suppositories. *Egyptian Journal of Pharmaceutical Sciences* (1995) 36**,** 31-35.

231. M.Gibaldi, S.Feldman. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. *Journal of Pharmaceutical Sciences* (1967) 56**,** 1238-1242.

232. T.Higuchi. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences* (1963) 52**,** 1145-1149.

233. T.Higuchi. Rate of release of medicaments from ointment bases containing drugs in suspension. *Journal of Pharmaceutical Sciences* (1961) 50**,** 874-875.

234. R.W.Korsmeyer, R.Gurny, E.Doelker, P.Buri, N.A.Peppas. Mechanism of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics* (1983) 15**,** 25-35.

235. N.A.Peppas. Analysis of Fickian and non-Fickian drug release from polymers. *Pharmacy Acta Helv* (1985) 60**,** 110-111.

236. S.K.El-Arini, H.Leuenberger. Modelling of drug release from polymer matrices: effect of drug loading. *International Journal of Pharmaceutics* (1995) 121**,** 141-148.

237. H.Kim, R.Fassihi. Application of binary polymer system in drug release rate modulation. 2. Influence of formulation variables and hydrodynamic conditions on release kinetics. *Journal of Pharmaceutical Sciences* (1997) 86**,** 323-328.

238. V.Pillay, R.Fassihi. *In vitro* release modulation from cross linked pellets for site-specific drug delivery to the gastrointestinal tract. I. Comparison of pH-responsive drug release and associated kinetics. *Journal of Controlled Release* (1999) 59**,** 229-242.

239. F.Langenbucher. Linearization of dissolution rate curves by the Weibull distribution. *Journal of Pharmacy and Pharmacology* (1972) 24**,** 979-981.

240. E.Karasulu, H.Y.Karasulu, G.Ertan, L.Kirilmaz, T.Güneri. Extended release lipophilic indomethacin microspheres: formulation factors and mathematical equations fitted drug release rates. *European Journal of Pharmaceutical Sciences* (2003) 19**,** 99-104.

241. Rectal and viginal products. The Pharmaceutical Codex. Principles and practice of pharmaceutics 12th Edition. W. Lund (Ed). The Pharmaceutical Press, London. 1994, pp 170-175.