# CHAPTER 7
# PERMEABILITY

## 7.1 Introduction

The transport rate, as well as the extent to which a drug is carried across a membrane can be collectively described as the permeability of the drug through that membrane. The permeation process through the intestinal membrane is facilitated by different mechanisms (Hunter & Hirst, 1997:130). These mechanisms all have their unique ways of transporting drug molecules through the membrane, including passive diffusion, and active- and facilitated transport (Figure 7.1) (Hunter & Hirst, 1997:130). Permeation of a drug across membranes can be used to establish an estimation of its oral absorption within the human body. Of the most used *in vitro* methods for the assessment of drug permeability across the intestinal membrane include the parallel artificial membrane permeability assay (PAMPA), Caco-2 cell monolayer, Madin-Darby canine kidney (MDCK) cell monolayers, and excised tissue techniques. The two most commonly used tissue techniques are isolated sheets of intestinal mucosa, mounted in Ussing type chambers (Berggren, 2004:555) and everted intestinal rings (Ashford, 2002:259). These *in vitro* permeability assays are effective and insightful in the early screening of the pharmacokinetic properties of new and improved drug compounds. Permeability across the intestinal epithelium is a very important factor that determines the total amount of drug absorbed from the gastro-intestinal tract. Poor membrane permeability of a drug will ultimately result in its low bioavailability (Hunter & Hirst, 1997:130; Nožinić *et al*., 2010:323; Kerns & Di, 2008:90).

In this chapter, the *in vitro* permeation of azithromycin dihydrate (AZM-DH) and azithromycin glass (AZM-G), dissolved in different media (pH ranging from 4.5 to 7.2) and across excised pig intestinal tissue, is discussed. The permeability of these two forms of azithromycin (AZM) was compared to determine if the improved solubility of AZM-G over AZM-DH had an effect on its membrane permeability. The cumulative transport of AZM-DH and AZM-G was each expressed as a function of time and the apparent permeability coefficient ($P_{app}$) values were calculated for every transport test performed. Research article is in process for submission in due course (Annexure C).

## 7.2 Membrane permeability

Permeability can be described as the rate and extent of drug transport through a biological membrane. Permeation across the intestinal membrane depends on the release of the drug from the solid dosage form, the dissolution rate, the amount of drug dissolved, the stability of the drug inside of the lumen and the transit time through the lumen (Lennernäs, 2007:1105). Without permeability, the drug absorption process from the gastro-intestinal tract into the systemic circulation would not occur. Daugherty and Mrsny (1999:147) present the following guidelines for understanding drug transport across the intestinal membrane and for the drug absorption process:

- Amphipathic drugs with small particle size will be transported *via* the transcellular route. They will partition in and out of the lipid bilayers.
- Hydrophilic drugs with small particle size are limited to the route of paracellular diffusion, although they can also follow the aqueous route.
- Absorption of peptides and proteins are poor and therefore necessitates the use of special mechanisms for uptake, or the addition of enhancing agents.
- The further along the intestine the drug travels, the lesser drug permeability becomes.

For a drug molecule to reach the blood vessels surrounding the gastrointestinal tract, it must pass across the apical (bilayer) lipid membrane of the intestinal epithelium, then through the cytoplasm and ultimately through the basolateral membrane (Figure 7.1). In some cases, the drug molecule can move in a lateral direction and exit the cell membrane elsewhere. Membrane permeation occurs as a result of various mechanisms which facilitate this process, including passive transcellular or paracellular diffusion, active uptake, facilitated transport and endocytosis (Figure 7.1) (Hunter & Hirst, 1997:130; Kerns & Di, 2008:86).

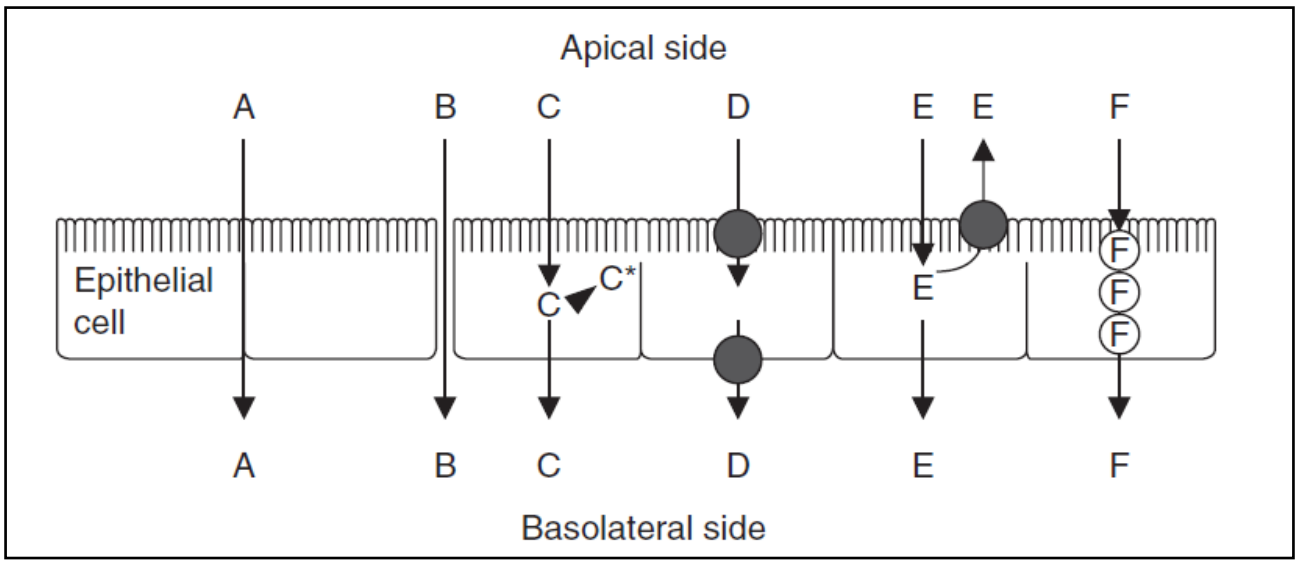


**Figure 7.1 Graphic illustration of the different mechanisms of intestinal absorption. (A) Paracellular diffusion; (B) Paracellular diffusion enhanced by a modulator of tight junctions; (C) Transcellular passive diffusion; (C* Intracellular metabolism); (D) Carrier-mediated active transcellular transport; (E) Transcellular diffusion coupled with an efflux mechanism; (F) Transcellular endocytosis (Taken from Hunter & Hirst, 1997:131).**

### 7.2.1 Transcellular and paracellular passive diffusion

Passive diffusion is accountable for almost 95% of drug absorption from the gastro-intestinal tract and is therefore one of the most studied mechanisms of drug transport during drug research and development. This mechanism is characterised by drug molecule movement from a high concentration in the gastro-intestinal lumen to a low concentration in the blood vessels (Figure 7.1). The main driving force behind passive diffusion is hence the existing concentration gradient. Passive diffusion can be generalised to be divided into two routes, according to the occurring pathway of diffusion. If the drug molecules move through the tight junctions between adjacent cells, it is referred to as paracellular diffusion, whereas diffusion through the cells is described as transcellular (Daugherty & Mrsny, 1999:147; Hayashi *et al*., 1997:192; Kerns & Di, 2008:87).

The permeability of polar molecules *via* passive diffusion is lower than that of lipophilic molecules. Molecules in their unionised or neutral forms are also more permeable than molecules in their ionic forms. This signifies the importance of pH and $pK_a$ for passive

diffusion of a drug across a biological membrane (Hayashi et al., 1997:192; Hunter & Hirst, 1997:132; Kerns & Di, 2008:87).

The passive diffusion of basic drug molecules at a low pH is relatively low across a membrane (transcellular diffusion), due to the fact that when in solution, molecules are charged. As the pH increases closer to the $pK_a$ value of the drug, the amount of neutral molecules increases and better conditions exist for its permeability. It is therefore noteworthy that passive diffusion of a basic drug molecule across a biological membrane is most favourable at a higher pH value (Hayashi et al., 1997:192; Hunter & Hirst, 1997:132; Kerns & Di, 2008:88).

Small polar molecules (usually with a molecular weight of less than 180 Da.) can pass through the intestinal membrane by way of paracellular diffusion. This mechanism relates to the transport of molecules between the epithelial cells (through intercellular spaces) to the other side of the epithelium (Figure 7.1). Due to the relatively small surface area, paracellular transport accounts for less than 5% of drug permeability in the intestines (Hunter & Hirst, 1997:132; Kerns & Di, 2008:89; Pade & Stavchansky, 1997:1210).

### 7.2.2 Endocytosis

Endocytosis relates to the entrapment of the drug molecule within a cell vesicle in the membrane, where after the vesicle releases the molecule beyond the membrane (Figure 7.1). The transport of drugs through the intestinal membrane by way of endocytosis seems to be less important and is therefore studied to a lesser extent (Hunter & Hirst, 1997:132; Kerns & Di, 2008:89).

### 7.2.3 Active transport

This mechanism of drug transport involves transmembrane proteins for which the drug molecule should have affinity and energy in the form of ATP (Figure 7.1). ATP supplies the energy needed for the transmembrane protein to carry the drug molecule through the membrane. Transport *via* this mechanism may take place from a low to a high concentration, thus resulting in transport against the concentration gradient (Hunter & Hirst, 1997:132; Kerns & Di, 2008:89).

Besides passive diffusion, efflux is also considered a significant permeability mechanism. Efflux transporters are utilised to actively transport the drug molecules from within the

epithelial cells back into the intestinal lumen (Figure 7.1). The efflux mechanism therefore reduces drug absorption and thereby also decreases the bioavailability of an orally administered drug that is a substrate of efflux transporters. P-glycoprotein (P-gp) is an active efflux transporter protein that is commonly associated with macrolide antibiotics. According to Nožinić *et al*. (2010:329), some macrolide antimicrobials are regarded as P-gp substrates. Azithromycin has proven to have the smallest affinity for P-gp, whereas telithromycin has the highest P-gp affinity (Hunter & Hirst, 1997:133; Kerns & Di, 2008:89; Nožinić*et al*., 2010:329; Pachot *et al.*, 2003:1).

## 7.3 Effect of membrane permeability on bioavailability

Permeability is a significant factor in the overall amount of drug that is absorbed from the gastro-intestinal tract into the systemic circulation after its oral administration. The permeation of a drug across the intestinal membrane is affected by its solubility, as well as by its dissolution rate. If these two factors are low, the permeability of a drug will be poor as a result of the low concentration of drug being dissolved and available for permeation. Characteristically, a drug compound with poor membrane permeability will result in its low bioavailability. On a typical blood plasma concentration/time curve, the first part of the curve, also referred to as the absorption phase, ascends, hence representing an increase in drug concentration due to absorption. Membrane permeation of the drug molecules in the absorptive direction therefore directly affects its bioavailability. The bioavailability of a drug to a large extent thus depends upon the permeability of a drug across the epithelial membrane of the gastro-intestinal tract after oral drug administration (Ashford, 2002:262; Hunter & Hirst, 1997:131; Kerns & Di, 2008:90; Nožinić*et al*., 2010:323; Pachot *et al.*, 2003:1).

## 7.4 Materials and methods

Azithromycin dihydrate (AZM-DH) (Batch no: IF-AZ-080506, DB Fine Chemicals) was used to prepare azithromycin glass (AZM-G). Krebs Ringer bicarbonate buffer (KRBB) (Batch no: 089K8300) was purchased from Sigma Aldrich (SA) and was used as physiological buffer. Sodium bicarbonate (Batch no: 444227/1) was purchased from Fluka. Pig intestinal tissue for this study was obtained from freshly slaughtered pigs at the

Potchefstroom abattoir and the study was approved by the North-West University's Ethics Committee (NWU-0018-09-A5).

### 7.4.1 Preparation of azithromycin solutions

The permeability of both AZM-DH and AZM-G was determined in order to establish any differences in permeability, due to the improved solubility and dissolution rate of AZM-G over AZM-DH (refer to Chapter 5 for improved solubility of AZM-G and to Chapter 6 for dissolution profiles). The *in vitro* transport study was performed in four media, i.e. in three KRBB solutions at pH 7.2, 6.8 and 4.5, and in water (double-distilled, pH 6.2 - 6.8). KRBB was prepared at 9.50 g/L and supplemented with 1.26 g of sodium bicarbonate. In order to establish the effect of solubility on the permeability of these two AZM forms, oversaturated solutions were prepared. To determine the oversaturation concentrations of AZM in each medium, the solubility of AZM-DH and AZM-G was determined in each. Upon analysis of the solubility samples, results exceeding the concentration range of the HPLC method were obtained. It was much higher than anticipated, and hence not within the analytical range. Since solubility data is semi-quantitative, it was decided to accept the data 'as is' and not widen the analytical range. Extrapolation was utilised to ultimately quantify the samples exceeding the range. These solubility results are summarised in Table 7.1.

**Table 7.1: Concentrations of AZM-DH and AZM-G in different transport media**

| Medium | AZM-DH (µg/mL) | AZM-G (µg/mL) |
|---|---|---|
| **Krebs Ringer bicarbonate buffer (pH 7.2)** | 480 | 710 |
| **Krebs Ringer bicarbonate buffer (pH 6.8)** | 1240 | 1500 |
| **Krebs Ringer bicarbonate buffer (pH 4.5)** | 8600 | 8400 |
| **Water (pH 6.2 - 6.8)** | 70 | 300 |

From the solubility results in Table 7.1, it is clear that AZM-G had a higher solubility than AZM-DH in those media with pH values of 6.8 and 7.2, but not at pH 4.5. Since AZM is a basic drug, it becomes more ionised as the pH drops to below its $pK_a$ value, which explains why these different forms had more similar solubility profiles at lower pH values.

From the saturated concentrations of AZM-DH and AZM-G, as per Table 7.1, it was decided that a concentration of 2000 µg/mL (oversaturated solution) would be used for the transport studies in two KRBB solutions (pH 7.2 and pH 6.8) and in water. The chosen concentration for oversaturation of the AZM-DH and AZM-G for the transport tests in pH 4.5 KRBB was 9000 µg/mL. All drug solutions were prepared by individually weighing the accurate amount of drug for each solution (7 mL). The solutions were all sonicated for 5 minutes prior to commencement of the study. The solutions of AZM-DH and AZM-G in each medium were prepared in 7 individual replicates, of which one was used to determine the initial concentration of AZM being dissolved in the medium. The other 6 solutions were each applied to the apical side of the excised pig intestinal segment within the diffusion chambers.

### 7.4.2 Preparation of tissue for *in vitro* transport

This *in vitro* transport study was based on measuring the passage of two forms of AZM (the dihydrate and the glass) across excised jejunal tissue of pigs from the apical- to the basolateral direction. The tissue section that was used in this study consisted of that part of the small intestine that exists beyond the first 60 cm from the stomach. A small piece (approximately 30 cm) (Figure 7.2) of this area of intestinal tissue was collected and carefully washed with cold KRBB (below 8˚C). This cleaned section of the small intestine was then placed in a container filled with cold KRBB, within which it was transported to the laboratory for commencement of the study.

Further preparation of the tissue for the transport study was done within one hour of collection at the abattoir and minimal handling of the tissue was important. On arrival at the laboratory, the acquired piece of small intestine was again washed with cold KRBB solution, where after it was carefully hauled over a glass tube for final preparation for experimental use. Throughout the preparation process the tissue was kept moist with KRBB to avoid alteration to the integrity of the tissue.

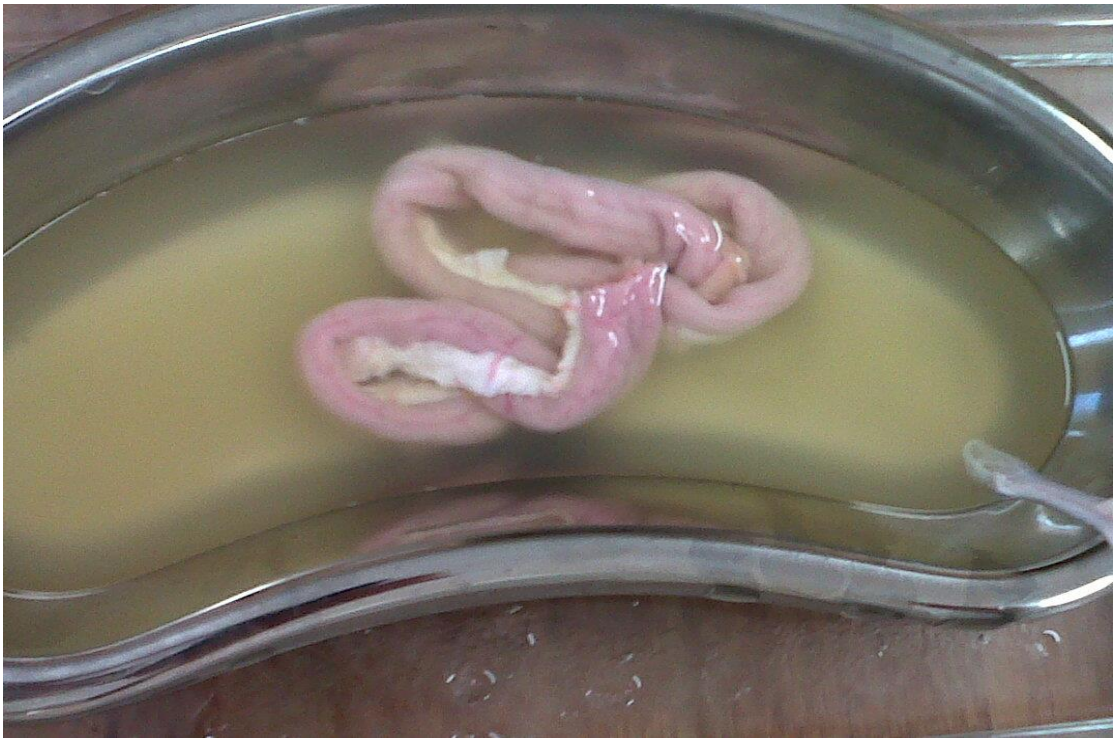

**Figure 7.2 Isolated section of pig intestinal tissue after being washed with cold Krebs Ringer bicarbonate buffer.**

The serosal layer on the outside of the small intestine was cautiously removed by making a superficial incision along the mesenteric border and then slowly pulling the whole serosal layer to separate from the mucosal layer (Tukker, 2000:60). The remaining tissue was then thoroughly inspected for weaknesses or thickened areas (e.g. Payer's patches) that may negatively influence the transport results of the study. After this, the mesenteric border was used as guideline to dissect the intestinal tissue and to produce a flat sheet of tissue (Figure 7.3), which was again rinsed with cold KRBB to enable removing it from the glass tube onto a strip of filtration paper (Figure 7.3).

The tissue was then cut into small pieces (approximately 3 cm in width) and mounted between two half cells (Ussing chambers) of a Sweetana-Grass diffusion apparatus (Tukker, 2000:60). The surface area of the mucosal tissue that was exposed for transport was 1.78 $cm^2$. The Ussing chambers were mounted onto the diffusion apparatus and coupled to a heating block (heat was generated from actively flowing water coming from the water bath having a temperature of 37˚C) (Berggren, 2004:555; Tukker, 2000:60).

**Figure 7.3 Isolated intestinal mucosa sheet after removal of the serosal layer, prior to cutting it into smaller pieces.**

**Figure 7.4 Diffusion apparatus with the six sets of Ussing chambers.**

The diffusion apparatus comprised of 6 diffusion chambers (Figure 7.4). Pre-heated (37˚C) KRBB (7 mL) was placed at both sides (apical and basolateral) of the tissue, whilst medical oxygen was constantly circulated through the chambers. Sufficient time was allowed as equilibration period (30 minutes), during which time the prepared drug solutions were heated to 37˚C. After reaching equilibrium, the buffer was removed from the chambers. The basolateral sides were refilled with 7 mL of fresh KRBB (also 37˚C). Upon filling the apical sides with 7 mL of drug solution, the integrity of the intestinal tissue was measured by taking trans-epithelial electrical resistance (TEER) readings with a Millipore™ Millicell® ERS-2 epithelial volt/ohm meter. These measurements were performed again at the end of each study, prior to the last withdrawing of samples, in order to verify the integrity of the tissues for the duration of the test (Berggren, 2004:555).

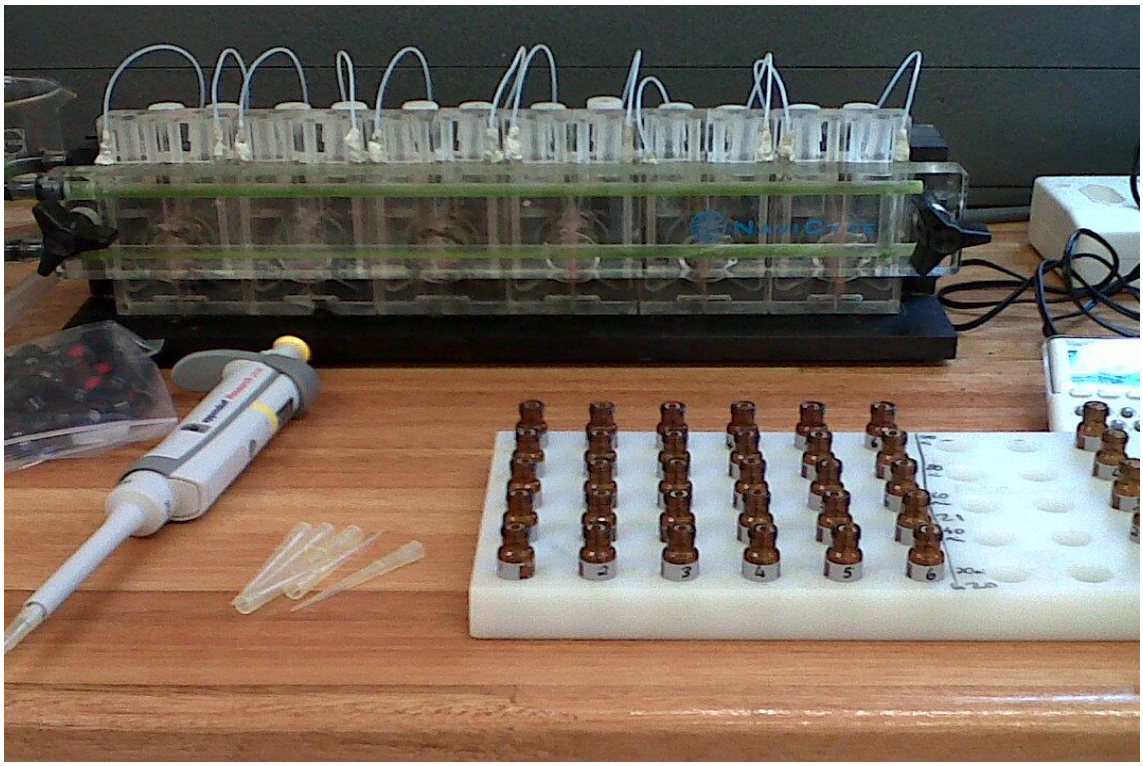

**Figure 7.5 Diffusion apparatus and HPLC vials used during this study.**

Two hundred (200) µL samples were accurately pipetted into HPLC vials containing inserts (Figure 7.5). Sampling times were 20, 40, 60, 80, 100 and 120 minutes. The 200 µL samples were withdrawn from the basolateral sides of the chambers and immediately replaced by 200 µL KRBB (37˚C). The concentration of AZM in the samples were analysed using the validated HPLC method developed during this study (Chapter 2.4).

## 7.5 Data analysis

The HPLC data being generated was processed to determine the amounts of AZM-DH and AZM-G that were transferred from the apical- to the basolateral sides of the tissue. The cumulative percentage of AZM being transported was expressed as a function of time. These results were used to calculate the apparent permeability coefficient ($P_{app}$) values obtained during each transport test. The $P_{app}$ value in this study represented the normalised amount of AZM being carried across the exposed membrane surface area (A = 1.78 $cm^2$) into the basolateral side of the diffusion chamber.

The $P_{app}$ values were calculated with the following equation (Hansen & Nilsen, 2009:88):

$$P_{app} = \frac{dC}{dt} \times \frac{1}{A \bullet C0 \bullet 60} \qquad (7.1)$$

Where: d*C*/d*t* represents the transport rate (slope), A is the surface area available for the transport and $C_0$ accounts for the initial AZM concentration (µg/mL) on the apical side of the diffusion chamber. The percentage difference between the $P_{app}$ values of AZM-DH and AZM-G were calculated using the following formula:

$$\%_{AZM\text{-}G} = \frac{AZM\text{-}G\ P_{app}}{AZM\text{-}DH\ P_{app}} \times 100\ \%_{AZM\text{-}DH} \qquad (7.2)$$

Where: the $P_{app}$ of AZM-DH was assimilated to 100% to serve as reference for calculating the percentage $P_{app}$ of AZM-G. According to the $\%_{AZM\text{-}G}$, the percentage improvement could be calculated by:

$$\%\ improvement = \%_{AZM\text{-}G} - 100 \qquad (7.3)$$

Statistical analysis (analysis of variance [ANOVA], single factor using Excel software) was performed to determine if a statistically significant difference existed between the transport values of the two AZM forms at each pH.

## 7.6 Results

### 7.6.1 Azithromycin transport at pH 7.2

The trans-epithelial electrical resistance (TEER) being recorded during this study ranged from 83 – 93 Ω.$cm^2$ (at commencement of the transport test) and 73 - 93 Ω.$cm^2$ (prior to withdrawal of the last sample). These values indicated that the integrity of the intestinal

tissue had remained intact, as the TEER value decreased by a mere maximum of 5.16 % throughout the duration of the study. The cumulative concentrations of transported AZM-DH and AZM-G being dissolved in KRBB at pH 7.2 are presented in Figure 7.6.

The measured concentration of AZM-DH in the oversaturated solution (sonicated for 5 minutes) within the apical chamber (Section 7.4.1) was 711.44 µg/mL. HPLC analyses of the AZM-DH samples showed no visible peaks, as was confirmed by repeating this test. It was therefore concluded that either no transport of AZM-DH had occurred, or that the amount of AZM-DH being transported had been below the detection limit (3.88 µg/mL) of the HPLC method.

The recorded TEER values for AZM-G were 80 - 97 $\Omega.cm^2$ (on commencement of the test) and 74 - 92 $\Omega.cm^2$ (prior to the last sampling). These values indicated that the integrity of the intestinal tissue had remained intact, as a relatively low decrease in TEER value (maximum of 3.29 %) was recorded. The measured AZM-G concentration in the oversaturated solution (sonicated for 5 minutes) within the apical chamber (Section 7.4.1) was 1381.34 µg/mL. This confirmed that AZM-G was in fact more soluble in KRBB (pH 7.2) than AZM-DH, as had been determined prior to the permeability study. In contrast to no transport of AZM-DH across the intestinal tissue being detected, AZM-G demonstrated considerable permeability (Figure 7.6). At 20 minutes after administration to the apical side, the concentration of transported AZM-G to the basolateral side was already 24.02 µg/mL, while its final concentration after 120 minutes was 51.23 µg/mL. The cumulative percentage of AZM-G that had ultimately been carried from the apical- to the basolateral sides of the intestinal tissue constituted 3.71 % of the initial concentration being added to the apical side, hence representing a 371 % increase in the transport of AZM-G. Since no AZM-DH permeation (0 %) across the intestinal tissue had been detected, the process of determining the exact percentage of improvement in permeability of AZM-G over AZM-DH had been complicated, due to the fact that 0% had constituted an unknown quantity for an effective comparison.

The $P_{app}$ value of AZM-DH was concluded as being $0.000 \times 10^{-6}$, since no permeation across the intestinal tissue was detected with HPLC analysis. The $P_{app}$ of AZM-G was $2.677 \times 10^{-6}$ cm/s, which represented a statistically significantly higher value than that of AZM-DH ($p < 0.05$). The permeability improvement of AZM-G over AZM-DH therefore was 267 %, but as mentioned, this value was merely an indication of the percentage improvement, compared to the 0 % of the reference, AZM-DH. These results indicated

that the transformation of AZM from its dihydrate into its glass form had not only increased its solubility significantly, but also its permeability across intestinal tissue. A higher solubility of AZM-G at pH 7.2, compared to that of AZM-DH, meant that a higher concentration was available in solution to provide a higher concentration gradient, which explained the higher permeability (possibly through passive diffusion) across the intestinal tissue.

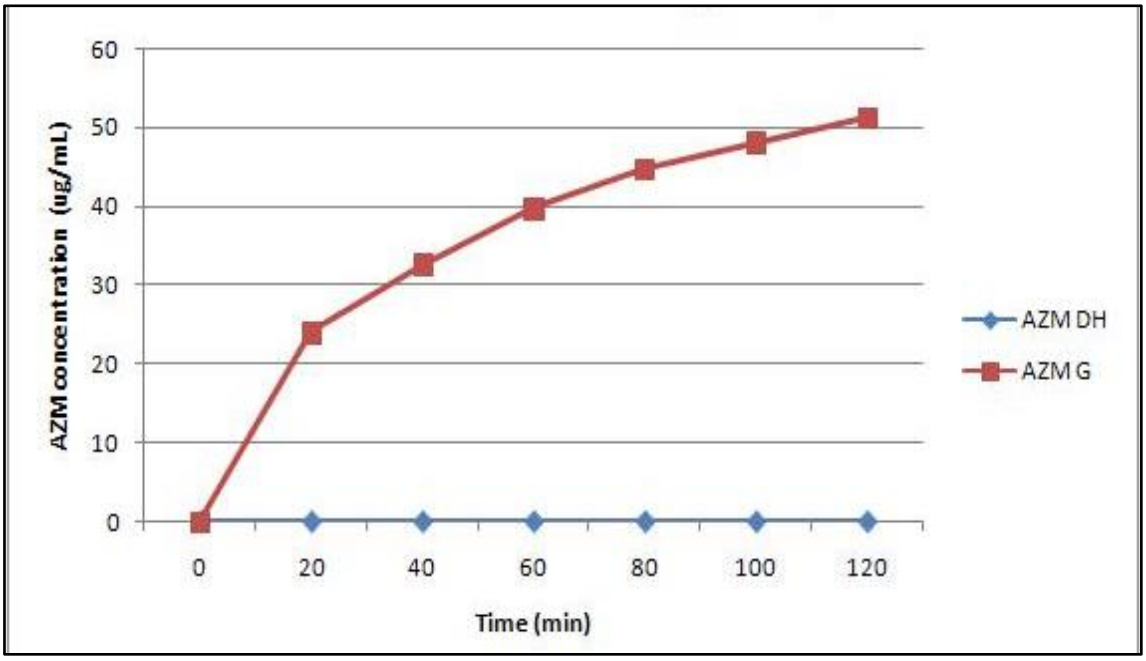


**Figure 7.6 Overlay of the concentrations of AZM-DH and AZM-G being transported across the intestinal mucosa, as a function of time (pH 7.2 buffer).**

### 7.6.2 Azithromycin transport at pH 6.8

The TEER values being generated during this transport study of AZM-DH ranged from 85 - 100 Ω.cm$^2$ (at commencement of the test) and 79 - 91 Ω.cm$^2$ (prior to withdrawing the last sample). These values were indicative that the integrity of the intestinal tissue had remained intact throughout the test, as a maximum decrease of 8.54 % in TEER value was reported for this study. The cumulative concentrations of transported AZM-DH and AZM-G in KRBB at pH 6.8 are presented in Figure 7.7.

The concentration of the initially oversaturated solution (sonicated for 5 minutes) within the apical chamber was 1447.64 µg/mL. The concentration of AZM-DH being carried across the intestinal tissue after 20 minutes was 12.56 µg/mL, with the concentration at the basolateral side after 120 minutes reaching 37.61 µg/mL. This represented a total

cumulative percentage of transported AZM-DH across the pig intestinal tissue at pH 6.8 of 2.60 %.

The recorded TEER values during the transport study of AZM-G were 85 - 93 $\Omega.cm^2$ (on commencement of the study) and 81 - 92 $\Omega.cm^2$ (prior to withdrawing the last sample). These values were indicative of the integrity of the excised intestinal tissue being maintained, as the TEER values decreased by a mere maximum of 1.92 % throughout the study. The concentration of the oversaturated solution (sonicated for 5 minutes) being applied to the apical chamber was 935.26 µg/mL. This concentration differs from the reported equilibrium concentration, and may be the result of experimental error.

As seen on Figure 7.7, AZM-G achieved a considerably higher concentration with transport across the intestinal tissue, than AZM-DH at pH 6.8. After 20 minutes AZM-G had already reached a concentration of 29.68µg/mL, while the final cumulative concentration of AZM-G after 120 minutes on the basolateral side was 47.08 µg/mL. The cumulative percentage of AZM-G that was ultimately carried from the apical- to the basolateral side over a period of 120 minutes amounted to 5.03 %. This related to a 93 % increase in transport of AZM-G, compared to that of AZM-DH at pH 6.8.

The calculated average $P_{app}$ of the 6 replicates of AZM-DH and AZM-G were $1.961 \times 10^{-6}$ cm/s and $3.365 \times 10^{-6}$ cm/s, respectively, which were statistically significantly different ($p < 0.05$).

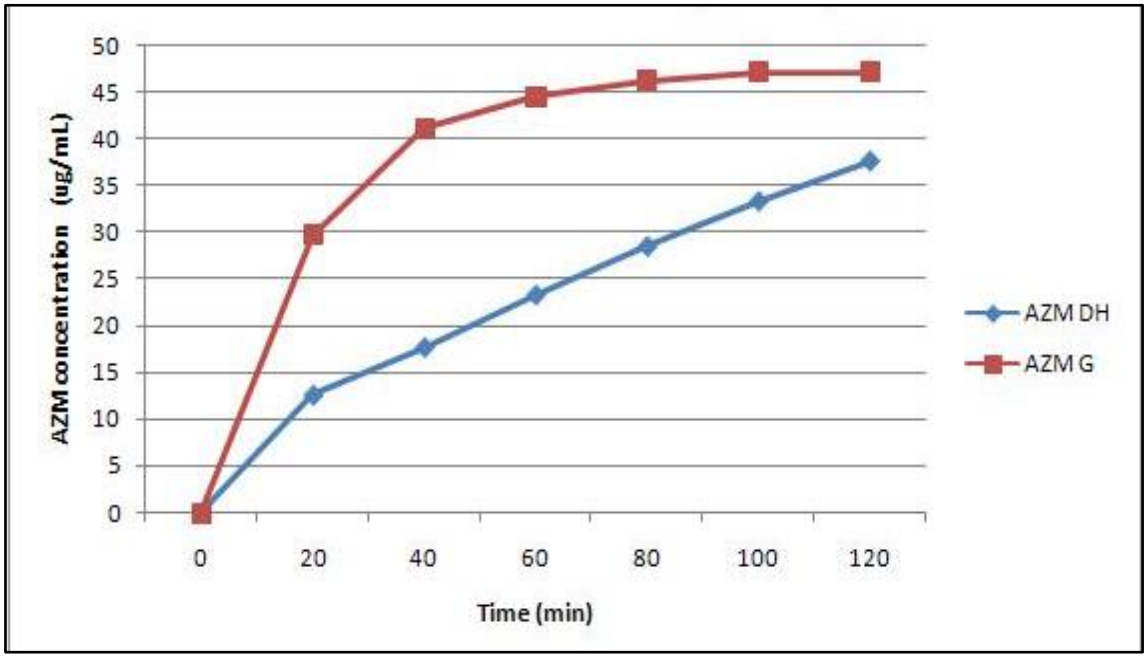


**Figure 7.7 Overlay of the concentrations of AZM-DH and AZM-G being transported across the intestinal mucosa, as a function of time (pH 6.8 buffer).**

### 7.6.3 Azithromycin transport at pH 4.5

The recorded TEER values during this study ranged from 88 - 94 Ω.cm$^2$ (at the start of the study) and 83 - 89 Ω.cm$^2$ (prior to withdrawing the last sample). These values were indicative of the integrity of the excised pig intestinal tissue having remained intact throughout the transport test, since the maximum recorded decrease in TEER value was only 5.59 %.

The cumulative concentrations of transported AZM-DH and AZM-G in KRBB solution (pH 4.5) are presented in Figure 7.8. The concentration of the initially oversaturated solution (sonicated for 5 minutes) within the apical chamber was 8873.22 µg/mL. The concentration of transported AZM-DH through the intestinal tissue after 20 minutes was 8.28 µg/mL, while it was 95.24 µg/mL after 120 minutes. This represented a total cumulative percentage of 1.07 % of AZM-DH being carried across the tissue over a period of 120 minutes.

The recorded TEER values during the transport study of AZM-G ranged between 81 - 90 Ω.cm$^2$ (on commencement of the test) and 75 - 85 Ω.cm$^2$ (prior to withdrawal of the last sample) and was representative of the integrity of the excised pig intestinal tissue having remained intact throughout the transport study, since the TEER value only

decreased by a maximum of 4.67 %. The concentration of the initially oversaturated solution (sonicated for 5 minutes) within the apical chamber was 9222.43 µg/mL. After 20 minutes AZM-G had reached a concentration of 9.22 µg/mL on the basolateral side, while the final concentration of AZM-G after 120 minutes was 44.57 µg/mL (Figure 7.8). The percentage of AZM-G that was ultimately transported from the apical- to the basolateral side amounted to 0.48 %.

The average $P_{app}$ values for the 6 replicates for AZM-DH and AZM-G were 0.874 x $10^{-6}$ cm/s and 0.367 x $10^{-6}$ cm/s, respectively, which were statistically significantly different ($p < 0.05$).

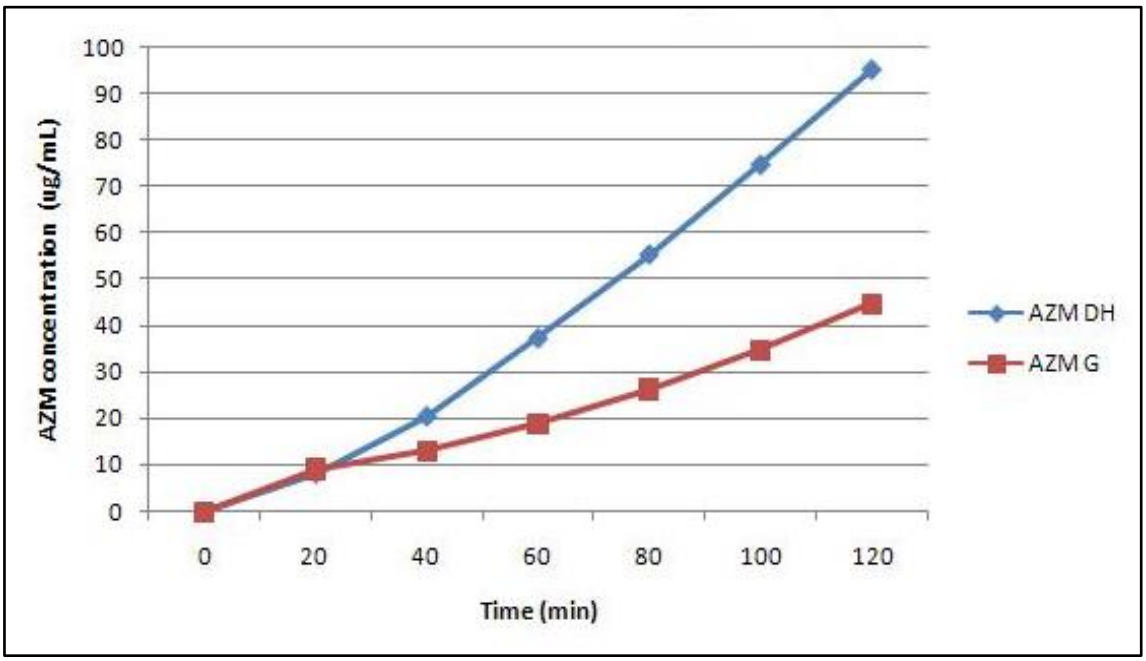


**Figure 7.8 Overlay of the concentrations of AZM-DH and AZM-G being transported across the intestinal mucosa, as a function of time (pH 4.5 buffer).**

### 7.6.4 Azithromycin transport in water

The concentration of the initially oversaturated AZM-DH and AZM-G standard solutions were, according to HPLC analyses, 8.13 µg/mL and 13.96 µg/mL, respectively. Unfortunately, these concentrations ranged between and near the validated detection- and quantification limits of the HPLC method. As a result thereof and because of the poor solubility of AZM-DH and AZM-G in water, the amount of drug being transported could not be determined.

## 7.7 Discussion

The apparent permeability coefficient of a drug is used to characterise it as highly permeable ($P_{app}$ > 10.0 x $10^{-6}$ cm/s), moderately permeable (10.0 x $10^{-6}$ > $P_{app}$ > 0.3 x $10^{-6}$ cm/s), or poorly permeable ($P_{app}$ < 0.3x$10^{-6}$ cm/s) (Fichert *et al*., 2003:720). This guideline for characterising permeability is specifically used for *in vitro* permeability studies, using Caco-2 cell monolayer assays and hence are only applicable to the Caco-2 cell monolayer model. These permeability characterisation guidelines were, however, used to approximate the level of permeability across excised pig intestine during this study. The $P_{app}$ values of AZM-DH and AZM-G, dissolved in Krebs Ringer bicarbonate buffer at different pH values, are presented in Figure 7.9. The results from the relevant statistical analyses are summarised in Table 7.2.

As illustrated in Figure 7.9 and depicted in Table 7.2, the transport of AZM-DH at pH 7.2 and pH 6.8 was relatively poor, according to the permeability criteria for Caco-2 cell monolayer transport studies, as proposed by Fichert *et al*. (2003:720). The transport of AZM was found to be pH dependent, which was consistent with the pH partition theory. At pH values above 6.8, the pH of the transport medium played an important role in the transport of the AZM forms (dihydrate or glass). This effect seemed less important at pH 4.5, due to the fact that the AZM was mainly in the ionised state at this pH value and therefore solubility played a less important role on permeability at this pH value.

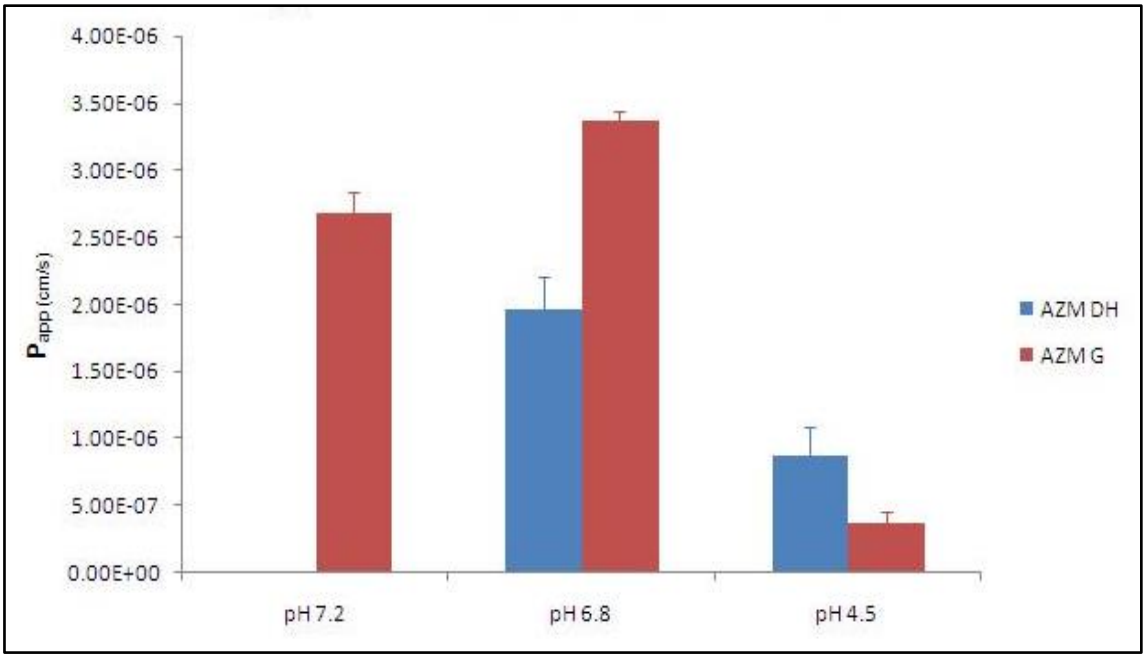


**Figure 7.9** **$P_{app}$ values of AZM-DH and AZM-G in different media at different pH values.**

**Table 7.2** **Results of statistical analyses for AZM-DH and AZM-G in different media at different pH values**

| | pH 7.2 | | pH 6.8 | | pH 4.5 | |
|---|---|---|---|---|---|---|
| | **AZM-DH** | **AZM-G** | **AZM-DH** | **AZM-G** | **AZM-DH** | **AZM-G** |
| **Replicates** | 6 | 6 | 6 | 6 | 6 | 6 |
| **Avg. $P_{app}$ ($x10^{-6}$ cm/s)** | 0 | 2.680 | 1.960 | 3.300 | 0.874 | 0.367 |
| **p-value** | $1.67 \times 10^{-12}$ | | $9 \times 10^{-8}$ | | $3.47 \times 10^{-4}$ | |

Contrary, the transport of AZM-G at pH 7.2 and pH 6.8 was much more favourable, according to the criteria by Fichert *et al*. (2003:720). Generally, when considering the solubility results (Table 7.1), the role that the improved solubility of AZM-G over AZM-DH had played in the permeability of these forms became very important. It was evident that the initial concentrations of both AZM-DH and AZM-G (milled) were higher than the respected concentrations recorded after the solubility determination. This could be attributed to the difference in experimental conditions with regards to a 24 hour equilibrium solubility determination and the supersaturated solutions prepared for the permeability

study. In essence, the improved solubility of AZM-G led to a significant increase in permeability across the intestinal tissue (Table 7.3), probably due to the increased concentration gradient as a result of its higher solubility. This same effect was demonstrated at pH 4.5, where the solubility of AZM-G was lower, which resulted in a lower permeability across excised pig intestinal tissue, compared to that of AZM-DH.

**Table 7.3 Correlation between the improved solubility of AZM-G and the permeability of AZM at variable pH values**

| | **Solubility % increase of AZM-G** | **$P_{app}$% increase of AZM-G** |
|---|---|---|
| **pH 7.2** | 47.92 % increase | 267 % increase |
| **pH 6.8** | 20.97 % increase | 71.60 % increase |
| **pH 4.5** | 2.33 % less soluble | 48 % less permeable |

The above results emphasised the pH dependence of the permeability of AZM. Because AZM is a base with $pK_a$ 8.74, it meant that with passive diffusion AZM would tend to move from the higher pH (unionised state) to the lower pH (ionised state). The better permeation of AZM at pH 7.2 and pH 6.8 during this study supported this hypothesis. The permeation from low pH (mainly in the ionised state, which implicates high solubility but low permeability) to the higher pH, may have been the reason for the very poor permeation of AZM in the buffer medium at pH 4.5 (Kerns & Di, 2008:86; Nožinić *et al*., 2010:327; Pachot *et al*., 2003:9).

## 7.8 Conclusion

Azithromycin (AZM) is known to show poor bioavailability (37 %) after oral drug administration, most likely due to its poor absorption across the intestinal epithelium. Probably one of the major factors affecting its absorption and ultimately its bioavailability, is the permeability of AZM across the human gastro-intestinal tract epithelium.

*In vitro* transport studies of AZM-G and AZM-DH were conducted in order to determine the possibility that the proven improved solubility of the AZM glass form (Section 5.4) may have had a positive effect on the permeability of AZM, in comparison with the dihydrate

form. Although the glass form exhibited an improved permeability across excised pig intestinal tissue, compared to the dihydrate form, it was further demonstrated during this study that the pH of the transport medium clearly impacted upon the permeability of AZM. The improved solubility of AZM-G at pH 7.2 and pH 6.8 caused a significant increase in the permeability of AZM-G over AZM-DH across excised pig intestinal tissue. This could possibly have been attributed to the higher concentration of AZM-G being applied to the intestinal tissue, compared to AZM-DH, due to the higher solubility that had driven passive diffusion across the biological membrane. It could thus be stated that the solubility, together with the pH of the final solutions, had impacted directly upon the permeability of AZM, and could therefore have had an enhancing effect on the absorption of AZM, and ultimately on its bioavailability. These research outcomes hence gave rise to the need for investigating the effect of administering lower dosages of azithromycin and to determine whether the same antimicrobial efficacy would possibly be achieved, due to maintaining the same tissue concentration levels at these lower dosages.

## 7.9 References


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