Effects of selected pharmaceutical excipients on drug permeation at different gastro-intestinal tract regions

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"...if you have faith as small as a mustard seed, you can say to this mountain, 'Move from here to there', and it will move. Nothing will be impossible for you."

Matthew 17:20

ABSTRACT

Although pharmaceutical excipients are considered pharmacologically inert, some excipients in dosage forms have shown an effect on the pharmacokinetics of certain drugs. This could be due to different mechanisms such as opening of tight junctions, inhibition of efflux transporters or interactions with the drug molecules. The presence of P-gp in the different regions of the gastrointestinal tract (GIT) is an important factor that may influence the absorption of drugs. Furthermore, the regions of the gastrointestinal tract have physiological differences that may have an impact on drug absorption, which is the reason why certain drugs have a limited absorption window after oral administration.

The aim of this study was to investigate whether selected excipients, Ac-di-sol® (ADS) and Pharmacel® (PC), at different concentrations would have an effect on the transport of the model compound, Rhodamine 123 (Rho123), across excised sections of different regions from the pig gastrointestinal tract (i.e. the duodenum, proximal jejunum, medial jejunum, distal jejunum and ileum).

The apical-basolateral transport of Rho123 over a period of 2 h across the different excised pig intestinal regions was measured in the absence (control group) and presence of ADS (0.005% w/v, 0.01% w/v and 0.02% w/v) and PC (0.015% w/v, 0.03% w/v and 0.06% w/v) respectively, using the Sweetana-Grass diffusion chamber technique. All the test solutions were prepared in Krebs-Ringer bicarbonate (KRB) buffer. Samples of 200 µl were withdrawn from the acceptor chamber at 20 min intervals for analysis by means of a flourometric analysis method.

Statistically significant ($p < 0.05$) differences were obtained in the transport of Rho123 for the different concentrations of the selected excipients in the duodenum, medial jejunum and ileum. Although enhanced transport of Rho123 was observed when applied with the selected excipients, the Rho123 transport was also reduced by certain concentrations of the excipients in some of the gastrointestinal tract regions. The reduction in the transepithelial electrical resistance (TEER) could indicate that the tight junctions opened, which explained the increase in Rho123 transport. The reduction in the transport of Rho123 could be explained by possible interactions between the excipients and the active ingredient molecules or interactions of the excipients with the intestinal tissue such as blocking of the paracellular spaces.

The results indicated that the addition of ADS and PC in certain concentrations had statistically significant effects on the intestinal transport of Rho123 across some of the regions of the gastrointestinal tract.

Keywords: *In vitro* model, Rhodamine 123, transport, excipients, region-specific transport, Sweetana-Grass diffusion chamber

UITTREKSEL

Farmaseutiese hulpstowwe word beskou as farmakologies inert, maar sommige hulpstowwe in doseervorms het ‘n effek op die farmakokinetika van verskeie geneesmiddels getoon. Dit kan toegeskryf word aan die verskeie meganismes wat betrokke is, soos die opening van digte aansluitings, inhibisie van effluks transporters of die interaksies met geneesmiddelmolekules. Die teenwoordigheid van P-glikoproteïen (P-gp) in die verskeie gedeeltes van die gastrointestinale kanaal is ‘n belangrike faktor wat moontlik die absorpsie van geneesmiddels kan beïnvloed. Daar is fisiologiese verskille tussen die verskillende gedeeltes van die gastrointestinale kanaal wat ‘n groot impak kan hê op geneesmiddelabsorpsie, wat dus die rede is waarom sekere geneesmiddels beperkte absorpsie toon na orale toediening.

Die doel van die studie was om die invloed van die hulpstowwe Ac-di-sol® (ADS) en Pharmacel® (PC), te ondersoek by verskillende konsentrasies op die transport van die modelgeneesmiddel Rhodamine 123 (Rho123). Uitgesnyde gedeeltes van die vark se gastrointestinale kanaal (duodenum, proksimale jejunum, mid jejunum, distale jejunum en ileum) was gebruik om die effek van die hulpstowwe op Rho123 transport te toets.

Die apikale tot basolaterale transport van Rho123 oor ‘n periode van 2 h oor die uitgesnyde weefsel van die verskillende gedeeltes van die vark se gastrointestinale kanaal was in die afwesigheid (kontrolegroep) en die teenwoordigheid van ADS (0.005% m/v, 0.01% m/v en 0.02% m/v) en PC (0.015% m/v, 0.03% m/v en 0.06% m/v) gemeet met behulp van die Sweetana-Grass diffusie-apparaat. Die toetsoplossings was voorberei in Krebs-Ringer bikarbonaatbuffer. Monsters van 200 µl was onttrek uit die ontvangerkant van die diffusiesel tydens 20 min intervalle, waarna dit geanaliseer was met behulp van ‘n fluorometriese analitiese metode.

Statisties betekenisvolle ($p < 0.05$) verskille was waargeneem met die transport van Rho123 in kombinasie met die hulpstowwe by verskeie konsentrasies in die duodenum, mid jejunum en ileum. Verhoogde transport, maar ook verlaging in transport van Rho123 was waargeneem in die verskillende gedeeltes van die gastrointestinale kanaal. Die afname in die transepiteel elektriese weerstand (TEEW) was ‘n moontlike aanduiding dat die digte aansluitings oopgemaak het, wat dus die verhoogde transport kan verduidelik. Die afname in die transport van Rho123 kan moontlik verduidelik word deur interaksies wat kan voorkom tussen die hulpstowwe en die aktiewe bestanddeel, asook die hulpstowwe se interaksies met die intestinale weefsel soos om die parasellulêre spasies te blokkeer.

Die resultate dui daarop dat die byvoeging van ADS en PC in sekere konsentrasies ‘n statistiese betekenisvolle effek toon op die transport van Rho123 oor sekere intestinale gedeeltes.

Trefwoorde*: In vitro* model, Rhodamine 123, transport, hulpstowwe, streek-spesifieke transport, Sweetana-Grass diffusieapparaat

ABBREVIATIONS

% RSD	Percentage relative standard deviation
3 R's	Reduce, Replace, Refine
ABC	ATP binding cassette
ADS	Ac-di-sol®
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under curve
Caco-2	Human Caucasian colon adenocarcinoma
Cham	Chamber
CYP3A4	Cytochrome P450 3A4
ELISA	Enzyme-linked immunosorbent assay
GIT	Gastrointestinal tract
IPEC	International Pharmaceutical Excipients Council
$K_{o/w}$	Oil/water partition coefficient
KRB	Krebs-Ringer bicarbonate
LOD	Limit of detection
LOQ	Limit of quantification
MDCK	Mandin-Darby canine kidney
P_{app}	Apparent permeability coefficient
PC	Pharmacel® PH101
PEG-400	Polyethylene glycol-400
P-gp	P-glycoprotein

pKa	Acid dissociation constant
QSAR	Quantitative structure-activity relationships
R^2	Correlation coefficient
Rho123	Rhodamine 123
RSD	Relative standard deviation
SA	South Africa
SD	Standard deviation
TEER	Transepithelial electrical resistance
TEEW	Transepiteel elektriese weerstand
USA	United States of America
USP	United States Pharmacopoeia
w/v	Weight per volume

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CHAPTER 1 INTRODUCTION

1.1 Background

1.1.1 Factors that may affect drug absorption from the gastrointestinal tract (GIT)

Drug absorption after oral administration can be defined as the movement of drug molecules from the lumen of the gastrointestinal tract into the blood surrounding the gastrointestinal tract including the hepatic portal vein, whereas bioavailability is the rate and extent to which drug molecules reach the systemic circulation (Ashford, 2007:267). There are several factors that may have an influence on the absorption of drugs from the intestine, which are summarised in Table 1-1.

Table 1-1: Factors influencing the absorption of drugs from the gastrointestinal tract (adapted from Deferme *et al*., 2007:183)

Factors	Description
Physiological factors	Emptying time/rate of the stomach Intestinal motility Permeability of the membrane in the specific intestinal region pH of the specific intestinal region Disease state Blood flow Luminal content and composition
Physicochemical factors	Aqueous solubility Molecular size/weight Aggregation/complexation pK_a H-bonding potential Surface area Hydrophobicity of drug Crystal lattice energy
Formulation factors	Type of dosage form Drug release Functional excipients (e.g. absorption enhancers)
Biochemical factors	Metabolism Efflux transporters Active uptake transporters

1.1.2 Excipient-drug interactions

There are two types of interactions that can occur between compounds (e.g. xenobiotics, excipients or food constituents) and drugs that are co-administered, namely pharmacodynamic interactions and pharmacokinetic interactions. Pharmacodynamic interactions refer to any modulation of the therapeutic effects or pharmacologic responses of a drug (Shargel *et al*., 2005:4). On the other hand, pharmacokinetic interactions refer to the modulation of the absorption, distribution, metabolism and elimination of the drug in the body (Ashford, 2007:304).

Although pharmaceutical excipients are considered pharmacologically inert, some effects of excipients on drug pharmacokinetics have been reported (Schulze *et al*., 2005:68). Examples of pharmacokinetic interactions that can occur between excipients and drugs exist in the scientific literature. For example, a combination of tolbutamide (anti-diabetic drug) with partly pre-gelatinised corn starch (disintegrant) in a dosage form showed an increased dissolution rate compared to that of a formulation containing regular corn starch (Jackson *et al.,* 2000:337). The combination of digoxin and polyethylene glycol 400 (PEG 400) showed a significant inhibition in the epithelial efflux of digoxin (Cornaire *et al.,* 2004:127). When a non-steroidal anti-inflammatory drug (aspirin) was formulated with gluconolactone (direct compression diluent), less hydrolysis was found compared to the formulation containing anhydrous lactose (tablet diluent). The combination of erythromycin acetate and sodium bicarbonate as excipient in a gelatin capsule resulted in an increase of the stomach pH, which then resulted in an increased bioavailability of the erythromycin (Jackson *et al*., 2000:338).

1.1.3 *In vitro* permeation techniques

In the early stages of drug development, whole animal studies cannot be used as a cost effective screening tool, therefore different *in vitro* models/techniques for drug permeation were developed. There are different *in vitro* techniques that can be used to investigate drug permeation across membranes such as computerised models (i.e. *in silico* techniques), physicochemical methods (e.g. determination of log P), brush border membrane vesicles, artificial membranes (e.g. Parallel artificial membrane permeation assay), everted intestinal rings/sacs, cell culture-based models (e.g. Caucasian adenocarcinoma cell line) and excised tissues in Ussing type diffusion chambers (Deferme *et al*., 2007:187-189). The excised tissue technique will be discussed in more detail, for it is being used in this study.

1.1.3.1 Excised tissue techniques

The side-by-side Ussing type diffusion apparatus (i.e. the Sweetana-Grass diffusion apparatus) was adapted to investigate *in vitro* transport of drugs across excised animal intestinal segments. In this apparatus, the isolated intestinal tissue strips are clamped in between half cells and the

drug in solution is introduced to the donor chamber where after samples are collected from the acceptor chamber (Balimane *et al*., 2000:305). Table 1-2 summarises the advantages and limitations of the excised tissue diffusion technique as used in Ussing type diffusion systems.

Table 1-2: The strengths and limitations of the Ussing type diffusion system for drug permeation across excised animal tissues (adapted from Deferme *et al*., 2007:189)

Advantages/Strengths	Disadvantages/Limitations
Good screening model	Tissue viability is limited to a certain period
Good correlation with *in vivo* permeability data	Under-estimation of permeability caused by the presence of circular muscle
Evaluation of permeation in different gastrointestinal tract regions	Difficulties with the unstirred water layer
No bio-analysis needed	Sometimes tissue availability
Evaluation of transport mechanisms (e.g. passive diffusion or active transport)	
Evaluation of absorption enhancing strategies on a mechanistic basis and well-defined absorptive area	
Good oxygenation of tissue	
A more realistic representation of the intestinal tract compared to single cells used in cell culture models	

Figure 1-1 illustrates the half cells of the Sweetana-Grass diffusion apparatus and the tissue mounting pins are indicated where the excised tissue strips are mounted. At the gas inlet in the direction of the arrows, the buffer (usually Krebs-Ringer bicarbonate buffer) is circulated by gas lift (O_2/CO_2 mixture), which is contained in both the chambers parallel to the tissue's mucosal and serosal surfaces (Bohets *et al*., 2001:372).

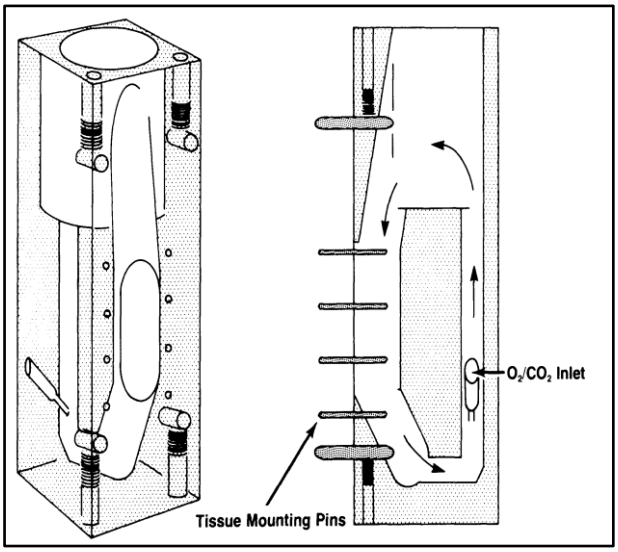

Figure 1-1: Illustration of the half cells of the Sweetana-Grass diffusion apparatus

The tissue strips mounted in the half cells are maintained at 37°C by a diffusion-cell heating block. Heated water is circulated through the aluminium block in which the assembled half cells are kept (Grass & Sweetana, 1988:374).

1.2 RESEARCH PROBLEM

Active ingredients are almost never administered as pure compounds, but are administered in dosage forms that also contain excipients. Excipients are meant to be pharmacologically inert, but they may have an impact on drug delivery. It is important to identify potential excipient-drug interactions that may affect the pharmacokinetics of drugs in order to prevent potential side-effects. On the other hand, beneficial excipient-drug interactions may include enhancement of drug absorption and bioavailability. Although region specific absorption has been shown for certain drugs, it is also important to understand if excipients have region specific effects on drug permeation in the gastrointestinal tract.

1.3 AIM AND OBJECTIVES

The aim of this study is to identify pharmacokinetic interactions, especially the permeation, between selected excipients and a model drug (i.e. Rhodamine 123) by means of an excised tissue permeation technique. Furthermore, it is important to determine if the effect of the excipients on drug permeation is region/site specific within the gastrointestinal tract.

To reach this goal, the following objectives need to be achieved:

- To select pharmaceutical excipients for the *in vitro* pharmacokinetic studies based on a literature review.
- To validate a fluorometric analysis method for the model compound (Rhodamine 123).
- To collect intestinal tissues from different anatomical regions (i.e. the duodenum, proximal jejunum, mid jejunum, distal jejunum and ileum) of the pig gastrointestinal tract.
- To conduct transport studies across the excised pig intestinal tissues of Rhodamine 123 in the presence and absence of the selected excipients.
- To process and interpret the transport data in order to determine if the selected excipients had any effect on the transport of the model compound in the different gastrointestinal regions.

1.4 SELECTION OF A MODEL COMPOUND

Rhodamine 123 (Rho123) is a commonly used flurone dye that can be used in fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA (enzyme-linked immunosorbent assay) (Forster *et al*., 2012:1). This dye is used in drugs, cosmetics, textiles, and in inks as colours. It has a high luminescence and was applied as fluorescent probe indicator of microscopic environments, like enzymes and membranes. Rho123 is a known substrate for the efflux transporter, P-glycoprotein (P-gp). For all these reasons Rho123 was used to observe any interactions with the selected excipients (Ac-di-sol® and Pharmacel®) (Mandal *et al*., 2010:78-79).

1.5 DATA ANALYSIS

Statistical analysis will be performed on the P_{app} values using analysis of variance (ANOVA). ANOVA will determine the overall variation between and within the groups. (Brown, 2005:90). There are different methods to calculate the test statistic for each post hoc test, for example using the Dunnett, Fisher (LSD), Scheffé and the Tukey (HSD) equation (Brown, 2005:91).

1.6 ETHICS

An ethics application for the use of excised pig intestinal tissues in the pharmacokinetic studies was submitted and approved by the Animal Ethics Committee (AnimCare) of the North-West University (NWU-00025-15-A5).

It is important to note that there are no ethical aspects related to the animals as they are routinely slaughtered for meat production purposes. The tissue will be obtained from a

registered abattoir (Potchefstroom abattoir) and used tissue will be disposed of according to approved protocol in the Vivarium.

CHAPTER 2 REGION SPECIFIC DRUG ABSORPTION

2.1 Introduction

Pharmacokinetics is a term that describes the relationship between the administered dose and the drug blood concentration profile over time, which is a function of drug absorption, distribution, metabolism and elimination (Thomson, 2004:769; Loftsson, 2015:48). The pharmacokinetic profile of the drug can be influenced by different physiological factors such as age, gender, weight, body mass index, hepatic and renal function (Wooten, 2012:437). In addition, the pharmacokinetics of a drug can also be altered by other factors such as interactions with drugs, excipients, herbs or food components (Laitenen *et al*., 2004:1904; Oga *et al*., 2016:94). Pharmacokinetic interactions cause changes in the pharmacokinetic parameters (i.e. absorption, distribution, metabolism and elimination) of a drug in the body (Ashford, 2007:304).

One example of a mechanism of an interaction that can interfere with drug absorption is inhibition or activation of the P-glycoprotein (P-gp) transporter, which is an active efflux transporter which pumps substrate molecules from the epithelium back into the gastrointestinal lumen. Absorbed drug molecules that are substrates for P-gp are continually cycled between the enterocyte cytoplasm and the intestinal lumen, which results in a reduction of the bioavailability of the drug (Chawla *et al*., 2003:52). Inhibition or activation of metabolic enzymes is another example of an interaction that can change the pharmacokinetic profile of a drug. Enzymes from the cytochrome P450 (CYP) system bind with drug molecules and catalyse phase I metabolic reactions that result in products that are more hydrophilic and water soluble to be easier excreted in the urine (Schonborn & Gwinnutt, 2010:2). The presence of intestinal metabolic enzymes in the gastrointestinal tract and liver such as CYP enzymes has a major influence on the bioavailability of many drugs by reducing the concentration of the drug that reaches the systemic circulation through metabolism, referred to as the first pass effect. The activity of CYP enzymes in the intestinal epithelium decreases along the small intestine in a distal direction away from the stomach (Kenneth *et al.,* 1997:110).

Some drugs display region specific absorption in the gastrointestinal tract due to differential solubility as a result of pH changes, differential stability as a result of changes in the types of enzymes present, interactions with endogenous components such as bile and variation in the presence of active transporters (uptake and efflux transporters). For these reasons, drugs have a preferred absorption site or ‘absorption window’ in the gastrointestinal tract (Davis, 2005:250).

2.2 Gastrointestinal tract (GIT)

The human GIT is a muscular tube with an approximate length of 6 m with varying diameters across the tube. The GIT consists of four main areas, namely the oesophagus, stomach, the small intestine and the large intestine. The surface area for absorption is increased by the rough surface of the GIT lumen (Ashford, 2007:271). The human GIT is illustrated in Figure 2-1 below.

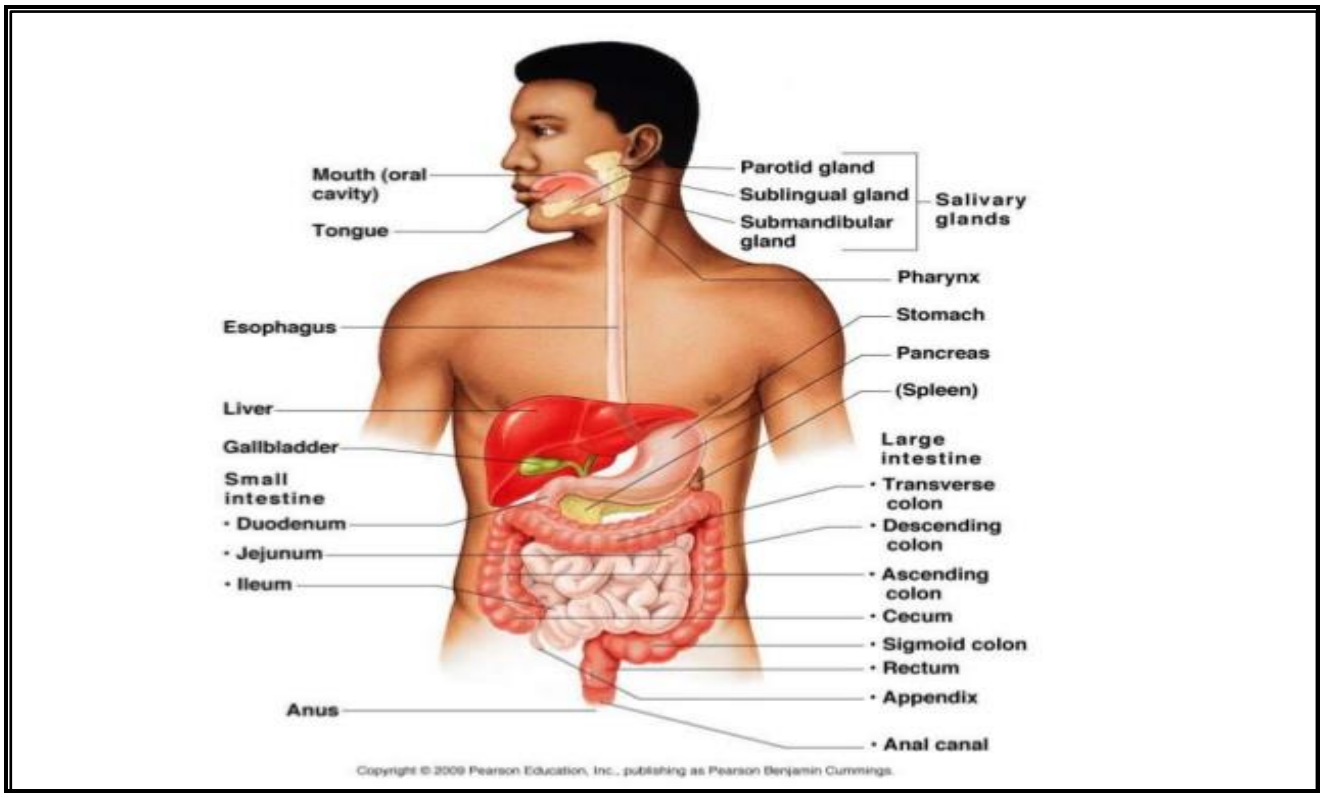

Figure 2-1: Illustration of the human gastrointestinal tract (Myrdal, 2013:4)

2.2.1 The intestinal epithelium

Viewed from the outer surface to the inner surface, the wall of the GIT consists of the serosa, muscularis externa, sub-mucosa and mucosa (Ashford, 2007:271) as schematically illustrated in Figure 2-2.

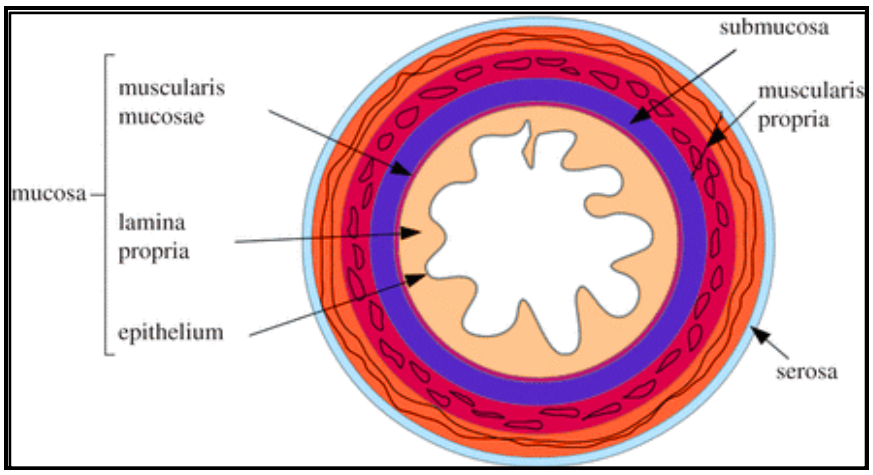

Figure 2-2: Schematic illustration of the intestinal wall (Balbi & Ciarletta, 2013:2)

2.2.1.1 Mucosa

The mucosa is composed of three layers, namely the muscularis mucosa, lamina propria and epithelium (Ashford, 2007:271). The epithelium has various structural features that increase the surface area, which supports the main function of the small intestine namely absorption of nutrient molecules and xenobiotics. Firstly, the surface area of the small intestine contains the Folds of Kerckring, which are sub-mucosal folds that extends in a circulary way around the intestine. Secondly, the intestinal mucosa forms villi which are approximately 1 mm in length. The villi increase the surface area by a factor of ten. The villi surface area is covered by simple columnar epithelial cells (also referred to as enterocytes). Thirdly, the enterocytes of the epithelium have numerous microvilli which are 1µm in length. The microvilli increase the surface area by a factor of hundred (Ashford, 2007:273; Slomianka, 2009).

2.2.1.1.1 Muscularis mucosa

The muscularis mucosa is a continuous thin sheet of smooth muscle cells, which can alter the local conformation of the mucosa (Ashford, 2007:271; Balbi & Ciarletta, 2013:1). The mucosa is located on the muscle layer where the muscularis mucosa fingers project into pits and villi of the mucosa (Ushida & Kamikawa, 2007:157).

2.2.1.1.2 Lamina propria

The lamina propria consists of cellular connective tissue (Ushida & Kamikawa, 2007:157). It supports the mucosal epithelium, where it allows the epithelium to move freely and it provides immune defence. The lamina propria contains different cell types supporting the immune function, namely the lymphocytes and other immune cells (King, 2009).

2.2.1.1.3 Epithelium

The epithelium is the innermost single cell layer of the mucosa that forms the surface area of the GIT lumen (Ushida & Kamikawa, 2007:157). The primary function of the epithelium is water and nutrient absorption. The epithelium forms a physical barrier against the uptake of luminal pathogens. The intestinal epithelium consists of four differentiated cell types, namely the goblet cells, entero-endocrine cells, Paneth cells and enterocytes (Van der Flier & Clevers, 2009:242).

2.2.2 Sub-mucosa

The sub-mucosa is situated between the muscularis externa and the muscularis mucosa (refer to Figure 2-2). The sub-mucosa consists of areolar connective tissue with many blood vessels and lymphatic vessels (Scanlon & Sanders, 2011:403). The sub-mucosa allows the mucosa to move flexibly during peristalsis (King, 2009).

2.2.3 Muscularis externa

The muscularis externa is arranged in two layers of smooth muscle tissue, namely a thin outer layer that is longitudinally orientated and a thicker layer inside which is circularly orientated. The contractions of the muscle in the muscularis externa provide peristalsis that is responsible for movement of the gastrointestinal contents (Ashford, 2007:271).

2.2.4 Serosa

The serosa is the outermost layer of the GIT and is a supporting connective tissue consisting of mesothelium. If the outermost layer is attached to the surrounding tissue it is called adventitia and when the layer lies adjacent to the peritoneal cavity it is called the serosa (King, 2009).

2.3 Anatomy and physiology of the small and large intestine

2.3.1 Small intestine

The small intestine is the longest and most convoluted part of the human GIT with a length of approximately 4-5 m and extends from the pyloric sphincter of the stomach to the ileoceacal junction. It consists of three main regions namely the duodenum, jejunum and ileum. The two main functions of the small intestine include the enzymatic digestion of food and the absorption of nutrients and other molecules into the blood circulation. The duodenum, jejunum and illeum comprise 5%, 50% and 45% of the length of the small intestine, respectively (Balimane & Chong, 2005: 336).

2.3.1.1 Duodenum

The duodenum is the first segment of the small intestine following the stomach and is also the shortest segment, approximately 25 cm to 30 cm long (Britannica, 2015; Meiring *et al*., 2006:252). The surface area of the duodenum is approximately 1.9 m^2 (Balimane & Chong, 2005:336). The duodenum has a pH of 6 to 6.5, which is ideal for digestion of peptide and protein food. Proteolytic enzymes that are present in the duodenum can cause instability of many protein drugs (Shargel *et al.*, 2005:385).

2.3.1.2 Jejunum

The jejunum is the middle segment of the small intestine, between the duodenum and the ileum, and is approximately 2 m in length (Ashford, 2007:273; Shargel *et al*., 2005:385). The jejunum's total surface area is approximately 184 m^2 (Balimane & Chong, 2005:336). The jejunum has a wider lumen, its walls are thicker and it has more mucous than the ileum (Meiring *et al*., 2006:252).

2.3.1.3 Ileum

The ileum is the last segment of the small intestine and is approximately 3 m long (Ashford, 2007:273). The total surface area of the ileum's lumenal lining is approximately 276 m^2 (Balimane & Chong, 2005:336). The pH of the fluids in the ileum is between 7 and 8. Acidic drugs dissolve relatively easy in the ileum due to the presence of bicarbonate secretions (Shargel *et al*., 2005:385).

2.3.2 The large intestine (colon)

The large intestine extends from the ileoceacal junction to the anus and is approximately 1.5 m in length. The large intestine is composed of sub-regions including the caecum, the ascending colon, hepatic flexure, the transverse colon, the splenic flexure, the descending colon, the sigmoid colon and the rectum. The two main functions of the large intestine are the maintenance of homeostasis in the body and the storage and compaction of faeces. The large intestine has limited drug absorption due to its relatively small surface area since it contains no villi (Ashford, 2007:274-275; Shargel *et al*., 2005:385).

2.4 Drug absorption

Drug absorption is defined as the permeation of a drug from its site of administration to the blood plasma (i.e. blood surrounding the site of administration) (Chillistone & Hardman, 2008:168). For drugs to become bioavailable (i.e. to reach the systemic circulation) after oral administration, it needs to be transferred across the intestinal epithelial lining into the blood.

The transport process may include passive diffusion across the apical membrane or through paracellular spaces, active transport (uptake and efflux), vesicular uptake (pinocytosis/endocytosis) as illustrated in Figure 2-3.

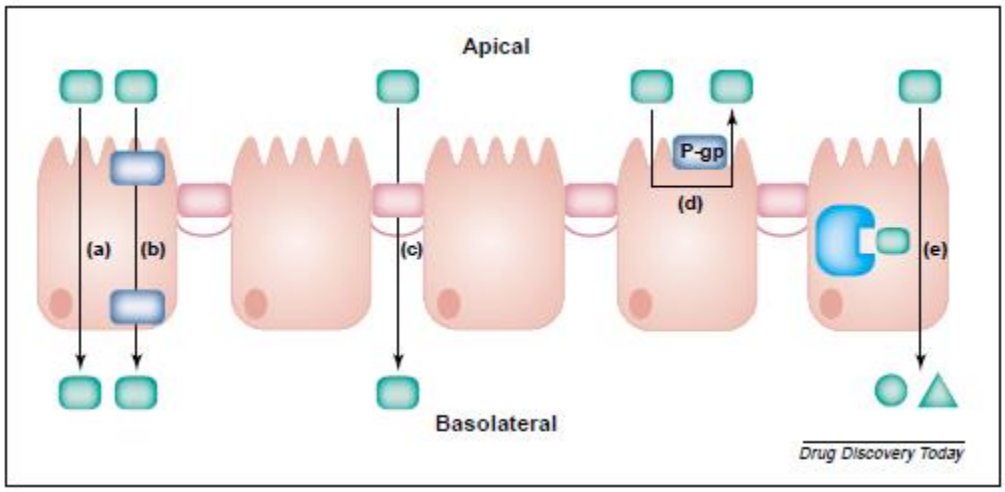

Figure 2-3: Routes and mechanisms of transport of molecules across the intestinal epithelium (Balimane & Chong, 2005:336). (a) Transcellular passive diffusion, (b) Active transport (uptake), (c) Paracellular passive diffusion, (d) Active transport (efflux), (e) Metabolism

2.4.1 Transcellular passive diffusion

Passive diffusion is the most common and important way by which drugs get absorbed after oral administration. During passive diffusion, there is movement of drug molecules from an area of high concentration to an area of lower concentration. The rate at which this diffusion takes place depends on the molecular size, concentration gradient, lipid solubility, degree of ionization and protein binding of the drug (Chillistone & Hardman, 2014:309).

The transcellular pathway of absorption involves the transport of drugs across the plasma membranes of the epithelial cells (Kawedia *et al*., 2007:3621). Unionized molecules are transported preferably by this mechanism (Vogel, 2006:439). Transcellular transport can be bi-directional, where the substance can move into the cytoplasm of the cell or back into the lumen. After diffusion across the membrane, the molecules can remain in the cell and accumulate or the substance can be degraded in the cell or it can be transported across the basolateral cell membrane where it is absorbed into the blood (Bellmann *et al*., 2015:612).

2.4.2 Carrier-mediated transport (active uptake and efflux transport)

Carrier-mediated transport mechanisms are used by drugs to cross cell membranes against a concentration gradient. It involves endogenous carrier proteins, which have specificity to particular compounds. Drugs that are subjected to carrier-mediated transport are usually structurally related to endogenous compounds of the human body (Bellmann *et* al., 2015:612; Chillistone & Hardman, 2014:310).

Efflux transport is mediated by active transporters where drugs are expelled back into the lumen after they have been absorbed into the epithelial cells, thus in a direction from the basolateral to the apical side of the intestinal epithlium. Drug absorption may be reduced by the presence of active efflux proteins. A well-known and documented drug efflux transporter is P-glycoprotein (P-gp). P-gp is expressed on the apical surface of the intestinal epithelial cells. The process of efflux requires a lot of energy and can therefore pump drugs back against a concentration gradient (Ashford, 2007:283-284; Shargel *et al*., 2005:399).

2.4.3 Endocytosis, pinocytosis, phagocytosis and transcytosis

Endocytosis occurs when a part of the membrane pinches off and engulf some of the luminal content in a vacuole or vesicle and thereby transporting it to the basolateral membrane. Endocytosis is triggered when chemical compounds that are ligands bind to receptor proteins on the exterior of apical cell membranes. Pinocytosis and phagocytosis is the process where small particles are brought into the cell within a small vacuole, without first binding to a receptor (Chillistone & Hardman, 2014:310). Pinocytosis is therefore considered to be the non-selective mechanism of endocytosis. Lysosomes and intracellular vesicles fuse to result in the enzymatic degradation of the vesicle contents. Phagocytosis differs from pinocytosis as it is the endocytosis of larger particles (bacteria, viruses and similar sized particles). Transcytosis is the process where intact macromolecules are transported across epithelial cells by means of pinocytosis but without getting degraded by enzymes in the vacuole (Bellman *et al*., 2015:612).

2.4.4 Paracellular passive transport

Paracellular transport is the passive movement of substances across the epithelium between the cells through the tight junctions and intercellular spaces (Bellmann *et al*., 2015:613; Kawedia *et al*., 2007:3621). The paracellular permeability of the epithelium is restricted by inter-cellular junctions, which consists of tight junctions, adherens junctions and gap junctions (Komarova & Malik, 2010:464; Widmaier *et al*., 2008:48). The paracellular pathway is more susceptible to positively charged, small molecuels (Vogel, 2006:439; Pade & Stavchansky, 1997:1210).

2.5 Models for determining drug permeability/transport

Four categories of methods are available to investigate the principle mechanisms of drug absorption in mammals namely *in vivo*, *in situ*, *in silico* and *in vitro*.

In Figure 2-4, various models for prediction of drug absorption are shown. The cell culture models, such as the Caco-2 cell lines, have the advantage of expressing many active drug transporter systems. However, the transport of hydrophilic drugs and the intestinal metabolism thereof is underestimated by this model. The *in situ* model, involving pre-clinical animal studies, gives a more complete picture of the absorption profile in humans, but high throughput screening is not possible with this model (Bohets *et al*., 2001:368).

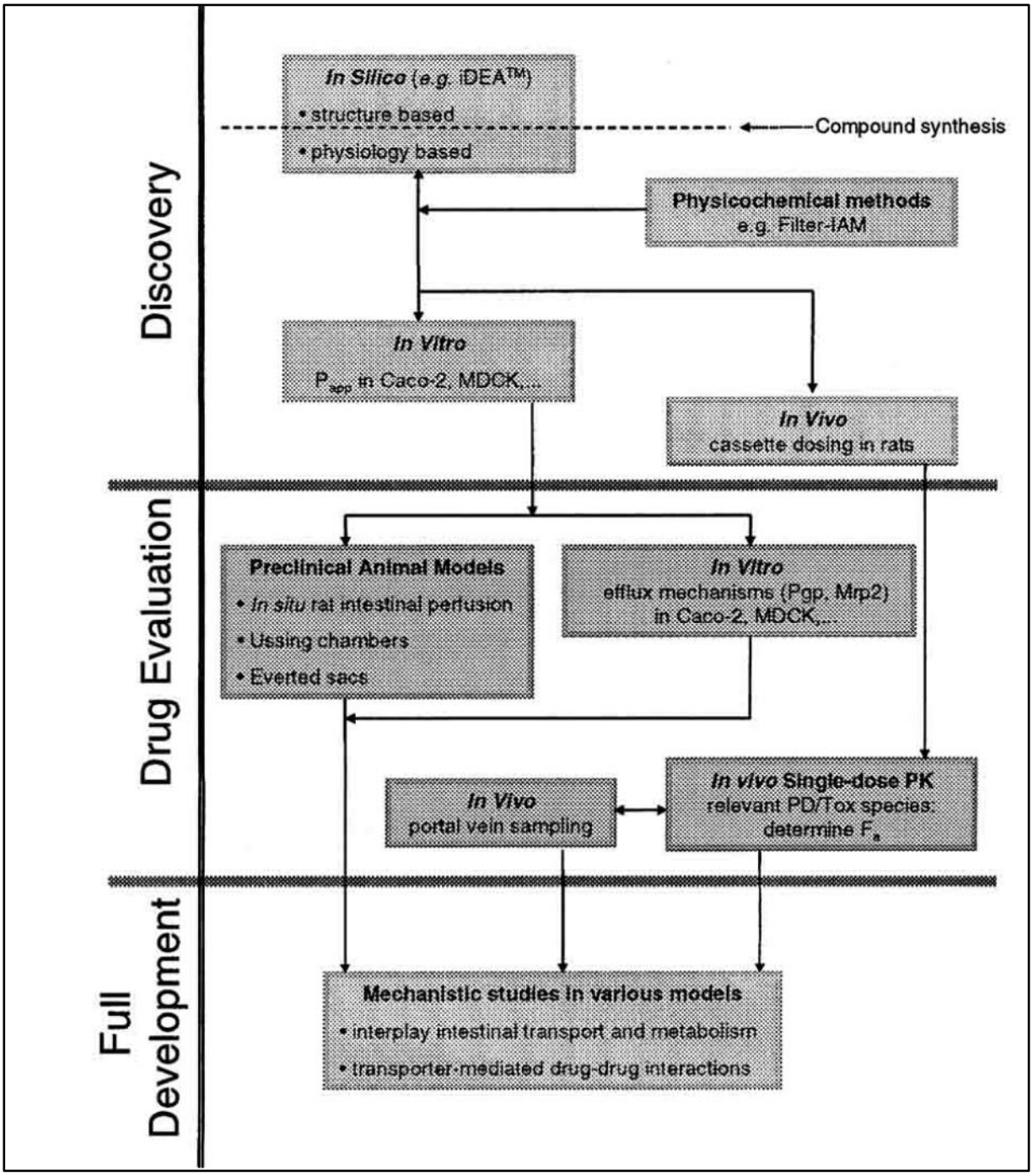

Figure 2-4: **Schematic illustration of various models for evaluation of drug absorption during drug development (Bohets *et al*., 2001:368)**

2.5.1 In vivo *models*

In vivo models are defined by the Merriam-Webster dictionary (2015) as experiments that take place in living organisms. *In vivo* models have the advantage that mesenteric blood circulation integrates with the gastrointestinal tract and other physiological factors that may influence drug dissolution and absorption.

The rat is the most frequently used animal for pre-clinical *in vivo* studies (Le Ferrec *et al*., 2001:653). The composition of epithelial cell membranes are similar across different mammal species, thus passive diffusion across the GIT epithelium should be similar. However, physiological factors such as pH, gastrointestinal motility, transit time and distribution of transporters can vary and should be taken into consideration when using data from animal experiments (Bohets *et al*., 2001:378).

2.5.2 In situ *models*

The term "*in situ"* refers to experiments that take place in an organ, which is still part of the animal, for example where the abdominal cavity of an anaesthetised animal is exposed to laparotomy. The *in situ* model has the advantage that the stomach can be bypassed, which means that acidic compounds are unlikely to precipitate and compounds that are normally degraded in the stomach are not affected. The limitation of using this model for experimenting is that the anaesthesia used may influence intestinal drug absorption (Le Ferrec *et al*., 2001:653).

2.5.3 *In silico* models

The term "*in silico"* refers to computational methods which can predict intestinal absorption based on the chemical structure. This computational method is based on experimental data obtained from other models for a wide range of diverse compounds (Bohets *et al*., 2001:369). There are different approaches for *in silico* modelling such as quantitative structure-activity relationships (QSAR); 3-dimensional QSAR; structure-based methods such as ligand-protein docking and pharmacophore modelling. The goal of *in silico* methods is to predict disposition behaviour of compounds in the body by taking all kinetic processes into account (Yamashita & Hashida, 2004:327).

2.5.4 *In vitro* models

The term "*in vitro*" is defined by the Merriam-Webster dictionary (2015) as experiments taking place outside of the living organism and in an artificial environment. *In vitro* techniques are specifically used in permeability studies because they are less labour and cost-intensive than *in*

vivo animal models (Balimane *et al*., 2000:305). The major limitation of using this model is that the effect of physiological factors (e.g. gastric emptying rate, GIT transit rate, pH, etc.) cannot be incorporated into the interpretation of the data (Balimane *et al*., 2000:305). Various *in vitro* techniques are available to predict drug absorption across the intestinal mucosa of which excised tissue and cell cultures are the most popular.

2.5.4.1 Excised animal tissues in Ussing-type chambers

Excised animal tissue models have been used since the 1950's to investigate the absorption of substances from the intestine (Balimane *et al*., 2000:305). For this method, the excised tissue must in some cases be stripped from the serosa (Deferme *et al.,* 2007:201). The side-by-side Ussing-type diffusion apparatus is used to test *in vitro* transport across excised animal intestinal tissue segments. This method involves the isolation of the intestinal tissue from an animal that is cut into appropriate size strips. Thereafter the strips are mounted on half cells of the diffusion apparatus and the drug in solution is introduced to the donor chamber where after samples are collected from the acceptor chamber (Balimane *et al*., 2000:305). Table 2-1 lists the advantages and limitations of the excised animal tissue technique in the Ussing-type chamber apparatus.

Table 2-1: The strengths and limitations of the Ussing-type diffusion chamber technique (adapted from Bohets *et al.,* 2001:373; Deferme *et al.,* 2007:189)

Advantages/Strengths	Disadvantages/Limitations
Good screening model with relatively high throughput	Tissue viability is limited to a restricted period of time
Relatively good correlation with *in vivo* permeability data	Under-estimation of permeability caused by the presence of circular muscle layer
Evaluation of different gastrointestinal tract regions possible	Challenges with variation in the unstirred water layer
No bio-analysis needed	Tissue availability
Transport mechanisms can be evaluated	Dissection of the epithelial tissue is challenging and serosa can be remove, but not the muscle layers
Evaluation of absorption enhancing strategies on a mechanistic basis is possible	
Good oxygenation	
Transepithelial drug transport can be investigated in combination with intestinal metabolism	
Amount of drug needed to perform study is relatively small	

Advantages/Strengths	Disadvantages/Limitations
Apical mucus layer is present	

Figure 2-5 illustrates the half cells of the Sweetana-Grass (Ussing-type) diffusion apparatus and the tissue mounting pins are shown where the excised tissue strips are fitted. At the inlet in the direction of the arrows, a buffer (usually Krebs-Ringer bicarbonate buffer) is circulated by gas lift (O_2/CO_2 mixture), which is contained in both the chambers parallel to the tissue's apical and basolateral surfaces (Bohets *et al*., 2001:372).

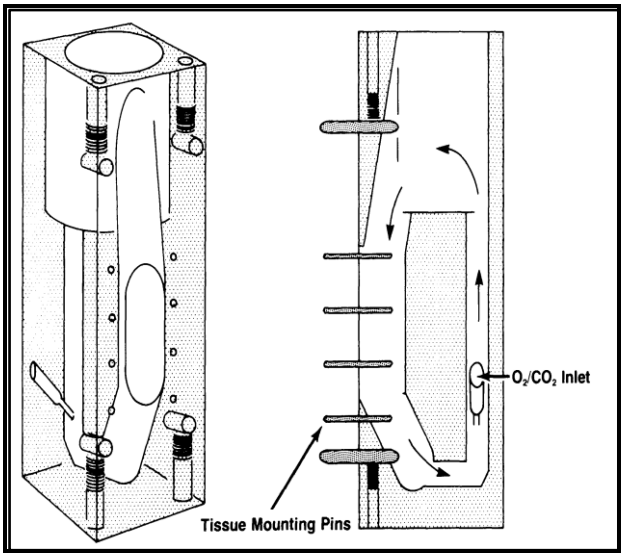

Figure 2-5: Illustration of the half cells of the Sweetana-Grass diffusion apparatus (Grass & Sweetana, 1988:374)

The tissue strips, mounted in the half cells, are maintained at 37°C by a diffusion-cell heating block. Heated water is circulated through the aluminum block in which the assembled half cells are kept (Grass & Sweetana, 1988:374).

2.5.4.2 Everted sac technique

The everted sac technique was first introduced in 1954, where the active transference of substances across the intestinal wall could be described (Wilson & Wiseman, 1954:116). Everted sacs are prepared when the intestine is removed from the animal (e.g. rat), flushed with saline solution, everted on a glass rod and oxygenated after being closed off at the ends to form sacs (Bohets *et al*., 2001:373). Previously this technique was used to study the transport of macromolecules and liposomes. In more recent studies, it has been used to quantify the paracellular transport of hydrophilic molecules and to investigate the effect of chemical enhancers on drug absorption (Le Ferrec *et al*., 2001:654). This model is ideal when studying

drug absorption mechanisms as both passive and active transport mechanisms can be studied (Balimane *et al*., 2000:305). The advantages and limitations of the everted sac technique is listed in Table 2-2.

Table 2-2: Advantages and limitations of the everted sac technique over other *in vitro* models (Balimane *et al*., 2000:305)

Advantages	Limitations
The sample volume on the serosal side can be small since drugs accumulate faster. Tissue can be obtained from animals slaughtered for other reasons than research (e.g. meat production) and therefore animals don't have to be specifically bred and euthanized for research purposes.	Lack of active blood and nerve supply which leads to rapid loss of viability (only viable for a limited period of time). Morphological damage is possible due to everting the intestinal tissue. Leakages may occur if sac is not closed off properly.

2.5.4.3 Cell culture models

Certain cell-based models mimic the *in vivo* intestinal epithelium in humans very well, thus making it a popular method to study absorption mechanisms. These models are based on the assumption that the epithelial monolayer of cells is the main barrier for drugs to reach the portal circulation. Regardless of the popularity of this method, it has certain disadvantages such as difficulties with culturing of the isolated epithelial cells, limited viability and potential of infections (Balimane *et al*., 2000:305; Bohets *et al*., 2001:373; Le Ferrec *et al*., 2001:655). Table 2-3 summarizes the most commonly used cell culture models to study drug permeabilty.

Table 2-3: The most commonly used cell culture models to assess drug permeability (Deferme *et al.,* 2007:193)

Cell line	Species or origin	Specific characteristics
Caco-2	Human colon adenocarcinoma	The model is well-established and differentiates spontaneously. Some efflux transporters are expressed (e.g., P-gp, MRP1-2)
MDCK	Dog kidney epithelial cells	The model has polarized cells with low intrinsic expression of transporters. It is suitable for transfections
LLC-PK1	Pig kidney epithelial cells	The model has polarized cells with low intrinsic expression of transporters. It is suitable for transfections

Cell line	Species or origin	Specific characteristics
2/4/A1	Rat fetal intestinal epithelial cells	The model has temperature-sensitive differentiation. It has leakier pores than the Caco-2 and therefore is more suitable for paracellular transport studies
TC7	Caco-2 sub-clone	This model has high taurocholic acid transport. It gives a stable CYP3A4 and CYP3A5 expression. It gives a low expression of P-gp.
HT29	Human colon	It is a co-culture containing mucus-producing goblet cells

Madin-Darby canine kidney (MDCK) cells are used to study drug metabolism, toxicity and transport at the distal renal tubule epithelial level (Le Ferrec *et al*., 2001:655). These cells differentiate into columnar epithelial cells and form tight junctions (Balimane *et al*., 2000:307). Unlike the Caco-2 cells, MDCK cells do not need 3 weeks to culture before they can be used, as they do not express P-glycoprotein (Le Ferrec *et al*., 2001:655).

The Caucasian adenocarcinoma cell line (i.e. Caco-2 cells) differentiates spontaneously into enterocytes that mimic the epithelium of the small intestine despite its colonic origin. Caco-2 cell monolayers can effectively be used to predict the active and passive transport of drugs across the human gastrointestinal epithelium (Le Ferrec *et al*., 2001:659). The apical and the basolateral surface of the cells are polarized, it forms domes, and it has a brush border and tight junctions (Bohets *et al*., 2001:373).

Table 2-4 displays the advantages and limitations of different cell culture models used for *in vitro* drug permeability studies.

Table 2-4: Advantages and limitations of using different cell culture models in drug permeation studies (Le Ferrec *et al*., 2001:662)

Technique	Advantages	Limitations
Caco-2 cells	Relatively fast and simple model. Used for determining mechanism of transport. The drug which is tested can be applied to the apical or basolateral side. It is human cells – no species	Physiological factors such as bile salts and mucous are not present which can influence permeation of drugs. These cells are of tumoral origin. This model has only one cell type (e.g. no goblet cells). The influence of P-

Technique	Advantages	Limitations
	differences. This model can be used for high throughput drug screening tests.	glycoprotein is difficult to estimate.
TC7 cells	These cells express CYP3A. Grow faster than Caco-2 cells. These cells need less glucose than Caco-2 cells.	
MDCK cells	This is a relatively fast and simple method. These cells can be used for high throughput screening tests. These cells can effectively be used to measure passive diffusion.	This is not an intestinal epithelial model. This is an animal model, which may present species related differences. Cells do not express P-glycoprotein.

2.6 Anatomy and physiology of the gastro-intestinal tract of the pig

The anatomy, morphology and physiology of the gastro-intestinal tract of the pig is closely associated with that of the human, thus making the pig a suitable model to investigate gastrointestinal related activities (Clouard *et al*., 2012:118; Patternson *et al*., 2008:652). The digestive system of the pig can be classified as mono-gastric or non-ruminant, the same as for humans. The size of the gastro-intestinal regions in relation to the body weight of the pig is generally considered to be very similar to those of humans (Sjögren *et al*., 2014:109). The anatomy of the GIT tract of the pig is schematically illustrated in Figure 2-6.

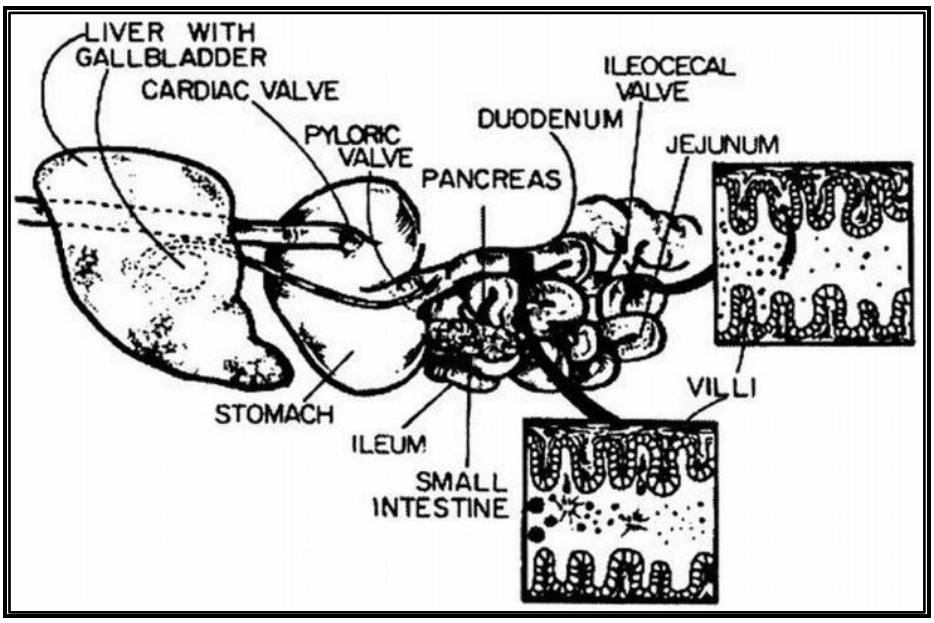

Figure 2-6: Schematic illustration of the gastro-intestinal tract of the pig (Rowan *et al*., 1997:2)

The average length of the different regions of the GIT tract of the pig compared to that of the human is shown in Table 2-5.

Table 2-5: Length of the different regions of the gastrointestinal tract of the pig compared to that of the human (Hatton *et al*., 2015:2750; Sjögren *et al*., 2014:102-103)

GIT parameter		Human	Pig
Small intestine Mucus thickness (µm)			
	Duodenum	15.5	25.6 ± 12.2
	Jejunum	15.5	35.3 ± 17.8
	ileum	15.5	53.8 ± 22.1
Length of small intestine (m)		6.25	14.16
Villi shape		Finger shaped	Finger shaped
Large intestine Mucus thickness (µm)			
	Cecum	313	37.2 ± 16.1
	Ascending colon	34.4	68.1 ± 36.5
	Transverse colon	50.5	83.6 ± 36.2
	Descending colon	62.0	76.3 ± 56.7
	Rectum	88.8	58.8 ± 27.9
Colon length (m)		1.5	4.27

GIT parameter		Human	Pig
Small intestine pH	Fasted	6.0–7.0(Duo); 6.0–7.7 (Jej); 6.5–8.0 (Ile)	7–8
	Fed	5.0–5.5 (Duo); 5.0–6.5 (Jej); Similar to fasted (Ile)	4.7–6.1 (Duo); 6.0–6.5 (Jej); 6.3–7.2 (Ile)
Transit time in small intestine	Fasted	3–4h	<1–3days
	Fed	3–4h	3–4h
Small intestine bile concentration	Fasted	2.0–10mM	
	Fed	8.0 (fed)	42–55mM
	After meal	10–20mM (after meal)	
Metabolic activities	Phase I	CYP3A4,2C9,2C19, 2D6,2J2	CYP1A1, 1A2, 2A6, 2B6, 2C9, 2D6, 2E1, 3A4
	Phase II	UGT, SULT, GST	UGT, SULT, GST
Major drug transporters		P-gp, MRP2, BCRP, PepT1, OATP	P-gp, MRP2, BCRP, OATP

2.7 Factors that may influence drug absorption

There are many factors that can affect the rate and extent of oral drug absorption. These factors can be divided into three categories namely, physiological, physico-chemical and dosage form factors.

2.7.1 Physiological factors

Many physiological factors are involved in the delivery of a drug after oral administration such as gastric emptying, pH, food, membrane permeability, surface area, intestinal metabolism, active efflux and first-pass hepatic extraction (Song *et al*., 2004:172).

2.7.1.1 Bile salts

The bile salts excreted into the lumen of the GIT increase the solubility and absorption of lipophilic molecules and fats via micellar solubilisation. The liver produces 0.5-1 litre of bile salt solution that is stored and concentrated in the gallbladder, which is released into the duodenum daily (Song *et al*., 2004:172; Zhou & Qiu, 2009:65).

2.7.1.2 Gastric emptying

The gastric emptying rate plays an important role in the onset of absorption of a drug, whereas the transit time plays a significant role in the residence time of the drug at the absorption site and therefore the extent of absorption (Song *et al*., 2004:172). The gastric emptying time is influenced by the type of food, size of components in the stomach contents and osmolality. The small intestine transit time is 3-4 h on average (Zhou & Qiu, 2009:66).

In a study conducted by Marathe and colleagues (2000:329), the effect of altered gastric emptying and motility on the absorption of metformin was measured. The results showed that the area under the curve (AUC) and the percentage of unchanged drug in the urine increased when the emptying rate increased. They further showed that when the gastrointestinal motility is slowed by the administration of pre-treatment with propantheline, the absorption of metformin could be improved.

2.7.1.3 Effect of food

The physiological changes induced by the intake of food play an important role in the absorption of drugs, as it slows the gastric emptying time and increases the gastric pH. The prolonged retention of drugs in the stomach may increase the portion of drug that is dissolved, prior to the passage into the small intestine where absorption will take place. The absorption of weakly acidic drugs and basic drugs can be influenced by a fasting or fed state. With an elevation in gastric pH after a meal, the dissolution of a weak acidic drug will increase in the stomach, but the dissolution rate of a weak base will be reduced (Song *et al*., 2004:174).

2.7.1.4 Intestinal metabolism and efflux

Drug loss can occur via Phase I and II metabolism inside the epithelial cells and degradation within the gut lumen. Cytochrome P450s are the major enzymes involved in drug metabolism in the GIT. CYP3A4 is a major Phase I metabolising enzyme, which is present in high amounts in the villus tip enterocytes of the small intestine (Song *et al*., 2004:173).

P-glycoprotein was first identified by Juliano and Ling in 1976 as a plasma membrane-bound ATP-dependant efflux transporter, which influences drug pharmacokinetics by pumping drug molecules back into the lumen after they have been taken up into the enterocytes (Bansal *et* al., 2009:47; Kagan *et al*., 2010:1560; Lin *et al*., 2003:60). P-gp is not limited to humans, but is also present in animals such as the pig (Childs & Ling, 1996:205). P-gp in the small intestine is located at the apical membrane of enterocytes, where it acts with CYP metabolizing enzymes to limit drug bioavailability (Hebert, 1997:201; Kagan *et al*., 2010:1560). P-gp levels increase along the small intestine with the highest level reached in the distal jejunum, while it is

decreased in the ileum (Sjögren *et al*., 2014:110). P-gp is composed of two homologous and symmetrical halves; each halve containing six transmembrane domains and two ATP-binding regions. These domains are separated by a flexible intracellular linker polypeptide loop (refer to Figure 2-7) (Aller *et al*., 2009:1718).

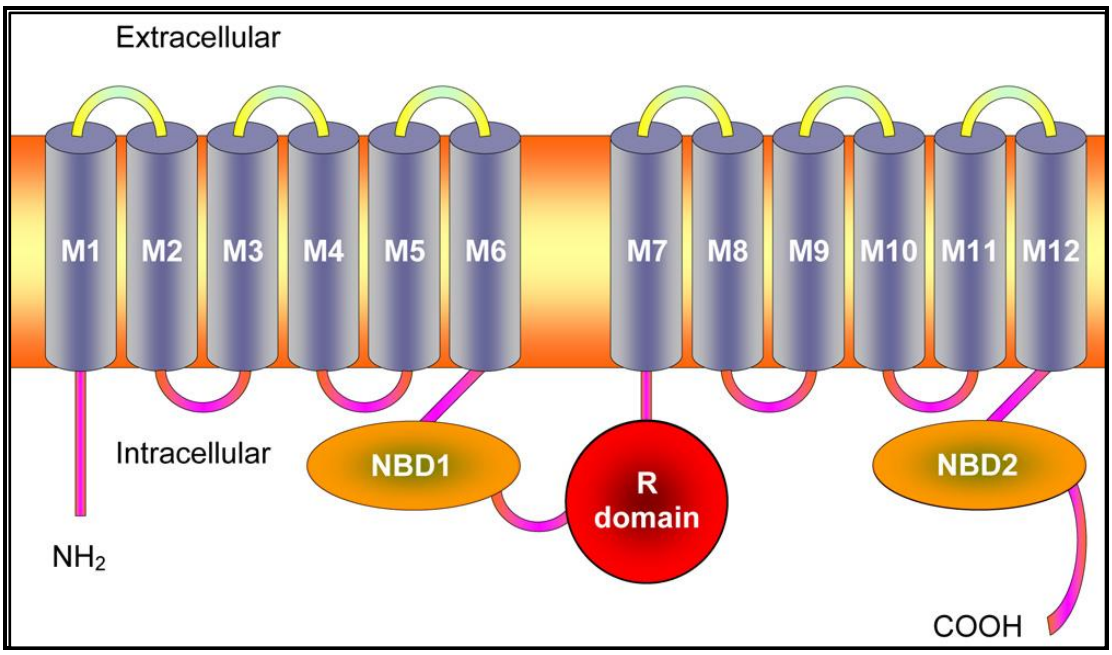

Figure 2-7: Schematic illustration of the P-glycoprotein efflux transporter structure

To inhibit the effect of P-gp, inhibitors can be introduced (Song *et al*., 2004:173). There are three mechanisms available through which P-gp can be inhibited (Amin, 2013:28), namely:

- Competitively or non-competitively blocking the drug binding site,
- Interfering with ATP-hydrolysis,
- Altering integrity of cell membrane lipids.

Table 2-6 displays P-gp inhibitors that can be classified into three groups according to their specificity, affinity and toxicity. The first generation inhibitors are pharmacologically active substances, which are used for specific treatments and have the ability to inhibit P-gp. These first generation inhibitors are also substrates to other transporters and enzymes, which can lead to unpredictable pharmacokinetic interactions. Second generation inhibitors lack the pharmacological activity compared to the first generation. They pose a higher affinity to P-gp and inhibit CYP3A4 enzymes and other ABC transporters, which include non-immunosuppressive analogues of cyclosporine A and the D-isomer of verapamil. The third generation are still under clinical development. They aim to inhibit P-gp with higher specificity and lower toxicity, compared to other generations (Amin, 2013:29).

Table 2-6: Classification of P-glycoprotein inhibitors (Thomas & Coley, 2003:160-161)

Generation	Examples	Specificity	Limitations
First generation	verapamil, cyclosporine A, tamoxifen	Non-selective and low binding affinity	Substrates to other transporters and enzyme systems. They are pharmacologically active. They are transported by p-glycoprotein themselves.
Second generation	dexverapamil, dexniguldipine	Higher specificity and interact with other systems	Substrates to CYP 3A4 enzyme and other ABC transporters.
Third generation	cyclopropyldibenzosuberanezosuquidar, biricodar	Highest specificity that specifically and potently inhibit p-glycoprotein	No limitations.

2.7.2 Physico-chemical factors

There are two major physico-chemical factors that affect the rate and extent of drug absorption of orally administered drugs, namely solubility and intestinal epithelial permeability. These two factors are interrelated to many molecular properties such as lipophilicity, hydrophilicity, molecular size, surface area, partition coefficient, hydrogen bond donors and hydrogen bond acceptors (Song *et al*., 2004:169).

2.7.2.1 Solubility

Solubility is a determinant of drug dissolution, which is a key requirement for absorption. If a drug is able to dissolve rapidly in the lumen, absorption will not be limited by the dissolution rate (Song *et al*., 2004:169; Zhou & Qiu, 2009:66).

Solubility can be defined as the amount of a compound that can dissolve in a certain volume of solvent and is related to the extent to which molecules are removed from the surface of the solid by a solvent (Song *et al*., 2004:169). Most drugs are weakly acidic of basic compounds, which are only partly ionized. This ionization is dependent on the pH of the solvent. According to the Henderson-Hasselbalch equation, weakly acidic drugs dissolve faster in solvents with a relatively high pH, whereas weakly basic drugs dissolve faster in solvents with a low pH (Martinez & Amidon, 2002:622; Song *et al*., 2004:170).

The surface area of drug particles has a great influence on the dissolution rate of the drug, thereby affecting the absorption. The particle size of a substance determines the surface area, thus the smaller the size of the particle the bigger the surface area, resulting in rapid dissolution. It was determined that particle size of high aqueous solubility drugs has little influence on the absorption. For optimal dissolution, the surface area can be increased by reducing the particle size or by adding surface-active agents to a dosage form (Granero & Polache, 2008:292; Song *et al*., 2004:171). The intermolecular forces present can influence the solubility of a substance. Crystals with weak attractive forces present has greater solubility compared to the counterpart (Granero & Polache, 2008:292).

2.7.2.2 Permeability

Epithelial cell membranes are lipoid, which means they are more permeable to drugs that are lipid-soluble. The presence of non-polar groups and ionisable groups in the chemical structure of a drug determines the lipid solubility of a drug. High lipid solubility must be accompanied by adequate water solubility in order for a substance to be absorbed. If the water solubility is too low, the required concentration of the drug cannot be achieved at the membrane surface. The oil/water partition coefficient ($K_{o/w}$) describes the lipophilic/hydrophilic balance of a drug molecule. This parameter predicts the absorption by passive diffusion across lipoid membranes. As the partition coefficient increases, the rate of absorption increases (Granero & Polache, 2008:292-293; Song *et al*., 2004:171; Zhou & Qiu, 2009:67).

2.8 Pharmaceutical excipients

Pharmaceutical excipients have traditionally been included in medicinal products to serve as a carrier, to provide the correct consistency, to make up volume and to give sufficient mass to the product. Since its inception, excipients evolved to fulfil certain important roles in modern dosage forms such as to assist with the manufacturing process, ensure that the drug is released at a specific rate, is absorbed at sufficient concentrations and is stable for a suitable period of time (Hamman & Steenekamp, 2012:219; Patel *et al*., 2015:15). The International Pharmaceutical Excipients Council (IPEC) defines excipients as substances other than the active ingredients that are included in dosage forms, which contribute to improve manufacturing of the medicinal product, increase stability, facilitate drug delivery and ensure patient acceptability (Panda *et al*., 2015:440).

2.8.1 Disintegrants

Disintegrants are excipients added to drug formulations to facilitate the breakup or disintegration of the solid dosage form (e.g. tablet) into smaller fragments, thereby enhancing the dissolution process (Antony & Sanghavi, 1997:413). Disintegrants have

different mechanisms of action in solid dosage forms such as tablets, which include swelling, wicking (porosity and capillary action), wetting, deformation, particle-particle repulsive forces, release of gasses and enzymatic reactions (Kaur & Mehara, 2016:40). Super-disintegrants are used at relatively low quantities (1-10% w/w) in solid dosage forms (Kaur & Mehara, 2016:39). Croscarmellose sodium (Ac-di-sol®) is an example of a crosslinked cellulose, which mechanism of action includes both swelling and wicking, resulting in a good superdisintegrant (Mohanachandran *et al*., 2011:108).

2.8.2 Diluents

Diluents/fillers are designed to make up the bulk of dosage forms when the drug itself is inadequate to produce a dosage form with sufficient size. Diluents are also included in tablets to improve cohesion to facilitate direct compression and to promote flow properties of drugs (Pandey *et al*., 2009:2273). Microcrystalline cellulose (Avicel®) is a diluent that is incorporated into tablet/ capsules to increase the volume or weight of the dosage form and can also be referred to as a dry binder, as it aids in the compactibility of the compression mix (Thoorens *et al*., 2014: 65).

2.8.3 Binders

A binder is included in solid dosage forms such as tablets to ensure that it can be manufactured so that it possesses acceptable mechanical strength to withstand abrasion during packaging and handling. Binders can be added to a powder as a dry powder before wet granulation, as a solution during wet granulation or as a dry powder mixed with other ingredients before compaction (Alderborn, 2007:452).

2.8.4 Glidants

The function of a glidant is to improve the flow-ability of a powder (Chaudhari & Patil, 2012:28). Powder flow plays an important role in the manufacturing process of solid dosage forms such as tablets as the flow of powder from the hopper into the die of the tablet determines the weight and content uniformity of the tablets (Shah *et al*., 2008:250).

2.8.5 Lubricants

Lubricants are added to tablet formulations to ensure that the tablet can easily be ejected from the die, causing low friction between the solid and the die wall. The ideal lubricant should fulfil the following requirements (Faldu & Zalavadiya, 2012:921):

- Reduce friction at low quantities,
- Be inert,

- Be water soluble,
- Be colourless,
- Be odourless.

2.9 Excipient-drug pharmacokinetic interactions

Pharmacokinetic interactions can be defined as potential adverse effects that can occur due to altered pharmacokinetics of a drug as a result of a co-administered compound that can alter the absorption, distribution, metabolism, and excretion of the co-administered drug (Li, 2008:855).

As mentioned before, excipients were originally developed to be pharmacologically and chemically inert, but some excipients may cause undesirable effects due to interactions with drugs. Excipient-drug interactions can be classified into three main categories namely physical, chemical and biopharmaceutical interactions (Chaudhari & Patil, 2012:23; Patel *et al*., 2015:15-16).

2.9.1 Physical interactions

Physical interactions can change the dissolution rate and dosage uniformity, but there are no chemical changes to the molecular structure of the active ingredient. This type of interaction can be beneficial or harmful to the product's performance. There are three typical physical interactions that can occur, namely complexation, adsorption and formation of solid dispersions. An example of a positive complexation interaction is the complexation of cyclodextrin with ursodeoxycholic acid that resulted in an increase in the bioavailability of this drug, which was triggered by an increased dissolution (Patel *et al*., 2015: 16). An example of a negative complexation interaction is the interaction of tetracycline with calcium carbonate, which resulted in a slower dissolution rate and consequently a lower bioavailability of tetracycline (Trovato *et al*., 1991:1652).

Formation of a solid dispersion usually improves dissolution and potentially also the bioavailability of hydrophobic drugs (Patel *et al*., 2015:16). Polyethylene glycol formulated with ibuprofen, nifedipine and piroxicam increased their dissolution rates (Chaudhari & Patil, 2012:30). A negative solid dispersion reaction between povidone and stearic acid resulted in a decreased dissolution of the drug (Patel *et al*., 2015:16; Pifferi & Restani, 2003:544). The adsorption of a drug to an excipient sometimes result in reduced bioavailability of the drug, but the adsorption of the drug on the excipient surface can also increase the surface area and consequently the drug's availability for dissolution and bioavailability (Chaudhari & Patil, 2012:30). Indomethacin adsorption to kaolin resulted in an increase in its dissolution and consequently its bioavailability. A detrimental adsorption interaction is, for example, between cetylpyridium chloride and magnesium stearate. The cetylpyridium chloride adsorbs on the

surface of the magnesium stearate and leads to a reduced antibacterial activity (Patel *et al*., 2015:16).

2.9.2 Chemical interactions

Generally, chemical interactions occur when active pharmaceutical ingredients react with excipients to form an unstable product (Patel *et al*., 2015:16). A list of potential chemical interactions that can occur between drugs and excipients are summarized in Table 2-7.

Table 2-7: Potential chemical interactions between active pharmaceutical ingredients and excipients (Chaudhari & Patil, 2012:30)

Interaction	Causing factors	Examples of drugs
Hydrolysis	In the presence of water, low and high pH levels, in the presence of alkaline metals and acids.	Drugs with functional groups such as esters, amides and lactones, e.g.: Antibiotics Anaesthetics Barbiturates
Oxidation	Oxygen, light, heavy metal ions, fumed metal oxides, fumed silica and zirconia are responsible for catalysing the reaction. These types of interactions of active Ingredients occur with oxidizing impurities in excipients or oxidative degradation products of excipients.	Steroids Antibiotics Epinephrine Aldehydes Alcohols Phenols
Maillard reactions	Carbonyl group of sugar reacts with amino-acid, which produces N-substituted glycosylamine and water. The unstable glycosylamine undergoes amidroid rearrange forming ketosamine. The ketosamines react to produce water and reductones or produce short chain hydrolytic fission products. The rate of Maillard reactions increases as water activity increases.	Primary amines undergo Maillard reactions, where they cause yellow brown coloration of drugs like chlorpromazine. Maillard reaction products are found in capsules containing lactose and in antidepressants such as Fluoxetine.

2.10 Summary

From the literature review, it is clear that the pig is a good animal model for use in drug transport studies. Excipients can influence the transport of drugs in the GIT. Various *in vitro* methods are available to study the transport of drugs across the intestinal mucosa. The Ussing chamber technique (i.e. Sweetana-Grass diffusion chamber) that will be used in this study requires the use of excised animal tissue from the pig (slaughtered for meat production purposes). This technique is cost-effective, relatively simple and complies with the three R's in ethics regarding use of animals in research (i.e. reduce, replace and refine). Excipient-drug interactions can be detrimental or beneficial to the bioavailability of drugs included in solid oral dosage forms. It is therefore important to obtain information regarding excipient-drug pharmacokinetic interactions.

CHAPTER 3 MATERIALS AND METHODS

3.1 Introduction

Pharmaceutical excipients were initially intended to be pharmacologically inert; however, some unexpected effects of excipients on drug pharmacokinetics have been reported (Schulze *et al*., 2005:68). Although functional excipients are now intentionally included in dosage forms to assist in drug delivery via different mechanisms, it is important to evaluate the effect of excipients on drug pharmacokinetics that were included in dosage forms purely for formulation purposes (Takizawa, *et al.,* 2013:363). Furthermore, very few studies have investigated the region specific effects of excipients on drug absorption in the gastro-intestinal tract (GIT). The Sweetana-Grass diffusion technique was used to investigate the effect of two selected excipients on the transport of a model compound across excised pig epithelial tissues of five different GIT regions. Pig intestinal tissue was used for the permeation studies as it has anatomical and physiological features similar to the human GIT (Westerhout *et al*., 2014:167).

Rho123 is a highly selective P-gp substrate that can be used as a model compound in permeation studies where the influence of compounds on P-gp related efflux need to be investigated (Crawford & Putnam, 2014: 1462). Furthermore, Rho123 has many advantages as a model transport compound namely it is commercially available, has a relatively low cost, provides high quantum yield, can easily be analysed by non-invasive detection and exhibits low interference with metabolic processes (Forster, *et al*., 2012:1).

Distinction was made between the different regions of the pig GIT as indicated in the photograph shown in Figure 3-1, which was based on illustrations and descriptions in a pathology text book with the assistance of a veterinary surgeon. For the *in vitro* permeability studies in this research project, the pig GIT was divided into five anatomical regions namely the duodenum, proximal jejunum, mid-jejunum, distal jejunum and ileum. Excised tissues from each region were used to investigate the effect of selected excipients on drug permeation.

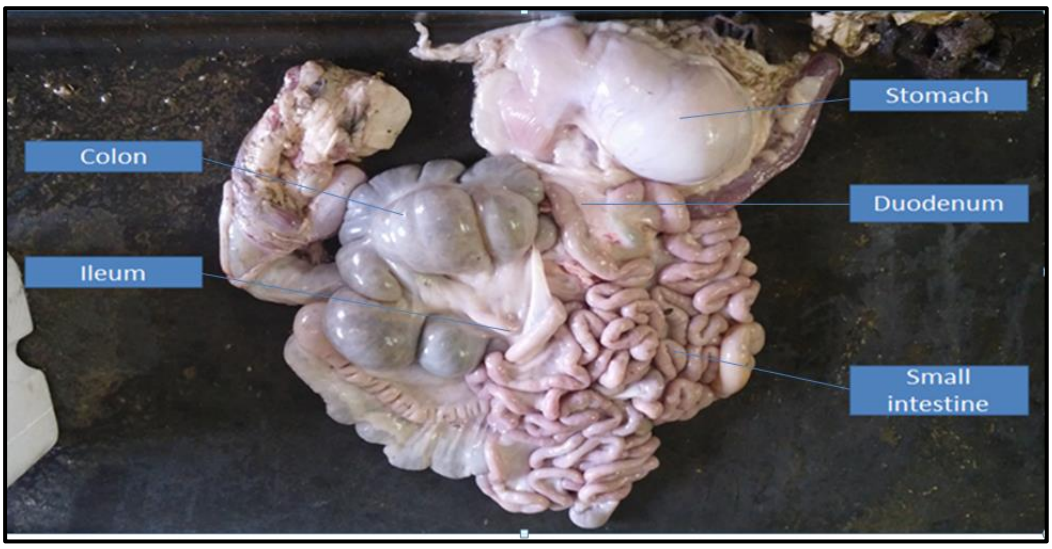

Figure 3-1: Photograph of the gastrointestinal tract of the pig labelled with the major anatomical regions

3.2 Materials

Rhodamine 123 and Krebs-Ringer bicarbonate (KRB) buffer were purchased from Sigma Aldrich (Johannesburg, South Africa). Ac-di-sol® and Pharmacel® PH-101 were purchased from Warren Chem Specialities (Johannesburg, South Africa). The pig intestinal tissues were collected at the local abattoir in Potchefstroom. Ethics approval was obtained for use of animal tissues in research (NWU-00025-15-A5). The Costar® 96 well plates were purchased from The Scientific Group (Randburg, South Africa).

3.3 Validation of the fluorometric analytical method

The samples withdrawn from the acceptor chambers during the permeation studies were quantitatively analysed for Rho123 content by means of a fluorometric method in a SpectraMax® Paradigm® Multi-Mode Detection Platform multi-plate reader (Separations, Johannesburg). This analytical method was validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantification as described below.

3.3.1 Linearity

Linearity of an analytical method can be defined as its ability to obtain test results that are directly proportional to the test sample concentration within a given range (USP-NF, 2016). Linearity of an analytical method would be accepted if a correlation coefficent (R^2) greater than 0.999 can be achieved (Shabir, 2003:64).

Two stock solutions of 5 µM and 2.5 µM respectively were prepared. The two stock solutions were diluted with KRB four times by a factor of ten. The regression line were calculated after the concentration range of 5 µM, 2.5 µM, 0.5 µM, 0.25 µM, 0.05 µM, 0.025 µM, 0.005 µM, 0.0025 µM and 0.0005 µM were analysed with the SpectraMax® Paradigm® multi-plate reader. A correlation coefficient (R^2) of 0.9998 was obtained.

3.3.2 Accuracy

To determine accuracy, the measured concentration values are often compared with referenced concentration values to show how close or accurate the instrument measures the concentration of the analyte (Riu & Rius, 1996:1851). According to the USP-NF (2016), the accuracy of an analytical method is determined by no less than nine readings of a minimum of three different concentrations. The accuracy of the method is then expressed as the percentage deviation from the theoretical concentration by the experimental values. The mean recovery of the analyte should be 100 ± 2% for the method to be considered accurate (Shabir, 2003:61).

Three solutions of Rho123 with different concentrations were prepared in KRB buffer (i.e. 5 µM, 2.5 µM and 0.0125 µM). Each solution was analysed three times with the SpectraMax® Paradigm® multi-plate reader at an excitation wavelength of 480 nm and emission wavelength of 520 nm. The recovery was calculated as the percentage obtained of the theoretical concentration of each of the three solutions.

3.3.3 Precision

Precision of an analytical method can be defined as how consistent the results are when measurements are repeated at different time points. Precision is therefore used to indicate the repeatability of the analytical method (USP-NF, 2016). Inter-day and intra-day precision were determined as described below.

3.3.3.1 Intra-day precision

Shabir (2003:62) stated that the percentage relative standard deviation (RSD) calculated from the intra-day precision measurements should be ≤ 5%.

Three solutions of Rho123 with different concentrations (i.e. 5 µM, 2.5 µM and 0.0125 µM) in KRB buffer were analysed at three different time points on the same day and the percentage RSD values were calculated for each concentration.

3.3.3.2 Inter-day precision

Three solutions of Rho123 in KRB buffer (i.e. 5 µM, 2.5 µM and 0.0125 µM) were analysed over three consecutive days and the percentage RSD values were calculated for each concentration.

3.3.4 Specificity

Specificity can be defined as the ability to assess an analyte in the presence of components that is expected to be present during the experiment (USP-NF, 2016).

Solutions were prepared with Rho123 only and with each of the selected excipients (i.e. Ac-di-sol® at concentrations of 0.005% w/v; 0.01% w/v and 0.02% w/v; Pharmacel® PH-101 at concentrations of 0.015% w/v; 0.03% w/v and 0.06% w/v). The mean fluorescence value obtained with the SpectraMax® Paradigm® multi-plate reader at an excitation wavelength of 480 nm and emission wavelength of 520 nm for the Rho123 only solution was compared with that of the solutions containing the excipients each at three different concentrations.

3.3.5 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) can be defined as the lowest amount of analyte that can be detected by the instrument, but not necessarily quantified as an exact value (USP-NF, 2016). The limit of detection (LOD) of Rho123 obtained with the fluorometric method used in this study was calculated by applying the following equation (Singh, 2003):

$$\mathrm{LOD} = 3.3 \times \frac{\mathrm{SD}}{\mathrm{S}}$$

Where SD is the standard deviation of the blank samples (KRB buffer) and S is the slope of the straight line (i.e. fluorescence plotted as a function of concentration for a range of solutions prepared)

The concentration range used for Rho123 solutions that were used to plot the linear regression line included 5 µM, 2.5 µM, 0.5 µM, 0.25 µM, 0.05 µM, 0.025 µM, 0.005 µM, 0.0025 µM, 0.0005 µM. The slope of the straight line was obtained after plotting the fluorescence values of the different Rho123 solutions as a function of concentration. The fluorescence of nine blank samples (KRB buffer only) was measured by the SpectraMax® Paradigm® multi-plate reader and represented the background noise. The standard deviation was calculated for three fluorescence readings of the blank samples.

The limit of quantification (LOQ) of an analytical method can be defined as the lowest concentration of an analyte that can quantitatively be determined with precision and accuracy (Singh, 2013:30). The same measurements were made as described for the LOD in terms of a

concentration range of Rho123 and blank samples. The LOQ was calculated by the following equation (Singh, 2013):

$$\mathrm{LOQ} = 10 \times \frac{SD}{S}$$

Where SD is the standard deviation of the blank samples (KRB buffer) and S is the slope of the straight line (i.e. fluorescence plotted as a function of concentration for a range of solutions prepared).

3.4 *In vitro* transport studies

The experimental design of the in vitro permeation studies are schematically illustrated in Figure 3-2.

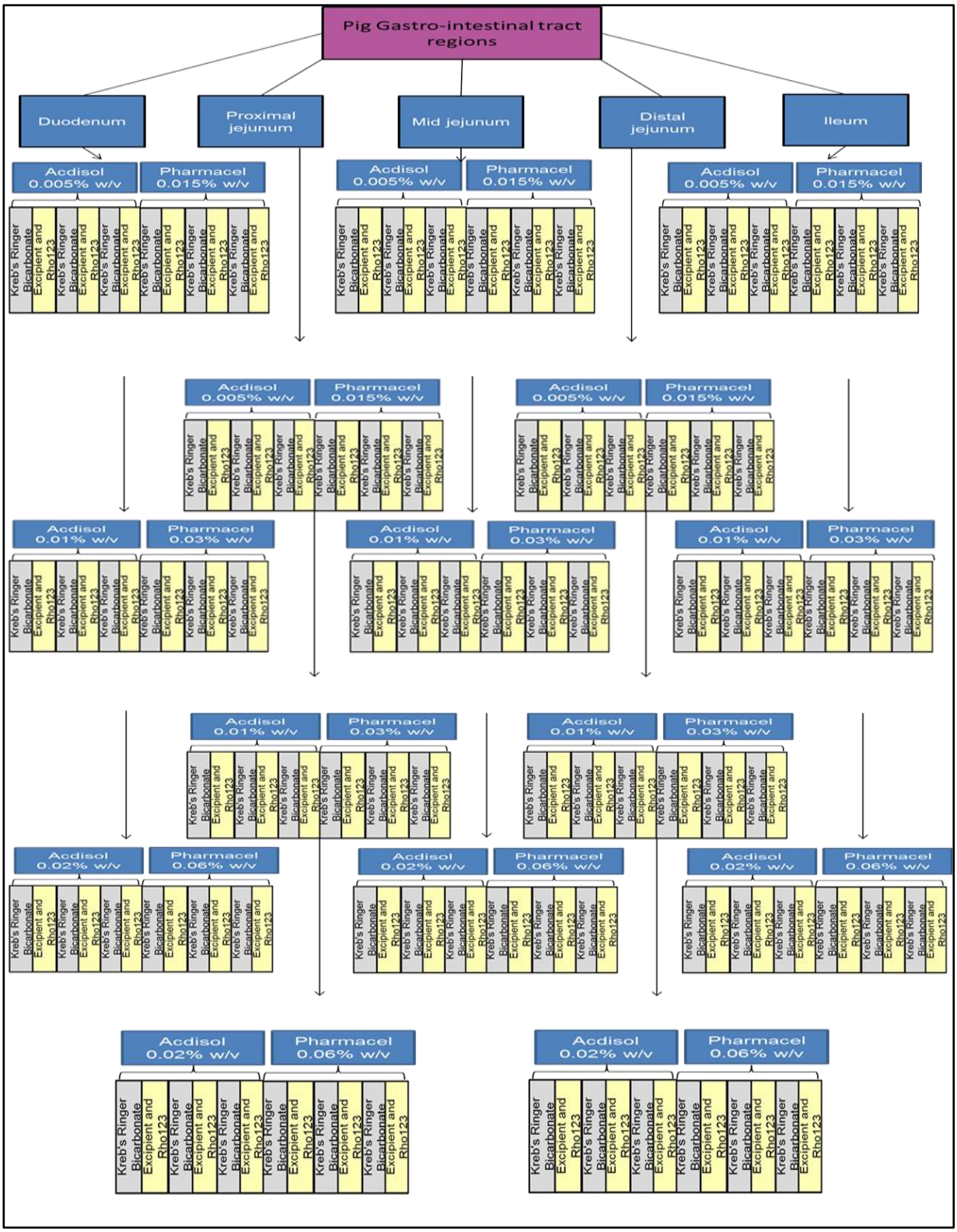

Figure 3-2: The experimental design of the in vitro permeation studies

3.4.1 Preparation of test solutions

To prepare Krebs-Ringer Bicarbonate (KRB) buffer, a single container of KRB buffer powder mixture (Sigma Aldrich, Johannesburg, SA) was mixed with 1.26 g sodium bicarbonate and added to approximately 500 ml distilled water in a 1000 ml volumetric flask. The solution was stirred with a magnetic stirrer. Thereafter, the solution was made up to volume (i.e. 1000 ml) with distilled water. The pH was measured and recorded. The KRB buffer solution was freshly prepared and used as the transport medium in all of the permeation experiments.

A stock solution of Rho123 (1312.96 µM) was prepared in KRB buffer and this stock solution was then diluted with KRB to the desired concentration for each of the experimental solutions. For example, to obtain a Rho123 solution with a concentration of 5 µM, a volume of 190.4 µl of the stock solution of was diluted with KRB to 50 ml. Suspensions of the selected excipients (i.e. Ac-di-sol® and Pharmacel PH101®) were each prepared in the 5 µM Rho123 solutions at three different concentrations as illustrated in Figure 3-2. Each of these experimental solutions was used in permeation studies at five different GIT regions.

3.4.2 Collection and preparation of pig intestinal tissue

Pig intestinal tissue was collected freshly from the local abattoir in Potchefstroom on each day of the *in vitro* permeation experiment. Immediately after the pigs were slaughtered, the correct intestinal region was identified and a piece of approximately 15 cm in length was excised from the specific region, rinsed and submerged in ice-cold KRB buffer in a cooler box for transport to the laboratory. It was ensured that the process of obtaining the intestinal tissue, dissecting the region of interest and transporting it to the laboratory for experimentation did not exceed 30 min from the time that the pig was slaughtered until the mounting of the tissue on the Sweetana-Grass diffusion apparatus.

A step-by-step description of the preparation of the excised pig intestinal tissue for conducting *in vitro* permeation studies is given below. The same method was used to prepare the tissue from each selected region of the pig GIT for each of the *in vitro* permeation studies.

In the laboratory, the excised pig intestinal segment was removed from the cooler box and carefully pulled over a wetted glass tube as shown in Figure 3-3.

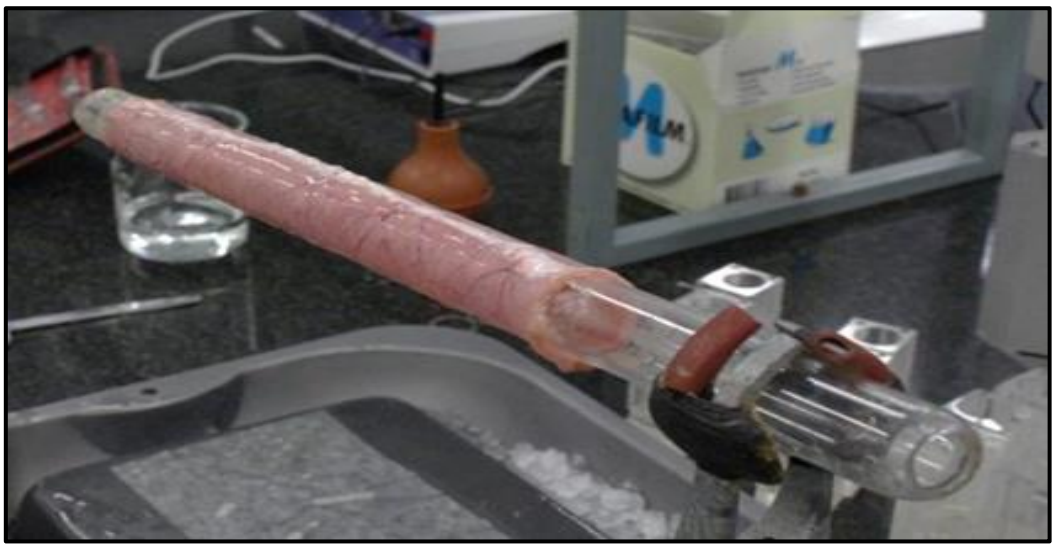

Figure 3-3: Photograph illustrating an excised segment of pig intestine pulled onto a glass tube

The serosal layer was stripped off by blunt dissection as illustrated in Figure 3-4 and thereafter the intestinal segment was cut along the mesenteric border with a scalpel.

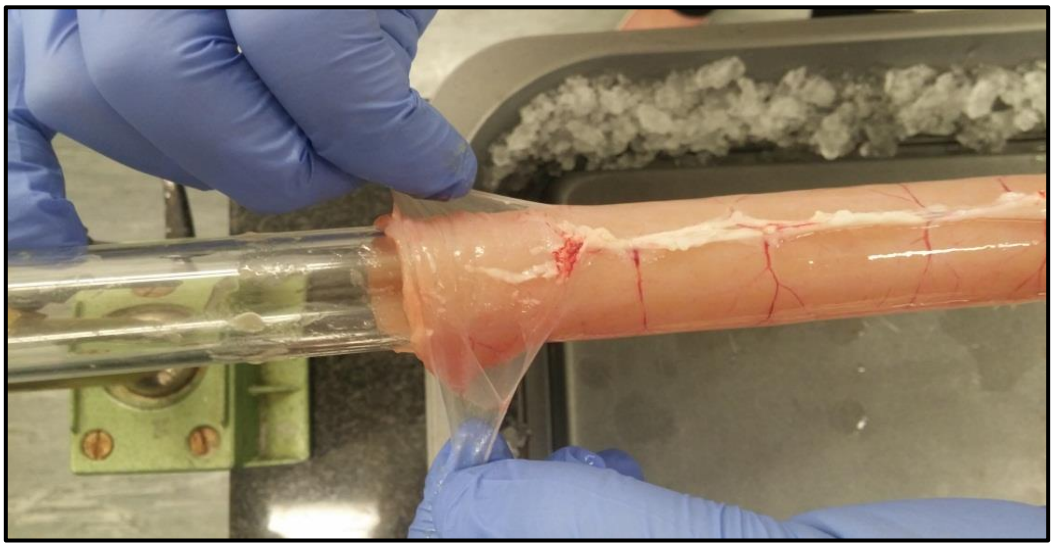

Figure 3-4: Photograph illustrating the serosa being removed from the intestinal segment by means of blunt dissection

The intestinal segment that was cut open in its entire length was washed off the glass tube onto a sheet of filter paper as illustrated in Figure 3-5.

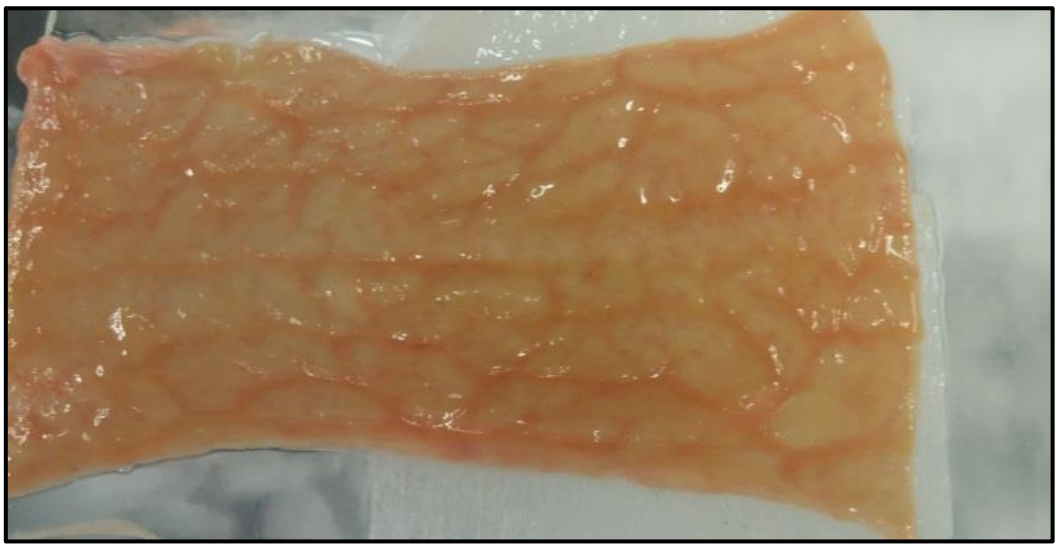

Figure 3-5: Photograph illustrating a spread open segment of excised pig intestinal tissue on a piece of filter paper

Subsequently, the excised segment was cut into smaller pieces of tissue of approximately 1 cm × 3 cm, which were then mounted onto the half-cells of the Sweetana-Grass diffusion apparatus as illustrated in Figure 3-6.

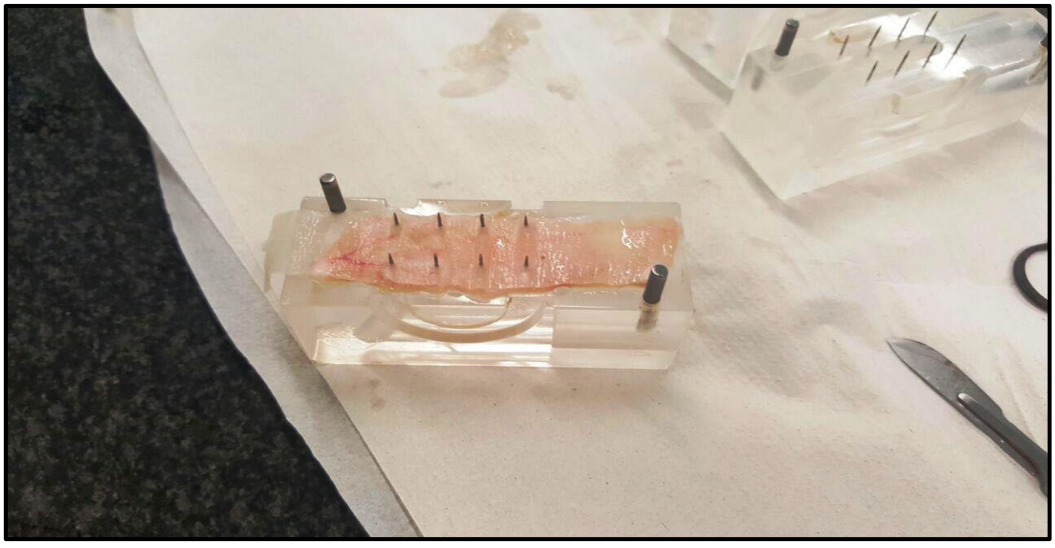

Figure 3-6: Photograph illustrating a piece of excised pig intestinal tissue mounted on a half-cell of the Sweetana-Grass diffusion chamber with a piece of filter paper still attached to the tissue

Peyer's patches (illustrated in Figure 3-7) on the excised intestinal tissue were avoided.

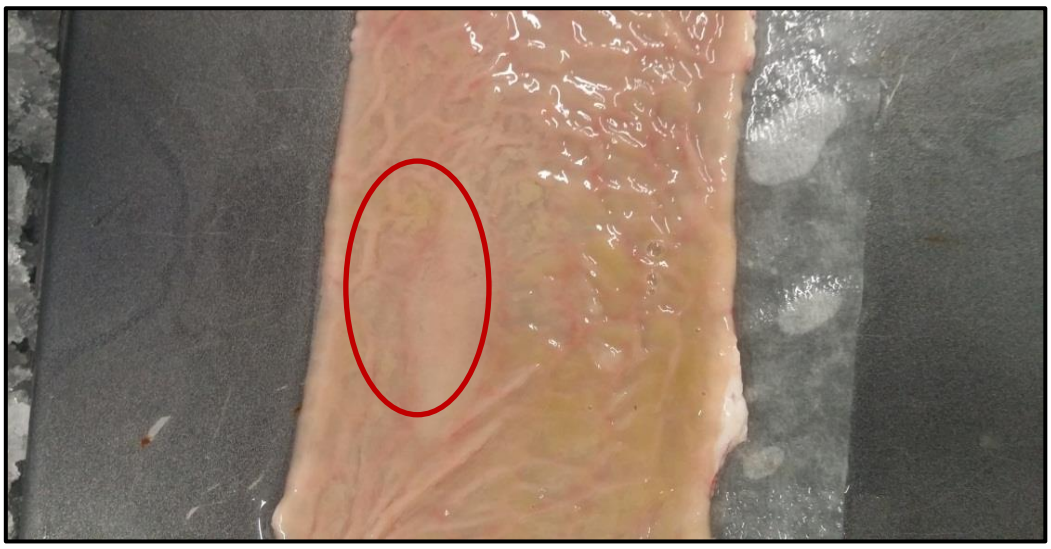

Figure 3-7: Photograph illustrating a Peyer's patch (within the red oval) on an excised pig intestinal tissue segment

The filter paper was removed from the tissue mounted onto the half-cell. The matching half-cell was clamped onto the half-cell containing the excised piece of tissue and the assembled chamber was secured by using metal rings as illustrated in Figure 3-8.

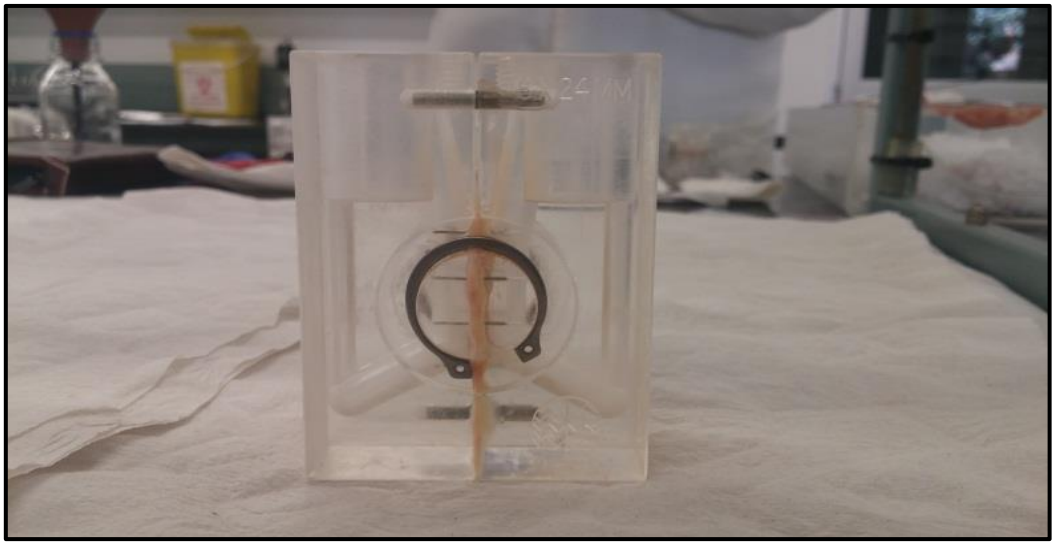

Figure 3-8: Photograph illustrating the two half-cells of the Sweetana-Grass diffusion apparatus clamped together and secured with a metal ring

All six of the assembled Sweetana-Grass diffusion chambers were linked to a heating block in order to maintain the KRB buffer at 37°C as illustrated in Figure 3-9. A tube supplying medical oxygen was attached (95% O_2: 5% CO_2) to each half-cell of the chambers, which ensured that

medical oxygen was bubbled through the KRB during the entire period of the permeation study (Lennernäs *et al.,* 1997:667)

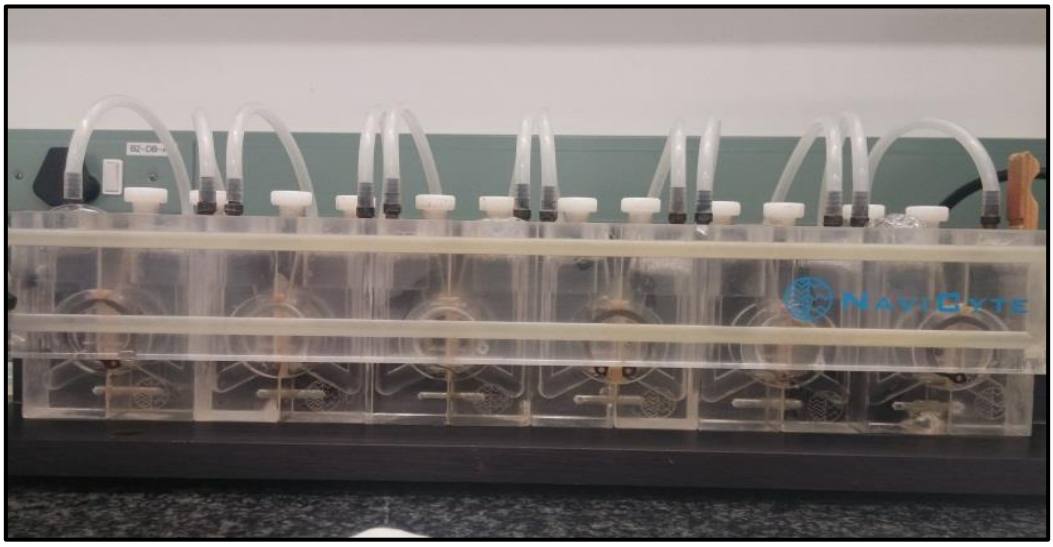

Figure 3-9: Photograph illustrating assembled Sweetana-Grass diffusion chambers clamped in the heating block and medical oxygen tubes attached to each half-cell

Each donor chamber (i.e. apical side) was filled with 7 ml of KRB buffer, which circulated for a period of 15 min to equilibrate the tissue with the new environment. Thereafter, the KRB buffer in the donor chamber was removed by aspiration with a vacuum system (Vacusafe®, Hudson, USA) and it was replaced with 7 ml of pre-heated (37°C) Rho123 test solution in the absence (control group) or presence of excipients (test solutions) in three different concentrations. The trans-epithelial electronic resistance (TEER) was measured with a Dual Channel Epithelial Voltage Clamp (Warner Instruments, Hamden, Connecticut, USA) every 20 min for a period of 120 min to monitor membrane integrity over the entire period of the permeation study.

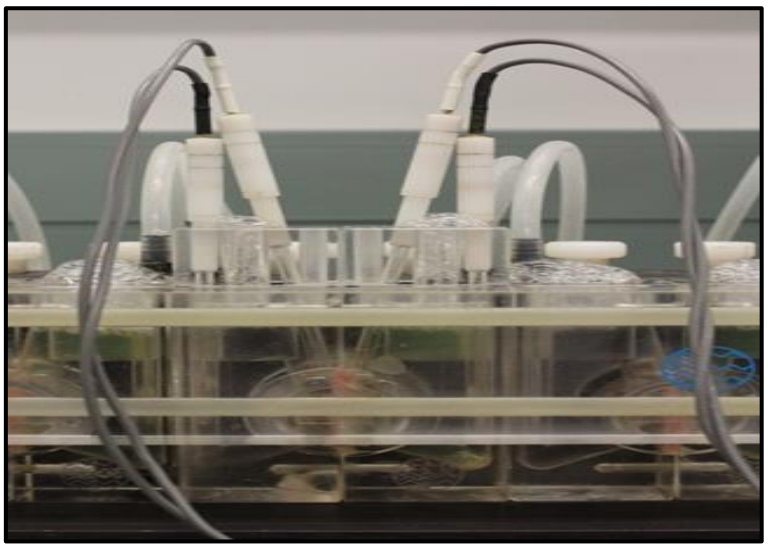

Figure 3-10: Photograph illustrating the electrodes inserted into the half-cells to measure trans-epithelial electrical resistance (TEER)

Samples (180 µL) were taken every 20 min from the acceptor chamber (i.e. basolateral side) over a period of 2 h. The extracted sample volume was replaced with the same volume of pre-heated KRB buffer after each withdrawal. The concentration of Rho123 in each sample was determined by means of a validated fluorescence spectroscopic method with the SpectraMax® Paradigm® multi-plate reader.

3.5 Data processing

3.5.1 Apparent permeability coefficient (P_{app})

Volpe (2010:673) defined apparent permeability coefficient (P_{app}), as the rate of accumulated drug in the receiver chamber normalised for tissue surface area.

The Rho123 concentrations obtained in the transport samples were corrected for dilution and expressed as cumulative drug transport (percentage of initial dose) and the apparent permeability coefficient (P_{app}) values for the test compound from samples withdrawn from the receiver compartment were calculated with the following equation. The values are expressed as cm/second (Le Ferrec *et al*., 2001:657)

$$P_{app} = \frac{dQ}{dT} \left(\frac{1}{A \times C_0 \times 60} \right)$$

Where P_{app} represents the apparent permeability coefficient, dQ represents the quantity variations, dT is the time variations, thus dQ/dT is the permeability rate (amount of Rho123 per

minute), A represents the exposed surface in cm^2 and C_0 represents the initial concentration in the donor compartment (µg/ml)

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Introduction

A quantitative research methodology approach with an experimental design was used in this research study where *in vitro* transport experiments were conducted across excised tissue from different regions of the gastro-intestinal tract. The experimental setup included a control group where the transport of Rhodamine 123 (Rho123) alone (a known P-gp substrate) was measured and compared to the transport of Rho123 in the presence of selected excipients. The rationale for this study was based on reports that pharmaceutical excipients used in dosage forms may modulate the permeation of drug molecules across the intestinal epithelium. A change in drug permeation can be brought about by modulating the activity of active transporters, opening of tight-junctions or changing the membrane fluidity, which may result in decreased or enhanced drug absorption and systemic availability of the drug (Hetal & Jagruti, 2015:69).

In this study, the effects of Ac-di-sol® (ADS) and Pharmacel® PH 101 (PC) on Rho123 transport was investigated across excised pig intestinal tissue from different regions of the gastrointestinal tract. Excised tissue from the duodenum, proximal jejunum, mid jejunum, distal jejunum and ileum were used for the transport studies that were done in triplicate using the Sweetana-Grass apparatus. The samples withdrawn from the permeation experiments were analysed using a validated fluorescence spectroscopic analytical method.

Results obtained from the validation of the fluorometric analytical method are reported in this chapter to prove that the measurement of Rho123 concentrations in the transport samples produced reliable results. Both the control group (Rho123 alone) and the experimental groups (Rho123 in combination with ADS and PC respectively), were dissolved/dispersed in Krebs-Ringer Bicarbonate (KRB) buffer. The TEER was measured prior to the start of each individual transport experiment and at 20 min intervals during the experiment to ensure that membrane integrity and viability was maintained throughout, but was also used as an indicator of the opening of tight junctions to allow for paracellular transport of the model compound.

4.2 Validation of fluorescence spectroscopic analytical method

4.2.1 Specificity

Table 4-1 shows the fluorescence values of Rho123 alone and in the presence of the selected pharmaceutical excipients. The results showed that the detection of the Rho123 (5 μM) fluorescence was not affected by the presence of ADS (0.02% w/v with 106.75% recovery) and PC (0.06% w/v with 99.17% recovery).

Table 4-1: Percentage Rhodamine 123 (Rho123) recovered in the absence and presence of the selected excipients

Solution	Fluorescence values	Mean values	Actual Rho123 concentration (μM)	% Rho123 recovered
Rho123 alone (5 μM)	1037691435 1018611691 1070400619	1042234582	4.440	100
Rho123 (5 μM) with ADS 0.02% w/v	1117000000 1112000000 1108000000	1112333333	4.738	106.745
Rho123 (5 μM) with PC 0.06% w/v	1100041963 1043445227 957412906.6	1033633366	4.403	99.175

4.2.2 Linearity

Linear regression was performed on a graph where the fluorescence values were plotted as a function of Rho123 concentration for a series of standard solutions, as shown in Figure 4-1. A regression value (R^2) of 0.9998 was obtained and therefore a high degree of linearity was achieved with the standard curve over a relatively large concentration range (i.e. concentrations representing those that are expected in the experimental samples). The requirement for linearity (i.e. R^2 of at least 0.999) was therefore achieved (Shabir, 2003:64). A relatively large concentration range of 0.0005 μM – 5 μM was used to confirm the linearity.

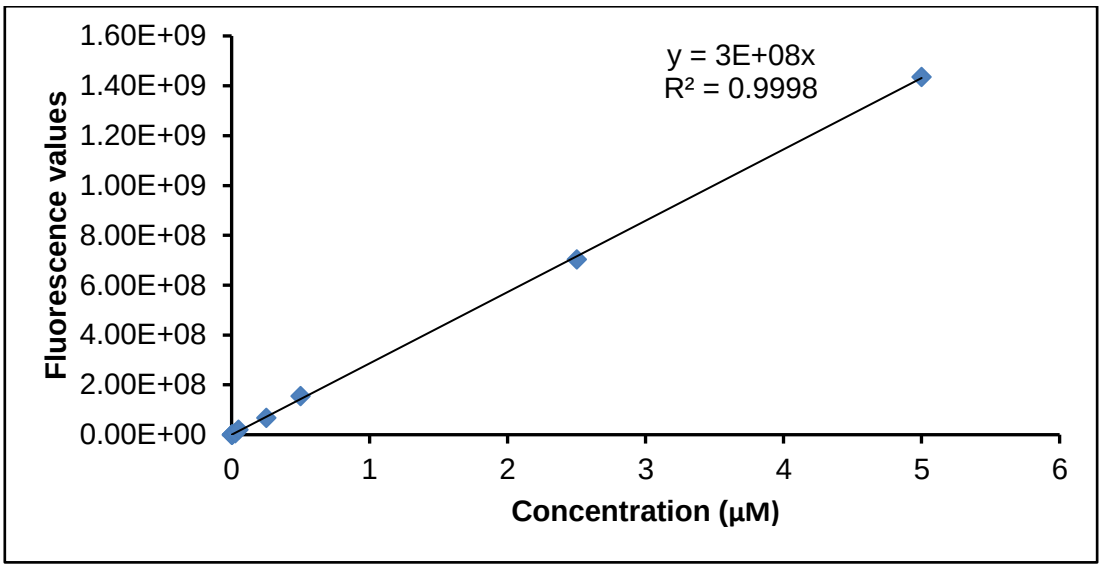

Figure 4-1: **A standard curve where fluorescence values were plotted as a function of Rhodamine 123 concentration for a large concentration range. The linear regression (R^2) value and a mathematical equation describing the straight line are shown**

Table 4-2: **Fluorescence values obtained for Rhodamine 123**

Concentration (µM)	Mean fluorescence value
5	1435694272
2.5	704479072
0.5	155905944
0.25	68064503
0.05	20477112.5
0.025	7131501
0.005	1953453
0.0025	953950
0.0005	389924.25
R^2	0.999861626
y-intercept	0
Slope	286255811.9

4.2.3 Limit of quantification (LOQ) and limit of detection (LOD)

LOQ is the lowest concentration of an analyte (Rho123) that can quantitatively be determined with precision and accuracy (Singh, 2013:30). The LOQ for Rho123 using the fluorometric

method was calculated to be 0.00048 μM. The LOQ value is far below the concentration of the Rho123 solutions applied to the excised tissues and that is expected to be transported across the excised tissues. This method is therefore suitable for analysis of the transport samples in terms of its ability to quantify the analyte at the concentrations investigated.

LOD is the lowest amount of analyte (Rho123) that can be detected by the instrument, but not necessarily quantified as an exact value (USP-NF, 2016). The LOD for Rho123 using the flourometric method was calculated to be 0.00016 μM.

4.2.4 Accuracy

Table 4-3 shows a mean recovery of 99.29% obtained by the flourometric analytical method with relative standard deviations (% RSD) of 0.007%, 0.01% and 0.05% for each of the three concentrations, respectively. Shabir (2003:61) stated that the mean recovery should be between 98 to 102% to be accurate; therefore this fluorescence spectroscopic analytical method complies with this requirement.

Table 4-3: Rhodamine 123 recovery from three solutions with different concentrations

Theoretical concentration (μM)	Mean value	SD	%RSD	Actual concentration (μM)	Percentage recovery
5	1410747548	10355034	0.007	4.928	98.57
2.5	706380061	9870269	0.014	2.468	98.71
0.125	35992194	2010230	0.056	0.126	100.59
				Mean % recovery	**99.29**

4.2.5 Precision

Both the intra-day and inter-day precision of the fluorescence spectroscopic analytical method were determined and the results are displayed below.

4.2.5.1 Intra-day precision

Table 4-4 shows that the %RSD values calculated for the fluorescence values of the three different Rho123 concentrations at three different time points on the same day (i.e. intra-day precision) was lower than 4%. The fluorescence spectroscopic analytical methods therefore complies with the requirement stated by Shabir (2003:62) for intra-day precision of %RSD ≤ 5%.

Table 4-4: Intra-day precision of Rhodamine 123 at three different concentrations

Concentration (µM)	Mean fluorescence value			SD	%RSD
	9:00	13:00	17:00		
5	1319619948	1384864435	1258884388	51442118	3.869
2.5	656665377	653428782	656490147	1486166	0.227
0.125	36397683	36565216	36905408	211234	0.577

4.2.5.2 Inter-day precision

Table 4-5 shows the % RSD values obtained for the inter-day precision were lower than 5% and therefore the fluorescence spectroscopic analytical method complies with the requirement for precision.

Table 4-5: Inter-day precision of Rhodamine 123 at three different concentrations

Concentration (µM)	Mean fluorescence value			SD	% RSD
	Day 1	Day 2	Day 3		
5	1329647180	1242584880	1382952653	57854635	4.388
2.5	655528102	621546757	697795789	31189748	4.738
0.125	36622769	35817597	39563309	1609880	4.312

4.2.6 Conclusion

The validation of the flourometric method used for the analysis of Rho123 in the transport samples was sufficiently sensitive as demonstrated by the LOQ and LOD and sufficiently selective for the determination of Rho123 concentrations in the presence of the selected excipients. The response correlated well with the concentration of Rho123 as demonstrated by the correlation coefficient (R^2) obtained for the standard curve. All the criteria were met for the validation parameters tested for Rho123 analysis and the method is therefore acceptable for use in the determination of Rho123 concentrations in the transport samples.

4.3 *In vitro* transport studies across excised pig intestinal tissues

4.3.1 Duodenum

The percentage transport of Rho123 across excised duodenum tissue in the absence (control) and presence of ADS (in three different concentrations) plotted as a function of time is shown in

Figure 4-2. All three the concentrations of ADS increased the transport of Rho123 when compared to the control (Rho123 alone) group.

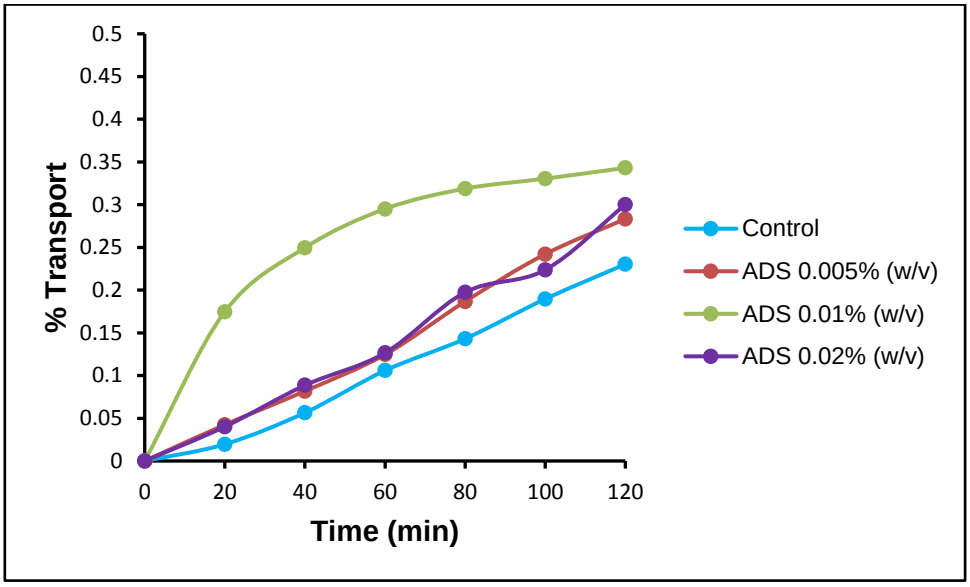

Figure 4-2: Graph of the percentage transport of Rho123 in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations across excised pig duodenum tissue

The difference in the TEER readings of the duodenum tissues before and after application of the three different concentrations of ADS over 120 min are shown in Table 4-6. The TEER results suggested that the tight junctions between epithelial cells were opened by the ADS, which is one explanation for the increased transport of Rho123 in the presence of ADS, namely increased paracellular transport.

Table 4-6: TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Ac-di-sol® (ADS) concentrations across excised duodenum tissue

ADS concentration (% w/v)	TEER		% difference in TEER
	Time 0 min	Time 120 min	
0.005	69.66	54.66	21.52
0.01	67.66	51.33	24.13
0.02	49	34	30.61

The higher average P_{app} values for Rho123 in the presence of ADS as indicated in Figure 4-3 are also a reflection of the increase in the transport of Rho123 when compared to the control

group. Although ADS 0.01% w/v yielded the highest P_{app} value, it was relatively close to the P_{app} values of the other two ADS solutions and was not statistically significantly different ($p > 0.05$).

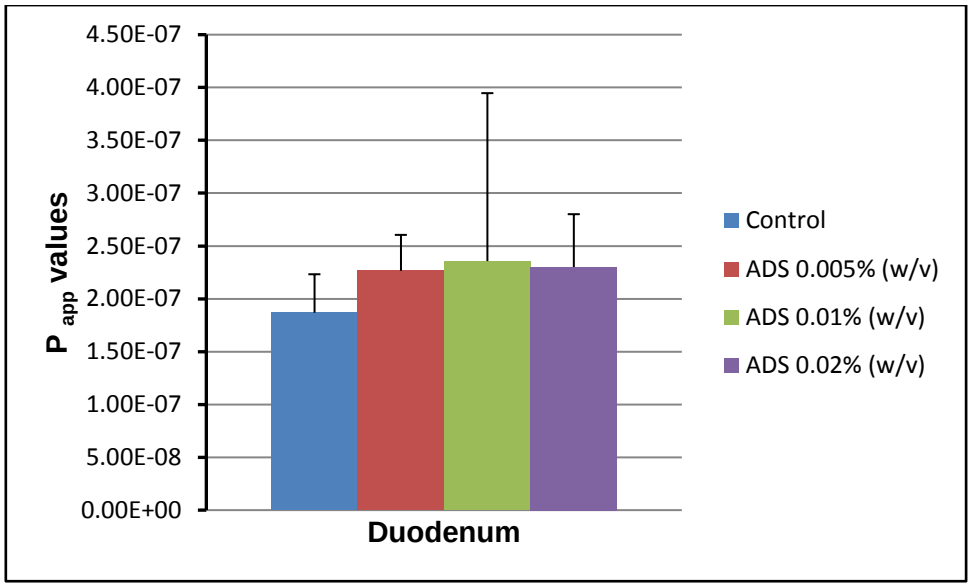

Figure 4-3: P_{app} values of Rho123 across excised duodenum tissue in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations

ADS therefore exhibited potential to interact *in vitro* with Rho123 transport across excised duodenum tissue through opening of tight junctions. This effect is, however, not statistically significant ($p > 0.05$) and will therefore most likely not increase absorption in this part of the gastro-intestinal tract to such an extent that side-effects would occur.

The percentage transport of Rho123 across excised duodenum tissue in the absence (control group) and presence of PC in three different concentrations plotted as a function of time is shown in Figure 4-4. PC at a concentration of 0.015% w/v had a statistically significant ($p \leq 0.05$) decrease on the Rho123 transport, while PC at 0.03% w/v and 0.06% w/v increased Rho123 transport, albeit not statistically significantly ($p > 0.05$). PC 0.06% w/v had the highest transport enhancing effect, with a 1.41 fold increase in Rho123 transport.

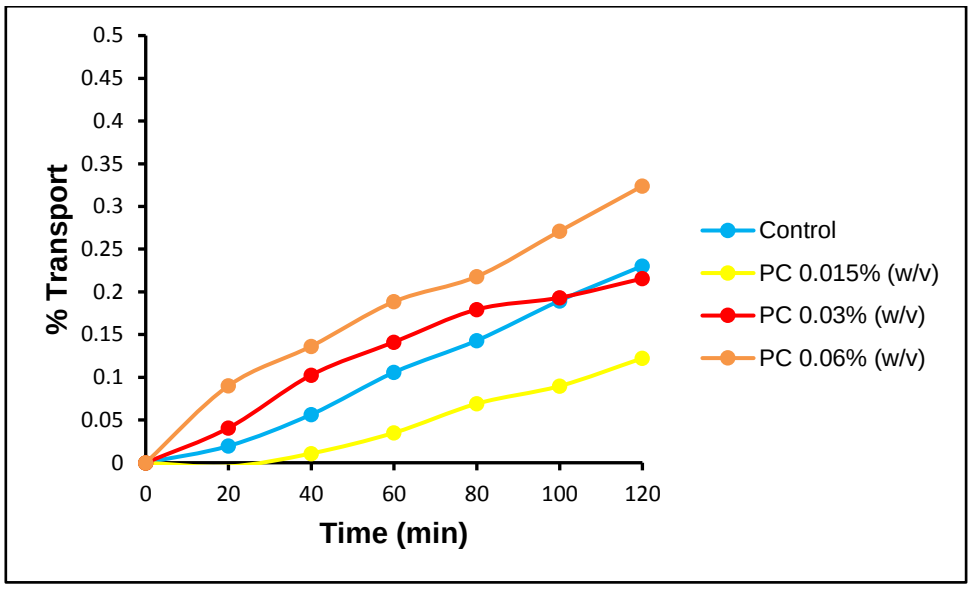

Figure 4-4: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Pharmacel® PH101 (PC) in three concentrations across excised pig duodenum tissue**

The difference in the TEER readings of the duodenum tissues before and after application of the three different concentrations of PC over 120 min are shown in Table 4-7. The reduction in the TEER readings of the excised duodenum after exposure to the PC dispersions suggested that the tight junctions were opened. Although PC at a concentration of 0.015% w/v caused a statistically significant decrease in the transport of Rho123, it also decreased the TEER, which indicated opening of tight junctions. This discrepancy between the transport and TEER results may indicate an apparent decrease in transport that may have been caused by other factors such as movement of PC molecules into the intercellular spaces and thereby blocking the paracellular route for transport of the Rho123 molecules. A similar trend was observed when the duodenum was exposed to PC at a concentration of 0.03% w/v, especially towards the end of the transport experiment at 100 – 120 min where the transport decreased below that of the control group (Rho123 alone). However, this phenomenon was not observed with the highest concentration of PC applied (0.06% w/v).

Table 4-7: TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Pharmacel® PH101 (PC) concentrations across excised duodenum tissue

PC concentration (% w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
PC 0.015	72	47.66	33.79
PC 0.03	43.3	34	21.50
PC 0.06	58.66	45.33	22.71

The apparent permeability coefficient (P_{app}) values of Rho123 in the absence (control group) and presence of the PC dispersions are shown in Figure 4-5.

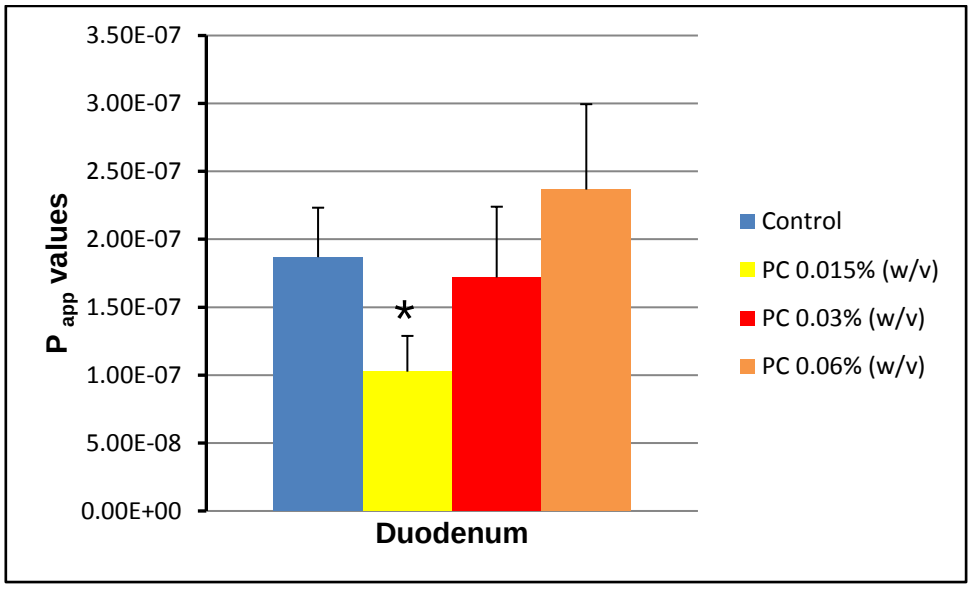

Figure 4-5: P_{app} values of Rho123 across excised duodenum tissue in the absence (control) and presence of Pharmacel® PH101 (PC) in three concentrations (* statistically different from control)

From Figure 4-5 it is clear that PC had an apparent concentration dependent increase in Rho123 transport, however, when the P_{app} values are compared with that of the control group, Rho123 transport was decreased by PC at concentrations of 0.015% w/v and 0.03% w/v. As mentioned before, other factors were probably responsible for the decrease in Rho123 transport as the TEER values suggested opening of the tight junctions for all three PC concentrations.

4.3.2 Jejunum

In this study, the effect of the selected excipients on Rho123 transport was tested across three regions of the jejunum namely, proximal jejunum, medial jejunum and the distal jejunum.

4.3.2.1 Proximal jejunum

The percentage transport of Rho123 across excised proximal jejunum tissue in the absence (control) and presence of ADS in three different concentrations plotted as a function of time is shown in Figure 4-6. The lowest concentration of ADS applied (i.e. 0.005% w/v) decreased Rho123 transport compared to the control, while ADS 0.01% w/v and 0.02% w/v increased Rho123 transport across the excised proximal jejunum tissue. Although not statistically significant ($p > 0.05$), the transport enhancement can be interpreted as a potential pharmacokinetic interaction that ADS can cause to increase drug absorption in this part of the jejunum.

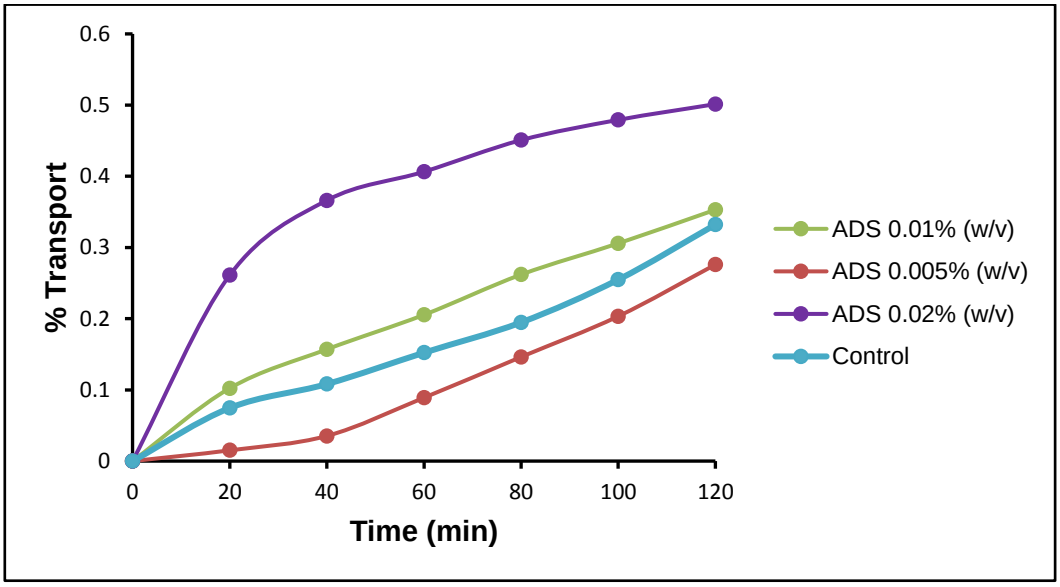

Figure 4-6: Graph of the percentage transport of Rho123 in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations across the excised pig proximal jejunum tissue

The difference in the TEER reading of the three different concentrations over 120 min is shown in Table 4-8 and this reduction in TEER could suggest that the tight junctions opened. ADS 0.02% w/v TEER reduction was the highest of the three concentrations, which could explain the high increased effect on Rho123 transport.

Table 4-8: TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Ac-di-sol® (ADS) concentrations across excised proximal jejunum tissue

ADS concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
ADS 0.005%	40.66	30	26.21
ADS 0.01%	80.8	65.8	18.56
ADS 0.02%	60.33	43	28.72

The lowest concentration (ADS 0.005% w/v) had a slight decreased effect on the transport, but as the concentration increased, the transport enhancing effect also increased. Figure 4-7 show an apparent concentration dependant increase on Rho123 transport (ADS 0.01% w/v and ADS 0.02% w/v), where ADS 0.005% w/v had a slight apparent decrease in transport. The higher average P_{app} values indicated in Figure 4-7 are also a reflection of the increase in the transport of Rho123. ADS 0.02% w/v had the highest apparent increase in the transport of Rho123.

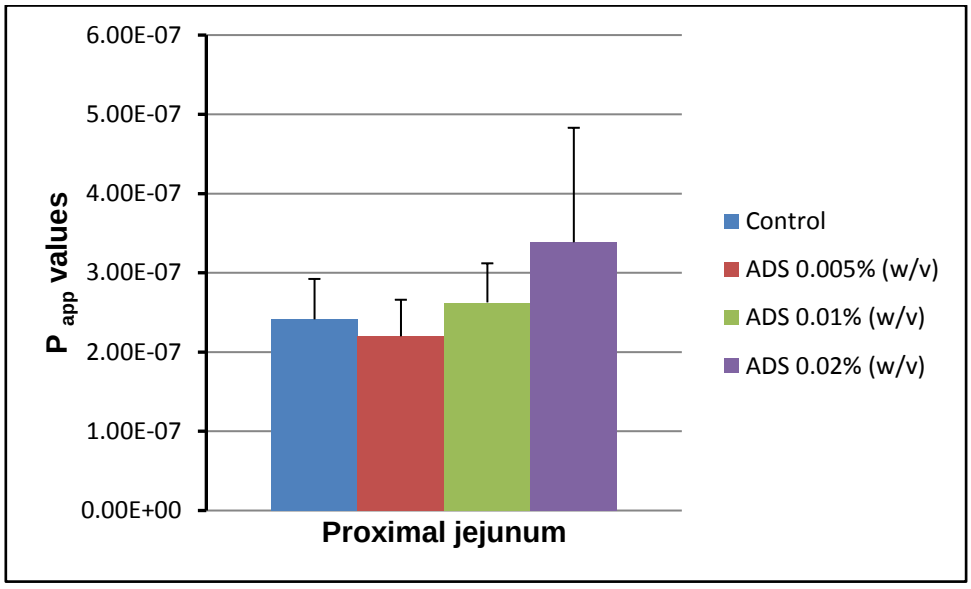

Figure 4-7: P_{app} values of Rho123 across excised proximal jejunum tissue in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations

The percentage transport of Rho123 across excised proximal jejunum tissue in the absence (control) and presence of PC (in three different concentrations) plotted as a function of time is shown in Figure 4-8. PC increased Rho123 transport that was inversely related to its

concentration, where the lowest concentration (PC 0.015% w/v) had the biggest effect and highest concentration (PC 0.06% w/v) had the smallest effect on Rho123 transport.

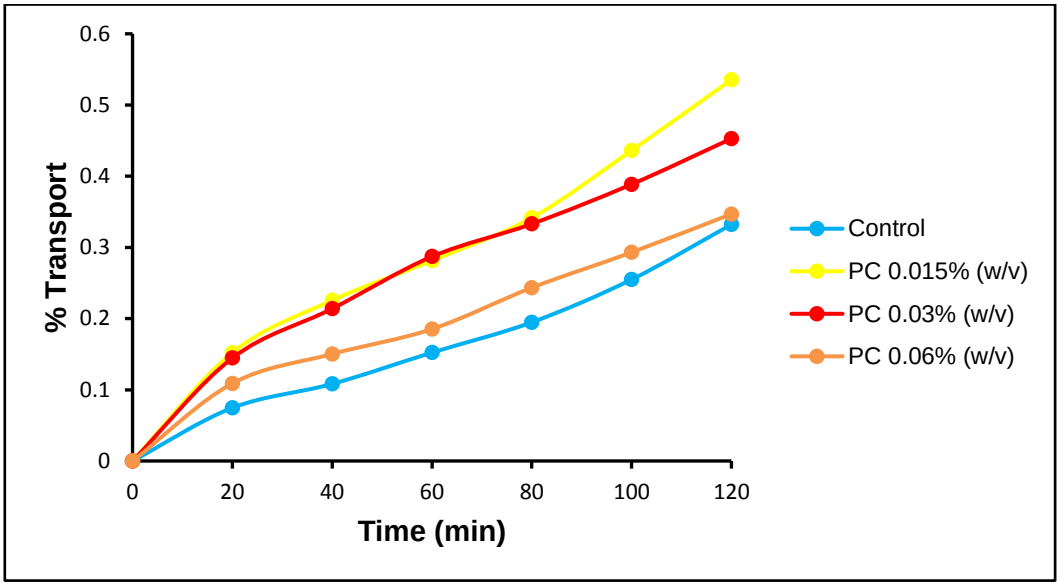

Figure 4-8: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Pharmacel® PH101 (PC) in three concentrations across excised pig proximal jejunum tissue**

Table 4-9 shows that the reduction in TEER was directly proportional to the concentration. Although the TEER reduction was proportional to the concentration of PC applied, the transport enhancing effect was inversely proportional to the concentration. As mentioned before, this could be due to the blockage of intercellular spaces by the increased concentrations of PC, but further experimentation is needed to be conclusive.

Table 4-9: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Pharmacel® PH101 (PC) concentrations across excised proximal jejunum tissue**

PC concentration (% w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
PC 0.015	42.6	31.3	26.44
PC 0.03	53.66	36.66	31.68
PC 0.06	56.66	33.66	40.59

Figure 4-9 shows the P_{app} values for Rho123 in the presence of PC with a clear inverse effect on Rho123 transport in terms of PC concentration.

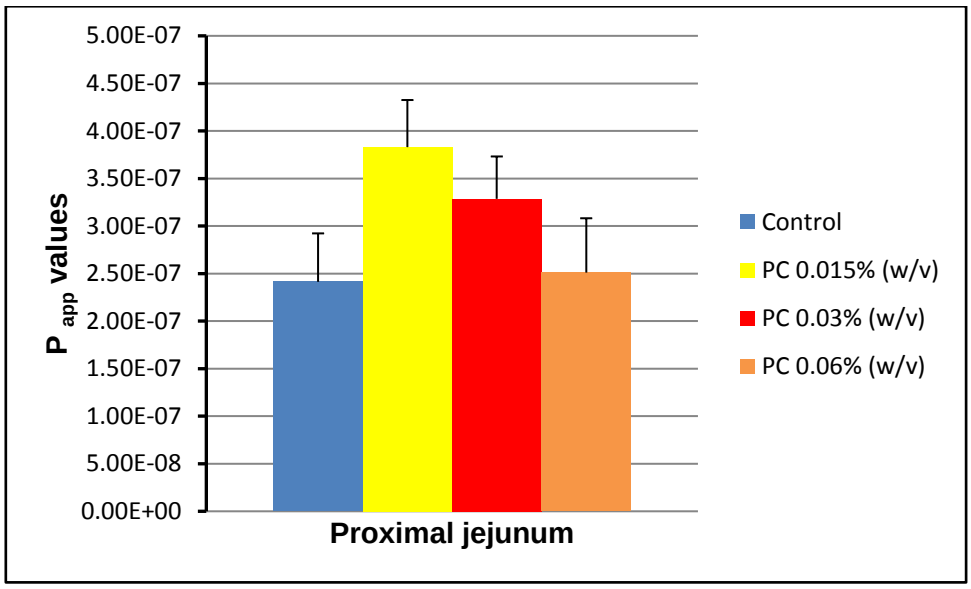

Figure 4-9: **P_{app} values of Rho123 across excised proximal jejunum tissue in the absence (control) and presence of Pharmacel® PH101 in three concentrations**

The selected excipients, ADS and PC, both mediated a reduction in the TEER of excised proximal jejunum tissue, corresponding with the increase in the Rho123 transport across the proximal jejunum, indicating the capacity of the excipients to open paracellular tight junctions. The transport and TEER data did not always correspond and the factors that could cause this should be investigated further to understand the mechanism behind this phenomenon.

In general, the results indicated that ADS and PC may exhibit excipient-drug pharmacokinetic interactions in the proximal part of the jejunum, but since it was not statistically significant, it may not cause blood levels to change to such an extent that side-effects are expected. However, this needs to be investigated *in vivo* to be conclusive with regards to the clinical significance of these interactions.

4.3.2.2 Medial jejunum

The percentage transport of Rho123 across excised medial jejunum tissue in the absence (control) and presence of ADS (in three different concentrations) plotted as a function of time is shown in Figure 4-10. Figure 4-10 illustrates that all the ADS concentrations had a decrease in transport of Rho123 in the medial jejunum when compared to the transport of Rho123 in the control group, where the mid concentration (ADS 0.01% w/v) had the biggest decreased effect in Rho123 transport.

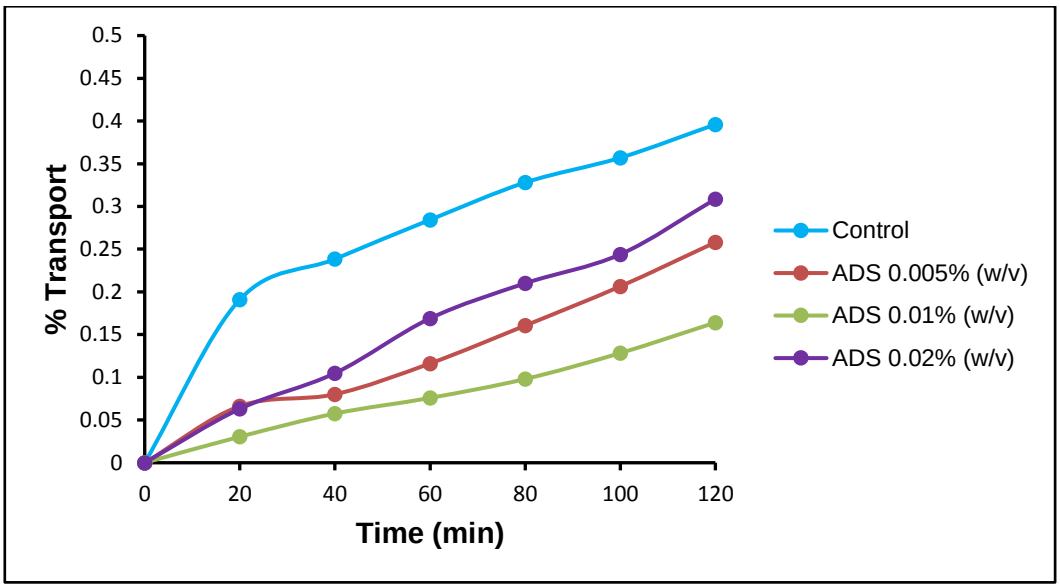

Figure 4-10: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations across the excised pig medial jejunum tissue**

Table 4-10 show that the TEER of the medial jejunum tissue were reduced by the ADS, but yet no transport enhancing effect occured. The difference in the TEER reading of the three different concentrations over 120 min could suggest that the tight junctions opened, but the ADS molecules could have blocked the opening and therefore no increased Rho123 transport was obtained via the paracellular pathway.

Table 4-10: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Ac-di-sol® (ADS) concentrations across excised medial jejunum tissue**

ADS concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
ADS 0.005%	51.5	34.3	33.39
ADS 0.01%	46.66	37	20.70
ADS 0.02%	49	32.8	33.06

ADS 0.005% w/v and ADS 0.01% w/v had a significant apparent decrease ($p < 0.05$) in Rho123 transport, where ADS 0.01% w/v had the largest decreasing effect on the transport, that correspond with the lowest reduction in TEER readings. ADS 0.02% w/v did not have a

statistically significant effect ($p > 0.05$) and had the lowest decreasing effect on the Rho123 transport.

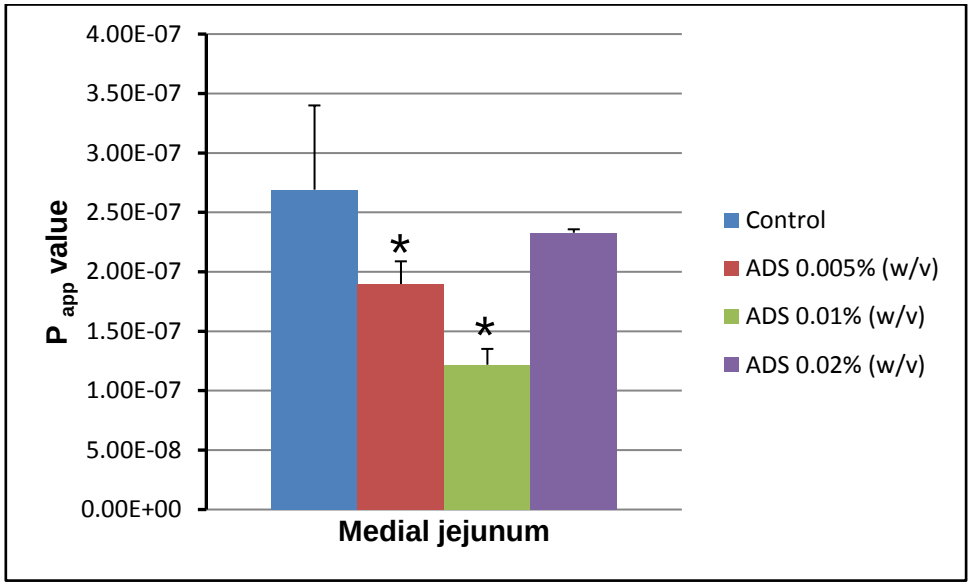

Figure 4-11: **P_{app} values of Rho123 across excised medial jejunum tissue in the absence (control) and presence of Ac-di-sol® in three concentrations (* statistically different from control)**

The percentage transport of Rho123 across excised medial jejunum tissue in the absence (control) and presence of PC (in three different concentrations) plotted as a function of time is shown in Figure 4-12. All the PC concentrations had a statistically significant ($p< 0.05$) decrease in transport of Rho123 across the excised medial jejunum tissue. PC 0.03% w/v had the largest decreasing effect on the Rho123 transport after 120 min and this could state that the maximum decreased effect was at the mid concentration. This is similar to the results obtained for ADS in the medial jejunum, both in terms of a transport decreasing effect as well as in terms of the trend obtained for concentrations namely the largest effect for the mid concentration (i.e. a U-shaped curve is formed when the P_{app} values are plotted).

This region specific effect on Rho123 transport should be investigated further in future studies to elucidate the mechanism by which this interaction occurred. It is specifically recommended that bi-directional transport studies are conducted to determine the role of active efflux modulation on transport in this region.

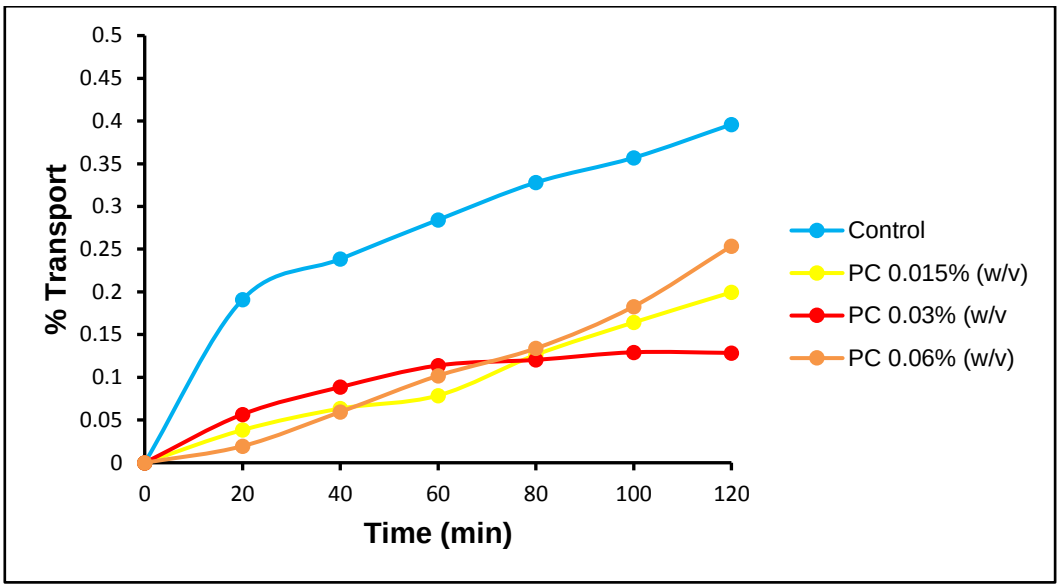

Figure 4-12: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Pharmacel® PH101 (PC) in three concentrations across the excised pig medial jejunum tissue**

The difference in the TEER readings over 120 min (Table 4-11) could suggest that the tight junctions opened, but the TEER reduction by PC 0.03% w/v was relatively low (compared to the reduction caused by the other two concentrations) and correspond with the biggest decrease on Rho123 transport.

Table 4-11: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Pharmacel® PH101 (PC) concentrations across excised medial jejunum tissue**

PC concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
PC 0.015%	39.3	29.7	24.5
PC 0.03%	90	84.33	6.3
PC 0.06%	66	50	24.24

Figure 4-13 illustrates that all the PC concentrations had a significant apparent decrease in the transport of Rho123 (as expressed by P_{app} values), where the mid concentration (PC 0.03% w/v) had the biggest decrease in the Rho123 transport. PC 0.06% w/v had the lowest decreased effect on the transport of Rho123.

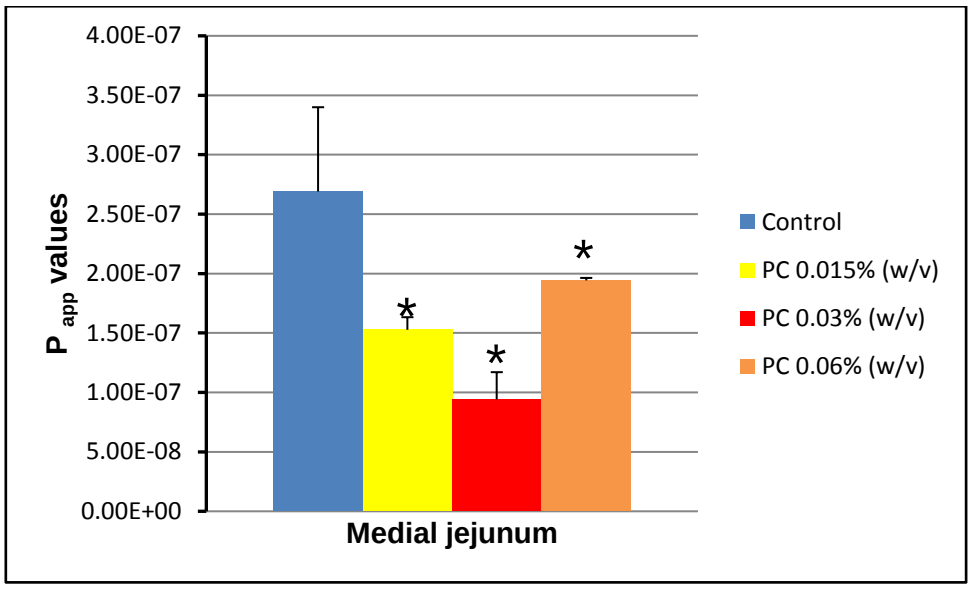

Figure 4-13: **P_{app} values of Rho123 across excised medial jejunum tissue in the absence (control) and presence of Pharmacel® PH101 in three concentrations (* statistically different from control)**

4.3.2.3 Distal jejunum

The percentage transport of Rho123 across excised distal jejunum tissue in the absence (control) and presence of ADS (in three different concentrations) plotted as a function of time is shown in Figure 4-14. Only ADS 0.01% w/v and 0.02% w/v had an increasing effect on the Rho123 transport after 120 min. No statistical significance for the effect of ADS on Rho123 transport was observed in the distal jejunum. The effect of ADS on Rho123 transport across excised distal jejunum tissue was concentration dependent, taking into consideration that the lowest concentration (ADS 0.005% w/v) had a decreasing effect on Rho123 transport when compared to the control group (Rho123 alone). As the concentration of ADS was increased, the Rho123 transport increased when compared to the control group and ADS 0.02% w/v had the best transport enhancing effect.

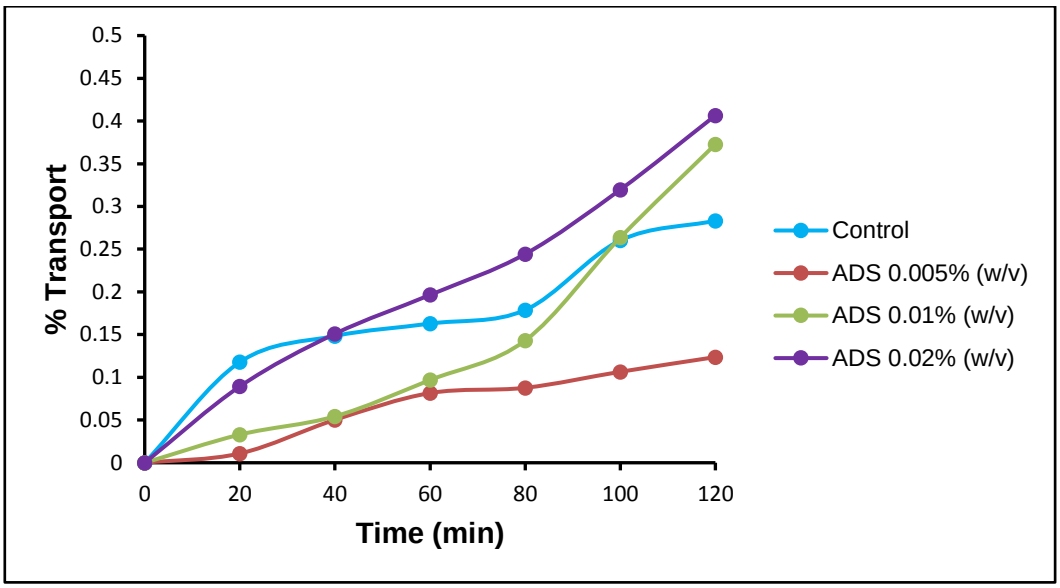

Figure 4-14: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Ac-di-sol® in three concentrations across the excised pig distal jejunum tissue**

The difference in the TEER reading over 120 min (Table 4-12) indicated that the tight junctions were opened by ADS 0.01% w/v and ADS 0.02% w/v to a larger extent than by ADS 0.005% w/v.

Table 4-12: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Ac-di-sol® (ADS) concentrations across excised distal jejunum tissue**

ADS concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
ADS 0.005%	62.33	49.33	20.85
ADS 0.01%	53	38.66	27.05
ADS 0.02%	61.66	45	27.01

No statistical significance ($p > 0.05$) in terms of Rho123 transport as modulated by ADS was observed in the distal jejunum. ADS 0.01% and ADS 0.02% had an increase in the transport of Rho123 as seen in Figure 4-15. ADS 0.005% had a decreasing effect on Rho123.

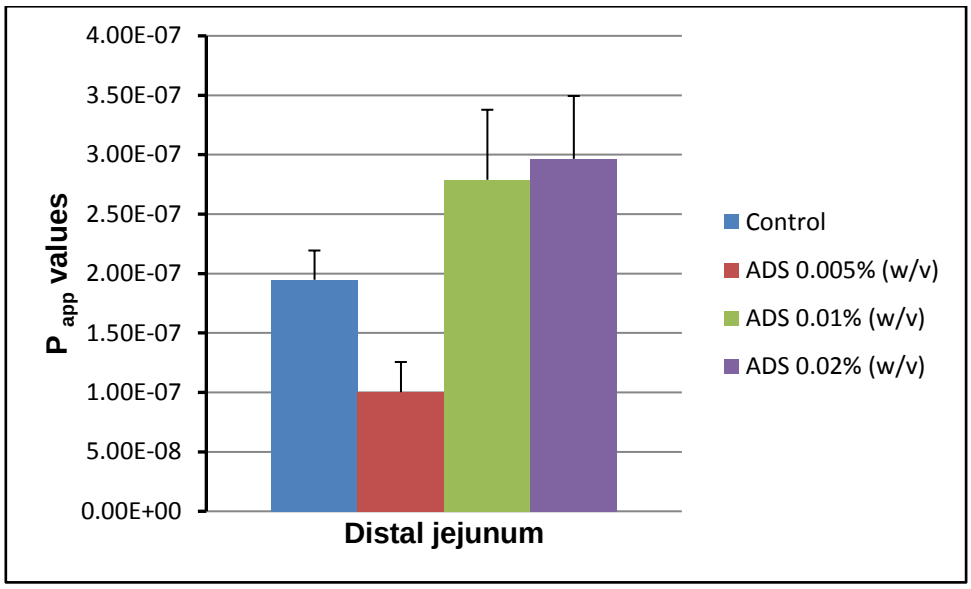

Figure 4-15: **P_{app} values of Rho123 across excised distal jejunum tissue in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations**

The percentage transport of Rho123 across excised distal jejunum tissue in the absence (control) and presence of PC (in three different concentrations) plotted as a function of time is shown in Figure 4-16. All the PC dispersions exhibited a decreasing effect on the transport of Rho123, albeit not statistically significant ($p > 0.05$). The effect on the Rho123 transport was inversely related to the concentration of PC applied to the excised distal jejunum tissue. After 120 min PC 0.015% w/v had the highest decrease in Rho123 transport and PC 0.06% w/v had the lowest decreasing effect on the transport.

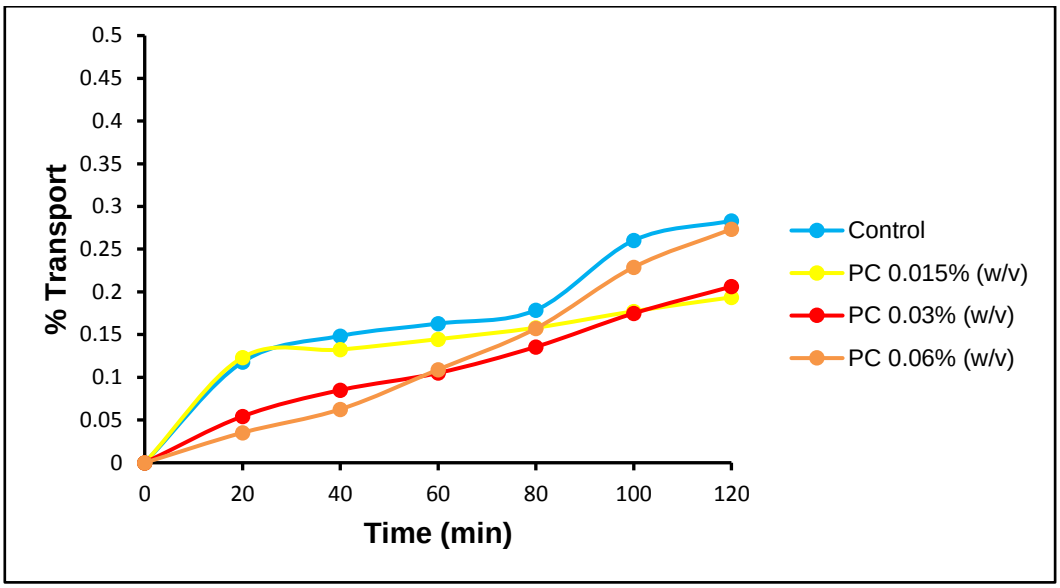

Figure 4-16: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Pharmacel® PH101 in three concentrations across the excised pig distal jejunum tissue**

The TEER reduction was similar for all three PC concentrations and this could suggest that not one of these concentrations could open the tight junctions enough for Rho123 transport to increase. Other factors may have also played a role in the effect of the PC on Rho123 transport such as blocking of the paracellular route, modulation of efflux transport and/or physical interactions with the Rho123 molecules such as adsorption to prevent transport across the tissue.

Table 4-13: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Pharmacel® PH101 (PC) concentrations across excised distal jejunum tissue**

PC concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
PC 0.015%	49.33	38.33	22.29
PC 0.03%	33.33	26	21.99
PC 0.06%	45.66	34	25.53

As seen in Figure 4-17, no significant differences in the P_{app} values of Rho123 were observed when PC was applied to the tissues together with the Rho123. Only the PC at a concentration of 0.06% w/v had an apparent increase on the transport, which may indicate a concentration

dependent effect and that a certain threshold concentration must be reached before transport enhancement can be achieved. The PC 0.015% w/v and PC 0.03% w/v had a decreasing effect on Rho123 transport.

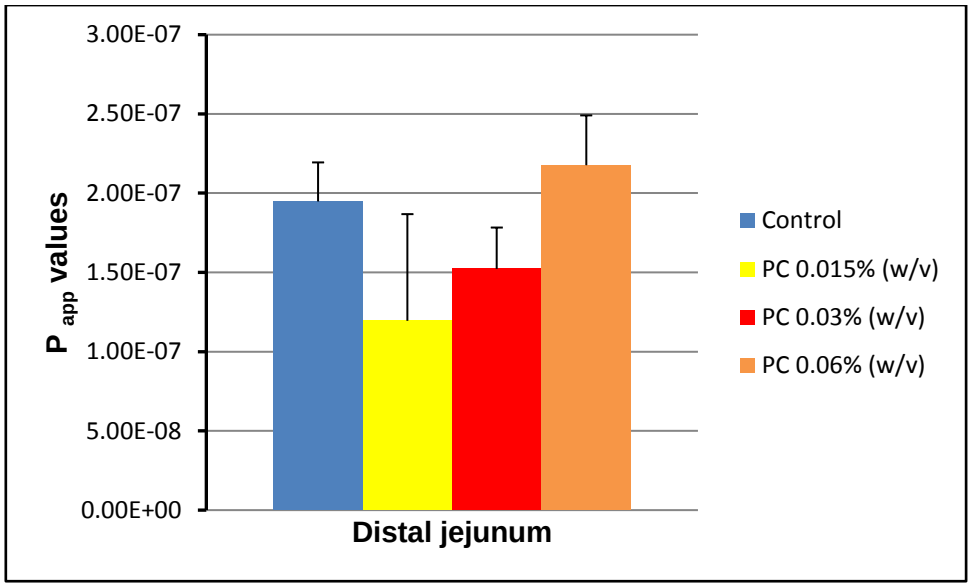

Figure 4-17: **P_{app} values of Rho123 across excised distal jejunum tissue in the absence (control) and presence of Pharmacel® PH101 in three concentrations**

4.3.3 Ileum

The percentage transport of Rho123 across excised ileum tissue in the absence (control) and presence of ADS (in three different concentrations) plotted as a function of time is shown in Figure 4-18. ADS exhibited a decreasing effect on Rho123 transport across excised ileum tissue as seen in Figure 4-18. The decreasing effect on Rho123 transport by ADS was concentration dependent where the lowest concentration (ADS 0.005% w/v) had the lowest decrease on Rho123 transport. The highest concentration (ADS 0.02% w/v) had the biggest decrease in the transport of Rho123.

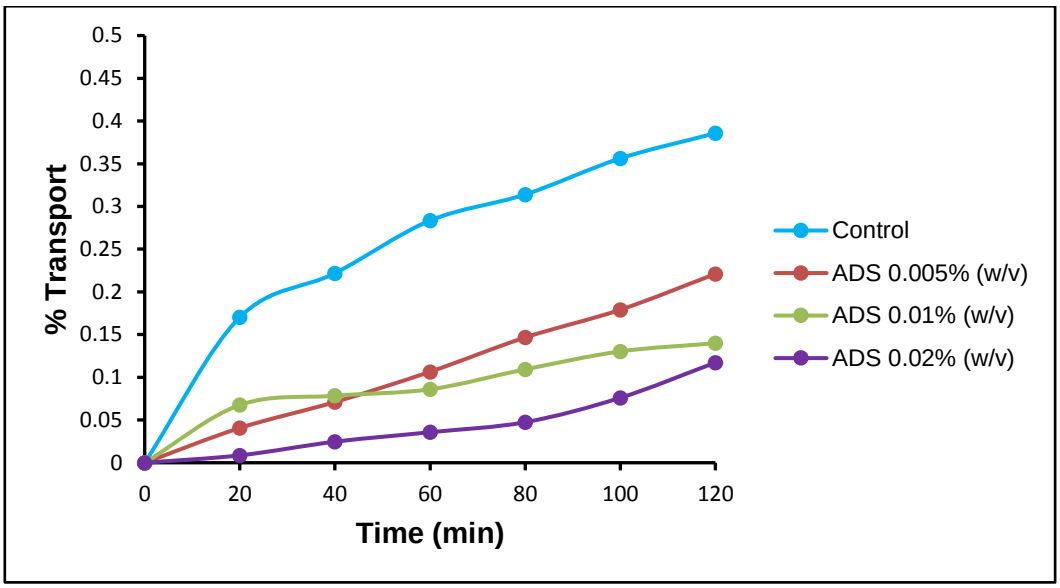

Figure 4-18: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Ac-di-sol® (ADS) in three concentrations across the excised pig ileum tissue**

The TEER reduction caused by ADS in the ileum was relatively low and could be due to the high quantity of Peyer's patches in this region.

Table 4-14: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Ac-di-sol® (ADS) concentrations across excised ileum tissue**

ADS concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
ADS 0.005%	73	57	21.91
ADS 0.01%	61.6	50.5	18.0
ADS 0.02%	60	52.66	12.23

Figure 4-19 clearly shows that there was a statistically significant ($p < 0.05$) decrease in Rho123 transport, that was concentration dependent.

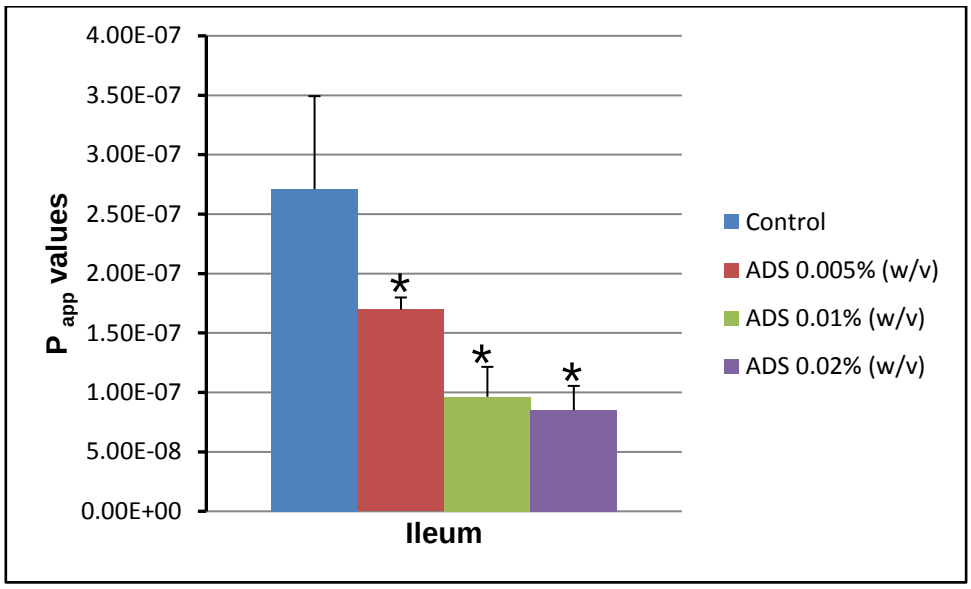

Figure 4-19: **P_{app} values of Rho123 across excised ileum tissue in the absence (control) and presence of Ac-di-sol® in three concentrations (* statistically different from control)**

The percentage transport of Rho123 across excised ileum tissue in the absence (control) and presence of PC (in three different concentrations) plotted as a function of time is shown in Figure 4-20. All the PC concentrations had a decreasing effect on Rho123 transport over the entire period of 120 min. PC 0.03% w/v and PC 0.06% w/v had a statistically significant ($p < 0.05$) decrease on the transport of Rho123. The decrease in Rho123 transport was concentration dependent, where the lowest concentration (PC 0.015% w/v) had the smallest decreasing effect on the transport of Rho123 after 120 min and the highest concentration (PC 0.06% w/v) had the highest decreasing effect.

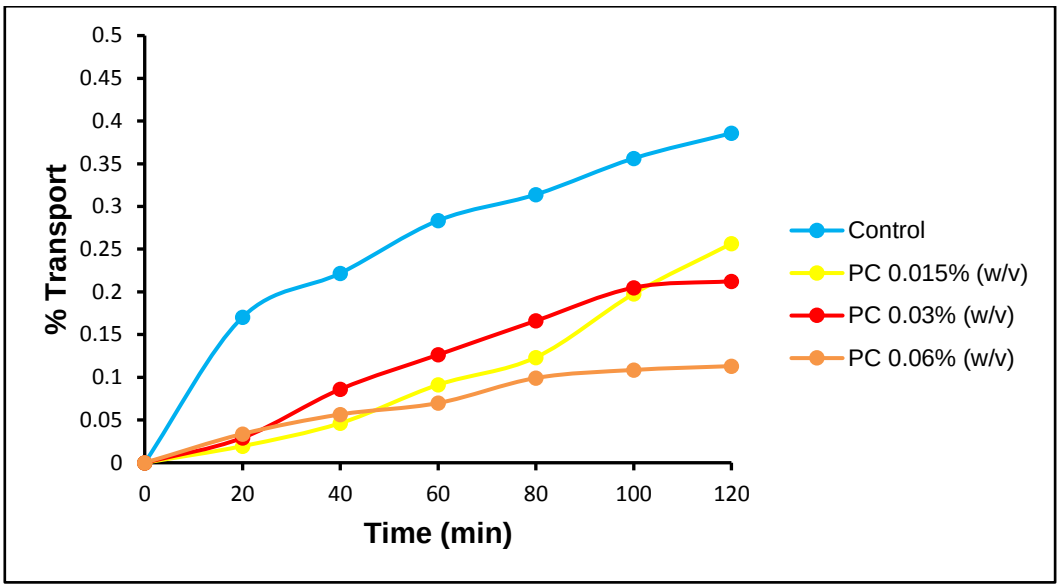

Figure 4-20: **Graph of the percentage transport of Rho123 in the absence (control) and presence of Pharmacel® PH101 in three concentrations across the excised pig ileum tissue**

As mentioned before, the relatively high number of Peyer's patches in this part of the gut wall could be the reason for the relatively low TEER reduction readings.

Table 4-15: **TEER readings at the start (time 0 min) and end (time 120 min) of the Rho123 transport experiment in the presence of three different Pharmacel® PH101 (PC) concentrations across excised ileum tissue**

PC concentration (w/v)	TEER		% difference in TEER
	Time 0 min	After 120 min	
PC 0.015%	61.66	55	10.80
PC 0.03%	70.66	65	8.01
PC 0.06%	79	68.66	13.08

Figure 4-21 shows that PC 0.03% w/v and PC 0.06% w/v had a significant decrease in the transport of Rho123. The decrease in Rho123 was concentration dependent, where the lowest concentration (PC 0.015% w/v) had the smallest effect and the highest concentration (PC 0.06% w/v) had the biggest effect on Rho123 transport.

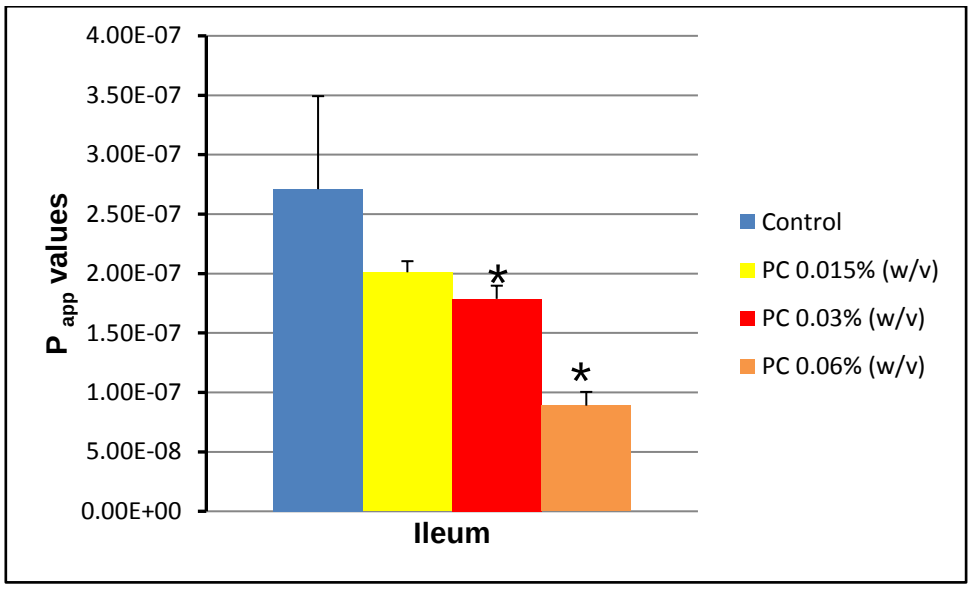

Figure 4-21: **P_{app} values of Rho123 across excised ileum tissue in the absence (control) and presence of Pharmacel® PH101 in three concentrations (* statistically different from control)**

4.4 Summary of results and conclusions

Figure 4-22 illustrates the P_{app} values of Rho123 transported in the absence (control group) and in the presence of Ac-di-sol® and Pharmacel® PH101 across excised tissue from the five different gastro-intestinal regions.

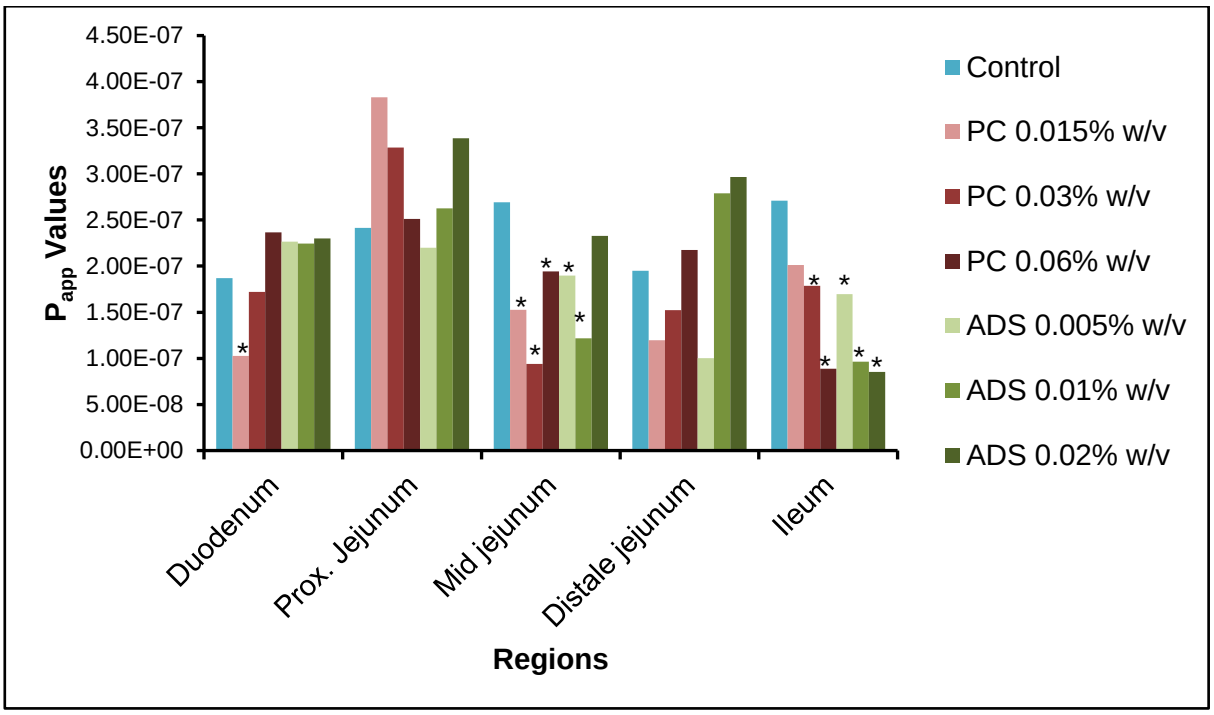

Figure 4-22: **P_{app} values of Rhodamine 123 in the absence (control group) and in the presence of the selected excipients (Ac-di-sol® (ADS) and Pharmacel® (PC)) across the different excised pig gastrointestinal tract regions**

The transport of Rho123 was higher in the presence of ADS and PC in the proximal jejunum when compared to the control. Overall, ADS increased the transport of Rho123 in three of the five regions used in this study and PC only increased Rho123 transport in two of the regions. ADS and PC showed a reduction in the measured TEER values over a period of 120 min, which indicated that the tight junctions opened in the intestinal tissue. The good transport of Rho123 in the proximal jejunum can be due to the opening of tight junctions to a relatively large extent that was indicated by the relatively large reduction of the TEER values. The decrease in transport in the ileum can be due to the high frequency of Peyer's patches in this region.

If all the transport data is considered, it can be concluded that the effect of the selected excipients on Rho123 transport was gastrointestinal region dependent and concentration dependent in some instances.

CHAPTER 5 FINAL CONCLUSIONS AND FUTURE RECOMMENDATIONS

5.1 Introduction

This *in vitro* study aimed at identifying potential interactions of selected excipients (Ac-di-sol® (ADS) and Pharmacel PH101® (PC) on the transport of Rho123 across specific regions of the pig intestine. The Sweetana-Grass diffusion chamber technique was used to conduct the *in vitro* transport studies across tissues obtained from the duodenum, proximal jejunum, medial jejunum, distal jejunum and ileum of the pig gastro-intestinal tract. The transport samples were analysed by means of a fluorometric method (SpectraMax® multi-plate reader) to determine the rate and extent of Rho123 transport in the absence and presence of the selected excipients.

5.2 Final conclusions

Both excipients, ADS and PC had an effect on the *in vitro* transport of Rho123 across the different gastro-intestinal regions investigated in this study. In some regions, the transport of Rho123 was increased, while it was decreased in other regions, which was in some of the experimental groups concentration dependent. A combination of transport decrease and increase also occurred in some of the transport experiments. Although some of the transport effects were statistically significant ($p < 0.05$), no statistical significance was observed in the proximal jejunum and distal jejunum.

The Rho123 transport was statistically significantly ($p < 0.05$) decreased by ADS 0.005% w/v, ADS 0.01% w/v, PC 0.03% w/v and PC 0.06% w/v across the medial jejunum and ileum. ADS 0.02% w/v had a significant ($p < 0.05$) decrease on Rho123 transport only in the ileum. PC 0.015% w/v had a statistical significant ($p < 0.05$) decrease on Rho123 transport across the duodenum and medial jejunum.

Using the Sweetana-Grass diffusion technique, the highest transport of Rho123 in combination with ADS 0.02% w/v and PC 0.015% w/v respectively, was achieved in the proximal jejunum.

The results from this study therefore indicated a region specific and in some instance also a concentration dependent effect on Rho123 transport by the selected excipients.

5.3 Future recommendations

Future research must focus on the following aspects:

- Other factors than excipients that could influence the rate and extent of drug transport, such as the physiological differences in the different regions of the gastrointestinal tract (including thickness of mucosa, surface area, pH, enzyme activity and residence time) should be investigated.

- The mechanisms by which Ac-di-sol® and Pharmacel® modulate Rho123 transport should be elucidated.

- More excipients should be tested for their effects on Rho123 transport.

- To verify the results of *in vitro* studies, *in vivo* studies should be conducted to determine if the pharmacokinetic interactions identified are clinically significant.

- Drug transport studies should be tested on human intestinal tissue or cell culture in order to compare the anatomical, biochemical and physiological differences and results to those of laboratory animals.

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ANNEXURE A: ETHICS APPROVAL

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ETHICS APPROVAL OF PROJECT

The North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby approves your project as indicated below. This implies that the NWU-RERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: Excised pig buccal and intestinal tissues as in vitro models for pharmacokinetic studies

Project Leader: Prof Sias Hamman

Ethics number: N W U - 0 0 0 2 5 - 1 5 - A 5
(Institution / Project Number / Year / Status)

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2015-04-16 **Expiry date:** 2020-04-15

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-RERC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-RERC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-RERC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-RERC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-RERC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU, Vaal Triangle Campus, ou=Vice-Rector: Academic, email=linda.duplessis@nwu.ac.za, c=US
Date: 2015.04.20 20:35:13 +02'00'

Prof Linda du Plessis
Chair NWU Research Ethics Regulatory Committee (RERC)

ANNEXURE B: CONTROLS OF SPECIFIC REGIONS

Duodenum Control					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0
20	0.03258499	0.011545759	0.0147754	0.01963538	0.009251195
40	0.081908061	0.057708316	0.029890464	0.05650228	0.021253212
60	0.162029618	0.090427321	0.065083528	0.105846823	0.041052461
80	0.188267944	0.143002071	0.098055791	0.143108602	0.036829034
100	0.226937399	0.197746172	0.144166998	0.189616856	0.03427632
120	0.271935275	0.234126057	0.185015047	0.230358793	0.035584882

Distal jejunum Control					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0
20	0.13044396	0.14207652	0.080866148	0.1177955	0.026541342
40	0.192227457	0.160247046	0.092752869	0.1484091	0.041464046
60	0.19960017	0.180245025	0.108845204	0.1628968	0.039028508
80	0.208405717	0.199690673	0.128229869	0.1787754	0.035917754
100	0.269104959	0.243608667	0.268014559	0.2602427	0.011770478
120	0.281650734	0.263472382	0.304619594	0.2832476	0.016836185

Prox. jejunum Control					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0
20	0.03933778	0.05088683	0.133614506	0.074613	0.041985908
40	0.057265513	0.089816908	0.177467028	0.1081831	0.050761469
60	0.110736034	0.118018311	0.227959395	0.1522379	0.053625647
80	0.150051672	0.147939504	0.286413764	0.1948016	0.064785288
100	0.18917788	0.240758667	0.334739354	0.254892	0.060259706
120	0.256632511	0.321180285	0.418516872	0.3321099	0.066539356

Ileum Control					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0
20	0.11014	0.14430026	0.256471975	0.1703041	0.062505523
40	0.136457925	0.194333722	0.334734526	0.2218421	0.083250361
60	0.170298983	0.289793733	0.390333475	0.2834754	0.089939741
80	0.195914886	0.342302164	0.403757087	0.3139914	0.087180734
100	0.229055573	0.374869823	0.46477294	0.3562328	0.097129377
120	0.262940821	0.405027673	0.489123252	0.3856972	0.09334478

Medial jejunum Control					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0
20	0.14201657	0.2346638	0.196370193	0.1910169	0.038012022
40	0.174654306	0.281437543	0.259169139	0.2384203	0.045996737
60	0.194157705	0.32806309	0.330747799	0.2843229	0.063765816
80	0.220731018	0.37285281	0.390402093	0.3279953	0.076184929

Time (min)	% trans Cham 1	% trans Cham 2	% trans Cham 3	Average trans	SD
100	0.244657711	0.406578737	0.419787376	0.3570079	0.07962641
120	0.291184933	0.452185665	0.444485103	0.3959519	0.074148107

ANNEXURE C: TRANSPORT IN DUODENUM

ADS 0.005%						PC 0.015%					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0.00	0.00	0	0	**0**	0	0	0	0	0
20	0.06146358	0.04	0.03	0.0424324	0.014834775	**20**	-0.00481328	-0.00501771	-0.003198937	-0.0043433	0.000813484
40	0.08642964	0.10	0.06	0.0817898	0.018880432	**40**	0.00404478	-0.00037413	0.02901288	0.0108945	0.012938011
60	0.11221938	0.17	0.09	0.1246275	0.037093046	**60**	0.039198486	0.009254264	0.056827915	0.0350936	0.019637564
80	0.19637687	0.23	0.13	0.1867202	0.039597531	**80**	0.071086116	0.044737346	0.091604078	0.0691425	0.019182559
100	0.26789114	0.27	0.19	0.2420963	0.03556787	**100**	0.070066493	0.070399218	0.128848476	0.0897714	0.027632002
120	0.30430344	0.31	0.24	0.2833343	0.032591174	**120**	0.114109101	0.09823482	0.154635624	0.1223265	0.02374738
ADS 0.01%						**PC 0.03%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.361421756	0.0831996	0.079614462	0.1747453	0.132008321	**20**	0.08736805	0.00502901	0.029870388	0.0407558	0.034484768
40	0.513476603	0.12345135	0.112021729	0.2496499	0.186612001	**40**	0.1310178	0.06573438	0.111197609	0.1026499	0.027328595
60	0.588299113	0.172061496	0.124688658	0.2950164	0.208282019	**60**	0.133849097	0.098489227	0.191410898	0.1412497	0.038294355
80	0.621164315	0.190497202	0.144578447	0.3187467	0.214661694	**80**	0.176506188	0.141946316	0.21974728	0.1793999	0.031827952
100	0.622298648	0.20353699	0.165616819	0.3304842	0.206923915	**100**	0.184135114	0.145543811	0.250001708	0.1932269	0.043126621
120	0.623313362	0.224133893	0.181740286	0.3430625	0.198921606	**120**	0.1811443	0.189118638	0.276454624	0.2155725	0.043173066
ADS 0.02%						**PC0.06%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.04479402	0.03097343	0.044641907	0.0401364	0.006479534	**20**	0.07596397	0.07716831	0.117297155	0.0901431	0.019207078
40	0.05513744	0.08482522	0.125860159	0.0886076	0.028996041	**40**	0.1598076	0.11671026	0.132718475	0.1364121	0.017787211
60	0.069051318	0.134405852	0.176069547	0.1265089	0.044045406	**60**	0.206861907	0.156925528	0.201894942	0.1885608	0.022461228

80	0.180068292	0.172068555	0.240354271	0.197497	0.03048011	**80**	0.236766808	0.17843098	0.238878255	0.2180253	0.028010713
100	0.203999427	0.208595877	0.258618983	0.2237381	0.024735791	**100**	0.300047576	0.201546709	0.311642142	0.2710788	0.049393948
120	0.320173208	0.262969761	0.316969348	0.3000374	0.026243421	**120**	0.360597167	0.22391076	0.387196226	0.3239014	0.071533075

ANNEXURE D: TRANSPORT IN PROXIMAL JEJUNUM

ADS 0.005%						PC 0.015%					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.01659805	0.0050486	0.023713594	0.0151201	0.007691286	**20**	0.25426237	0.07502993	0.126847223	0.1520465	0.075309672
40	0.039914012	0.020219439	0.045816136	0.0353165	0.010943807	**40**	0.328784708	0.186470337	0.161215968	0.2254903	0.073764223
60	0.135767684	0.055540722	0.075737509	0.0890153	0.034071653	**60**	0.378042329	0.244018308	0.222254622	0.2814384	0.068884691
80	0.194643281	0.111516276	0.132676835	0.1462788	0.035273075	**80**	0.442080944	0.306302958	0.276100231	0.3414947	0.072186074
100	0.252637952	0.165426154	0.192111575	0.2033919	0.036486606	**100**	0.510357148	0.424637972	0.373401707	0.4361323	0.056499484
120	0.335437815	0.244077209	0.248399178	0.2759714	0.042086108	**120**	0.61886289	0.54049535	0.447368044	0.5355754	0.070098858
ADS 0.01%						**PC 0.03%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.10602903	0.07193464	0.12887818	0.1022806	0.023397716	**20**	0.09533328	0.25409991	0.084163511	0.1445322	0.077610125
40	0.154630999	0.132681532	0.183694556	0.1570024	0.020893375	**40**	0.167687109	0.334351135	0.139544349	0.2138609	0.085970664
60	0.196680986	0.176452426	0.243166922	0.2054334	0.027930391	**60**	0.254014852	0.396875196	0.21031505	0.2870684	0.079668367
80	0.283358898	0.230882662	0.271549116	0.2619302	0.022477116	**80**	0.299269538	0.430972023	0.268668784	0.3329701	0.070414875
100	0.353333088	0.251945399	0.312079318	0.3057859	0.041629884	**100**	0.344567043	0.495663033	0.325558006	0.388596	0.076104506
120	0.423215249	0.285397092	0.350344326	0.3529856	0.056295016	**120**	0.402322612	0.56398679	0.391810839	0.4527067	0.078803809
ADS 0.02%						**PC 0.06%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.0400549	0.18842223	0.554849426	0.2611089	0.216357513	**20**	0.0821451	0.05310563	0.191624114	0.1089583	0.059643679
40	0.139737368	0.318075962	0.640850561	0.3662213	0.20739188	**40**	0.103094551	0.091004346	0.256843523	0.1503141	0.075489184
60	0.187022487	0.342126502	0.690133141	0.4064274	0.210366402	**60**	0.142489871	0.123843501	0.289430583	0.1852547	0.074055791
80	0.234189522	0.378131633	0.740086513	0.4508026	0.21282816	**80**	0.189389143	0.190378482	0.350269056	0.2433456	0.075607408

100	0.255210043	0.400883645	0.781641086	0.4792449	0.221942603	**100**	0.247675115	0.235549595	0.395917346	0.2930474	0.072908316
120	0.266289934	0.425522429	0.812271539	0.5013613	0.229256231	**120**	0.267381253	0.313418927	0.45971586	0.3468387	0.081999228

ANNEXURE E: TRANSPORT IN MEDIAL JEJUNUM

ADS 0.005%						PC 0.015%					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.08542592	0.06073099	0.052570767	0.0662426	0.01396778	**20**	0.04894065	0.0252441	0.040755097	0.0383133	0.009826949
40	0.100608011	0.07897047	0.060905007	0.0801612	0.016230536	**40**	0.064043153	0.058294911	0.067944924	0.0634277	0.003963568
60	0.115022113	0.115601865	0.118019459	0.1162145	0.001298074	**60**	0.085255857	0.070920726	0.079881733	0.0786861	0.005913044
80	0.160423561	0.166923819	0.154376464	0.1605746	0.00512355	**80**	0.110034053	0.135800443	0.133188907	0.1263411	0.011580032
100	0.229596737	0.206283773	0.183496828	0.2064591	0.018820617	**100**	0.156629991	0.164597657	0.171404666	0.1642108	0.006037936
120	0.300440821	0.251461931	0.222073801	0.2579922	0.032324713	**120**	0.193822925	0.203406842	0.201156514	0.1994621	0.004091956
ADS 0.01%						**PC 0.03%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.04298463	0.02329913	0.025179849	0.0304879	0.008869836	**20**	0.05252243	0.05523103	0.062247241	0.0566669	0.004097907
40	0.063188079	0.054445921	0.055101982	0.0575787	0.00397549	**40**	0.089671583	0.083057296	0.092867607	0.0885322	0.004085279
60	0.081379529	0.068132721	0.078079338	0.0758639	0.005630318	**60**	0.114581221	0.094455031	0.132532479	0.1138562	0.015553504
80	0.106874824	0.09147255	0.095661255	0.0980029	0.006502302	**80**	0.116193148	0.099131309	0.145934474	0.1204196	0.019339623
100	0.152306356	0.121171543	0.111717461	0.1283985	0.01734043	**100**	0.123901513	0.103513449	0.160504081	0.1293063	0.023578128
120	0.184033281	0.160379789	0.147749369	0.1640541	0.015038977	**120**	0.128091522	0.105978328	0.151610262	0.12856	0.018632105
ADS 0.02%						**PC 0.06%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.06000497	0.04258135	0.086690501	0.0630923	0.018139328	**20**	0.01461215	0.03577619	0.007989234	0.0194592	0.011850431
40	0.079483679	0.113507753	0.121517481	0.1048363	0.0182228	**40**	0.058581202	0.058941399	0.060950478	0.059491	0.001042412
60	0.158109226	0.180911173	0.16787156	0.168964	0.009340851	**60**	0.116300097	0.100128854	0.089175378	0.1018681	0.011141704
80	0.218838066	0.215823596	0.195413604	0.2100251	0.010404914	**80**	0.155194211	0.133769999	0.112663663	0.133876	0.017363185

100	0.234861	0.253962222	0.242973865	0.2439324	0.007827439	**100**	0.200820224	0.186690095	0.161073962	0.1828614	0.01645064
120	0.294652161	0.291744841	0.339480309	0.3086258	0.021849715	**120**	0.235713627	0.259570164	0.265724519	0.2536694	0.012942883

ANNEXURE F: TRANSPORT IN DISTAL JEJUNUM

ADS 0.005%						PC 0.015%					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.00843184	-0.0058131	0.02991833	0.0108457	0.014686814	**20**	0.20161913	0.06389893	0.104169529	0.1232292	0.057816763
40	0.008812416	0.038996149	0.102991058	0.0502665	0.03926551	**40**	0.204119642	0.067351077	0.125745605	0.1324054	0.056033771
60	0.036806462	0.077157569	0.130776893	0.0815803	0.038490527	**60**	0.197848505	0.0667035	0.169506722	0.1446862	0.056342969
80	0.044772834	0.084847341	0.133539933	0.08772	0.036295902	**80**	0.204920694	0.07477177	0.194507648	0.1580667	0.059051629
100	0.063058403	0.106026914	0.150319827	0.1064684	0.035625695	**100**	0.209807341	0.078355734	0.243734037	0.177299	0.071321283
120	0.098138942	0.116418673	0.156462955	0.1236735	0.024357029	**120**	0.223997681	0.096362397	0.260407354	0.1935891	0.070338213
ADS 0.01%						**PC 0.03%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.03484121	0.02359519	0.040410907	0.0329491	0.006994147	**20**	0.04847932	0.0669742	0.047459324	0.0543043	0.008968658
40	0.059528416	0.049730986	0.053635655	0.0542984	0.00402714	**40**	0.09130132	0.092472064	0.07138476	0.0850527	0.009676514
60	0.097111399	0.104945843	0.088457052	0.0968381	0.006734294	**60**	0.107695303	0.124645327	0.082822437	0.1050544	0.017175942
80	0.118797694	0.163653535	0.146723742	0.1430583	0.01849483	**80**	0.136224696	0.169078115	0.101755953	0.1356863	0.027486794
100	0.269814692	0.298685876	0.22207466	0.2635251	0.031591023	**100**	0.175859681	0.207928965	0.140671546	0.1748201	0.027467566
120	0.338490136	0.467171432	0.312620972	0.3727608	0.067588571	**120**	0.207248098	0.233989079	0.17735075	0.206196	0.023134467
ADS 0.02%						**PC 0.06%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.07257204	0.11226642	0.083118309	0.0893189	0.016787824	**20**	0.02268754	0.06122347	0.021625474	0.0351788	0.018421447
40	0.144546535	0.137693787	0.170664182	0.1509682	0.014205392	**40**	0.061108321	0.083577351	0.043153662	0.0626131	0.016537169
60	0.212847819	0.166519087	0.209959807	0.1964422	0.021191687	**60**	0.126492025	0.113711565	0.087148734	0.1091174	0.01638705
80	0.249896175	0.215038459	0.267947557	0.2442941	0.021960281	**80**	0.142309499	0.188873166	0.140298026	0.1571602	0.022439463

100	0.336682158	0.26349252	0.358809975	0.3196616	0.040731888	**100**	0.252404303	0.251008771	0.183086978	0.2288334	0.032352587
120	0.453777051	0.34384401	0.421836128	0.4064857	0.046173906	**120**	0.294965906	0.303111625	0.221878079	0.2733185	0.036525596

ANNEXURE G: TRANSPORT IN ILEUM

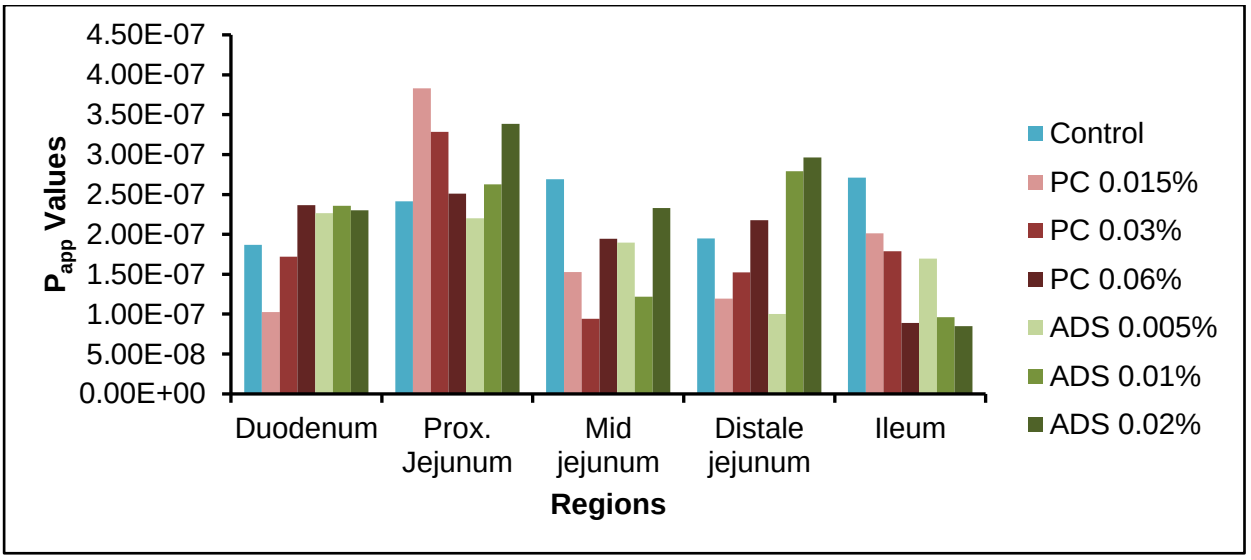
ADS 0.005%						PC 0.015%					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.01863436	0.01733424	0.086188793	0.0407191	0.032156287	**20**	0.01572855	0.00423319	0.038498016	0.0194866	0.01423872
40	0.050473458	0.053898857	0.108826459	0.0710663	0.026737089	**40**	0.030837667	0.047359115	0.060788969	0.0463286	0.012249262
60	0.078485309	0.099800465	0.141188895	0.1064916	0.026032197	**60**	0.082800981	0.099550077	0.091431875	0.091261	0.006838857
80	0.115854561	0.149851193	0.174209975	0.1466386	0.023931559	**80**	0.104890246	0.127552201	0.137613859	0.1233521	0.013685499
100	0.159948726	0.179563776	0.197773281	0.1790953	0.015445363	**100**	0.205475936	0.195364984	0.192293946	0.1977116	0.005631533
120	0.207325369	0.221226537	0.234375103	0.2209757	0.011044432	**120**	0.264016259	0.253483384	0.251783476	0.2564277	0.005410608
ADS 0.01%						**PC 0.03%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.07335685	0.08052818	0.049018275	0.0676344	0.013485253	**20**	0.02424375	0.02960235	0.033689696	0.0291786	0.003867916
40	0.101993181	0.0824941	0.050876403	0.0784546	0.021062916	**40**	0.064243677	0.126546028	0.067322129	0.0860373	0.028671569
60	0.11776984	0.086373401	0.053810057	0.0859844	0.026112921	**60**	0.104513548	0.165419422	0.10917094	0.126368	0.02767893
80	0.144027042	0.111453238	0.072408337	0.1092962	0.02927797	**80**	0.162658047	0.193261711	0.142571855	0.1661639	0.020842
100	0.156122072	0.134318267	0.100446068	0.1302955	0.022906935	**100**	0.20472935	0.21138561	0.198706038	0.2049403	0.005178563
120	0.156659568	0.170361007	0.092713626	0.1399114	0.033839374	**120**	0.224128766	0.215017765	0.198295199	0.2124806	0.010698015
ADS 0.02%						**PC 0.06%**					
Time (min)	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**	**Time (min)**	**% trans Cham 1**	**% trans Cham 2**	**% trans Cham 3**	**Average trans**	**SD**
0	0	0	0	0	0	**0**	0	0	0	0	0
20	0.00479159	0.02088463	0.000304251	0.0086602	0.008835999	**20**	0.07914511	0.01037873	0.01169654	0.0337401	0.03211068
40	0.012553236	0.036608514	0.024732306	0.0246314	0.009820785	**40**	0.09397929	0.041340579	0.034436688	0.0565855	0.026591183
60	0.031007487	0.048084832	0.02802734	0.0357066	0.008836918	**60**	0.100754311	0.052935193	0.055936027	0.0698752	0.021869186
80	0.041926908	0.052428968	0.048075683	0.0474772	0.004308284	**80**	0.123576536	0.098911679	0.075059435	0.0991826	0.01980795

100	0.05916916	0.084454349	0.084417598	0.0760137	0.0119109	**100**	0.133896141	0.106422916	0.085577653	0.1086322	0.019787705
120	0.082172578	0.161688036	0.107778731	0.1172131	0.033140436	**120**	0.132422381	0.116199521	0.090748766	0.1131236	0.017151651

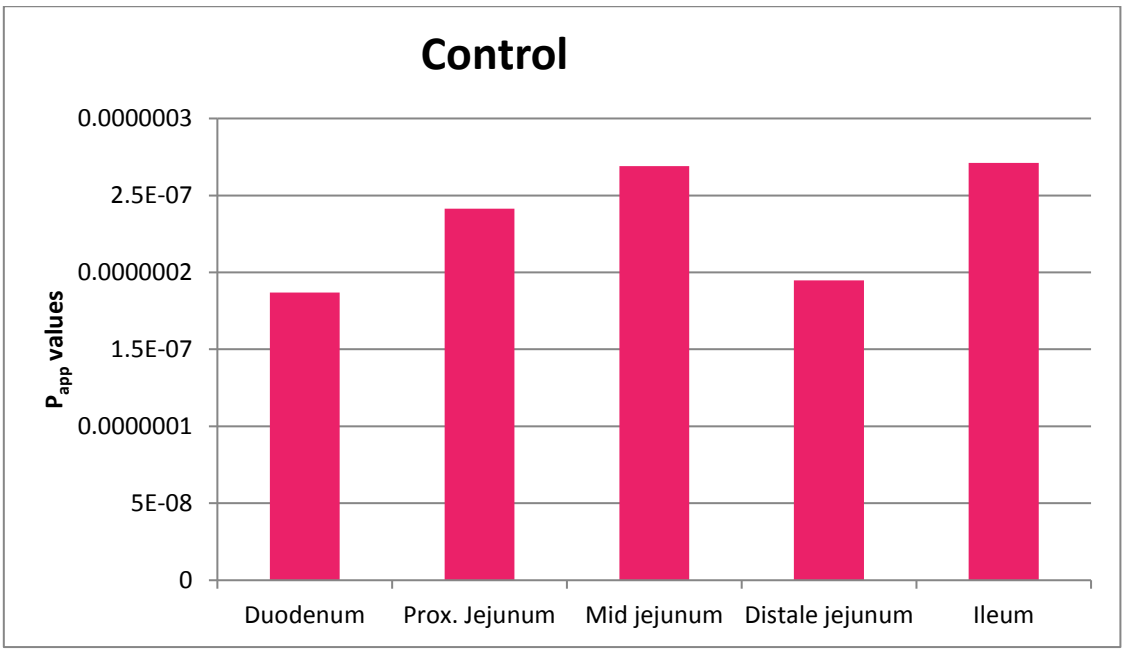
ANNEXURE H: P_{APP} IN GASTROINTESTINAL TRACT

P_{app} values of Rhodamine 123 in the abscence (control) and prescence of excipients

Region	Control	PC 0.015%	PC 0.03%	PC 0.06%	ADS 0.005%	ADS 0.01%	ADS 0.02%
Duodenum	1.86872E-07	1.03E-07	1.71952E-07	2.36622E-07	2.26435E-07	2.35715E-07	2.30104E-07
Prox. jejunum	2.41357E-07	3.83043E-07	3.28611E-07	2.51091E-07	2.1994E-07	2.62656E-07	3.38573E-07
Mid. jejunum	2.69097E-07	1.52671E-07	9.41089E-08	1.94321E-07	1.89744E-07	1.21791E-07	2.3287E-07
Distal jejunum	1.94791E-07	1.19477E-07	1.52196E-07	2.17665E-07	1.00274E-07	2.78925E-07	2.96527E-07
Ileum	2.7105E-07	2.01103E-07	1.78754E-07	8.89098E-08	1.69752E-07	9.62911E-08	8.51378E-08



Schematic illustration of P_{app} values of Rhodamine 123 in the abscence (control) and prescence of excipients



Schematic illustration of P_{app} values of Rhodamine 123 in different regions