THE *IN VIVO* EFFECTS OF PHARMACEUTICAL EXCIPIENTS ON GASTROINTESTINAL TRANSIT AND DRUG ABSORPTION

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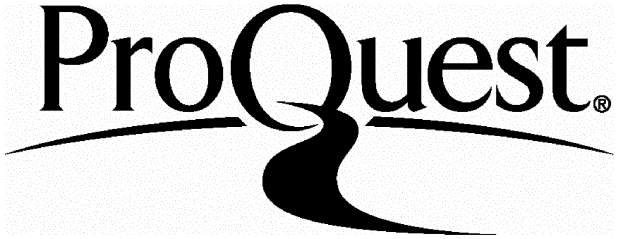

ABSTRACT

The objective of the project was to identify and characterize the *in vivo* effects of various pharmaceutical excipients, commonly employed as solubility enhancing agents, on gastrointestinal transit and drug absorption in humans and canines. Previous reports had indicated that high doses of the cosolvent polyethylene glycol 400 (PEG 400) accelerate the transit of oral liquid formulations through the small intestine leading to a reduction in drug bioavailability

In human subjects, the effects of PEG 400 were investigated at amounts of 0, 1, 2.5 and 5g using the non-invasive technique of gamma scintigraphy combined with simultaneous assessment of pharmacokinetic data. The transit effect of PEG 400 was established to be dose-dependent, decreasing the small intestinal transit time of the administered liquid formulations even at concentrations as low as 1g. Surprisingly, a degree of dose-dependency of PEG 400 was also found with respect to the absorption of the model drug ranitidine. At higher concentrations the bioavailability of ranitidine was markedly reduced most likely due to shorter transit times since PEG 400 was shown not to affect passive drug diffusion. The absorption of ranitidine, however, was significantly increased in the presence of 1g PEG 400, potentially due to modulation of intestinal permeability.

In separate scintigraphic studies the solubilizers propylene glycol, D-α-tocopheryl polyethylene 1000 succinate (VitE-TPGS), Labrasol® and Capmul® MCM were found not to affect small intestinal liquid transit but influenced the absorption of co-administered model drugs. The oral bioavailability of ampicillin was considerably reduced in the presence of propylene glycol, most likely as a result of the osmotic activity of the excipient, and Capmul® MCM, possibly via alterations in intestinal membrane function. VitE-TPGS was observed to increase the absorption of ranitidine by enhancing its permeation through the absorptive membrane.

The results obtained in an *in vivo* scintigraphic study in four beagle dogs correlated well with findings from the human studies regarding gastrointestinal transit. Differences observed in drug absorption were most likely a result of interspecies differences in the permeability of the intestinal mucosa.

These findings are expected to have ramifications for the use of these excipients in drug development and dosage form design.

Für meine Eltern

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LIST OF ABBREVIATIONS AND SYMBOLS

An	Absorption number
AUC	Area under the plasma concentration-time curve
BA	Bottom part of strip placed in acetone
BCS	Biopharmaceutics Classification Scheme
BNF	British National Formulary
BP	British Pharmacopoeia
BS	Bottom part of strip placed in saline
c_s	Solubility
CAT	Caecum arrival time
Cmax	Maximum plasma concentration
CMC	Critical micelle concentration
CYP450	Cytochrome-P450
Dn	Dissolution number
Do	Dose number
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
EMEA	European Medicines Evaluation Agency
F_{abs}	Fraction drug absorbed
FAB	Fast atom bombardment
FDA	Food and Drug Administration
GET	Gastric emptying time
GI	Gastrointestinal
HIV	Human immuno-defficiency virus
HLB	Hydrophile lipophile balance
HPLC	High performance liquid chromatography
HPMC	Hydroxypropylmethyl cellulose
log*P*	Octanol/water partition coefficient
MBq	Megabecquerels
MCAT	Mean caecum arrival time
MGRT	Mean gastric residence time
MMC	Migrating myoelectric complex
MS	Mass spectrometry

MSITT	Mean small intestinal transit time
NCE	New chemical entity
o/w	Oil-in-water
P_{eff}	Effective permeability
PEG	Polyethylene glycol
SAPP	Sodium acid pyrophosphate
SEDDS	Self-emulsifying drug delivery systems
SPE	Solid phase extraction
t_{50}	Time of 50 % activity within stomach or colon
t_{abs}	Absorptive time
t_{res}	Mean residence time
TA	Top part of strip placed in acetone
Tmax	Time of maximum plasma concentration
TcO_4^-	Pertechnetate
^{99m}Tc	Technetium-99m
TS	Top part of strip placed in saline
TLC	Thin layer chromatography
USP	United States Pharmacopoeia
UV	Ultraviolet
VCAT	Variance of caecum arrival time
VGRT	Variance of gastric residence time
VitE-TPGS	D-α-Tocopheryl polyethylene glycol-1000 succinate
w/o	Water-in-oil

CHAPTER 1

INTRODUCTION

1.1. Overview

The oral route still remains the most important and preferred means of drug administration. Oral dosage forms such as tablets, capsules, solutions, emulsions and suspensions allow easy and convenient self-medication and the resulting high acceptability in patients is reflected in an overall good compliance. Among these dosage forms solutions offer certain advantages. A liquid preparation allows individual dosing and easy swallowing, where tablets and capsules might present difficulty for elderly people and children. A further advantage of pharmaceutical solutions is that the active ingredient is already dispersed in its molecular form. Dissolution is well known to be a major factor controlling and limiting drug absorption, since a drug compound has to be completely dissolved in the gastrointestinal fluids in order to be absorbed from the lumen (Katchen and Symchowicz, 1967). Unfortunately, many drug compounds possess a low solubility in aqueous medium and as a result very often exhibit poor oral bioavailability.

Therefore, poorly soluble drugs comprise a great challenge in the design of pharmaceutical preparations. Over the years various strategies have been developed to improve the aqueous solubility and *in vitro* release rate of such compounds. Among these strategies are the synthesis of prodrugs, the formation of water-soluble salts and the reduction of drug particle size. Because of the ease of application the most popular approach, however, is regarded to be the solubilization of drug compounds. The process of solubilization may be best defined as the procedure whereby comparatively high amounts of poorly soluble substances are brought into aqueous solution by the addition of solubilizing agents or solubilizers to a pharmaceutical formulation.

Solubilizers play a very important role in the development of liquid dosage forms such as oral and parenteral solutions or liquid-filled soft gelatin capsules. A variety of drug formulations containing solubilizing agents can be found on the market ranging from painkillers, immunosuppressants, diuretics and antidepressants to drugs for the treatment of cancer, epilepsy and various bacterial infections as well as the human immunodeficiency virus (HIV) infection.

In addition, solubilizers are excessively used in the early stages of drug development in order to allow the formulation of a drug solution for the employment in intravenous or oral bioavailability studies. In many cases, the poor aqueous solubility of a potential drug candidate requires the addition of high amounts of solubilizers for complete drug dissolution. While for many years the influence of solubilizers on drug bioavailability was thought to be primarily the result of the solubility enhancing properties of the excipient it has recently been acknowledged that pharmaceutical excipients may exhibit various effects, positive or negative, in the *in vivo* environment. Such biopharmaceutical effects include, for instance, the impact on the membrane permeability of the drug or the transit of the dosage form through the gastrointestinal tract (GI tract), as well as interference with metabolising enzymes or efflux transporters.

With the introduction of new techniques in drug discovery such as molecular modelling and high throughput screening the need to assess the developability of a potential drug candidate as early as possible is crucial (Panchagnula and Thomas, 2000). As a result, the importance of biopharmaceutics has been significantly enhanced in recent years and pharmaceutical excipients, in particular, have become the focus for biopharmaceutical investigation. Hence, the purpose of this study was to investigate the influence of solubilizing agents on the *in vivo* performance of oral drug solutions.

1.2. Structure of the gastrointestinal tract

With its primary functions of food intake, digestion, nutrient absorption and elimination of unwanted material, the gastrointestinal (GI) tract is a highly specialized region of the body (Mayersohn, 1996). The GI tract comprises the oral cavity, oesophagus, stomach, as well as the small and large intestine. Each part of the gastrointestinal tract exhibits distinguishing features with respect to surface structure, pH, enzymes and electrolytes as a result of unique anatomical, biochemical and physiological properties (Table 1.1).

Table 1.1. Biological and physical parameters of the human gastrointestinal tract (adapted from Daugherty and Mrsny, 1999).

Segment	Approximate surface area	Approximate segment length	Approximate residence time	pH of segment	Catabolic enzymes
Oral cavity	100 cm^2		Seconds to minutes	6.5	Polysaccharidases
Oesophagus	200 cm^2	23-25 cm	Seconds		
Stomach	0.10-3.5 m^2 (variable)	0.25 cm (variable)	1.5 hrs (variable)	1.0-2.5	Protease, lipases
Small intestine			3-4 hrs		Polysaccharidases, oligosaccharidases,
Duodenum	1.9 m^2	0.3 m	0.5-0.75 hrs	5.0-6.0	peptidases, lipases,
Jejunum	184 m^2	2.8 m	1.5-2.0 hrs	6.0-7.0	nucleases,
Ileum	276 m^2	4.2 m	variable	7.0-7.5	nucleotidases
Large intestine	1.3 m^2	1.5 m	1-60 hrs	6.4-7.5	Broad spectrum of bacterial enzymes

Aside from dosage forms designed for drug delivery by the buccal and sublingual route, orally administered drug formulations usually pass the oral cavity and the oesophagus very quickly leaving these regions a rather negligible role in drug absorption. In general, the GI tract is a hollow muscular tube, which is composed of four concentric layers of tissue: the mucosa, the underlaying submucosa, the muscularis externa and the serosa. The three outer layers are similar throughout most of the GI tract, whereas the mucosa, which is located at the luminal surface of the gastrointestinal tract, has distinctive structural and functional characteristics and is of major importance with respect to drug and nutrient absorption. The intestinal mucosa itself includes three different layers of tissue. The muscularis mucosa consisting of a sheet of muscle fibres separates the mucosa from the submucosa. The middle layer, which is known as the lamina propria contains mostly connective tissue besides nerve fibres, blood capillaries and lymph vessels. Covering the lamina propria is a single layer of columnar epithelial cells, called the lining epithelium. The epithelium is in direct contact with the lumen and is directly involved in the absorption of drugs and nutrients, as well as processes of secretion and digestion and the erection of a physiological barrier against pathogens. The epithelial cells are connected to each other through direct contact between specific proteins across the intercellular space, the tight junctions. The tight junctions seal adjacent cells together forming a continuous sheet of cells, which prevents uncontrolled diffusion of fluids and compounds from the apical to the basolateral side of the cell and vice versa.

The mucosa of the **stomach** is folded into several rugae, which are covered with numerous pits. Both the rugae and the gastric pits increase the total surface area of the mucosa over that provided by a smooth lining. Gastric glands containing different types of secretory cells are located at the base of the pits and are mainly concerned with the secretion of hydrochloric acid, gastric hormones such as histamine and gastrin, as well as pepsinogen. A lining of simple columnar epithelial cells, also known as surface mucous cells cover the entire surface of the gastric mucosa and secrete mucous and bicarbonate to protect the epithelium from the aggressive gastric juice. Concerning drug absorption from oral dosage forms, the stomach seems to act more as a reception area than an absorptive organ itself, since the gastric epithelium is mostly dominated by secretive cells, whereas the absorptive cells are low in number. In addition, especially compared to the small intestine, the gastric surface area of the

mucosa is rather small and the blood flow in the stomach is very low with 150 mL passing each minute in relation to 1 Litre/min blood in the capillaries of the small intestine (Rowland and Tozer, 1995). However, some drug compounds with certain physicochemical structures such as weak acids might profit from the relatively long residence time in the stomach of sometimes up to 12 hours (Davis et al., 1984b) and may partly be absorbed from this region of the GI tract.

The **small intestine** is divided into three parts, namely the duodenum, jejunum and ileum, which differ in surface structure, as well as absorptive and secretive capacity. Because of its extremely large surface area the small intestine is the most important site for the absorption of drugs delivered by the oral route. The increase of the absorptive area in the small intestine is achieved by its unique mucosal surface structure. The intestinal mucosa and submucosa are thrown into several folds, known as plicae circulares or the folds of Kerckring, which are about 0.75 cm in height and may extend entirely around the circumference of the small intestine. These folds are further amplified with multiple finger-like projections, the villi with a length of 0.5 to 1.5 mm. Approximately 10 to 40 villi arise at 1 mm^2 of the duodenal mucosa, whereas the villi diminish in size and number towards the jejunum and ileum. Each villus is covered by a continuous layer of simple columnar epithelial cells, consisting mostly of absorptive cells, the so-called enterocytes.

Structural support of the numerous villi is provided by the lamina propria, which extends into each villus forming its core. From the luminal surface of the enterocytes projects a microscopic structure, the microvilli. Each villus cell is covered by approximately 600 microvilli, which are about 1 µm long and give the mucosa the hairy texture, which led to terming this region the "brush border" of the small intestine. Compared to a smooth lining the folds, villi and microvilli increase the effective surface area of the small intestine by the factors 3, 30 and 600, respectively, and provide a total absorptive area of 200 m^2, whereas the mucosal surface of the stomach is about 1 m^2 (Rowland and Tozer, 1995). The fact that drug absorption is highest in the proximal regions of the small intestine, i.e. the duodenum and jejunum (Aiache and Aiache, 1985), and decreases towards the ileum, where the plicae and villi of the mucosa are markedly reduced in size and number, underlines that the size

of the surface area is determinant for the absorptive capacity of the distinctive parts of the GI tract.

On top of the microvilli exists a coating of weakly acidic mucopolysaccharides, the glycocalyx, which presents a relatively impermeable barrier for foreign substances within the GI tract. The glycocalyx itself is covered with an unstirred layer of aqueous fluid, which tends to be an extra barrier especially for lipophilic drug molecules. The thickness of the unstirred water layer has been found to be about 40 μm and depending on the flow rate and viscosity of the luminal contents as well as on the morphology of the mucosal surface. The surface epithelial cells are renewed rapidly and regularly. The villous enterocytes are progressively replaced from the proliferative zone of the epithelium, the crypts of Lieberkuehn, which form pits adjacent to the villi. Normally, it takes about two days for cells to migrate from crypts to the surface of the villus, hereby renewing the duodenal surface completely. Cells between the villi form the crypts and their primary function beside the renewal of cells is the secretion of water and ions, as well as exocrine and endocrine secretions. There are also various specialized secretory cells located in the small intestine, especially in the duodenum. Despite the mucous secreting goblet cells situated between the columnar cells throughout the small intestine, Brunner's glands in the duodenum are involved in the secretion of alkaline mucus to protect the intestinal epithelium by neutralizing the acidic and pepsin containing chyme arriving from the stomach.

Besides the absorption of essential nutrients a major function of the GI tract is to impede the entry of pathogens. Therefore, a substantial part of the immune system is constituted in the intestinal lining, which is referred to as the gut-associated lymphoid tissue (GALT). There are organized structures of lymphoid cells, known as the Peyer's patches, which are particularly located in the ileum. As an immunologic response the epithelial cells covering the Peyer's patches differentiate into M cells (Kerneis, 1997), which have been speculated to transport particles across the luminal surface for antigen identification and adequate immunologic reaction (Daugherty and Mrsny, 1999).

The terminal region of the small intestine is called the ileocaecal junction. Papillary protrusions into the lumen form this kind of sphincter that prevents the spreading of bacteria from the colon into the small intestine (Phillips, 1983). At the ileocaecal junction the luminal contents are accumulated until digestion is largely completed.

The **large intestine** extends from the ileocaecal junction to the anus at an approximate length of 1.5 metres and is subdivided into four regions: caecum, colon, rectum and anal canal. The colon itself comprises the ascending, transverse, descending and sigmoid colon. The mucosal surface of the large intestine is relatively flat since it does not form such a villous structure as the small intestine. The wall of the caecum and ascending colon is folded into several sacs, the haustrae, which are formed by contraction of the circular muscle and allow the movement of the contents down towards the lower regions of the large intestine by distension or elongation. Only 10 % of the absorption of a drug passing through the entire GI tract occurs in the large intestine as a consequence of the comparatively small surface area and the increasing viscosity of the luminal contents. Nevertheless, besides storage and elimination of faecal material, the major function of the colon is the absorption of sodium and consequently water. It has been estimated that 1.5 Litres of chyme enter the colon each day, undergoing efficient water re-uptake on its way through the large intestine and leaving only about 200 mL for the elimination as faeces (Martini, 1995). Lubricating the surface of the intestinal mucosa in order to facilitate the transit of dehydrated faecal material, mucous is secreted into the large intestine by colonic goblet cells. One unique characteristic of the colon is its high, active bacterial microflora, which has been taken advantage of in the development of oral drug delivery systems targeting the colon (Van den Mooter and Kinget, 1995; Cummings et al., 1996).

1.2.1. Passage through the gastrointestinal tract

Material on its way through the GI tract faces remarkable differences in the transit times between the digestive organs. After ingestion and passage through the oesophagus food arrives at the stomach, where it is stored at first allowing relatively infrequent consumption. By relaxation of the fundus and body regions, the stomach is able to expand from accommodating mere 50 mL rest volume to up to 1.5 Litres when fed. This is achieved without significantly changing the intragastric pressure (Malagelada, 1981; Martini, 1995), which is steadily exerted by slow, segmental

contractions responsible for gradually pushing the gastric contents towards the distal stomach. In the antrum they are addressed by regular peristaltic contractions (3 per minute), which serve to mix and break down food particles and move them towards the pyloric sphincter, where they are ejected into the duodenum as a homogeneous, semi-solid chyme (Davis et al., 1986a). The pyloric sphincter acts as a sieve, allowing only liquids and small particles to pass. Larger particles are retropulsed back into the antrum for further digestion before gastric emptying (Meyer, 1980), while the residue of undigested solids remains in the stomach until the fasted pattern of motility is initiated (Phillips, 1983).

As eventually all digestible material is emptied, the motility pattern of the stomach changes distinctively. The interdigestive or fasted state begins comprising of a series of contractions referred to as the migrating myoelectric complex (MMC). The MMC lasts for about 2 hours and can be divided into four consecutive phases of activity (Szurszewski, 1969; Code and Marlett, 1975). Phase I, the basal phase, is regarded as the period of quiescence with rare contractions and little motor activity lasting up to an hour. It is followed by the preburst phase (phase II) of 20-40 minutes, comprising bile secretion into the intestines and irregular contractions, that increase in amplitude and frequency until phase III, the burst phase, is reached. This short 4-6 minute period performs intense, large regular contractions moving distally from the stomach to the large intestine. Hereby, the stomach lumen is cleared of undigested food fragments, which is the reason that the interdigestive cycle is also referred to as the "housekeeper wave". Phase IV, which is a short period of 0-5 minutes, separates phase III with intense activity from the quiescence of phase I. The MMC is thought to be modulated by motilin, gastrin, prostaglandins or signals from the central nervous system (Weisbrodt, 1984; Rao and Schulze-Derieu, 1993). Every two hours the whole cycle is repeated until food is ingested, which then immediately puts in the fed pattern of motility.

An orally administered dosage form will be subjected to the same physiological processes as described for foodstuffs. The emptying time of a pharmaceutical dosage form out of the stomach is highly variable and dependent on the type of the dosage form administered as well as the motility pattern at the time of administration. Liquids and small solids with a diameter of a few mm or less leave the fasted stomach within

an hour through the pylorus (Jenkins et al., 1983; Washington et al., 1986). However, the gastric residence time can be increased to up to 2 hours and more if a meal is ingested 30 minutes prior to administration (May et al., 1984). Larger solids remain in the stomach until they have been reduced to relatively small particles. Capsules or other large units, which do not disintegrate within the stomach, are treated as indigestible material, thereby requiring the strong contractions of phase III of the MMC to empty from the stomach (Wilson et al., 1984; Parker et al., 1988). This results in a high variability in gastric residence time lasting only 5 minutes if ingested while the stomach is performing the strong "housekeeper" contractions that sweep the contents out immediately or up to several hours if ingested during the digestive state.

In general, the release of the stomach contents into the small intestine is regulated by their nature with respect to pH (Hunt and Knox, 1972), temperature (Wilson and Washington, 1989), osmolality (Hunt, 1961) and viscosity. Significant deviations from the intestinal conditions will retard gastric emptying as observed for the presence of food (Davis et al., 1984a; Wilson et al., 1989) since they will have to be compensated for first (Kelly, 1981). Other factors known to delay gastric emptying comprise mental depression, strenous exercise, lying on the left side and diseases such as gastric ulcer, pyloric stenosis, gastroenteritis, gastroesophageal reflux and Crohn's disease. In addition, certain therapeutic agents have been found to cause prolonged stomach emptying times including anticholinergic, narcotic analgesics and antidepressants. Gender also appears to play a role in gastric emptying with women exhibiting slower emptying rates than man (Wald et al., 1981), as are age and obesity. A promotion of gastric emptying, on the other hand, is promoted by fasting or hunger, anxiety or stress, as well as lying on the right side and antiemetic drugs (Gibaldi, 1991). The volume of the administered fluid is also of importance. High amounts of fluids empty from the stomach markedly faster than small volumes of liquid showing first-order emptying kinetics compared to variable kinetics of low amounts of fluids (Hunt and McDonald, 1954; Hunt and Knox, 1968). Gupta and Robinson (1988) explained this phenomenon with an interruption of the fasted motility pattern when volumes of 100 mL and more are ingested, whereas the emptying of low amounts of fluid depends highly on the state of the fasting motility cycle.

In the small intestine the luminal contents are exposed to segmental and peristaltic movements, which depend on the current digestive state and are similar to those experienced in the stomach. The segmental contractions are initiated by the passage of the chyme into the small intestine and serve to optimise the contact of the intestinal contents with the absorptive area of the lining epithelium. The peristaltic contractions are responsible for moving the unabsorbed chyme distally, hereby slowing down in pace towards the lower regions of the small intestine as the viscosity of the chyme increases. This phenomenon is also observed in the fasted state, with the jejunum displaying an intestinal flow rate twice that of the ileum (2.52 mL/min against 1.23 mL/min) (Soergel, 1971; Kararli, 1995). In contrast to the highly variable residence times in the stomach the transit through the small intestine is remarkably constant at about 3 to 4 hrs. The small intestinal transit time was found to be unaffected by the type of the administered dosage form (Davis et al., 1986a), the presence or absence of food (Mundy et al., 1989) as well as by the time of dose administration, pathological conditions (Hardy et al., 1988), age (Davis et al., 1986b), body position or exercise (Ollerenshaw et al., 1987). The transit rate of chyme moving down the small intestine has been determined to be in the range of 1 to 4 cm per minute (Granger et al., 1985). This figure corresponds quite closely to the data published for the small intestinal transit speed of a non-disintegrating capsule formulation (4.2-5.6 cm/min) (Kaus et al., 1984) and the pace of migration of the MMC through the small intestine (4.7 cm/min) (Kerlin and Phillips, 1982).

The passage of material from the small intestine to the large intestine is regulated by the ileocaecal junction, which at the same time serves as a barrier for bacteria and faecal material in the other direction (Phillips, 1983). The opening of the junction was reported to occur in accordance with peristaltic waves reaching the terminal ileum, allowing about 2 mL of intestinal content to pass into the large intestine (Aiache and Aiache, 1985). Other reports, however, suggest that movement through the ileocaecal junction is independent of the MMC and instead related to the rate at which material accumulates within the ileum (Quigley et al., 1984). The nature of the intestinal contents also appears to be of importance concerning the passage across the junction. The presence of unabsorbed fat and protein within the terminal ileum was observed to delay the emptying of material from the ileum into the large intestine by releasing the so-called ileal-brake mechanism. The stagnation of transit at the ileocaecal junction

has the purpose to guarantee the efficient absorption of nutrients from the GI tract and may therefore last from 2 to 20 hours (Marvola et al., 1987). Dosage forms have also been shown to undergo stasis at the junction for considerable periods of time (Khosla and Davis, 1989; Clarke et al., 1993; Adkin et al., 1995a), which in turn may affect drug delivery to the large intestine.

The large intestine displays the same two distinct patterns of motility as the stomach and the small intestine. The segmental contractions, which are responsible for the mixing and spreading of the luminal contents and the slow movement of the chyme from the caecum to the transverse colon, predominate in the colon allowing time for water absorption. Powerful peristaltic waves occurring 3-4 times a day (Wilson and Washington, 1989) induce mass movement of the dehydrated chyme through the rest of the colon. Colonic transit is thus characterised by short bursts of activity followed by long periods of stasis (Hardy et al., 1985a). Therefore, unlike the small intestine, the residence time in the large intestine can be highly variable, both within and between individuals, ranging from 1 to more than 60 hours (Hardy et al., 1985a; Hardy, 1987). The average transit time from caecum to splenic flexure has been estimated to be about 14 hours (Metcalf et al., 1987). Peristaltic activity in the colon increases rapidly after the intake of food due to the gastrocolic reflex. This phenomenon is initiated by a receptor in the gastroduodenal mucosa (Sun et al., 1982), which appears to be sensitive to ingested fat and protein (Wright et al., 1980). Dietary and social habits appear to have a major influence on colonic transit times. Diets with a high content of fibre lead to a remarkable decrease in GI transit times (Cummings, 1984) demonstrated with the mouth to anus transit of a single unit lasting less than 6 hours in young vegetarians.

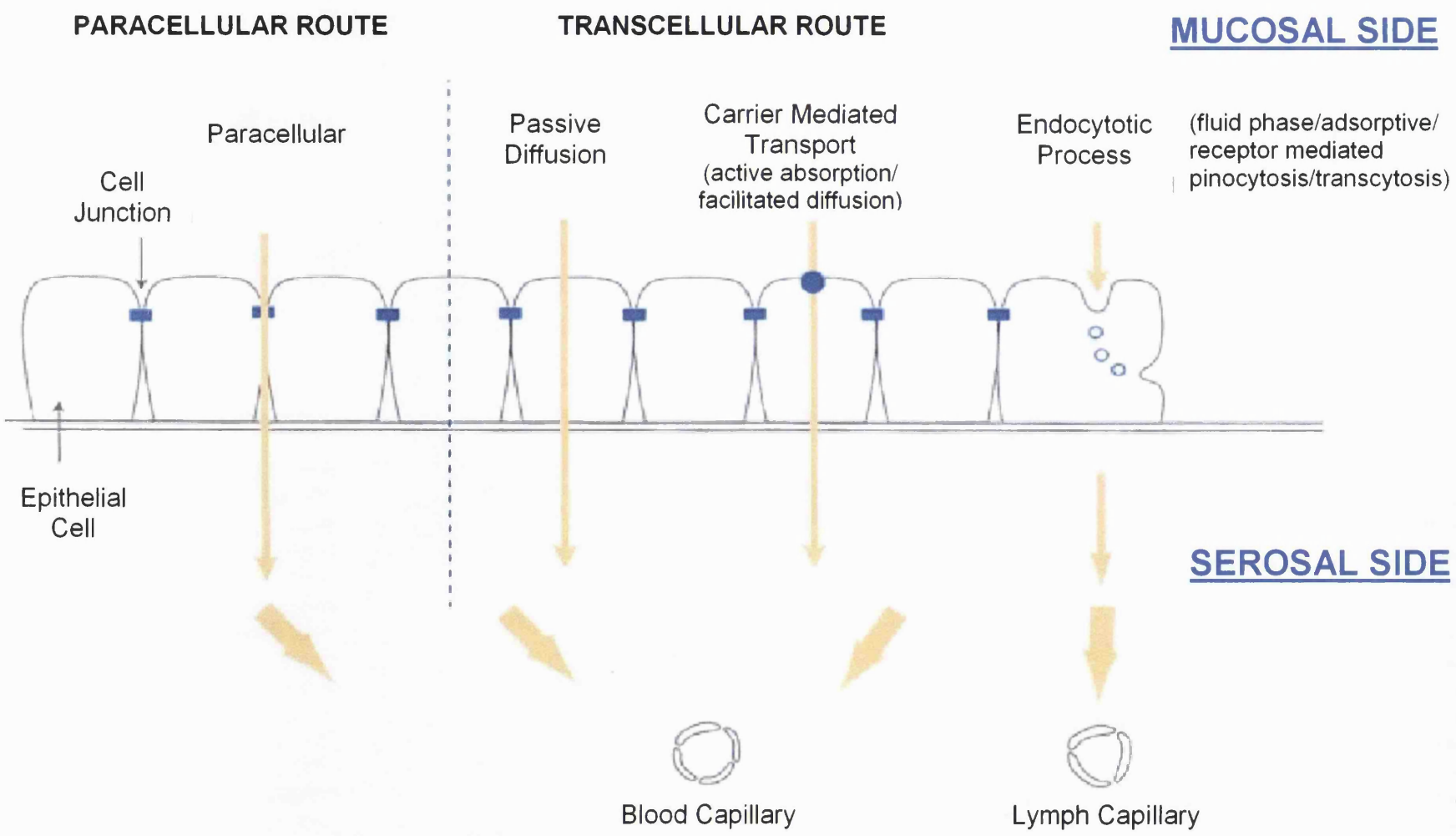
The total transit time from mouth to rectum ranges from 6 to 72 hours about an average value of 25 hours. Besides the variation between individuals GI motility was also shown to undergo circadian variation (Goo et al., 1987; Wilson and Washington, 1989) where the passage through the GI tract appears to take twice as long in the evening compared to the morning. This is in agreement with the findings of Kumar et al. (1986), who measured the velocity of the MMC in the upper intestine and found the daytime speed of the MMC to be more than twice that of the nocturnal speed in humans.

1.3. Absorption Pathways

Drug absorption from the GI tract requires the compound to pass through various barriers and regions before it reaches the blood vessels of the villi. A drug molecule at the luminal side of the intestinal mucosa first of all, has to diffuse through the unstirred aqueous layer, the mucous layer and the glycocalyx to reach the apical membrane of the enterocyte. Arriving at the apical membrane of the absorptive cells the molecule has the choice to either transverse the lining epithelium through the tight junctions between two adjacent cells, which is also referred to as the inter- or paracellular route, or transcellularly, meaning across the apical membrane of the enterocytes. The transcellular passage involves the penetration of the drug across the brush border, the intracellular space and the basolateral membrane before it can cross the basement membrane and reaches the lamina propria. Finally, the drug diffuses through the tissue region of the lamina propria and across the endothelium of the blood capillaries where it is then carried away in the blood to the systemic circulation via the liver, where it undergoes first-pass metabolism. Highly lipid-soluble drugs may have the potential to circumvent the first-pass metabolism by using fat absorption pathways via the lymphatic system of the villi (Porter and Charman, 1997). However, it is the epithelium lining that is considered the main cellular barrier to the absorption of drugs from the gastrointestinal tract (Blanchard, 1975). The various pathways a drug molecule can take to overcome the epithelium are illustrated in Figure 1.1.

Each pathway requires certain physicochemical properties of a drug as a pre-requisite for absorption. The **paracellular** passage of a molecule includes the overcoming of the tight junctions, which exist at the apical side of the intestinal membrane and seal adjacent epithelial cells together (Madara, 1998). Functionally, the tight junctions are not sealed completely but are permeable to water, electrolytes and certain other molecules. The size of the tight junctions lies in the range of 0.5 to 5 nm (Macheras et al., 1995) allowing only small molecules to pass and it decreases from the small intestine to the colon (Chadwick et al., 1977b). Therefore, the small intestine is regarded to be relatively leaky (Hayton et al., 1980) if compared to the colon and drug absorption via the paracellular route is most likely to occur in the upper part of the GI tract.

Figure 1.1. Routes and mechanisms for drug transport across the lining epithelium (adapted from Hillery, 2001).



Since the intercellular way of absorption is physiologically used for the uptake of water and electrolytes, molecules possessing a hydrophilic chemical structure and low molecular weights transverse the tight junctions preferentially (Chadwick et al, 1977a,b; Artursson et al., 1995). Meeting these requirements drugs including hydrochlorothiazide, atenolol, ranitidine, digoxin and small peptides (Gan et al., 1993; Macheras et al., 1995; Rouge et al., 1996) have been demonstrated to use paracellular absorption pathways. However, the role of the paracellular pathway for the absorption of drugs *in vivo* is controversial. Since the tight junctions occupy only 0.01 % of the total surface area of the GI tract (Madara and Pappenheimer, 1987), the uptake of hydrophilic drug compounds may not occur quantitatively via the paracellular route but also to a certain extent transcellularly. It was suggested that this is particularly true for hydrophilic drugs with a molecular weight of more than 200 Da (Lennernäs, 1995; Fagerholm et al., 1999).

If the uptake of water is increased, small hydrophilic molecules are thought to be dragged across the lining epithelium following the aqueous flow. This phenomenon of enhanced uptake is known as **solvent drag** or convective absorption (Karino et al., 1982a,b). Different opinions, however, exist whether water absorption and solvent drag occur mainly through the para- or transcellular route. *In vitro, in situ* and *in vivo* studies in the rat suggested that the presence of luminal nutrients enhances net fluid absorption and paracellular uptake of different hydrophilic solutes with molecular weights up to 5500 Da (Pappenheimer and Reiss, 1987; Sadowski and Meddings, 1993). The magnitude of the contribution of solvent drag to the transport of compounds through aqueous absorption pathways is dependent on the tonicity of the perfusing solution (Kitazawa et al., 1975; Hunt et al., 1992). Nylander et al. (2003) reported a hypotonicity-induced duodenal permeability enhancement in the rat *in situ*, which was suggested to fulfill the function of increasing the blood-to-lumen transport of sodium in order to facilitate adjustment of the hypoosmolar conditions in the lumen. Contrary to the findings in rats, *in vivo* permeability studies in human subjects infusing hypotonic glucose solutions did not result in increased jejunal permeability of hydrophilic compounds with molecular weights of more than 200 Da (Lennernäs, 1995; Fagerholm et al., 1995; Fagerholm et al., 1999). The controversies in the results of the different studies are likely to be the consequence of species differences between rats and humans. DeSesso and Jacobson (2001) recently confirmed that despite strong morphological

similarities between both species at the microscopic level gross, anatomical differences exist, for instance, in the relative absorptive surface areas. However, a non-invasive *in vivo* analysis of human small intestinal absorption via the paracellular route resulted in an increased uptake of the paracellular marker creatinine in the presence of luminal glucose (Turner et al., 2000), which correlates more closely with the findings obtained in rats. Therefore, it underlines the fact that the effect of fluid fluxes on drug absorption is not yet fully understood and further *in vivo* human studies are required.

The majority of drugs are believed to be absorbed **transcellularly** diffusing across the apical membrane of the epithelial cells. The drug molecules partition into the membrane, transverse the cytosol and diffuse across the basolateral membrane moving down a concentration gradient. Driven from their own kinetic energy, the molecules do not require an input of energy, which has led to the term of passive diffusion. The passive diffusion of a molecule across the intestinal membrane is dependent on various factors including the magnitude of the concentration gradient, the surface area of the membrane and the physicochemical properties of the molecule (Taylor, 1986). The cell membrane is formed of a bilayer of tightly packed phospholipids, which gives the membrane a lipid nature. The rate of passive transport across the membrane depends, therefore, on the solubility of the molecule in the lipid layer. Drugs with large octanol/water partition coefficients and unionized, non-polar drugs move efficiently through the apical membrane of the enterocytes by partitioning into and out of the lipid bilayer. These observations provided the basis for the pH partition hypothesis (Shore et al., 1957; Schanker et al., 1960; Hirtz, 1985), which stated that the pH of the gastrointestinal fluids is the determining factor for the rate and extent of the absorption of basic and acidic drugs. Weakly basic drugs were considered to be best absorbed from the small intestine and weak acids from the stomach (Hirtz, 1984), since the existing pH at these sites renders the largest fraction of the respective drug in an unionized state and with it in the least polar form. But, for the gastric absorption of acidic drugs, the validity of the pH partition hypothesis is limited, due to the fact that the large surface area of the small intestine still provides the best conditions for fast and efficient absorption of all drugs (Rowland and Tozer, 1995). Although hydrophobic characteristics are required of a drug to allow the diffusion into the lipid membrane, a too high lipophilicity might hinder the drug from leaving the lipid layer and moving into the more hydrophilic cytosol.

The lipid bilayer of the membrane is in fact not continuous but rather penetrated with numerous submicroscopic **aqueous filled pores**. The aqueous pores are responsible for the continuity between the interior and the exterior of the cells, which is mainly achieved by free water movement. They have an estimated radius of about 0.7 nm in the jejunum and 0.3 nm in the ileum of humans (Fordtran et al., 1965). Very small hydrophilic molecules such as water, urea and low molecular weight sugars are able to transcellularly cross the apical membrane by diffusing through the water filled channels. Most drugs, however, are too large to pass through these channels and the apical cell membrane and hence the gastrointestinal barrier behaves like a lipoidal sieve.

Other proteins located in the lipid bilayer of the apical cell membrane function as **carrier** systems performing an active role in the transport of many substances of nutritional interest and some lipid-insoluble drugs. A carrier, which may be an enzyme or some other component of the membrane, forms a complex with the drug molecule present at the luminal surface of a columnar absorption cell. The drug-carrier complex then moves across the membrane and liberates the drug on the other side of the membrane. The carrier, now free, returns to its initial position at the luminal surface of the epithelial lining of the enterocyte in order to await the arrival of another drug molecule. Active transport is a process, whereby compounds can be transported against a concentration gradient, which involves the consumption of energy. There appear to be several carrier-mediated transport systems in the small intestine. Many body nutrients such as sugars and amino acids are transported across the gastrointestinal membrane by active transport processes. Each carrier appears to be highly selective with respect to the chemical structure of its substrates. Thus, if the molecular structure of a drug is related to an actively transported nutrient or other natural compound, it is likely to be absorbed by the same carrier mechanism. For instance, the resemblance of the amino acid tyrosine and phenylalanine equips levodopa to use the carrier system, which transports amino acids from the lumen to the systemic circulation. The dipeptide carrier in the upper small intestine has been shown to be essential for the uptake of β-lactam antibiotics such as ampicillin (Oh et al., 1992; Lee, 2000). Generally, these transport carriers are not evenly distributed throughout the entire GI tract but are rather concentrated in specific segments. Therefore, the absorption of a substrate of such a transporter preferentially occurs at a location in the intestine where the density of the carrier is high. In contrast to the passive absorption processes, where the rate of absorption is directly proportional to the

concentration of the drug molecule present at the absorption site, carrier-mediated drug uptake is not inexhaustible as it is dependent on the capacity of the transport system. High concentrations of drug substrates are able to saturate the carrier mechanism, which keeps the absorption rate at a constant level.

Besides active transport mechanisms some carrier proteins do not require an energy input for the transport of a drug substance. Their driving force is the concentration gradient as it is the case for passive diffusion. This carrier-mediated transport system is therefore known as **facilitated diffusion.** Certain drugs such as quaternary ammonium compounds and tetracyclines, which are ionized over the entire gastrointestinal pH range, are proposed to use an absorption mechanism, which is referred to as **ion-pair transport.** As these drugs are too lipid-insoluble to pass the lipid bilayer and too large to cross the membrane through the aqueous pores or the tight junctions they interact with endogenous organic ions of opposite charge forming a neutral ion-pair. The ion-pair is less polar and hereby able to partition into the lipoidal cell membrane by passive diffusion.

In addition to carrier systems responsible for the uptake of certain drugs from the lumen into the cell, the lining epithelium also contains carriers that direct the transport of drugs in the opposite direction. Polarized **efflux transporters** such as P-glycoprotein transport drug compounds out of the cell and back into the lumen after they have been absorbed. P-glycoprotein is part of an active defence mechanism of the body protecting it from the invasion of xenobiotic agents (Hunter and Hirst, 1997). A wide range of drugs including digoxin, cyclosporine A and amprenavir have been discovered to be substrates to intestinal efflux and it is suggested that such protein transporters significantly contribute to the reduction of their oral bioavailability. Carrier-mediated secretion increases in the distal direction towards the large intestine, being lowest in the jejunum and highest in the colon (Wagner et al., 2001). Recently, it has been recognized that the intestinal efflux of drugs is connected to the metabolic system located in the enterocytes and both elimination mechanisms have been found to have a broad overlap in substrate and inhibitor specificity (Benet and Cummins, 2001; Wacher et al., 2001).

Besides carrier-mediated transport and diffusional absorption processes there are two further uptake pathways worth mentioning, which drug molecules might use to reach the systemic circulation. In both cases, the drug does not have to be in solution to be absorbed. **Pinocytosis** involves a mechanism by which the apical membrane invaginates the drug or particle present at the site of absorption forming vacuols around it and hereby uptaking the material into the enterocytes. The vacuols then release the material either into the cytosol or at the basolateral membrane into the lamina propria. The second mechanism is called **persorption** and it takes advantage of the fact that during digestion the epithelium lining suffers from sloughing off of cells from the top of the villi. These cells are soon replaced by the continuous migration of cells from the crypts of Lieberkühn. But the sloughing off of cells causes a temporary gap in the epithelium lining giving large molecules the opportunity to pass through and reach the lamina propria and systemic circulation. Both absorption processes may not to be of major importance for the majority of drug compounds but macromolecules such as proteins and peptides might successfully be absorbed as they are too large to be absorbed otherwise.

1.4. Drug absorption

The bioavailability of a drug has been defined as "the extent and the rate to which a substance or its therapeutic moiety is delivered from a pharmaceutical form into the general circulation" according to the "Note for guidance on the investigation of bioavailability and bioequivalence" of the Committee for Proprietary Medicinal Products of the European Medicines Evaluation Agency (EMEA) (EMEA, 1998). It is dictated by two major parameters: the fraction of the drug absorbed from the lumen across the intestinal epithelium and the first-pass metabolism in the liver every drug molecule is exposed to after reaching the blood vessels of the lamina propria. The absorption of a drug compound from the GI tract is known to be a highly complex process, which has been under investigation for many years and is still not fully understood (Helliwell, 1993). Various factors including the physicochemical properties of the drug, the physiological conditions in the GI tract and the coadministration of food have been shown to influence the absorption and bioavailability of drugs. The importance of magnitude of the absorptive area must not be underestimated. Although for some drugs

the absorption conditions might be better in the stomach, e.g. as a result of low pH, the small intestine remains the favourite site of drug absorption due to a luminal surface area of approximately 200 m^2 (Rowland and Tozer, 1995).

Among all the parameters that influence oral drug bioavailability, two key parameters seem the most important: solubility and permeability. The majority of drugs are considered to be absorbed by passive diffusion through the lining epithelium. The importance of both solubility and permeability becomes clear when Fick's First Law is applied to the diffusion process of a drug molecule across a membrane as suggested by Amidon et al. (1995):

$$J_w = dM/dt \times 1/A = P_w \times c_w \qquad \text{(Equ.1.1)}$$

where J_w is the mass transport across the gut wall, A is the surface area, P_w is the intestinal wall permeability and c_w is the concentration of the drug at the membrane.

For poorly soluble compounds the concentration of the drug at the membrane, c_w, is determined by the solubility of the drug in the gastrointestinal fluids and is often equal to the saturation concentration.

The general importance of drug dissolution and GI permeability with respect to the rate and extent of drug absorption has been recognized with the establishing of a biopharmaceutics classification system (BCS) (Amidon et al., 1995). The BCS categorizes drug compounds in four different groups according to their aqueous solubility and permeability properties (Table 1.2). Most recently a simplification of the BCS has been introduced in an FDA guideline.

In order to decide upon appropriate borderlines between the different drug classes the BCS defines three dimensionless numbers characterizing the solubility and permeability profile of the drug compounds: the absorption number, *An*, the dissolution number, *Dn*, and the dose number, *Do*. These numbers combine the physicochemical and physiological drug properties in a simplified form, thereby allowing the assessment of the *in vivo* behaviour of the drug (Amidon et al., 1995).

Table 1.2. The Biopharmaceutics Drug Classification Scheme (Amidon et al., 1995)

	Class I	Class II	Class III	Class IV
Solubility	High	Low	High	Low
Permeability	High	High	Low	Low

The absorption number is the ratio of the mean residence time, t_{res} and the absorptive time, t_{abs}, which equals the ratio of the radius R of the intestinal tube and P_{eff}, the effective permeability:

$$An = P_{eff}/R \times t_{res} = t_{res}/t_{abs} \qquad \text{(Equ.1.2)}$$

The dissolution number can be described as the ratio of the mean residence time, t_{res}, to the dissolution time, t_{diss}, which includes the solubility, c_s, diffusivity, D, density, ρ, and the initial particle radius, r, of the drug:

$$Dn = (c_s \times 3D) / (r^2 \times \rho) \times t_{res} = t_{res} / t_{diss} \qquad \text{(Equ.1.3)}$$

The dose number is the ratio of dose concentration to drug solubility, c_s,:

$$Do = (M / V_0) / c_s \qquad \text{(Equ.1.4)}$$

with the dose M and V_0 as the volume of water taken with the dose.

Exhibiting high solubility and permeability characteristics, class I drugs are expected to display excellent bioavailability profiles unless they are susceptible to enzymatic or metabolic degradation. Examples of class I compounds include acetaminophen

(paracetamol) and metoprolol (Galia et al., 1998). They display high absorption (*An*) and dissolution (*Dn*) numbers, but the dose number (*Do*) appears to be rather small as a result of the good solubility of the drug in aqueous medium (Equ.1.2-1.4).

Class II drugs, which include compounds such as griseofulvin (Dressman et al., 1998), danazol and mefenamic acid (Galia et al., 1998) exhibit high membrane permeability but poor water solubility because of their hydrophobic molecular structure. These characteristics are reflected in a high absorption ($An \geq 6$), a low dissolution ($Dn < 1$) and a high dose number underlining that the rate of absorption of class II drugs is determined by their dissolution rate (Oh et al., 1993). The extent of the difficulties in achieving efficient solubility for class II drugs becomes apparent in the case of griseofulvin. Here, a single dose requires a fluid volume of 33 Liters for complete dissolution of the compound in the GI tract (Katchen and Symchowicz, 1967; Dressman et al., 1998). In order to increase the aqueous solubility and hence the amount of drug absorbed of class II drugs, pharmaceutical preparations often contain solubility-enhancing agents such as cosolvents or surfactants.

Like compounds belonging to class I, class III drugs have a high aqueous solubility. The permeability across the lipid barrier of the intestinal epithelium, however, is poor and hence the rate-limiting step in the absorption of class III drugs. *An* is significantly low due to low P_{eff} values and a very high absorption time. Therefore, an increase in oral bioavailability can be achieved by maximizing the contact time between the dissolved drug and the absorbing surface and/or by increasing the effective permeability with the use of penetration enhancing excipients. Neomycin B and acyclovir are examples for class III compounds as is ranitidine.

Class IV drugs with low aqueous solubility and low intestinal permeability exhibit low *An*, low *Dn* and high *Do* resulting in overall poor bioavailability. Normally, these drugs rarely reach the development stage but some drugs including taxol and frusemide (Devane, 1998) can be found on the market.

1.4.1. Gastrointestinal Transit

The transit of a dosage form through the GI tract plays a crucial role in the oral bioavailability of a therapeutic agent since it is the passage of the drug along its sites of absorption. Due to the large surface area of the lining epithelium most orally administered drugs are best absorbed from the small intestine. Therefore, the small intestinal transit time is thought to be the important figure in gastrointestinal transit with respect to the amount of drug reaching the systemic circulation. The longer a drug molecule resides in the small intestine the more time it has to be absorbed and the higher is the bioavailability of the compound.

The amount of time a drug molecule is present at its absorption site is referred to as the mean residence time of the drug (t_{res}). The importance of t_{res} for the extent of drug absorption becomes clear in Equation 1.2 and 1.3 as the absorption (*An*) and dissolution number (*Dn*) of a drug compound are directly dependent on the mean residence time (t_{res}) of the drug formulation. *An* of a drug is defined as the relation of the amount of time the drug is present at the site of absorption and the time the drug requires to be absorbed. The faster the transit through the GI tract, the lower t_{res}, the lower is *An*. The same applies for the dissolution number: if there is limited time for drug dissolution to occur less drug will be able to diffuse across the lining epithelium. Except in the case of class I compounds, drug absorption appears to be especially sensitive to changes in t_{res} and hence the small intestinal transit time.

Dressman et al. (1998) defined the fraction of drug absorbed as:

$$F_{abs} = 2\,An \,/\, Do \qquad \text{(Equ.1.5)}$$

for dissolution rate independent drug compounds, which can be transformed into Equation 1.6 visualizing the overall importance of the mean residence time:

$$F_{abs} = \frac{2\,t_{res} \times c_s \times V_0}{t_{abs} \times M} \qquad \text{(Equ.1.6)}$$

where c_s is the solubility and M the dose of the drug, V_0 the volume of water taken with the dose and t_{abs} the absorptive time.

Pharmaceutical preparations exhibiting dissolution rate independent bioavailabilities include formulations of drugs belonging to class I and III of the Biopharmaceutics Classification Scheme (BCS). These drugs are highly soluble in aqueous media ruling out that drug solubility and dissolution are the rate-limiting step in their absorption. A different situation arises for class II compounds, which exhibit poor aqueous solubility and are, therefore, prone to develop dissolution rate controlled bioavailabilities. However, Equation 1.6 will also be valid for class II compounds when the drug is administered as an oral solution or soft gelatin capsule, which contains the active agent completely dissolved in the formulation. In such cases, the fraction of drug absorbed will be independent of the dissolution rate.

The specific sensitivity of class II and III compounds with respect to t_{res} can be seen in Equation 1.6. For class II compounds the solubility, c_s, is very small resulting in low F_{abs} values. An increase in t_{res} is, therefore, expected to lead to an increase in their bioavailability particularly when the dose is comparatively high. A similar situation can be seen for class III drugs, which exhibit high t_{abs} values due to their poor permeability. With Equation 1.6 the direct dependency between the fraction of drug absorbed and t_{res} or small intestinal transit time has been established. An accelerated transit through the small intestine could, therefore, lead to a significant decrease in the bioavailability of the therapeutic agent and as a result jeopardising the success of the treatment.

This effect has been recognized for a long time and warnings are given out for the simultaneous administration of laxatives with drugs such as antibiotics and contraceptives. Osmotic laxatives are non-absorbable macromolecules, which maintain an increased amount of fluid inside the lumen of the small intestine in an attempt of the body to compensate for the high osmotic pressure (Riley et al., 1992). An increased osmotic load of the GI tract results in increased bulk, which stimulates peristalsis and as a result induces the acceleration of the transit of the gut contents through the small intestine. Although sorbitol, which is employed in pharmaceutical preparations mainly as a sweetener but also acts as a cosolvent, has been shown to cause diarrhoea when administered in high doses (Charney and Bodurtha, 1981), there are only a few studies, which have investigated the effect of pharmaceutical excipients on GI transit. An acceleration of small intestinal transit in healthy volunteers has been found for excipients

employed in effervescent dosage forms such as sodium acid pyrophosphate (SAPP) and mannitol (Koch et al., 1993; Adkin et al., 1995a,b). The enhanced passage through the small intestine in the presence of SAPP or mannitol resulted in a significant reduction in the oral bioavailability of the coadministered class III model drugs ranitidine and cimetidine, respectively (Koch et al., 1993; Adkin et al., 1995c). Furthermore, polyethylene glycol 400 (PEG 400), a commonly used solubilizing agent, was observed to cause a 35-37 % decrease in the small intestinal transit time of oral liquids when administered at an amount of 10 g to human subjects (Basit et al., 2001; 2002). As a result, ranitidine oral bioavailability was significantly reduced in the presence of PEG 400 by 31 % compared to the control solution of drug in water.

For many years, pharmaceutical excipients have been accepted as pharmaceutically inert with respect to gastrointestinal transit but now, it appears that this is not necessarily true in all cases. In the preceding paragraphs it has been established that there is a direct dependency of the oral bioavailability of class II and III compounds on the mean residence time of the drug, t_{res}, and hence a moderate passage rate through the small intestine. However, it is these drug compounds, in particular, that require the addition of often-high amounts of pharmaceutical excipients enhancing the solubility and/or permeability.

An example for such adverse effects of pharmaceutical excipients is the anti-HIV preparation Agenerase®, which is on the market as an oral solution and a soft gelatin capsule formulation. The active agent is amprenavir, a protease inhibitor that has been classified as a class II compound with poor aqueous solubility and good intestinal permeability. The solution and the capsule formulation, however, have been found not to be bioequivalent as amprenavir exhibits a 14 % lower bioavailability from the oral solution compared to the soft gelatin capsule (BNF, 2003). The major difference between the two dosage forms is their content of solubility enhancing agents. According to the manufacturer GlaxoSmithKline a maximum single dose of the capsule treatment (eight capsules) contains approximately 1 g PEG 400, 2.2 g D-α-tocopheryl-polyethylene glycol 1000-succinate (VitE-TPGS), a solubilizing surfactant, and 0.4 g propylene glycol, whereas a single dose of Agenerase® oral solution (60 mL) contains 10 g PEG 400, 7 g VitE-TPGS and 33 g propylene glycol. The reduced bioavailability of

amprenavir from the oral solution may well be the result of an accelerated transit of the formulation through the GI tract due to the presence of 10 g PEG 400 (Basit et al., 2002). However, the solubilizing agents VitE-TPGS and propylene glycol are also contained to a considerably high degree in the solution and unlike PEG 400, their effect on GI transit has not yet been investigated. The particularly high content of propylene glycol has recently been recognized as potentially toxic to children under the age of four, pregnant women and patients requiring chronic exposure. As a result GlaxoSmithKline published a toxicity warning of propylene glycol suggesting that the treatment Agenerase® oral solution is only to be given to patients in cases that do not allow the administration of the capsule treatment. Although the addition of solubilizing agents to formulations of poorly soluble drugs is intended to achieve an enhancement in oral bioavailability additional adverse effects need to be considered especially in cases when high amounts of such excipients are employed.

On the other hand, it would be highly desirable to develop a dosage form exhibiting prolonged residence times in certain parts of the GI tract. One research strategy focuses on the adsorption of polymers to the intestinal mucosa – namely bioadhesive drug delivery systems (Carino et al., 1999). In addition, an increase in gastric residence time can be achieved by the inclusion of gases in solid dosage forms, which cause the formulation to float on the stomach contents resulting in a delaying of emptying into the small intestine (Thanoo et al., 1993). Yet another approach is to exploit the body's own pace-making systems with initiating, for instance, the ileal-brake. The ileal-brake serves as a feedback mechanism of the body. Activated with the ingestion of fat and/or proteins it slows down the passage of luminal contents through the GI tract to allow for sufficient digestion. Perfusing the lower small intestine with a lipid emulsion was found to slow down the transit of a test meal in healthy volunteers (Spiller et al., 1984). Various studies have been performed since, investigating the effect of fatty acids such as oleic acid. In patients with chronic diarrhoea oleic acid was found to significantly delay bowel movement almost 3-fold when administered at a dose of 3.2 mL (Lin et al., 2001). A prolonged small intestinal transit was also obtained for solid oral dosage forms (Dobson et al., 1999). The effects, however, were only found at high doses of oleic acid (1200 mg) and showed considerable variability between subjects. However, apart from the ileum it has also been demonstrated that this braking mechanism can be activated along

the entirety of the small intestine (Dobson et al., 2000). Recently, mixtures of medium chain glycerides including Labrasol® and Capmul® MCM have been introduced to pharmacopoeial standard. As surfactants these excipients are mainly employed for the solubilization of hydrophobic drugs but they also exhibit permeation-enhancing effects on poorly absorbable compounds. Chemically, this promising class of solubilizers are fat digestion products as they contain mono-, di- and triglycerides of fatty acids. It has yet to be proven whether an employment of these excipients allows a slowing of GI transit via an initiation of the body's braking mechanism.

The mean residence time of a drug compound at its absorption site can be regarded as a key figure in the overall absorption of the drug. Some excipients have been found to have a significant influence on the transit of pharmaceutical preparations through the GI tract with the consequence of decreasing the oral bioavailability of therapeutic agents. Since excipients such as solubilizers are commonly employed in the design of oral liquid formulations and often at high amounts, an investigation and characterisation of solubilizing agents with respect to their impact on small intestinal transit times is suggested to be of considerable significance.

1.4.1.1. Measurement of gastrointestinal transit times

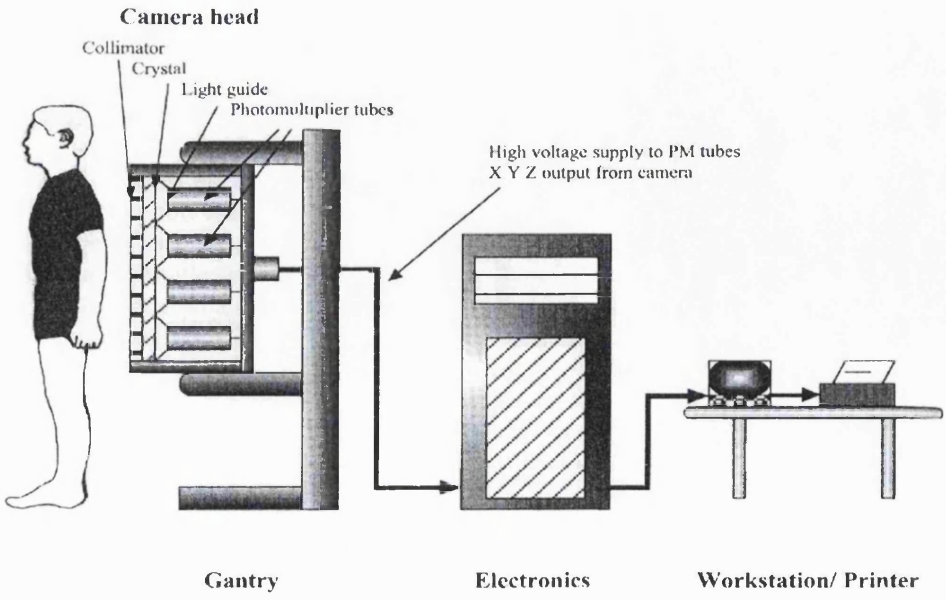
Over the last century several methods have been developed and employed to assess the GI transit time of oral dosage forms and foodstuffs. Among the most commonly used techniques to determine the transit of orally administered liquids are hydrogen breath analysis and gamma scintigraphy. The former involves the co-administration of unabsorbable carbohydrates such as lactulose, which will be rapidly fermented by anaerobic bacteria once the formulation enters the colon. During fermentation hydrogen is released, which after diffusion into the systemic circulation is exhaled in the breath, where a rise in hydrogen concentration can be detected signalling the arrival of the preparation in the colon. Although the hydrogen breath test is very sensitive, non-invasive, inexpensive and easy to perform it comprises of certain disadvantages. One major factor is, for instance, that this method does not detect the emptying of the formulation from the stomach as it only measures the mouth to caecum transit time. As a result it is not a useful tool to determine the time a preparation spends in the small intestine. Additionally, in cases of overgrowth of the small bowel by colonic bacteria the

hydrogen breath concentration increases too early leading to an underestimation of the time the preparation reaches the colon. Another disadvantage of this technique is the dependence on the ingestion of unabsorbable carbohydrate, which itself may alter GI transit. Lactulose, for instance, has been shown to have an accelerating effect on the passage of a meal through the GI tract (Read et al., 1980).

A superior technique has been found in gamma scintigraphy, which allows visual monitoring of oral dosage forms *in vivo*. With introducing a gamma-emitting radionuclide into the formulation a gamma camera records the distribution of radioactivity in the body creating scintigraphic images. Although this approach has been used for many years by GI physiologists an application for investigating the *in vivo* fate of pharmaceutical preparations was only reported in 1976 (Alpsten et al.; Casey et al.). Over the last few decades this application has been developed and its invaluable role in pharmaceutical research has been established (Wilson and Washington, 1988; Davis et al., 1992; Wilding et al., 2001).

Following the ingestion of a radiolabelled dosage form the human or animal subject is positioned upright in front of the gamma camera or, in a supine position (Fig.1.2).

Figure 1.2. Schematic diagram of gamma camera and computer system (Perkins, 1999).



The detector of a gamma camera can either be of a single variety or dual variety comprising of two detector heads that allow simultaneous acquisition of anterior and posterior images. The detector, which is shielded in lead to reduce the ingress of background radiation, consists of a large crystal of thallium activated sodium iodide, ($NaI_{(Tl)}$). On the face of the crystal a lead collimator is mounted comprising of specific holes to channel the arriving gamma rays onto the detector crystal, hence allowing the projection of an image of the radioactive source onto the crystal. Absorbed gamma photons produce visible light pulses, which are collected by an array of photomultiplier tubes coupled to the crystal by means of a light guide. Here, the light pulses are converted into electronic signals, which are recorded by the image forming electronics. The relative strengths of the photomultiplier signals allow the determination of the accurate position and intensity of the gamma radiation. An electronics package links the detector to a computer for storing and processing of the obtained images.

To choose a suitable radionuclide for the incorporation into the dosage form certain factors are taken into consideration such as the radiation energy, half-life, extent of particulate radiation, cost and availability. The most commonly employed radionuclide is technetium-99m (^{99m}Tc) as a result of its versatile chemistry, low radiation dose and short half-life of 6 hours. It also possesses a gamma ray energy of 140 keV, which is close to ideal for radionuclide imaging. This is due to the fact that the gamma rays are readily detected by the gamma camera (detection range 100-200 keV) but not easily absorbed by the participating subject. An additional advantage of ^{99m}Tc is that it is readily available through the use of portable generators. Other radionuclides such as indium-111 (^{111}In) are also commonly employed, for instance when an additional radionuclide is required (Davis et al., 1984b; Basit et al., 2001) or one with a longer half-life (67 hours). Incorporation of the radionuclide into the oral preparation is generally undertaken after the radionuclide has been bound to either an ion-exchange resin or a non-absorbable chelating agent such as diethylenetriaminepentaacetic acid (DTPA). These approaches provide that the radionuclide remains associated with the dosage form throughout the passage through the GI tract or that it is released in the same manner as the drug itself without being absorbed into the systemic circulation. These conventional methods of radiolabelling require the radionuclide to be incorporated as late as possible to minimize the handling of radioactive material, which might prove difficult if complex

dosage forms such as an enteric-coated tablet is to be labelled. These problems can largely be overcome by incorporating stable isotopes such as samarium-152 and erbium-170 into the formulation, which will be converted into their gamma-emitting radioactive forms samarium-153 and erbium-171 by exposure to a neutron flux (Parr et al., 1985; Digenis and Sandefer, 1991). This approach of neutron activation offers a safe and easy method of efficiently radiolabelling more complex delivery systems.

Despite the many advantages of gamma scintigraphy there are also limitations. Since the technique provides little or no anatomical detail it is often difficult to accurately locate the position of the dosage form within the body unless the preparation outlines the GI organs. In addition, the movement of a radioactive source backwards and forwards in the stomach and intestines causes problems in quantification. In a gamma scintigraphic study planar images are acquired, which do not account for the fact that the GI tract does not lie in a vertical plane throughout its entirety since the radioactivity near one of the detectors is detected more efficiently by way of less attenuation. This complication can largely be overcome by calculating the geometric mean of the anterior and posterior detector counts, thus giving a value, which is virtually independent of changing depth of source (Tothill et al., 1978; Hardy and Perkins, 1985). In recent years, new imaging techniques including single photon emission computed tomography (SPECT), positron emission tomography (PET) and magnetic resonance imaging (MRI) have emerged, which allow a three-dimensional visualization of the body and as a result their potential has been recognized to add to the diagnostic information provided by nuclear medicine. At present, however, the chance of a regular application of these techniques for the assessment of drug delivery is small due to the major disadvantages of these methods, which include high costs and high radiation doses as well as long imaging times.

Despite its limitations gamma scintigraphy remains the imaging technique of choice for evaluating the *in vivo* fate of pharmaceutical dosage forms (Newman and Wilding, 1999; Newman et al., 2003). In particular, the combination of scintigraphy with pharmacokinetic studies (pharmacoscintigraphy) has established itself as an invaluable means of providing information about transit and release behaviour of oral dosage forms and subsequent drug absorption (Wilding et al., 2001).

1.5. Solubilizing Agents

The process of solubilization may be best defined as the procedure whereby comparatively high amounts of poorly soluble substances are brought into aqueous solution. Pharmaceutical excipients that are capable of a significant solubility enhancement of poorly soluble compounds are referred to as solubilizers or solubilizing agents. With respect to the differences in the solubilization-mechanism, solubilizing agents can be categorized into three different groups, namely complexants, cosolvents and surfactants.

1.5.1. Complexants

Complexants are hydrophilic molecules, which undergo a chemical reaction with the poorly soluble drug compound forming an intermolecular complex of increased aqueous solubility. This complex formation is specific and must be reversible in order to release the therapeutic agent and guarantee efficient and unimpeded absorption. Mainly water-soluble macromolecules such as polyvinylpyrrolidone, polyethylene glycols and cyclodextrins, and excipients like sodium salicylate and benzoate are used as complexing agents and their solubilizing qualities have been proven for various drugs (Doherty and York, 1989; Law et al., 1992; Tenjarla et al., 1998).

1.5.2. Cosolvents

Unlike complexants, cosolvents operate in an unspecific way with respect to the solute compound as they interfere directly with the solvent itself. Water is known to constitute of so-called clusters (Nemethy and Scheraga, 1962), which are relatively rigid associations of water molecules accomplished with strong hydrogen bonds between individual water molecules. The cluster structure is held responsible for the characteristic solubility properties of water such as high polarity and high surface tension, which results in a low wettability as well as a low dissolution potential of less polar compounds. Cosolvents are thought to be able to penetrate into the aqueous clusters and disturb the inflexible water structures by interfering with the strong water/water interactions (Yalkowsky, 1999). The inclusion of cosolvents into the water associations affects the polarity of the solvent system. Increasing concentrations of cosolvents decrease the dielectric constant of the solvent system thereby reducing the strong dipole

characteristics of pure water, which appears to result in an enhanced dissolution and solubility of non-polar, poorly soluble drugs (Chellquist and Gorman, 1992). Solubilization by cosolvency is highly efficient and easy to achieve and therefore very often considered in the early stages of drug development (Li et al., 1999). The combination of certain cosolvents may increase the solubility of some drugs better than the individual cosolvent alone (Pramar and Gupta, 1994). This so-called synergistic effect also seems to work for the combination of cosolvents with surfactants (Hamza and Kata, 1990).

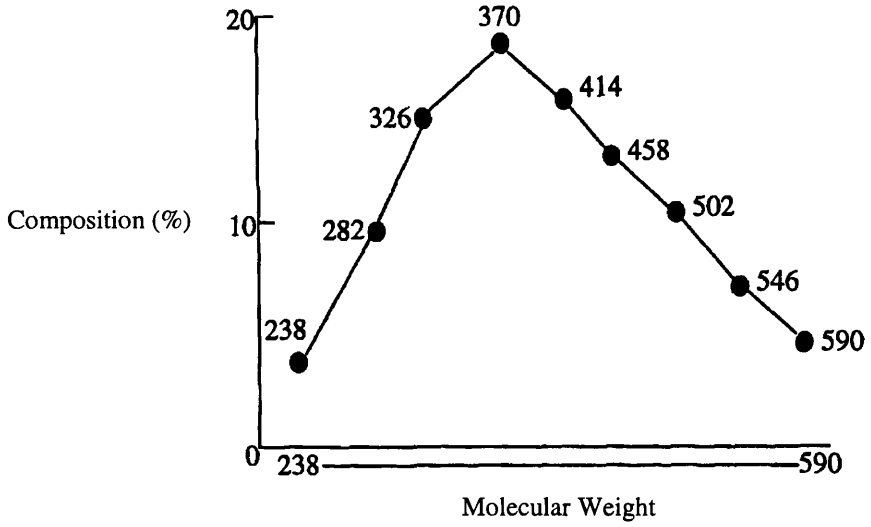
Safe and convenient administration of cosolvents seems to be limited at extremely high concentrations as toxicity and irritancy may occur (Spiegel and Noseworthy, 1963; Kelner and Bailey, 1985) or the final formulation becomes too bitter and viscous. Nevertheless, cosolvents are declared to be very safe over a wide range of concentrations in pharmaceutical use, thus, they are employed in approximately 10% of FDA approved parenteral products (Sweetana and Akers, 1996). In addition, cosolvency is particularly popular in the formulation of poorly water-soluble drug compounds in the soft gelatin capsule technology (Shelley, 1996). Examples of commonly used cosolvents include ethanol, glycerol, sorbitol, polyethylene glycol and propylene glycol. Two of the most popular cosolvents are polyethylene glycol 400 (PEG 400) and propylene glycol, which will be discussed in more detail.

1.5.2.1. Polyethylene glycol 400

Polyethylene glycol 400 (PEG 400) comprises a mixture of at least nine homologous polymers of ethylene glycol with the number of ethylene oxide units, n, ranging from 5 to 13. All nine PEG compounds in PEG 400 have a molecular weight range from 238 to 594 Da following a near gaussian distribution about an average molecular weight of 400 Da (Fig. 1.3).

PEG 400 is highly water-soluble, hygroscopic and non-toxic and like other low molecular weight PEGs it is liquid at ambient temperatures and therefore most commonly used in soft gelatin capsule formulation, as well as pharmaceutical oral liquids and parenteral dosage forms (Yalkowsky and Roseman, 1981).

Figure 1.3. Composition of PEG 400, illustrating the approximate gaussian distribution of the different polymers (adapted from Chadwick et al., 1977a).



Over the years many investigators have demonstrated the solubilizing qualities of PEGs and a closer look at recent literature reveals that PEGs continue to be of interest for the enhancement of drug solubility in aqueous medium. For drugs like allopurinol (Hamza and Kata, 1990), chlorthalidone (Williams et al., 1982), spironolactone (Pramar and Gupta, 1994) and stachyflin (Yagi et al., 1999), PEG 400 has been shown to successfully increase the solubility and thereby the bioavailability.

After oral administration PEG 400 itself is absorbed from various regions in the GI tract, e.g. the jejunum, ileum and colon (Fordtran et al., 1965). The permeation rate of PEG 400 was found to be directly dependent on the amount of PEG 400 present at the site of absorption leading to the assumption that passive diffusion is involved in the absorption of PEG 400 (Krugliak et al., 1989). A few years later, Ma et al. (1993) were able to prove that PEG 400 makes use of paracellular absorption pathways. Besides the employment as a cosolvent PEGs are also used in pharmaceutical research and dosage form design for purposes such as permeability probes and paracellular markers (Chadwick et al., 1977a; Artursson et al., 1993).

1.5.2.2. Propylene Glycol

Propylene glycol, chemically known as 1,2-propanediol, is another of the most frequently used cosolvents. It is inexpensive, stable, non-toxic and well tolerated and therefore preferentially employed in the formulation of parenteral dosage forms (Yalkowsky and Rubino, 1985). Propylene glycol is also commonly used in oral preparations, even though the propylene glycol concentrations in soft gelatin capsules must not exceed 5-10 % in order to prevent softening of the capsule shell caused by propylene glycol migration into the gelatin.

Besides its qualities as a cosolvent, propylene glycol is used as a penetration enhancer for the percutaneous absorption of drugs like melatonin (Lee et al., 1997). Its solubilizing qualities, however, were found to be dependent on the polarity of the solute: the more hydrophobic the drug, the greater the increase in solubility (Gould et al., 1984; Yalkowsky and Rubino, 1985). The aqueous solubility of oxfenicine, a polar hydrophilic drug, for instance, was markedly reduced in the presence of the cosolvent while thioconazole had an enhanced solubility in propylene glycol-water systems (Gould et al., 1984). The exquisite solubilizing qualities of propylene glycol, however, have been shown for many drugs including chloramphenicol (Rogers and Nairn, 1973), alpha styryl carbinol antifungal agents (Maurin et al., 1993), parabens (Darwish and Bloomfield, 1995), a Vitamin D_2 analogue (Stephens et al., 1999) and flavopiridol (Li et al., 1999). Propylene glycol was found to exhibit synergistic effects in mixtures with other additives such as a castor-oil derivative or Tween® 80 (Sugahara et al., 1995).

1.5.3. Surfactants

Surface-active agents or surfactants are substances, which alter the conditions prevailing at the interface of lipophilic and a hydrophilic region causing a marked decrease in the surface tension. An amphiphatic chemical structure comprising a hydrophobic group such as a hydrocarbon chain and a hydrophilic group such as an ion or a group with a large permanent dipole are the pre-requisites of surfactants allowing them to concentrate at hydrophilic/lipophilic interfaces, e.g. water/air or water/solute.

With increasing concentrations the surfactant molecules tend to aggregate together in order to remove the hydrophobic groups from the aqueous phase and hence achieve a state of minimum free energy. These aggregations of amphiphatic molecules, where the hydrophilic head groups interfere with the aqueous solvent and the lipophilic regions are located in the core, are better known as micelles and the surfactant concentration at which micelle formation begins is referred to as the critical micelle concentration (CMC).

Besides the employment as solubilizing agents, surfactants are established in a wide variety of fields as emulsifying agents, detergents, wetting agents, foaming and antifoaming agents, flocculants and deflocculants.

Surfactants have been classified into four different groups according to the nature of the ionic type of the hydrophilic group: anionic, cationic, nonionic and ampholytic. Due to their low toxicity and irritancy on the GI tract, the nonionic surfactants have proven to be the most suitable for orally administered drug formulations and in some cases even for parenteral preparations. Compared to anionic or cationic emulgents nonionic surfactants also have the advantage of a higher degree of compatibility with other materials and they are less sensitive to changes in pH or the addition of electrolytes. For the characterization of amphiphatic molecules the hydrophile lipophile balance (HLB) value was introduced, which describes the relative polarity of the surfactant. HLB values range from 0 to 20 where the higher the HLB the more hydrophilic the surfactant.

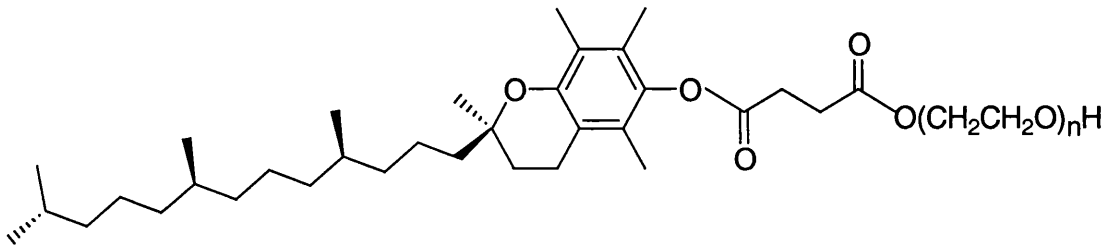
Three newly introduced surfactants, namely vitamin E-TPGS, Labrasol® and Capmul® MCM were investigated in the course of the project and are discussed in more detail below.

1.5.3.1. Vitamin E-TPGS

D-α-Tocopheryl polyethylene glycol 1000 succinate (Figure 1.4), also known as vitamin E-TPGS (VitE-TPGS), is a pale yellow waxy solid of a molecular weight of 1536 Da, which is prepared from crystalline D-α-tocopheryl acid succinate by esterification of the acid group with polyethylene glycol 1000 (PEG 1000). VitE-TPGS is a water-soluble derivative of natural-source vitamin E and its monograph was recently adopted by the

United States Pharmacopoeia as a new excipient (USP, 1998). Having an acute LD50 value of >7 g/kg in young adult rats (Krasavage and Terhaar, 1977) VitE-TPGS is considered safe and non-toxic for the use in humans even at a dose of 1.0 g/kg/day (Wu and Hopkins, 1999).

Figure 1.4. Molecular structure of D-α-Tocopheryl polyethylene glycol 1000 succinate (VitE-TPGS).



n = 22 (av.)

The chemical structure shows the characteristics of a typical surface-active agent, comprising the dual nature within the molecule consisting of a hydrophilic polar head, provided by the succinate and polyethylene glycol portion, and a lipophilic tail composed of the tocopherol portion. The HLB value for VitE-TPGS has been determined to be 13.2 and as a non-ionic surfactant VitE-TPGS was found to be suitable as a solubilizing agent (Ismailos et al., 1994). The amphiphilic character of the molecule leads to its self-association in water and micelle formation occurs when the surfactant concentration exceeds 0.02 % (w/w), the CMC of VitE-TPGS in water. With increasing concentrations VitE-TPGS was observed to form relatively low-viscosity solutions with water until a concentration of 20 % (w/w) is obtained, beyond which liquid crystalline phases may form.

The tocopherol contained in VitE-TPGS was found to be readily absorbed in the gastrointestinal tract even in the absence of detectable bile flow (Traber et al., 1986), since VitE-TPGS functions as a bile salt substitute owing to its amphiphatic nature (Ismailos et al., 1994). But there have been controversial reports in the literature about the fate of PEG 1000 of the VitE-TPGS molecule in the GI tract. Sokol et al. (1987), for instance, only found a small amount (< 5%) of PEG 1000 to be absorbed and promptly

excreted in the urine, whereas a study by Traber et al. (1988) on human cells demonstrated the complete uptake of the intact VitE-TPGS complex into the cell where it is then hydrolyzed to release vitamin E into the cytosol.

The high solubilizing qualities of VitE-TPGS have been demonstrated to enhance the bioavailabilities of various poorly soluble and/or poorly absorbable compounds including cyclosporine A (Sokol et al., 1991; Boudreaux et al., 1993), Vitamin D (Argao et al., 1992), and amprenavir, a protease inhibitor (Yu et al., 1999). Apart from micellar solubilization of poorly soluble drug compounds VitE-TPGS was also found to have a profound effect on polarized efflux transport in the GI epithelium via an inhibition of P-glycoprotein (Dintaman and Silverman, 1999; Yu et al., 1999).

1.5.3.2. Labrasol®

Labrasol® has recently been introduced as new pharmaceutical surfactant and reached European Pharmacopoeial standard in 1998. Also known as caprylcaproyl macrogolglyceride Labrasol® is a mixture of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethylene glycol (PEG). In detail it was found to contain saturated polyglycolysed C_6 to C_{10} glycerides, where caprylic acid (C_8) is 58.1 % and capric acid (C_{10}) is 39.8 %. Characterisation of Labrasol® using NMR indicated a mixture of 30 % mono-, di- and triglycerides of C_8 and C_{10} fatty acids, 50 % of mono- and di-esters of PEG and 20 % of free PEG 400 (Kreilgaard et al., 2000). Labrasol® is obtained from coconut oil and comprises of high tolerance and low toxicity, which makes it suitable as pharmaceutical ingredient. Its LD_{50} was determined to be 22 g/kg in rats.

As a mixture of non-ionic surfactants with an HLB of 14, Labrasol® was originally developed for the solubilization of hydrophobic drugs. Very recently, *in vitro* and *in situ* it has been found to also comprise of permeability enhancing properties for drugs such as gentamicin (Hu et al., 2001), vancomycin (Prasad et al., 2003), cephalexin (Koga et al., 2002) and insulin (Eaimtrakarn et al., 2002). It is employed in transdermal drug delivery systems as well as self-emulsifying or self-microemulsifying drug delivery systems.

1.5.3.3. Capmul® MCM

Capmul® MCM is also described as glyceryl caprylate/caprate as it comprises of a mixture of mono- and diglycerides of medium chain length fatty acids, which are primarily caprylic (C_8) and capric acid (C_{10}), as well as free glycerol.

If administered orally, Capmul® MCM is non-toxic (LD_{50} > 5 g/kg in rats) and has been generally recognized as safe according to FDA standards. Excellent oxidative stability is obtained due to the lack of unsaturated fatty acids. As a partial ester of glycerol Capmul® MCM exhibits the characteristics of a water-in-oil (w/o) emulsifier. With a lipophilic structure, Capmul® MCM is insoluble in water and soluble in oil at elevated temperatures and possesses a HLB value of about 5.

Capmul® MCM has been used as a surface-active agent in self-emulsifying drug delivery systems (SEDDS), which have recently been shown to improve the dissolution and absorption of lipophilic drugs (Pouton, 1985; Charman et al., 1992; Shah et al., 1994). SEDDS are formulated in the absence of water by mixing an oil with a non-ionic surfactant and a lipophilic drug to give a clear and transparent isotropic solution. When exposed to aqueous media SEDDS form oil-in-water microemulsions with uniform fine particle size droplets of less than 3 μm (Charman et al., 1992). Fine oil droplets may be able to empty rapidly from the stomach, distribute throughout the GI tract and provide a large surface area for drug partitioning. Additionally, bile salts are secreted in the presence of fatty acids and monoglycerides and may contribute to the absorption enhancement of drugs formulated with medium chain glycerides such as Capmul® MCM or Labrasol® (Kakemi et al., 1970). Class II and Class IV compounds of the BCS are expected to profit significantly from improved solubilization and dissolution in the presence of medium chain glycerides and exhibit enhanced bioavailabilities (Constantinides, 1995). However, Capmul® MCM, with an HLB value of 5, was shown to be not hydrophilic enough to provide for an efficient SEDDS and, therefore, it required the presence of an auxiliary non-ionic surfactant with a higher HLB to attain a more hydrophilic environment (Shah et al., 1994).

In addition to the solubility-enhancing properties, medium chain glycerides have the potential to increase the membrane permeability of poorly absorbable drugs. Controversy

exists about the mechanism behind this permeation-enhancing effect. Several processes have been reported to occur in the presence of these excipients including dilatation of tight junctions (Anderberg et al., 1993), inhibition of efflux carrier (Hu et al., 2001) and interactions with membrane phospholipids causing defects in the bilayer structure (Muranushi et al., 1981; Swenson and Curatolo, 1992). It is suggested that the exact mechanism may vary between the different mixtures of medium chain glycerides, e.g. Labrasol® and Capmul® MCM, and is likely to be a combination of different effects. Medium chain glycerides have also been employed in formulations of w/o-microemulsions, which undergo phase inversion *in vivo* to release the encapsulated drug (Ritschel, 1993). The lipid-based microemulsion was found to protect the hydrophilic active ingredient against enzymatic hydrolysis, which is known to be crucial for the delivery of peptide drugs.

1.6. Scope and purpose of the study

Solubilizing agents are common additives in the development and design of oral liquid formulations. On a regular basis, high amounts of solubilizers are employed in early phase I studies but some preparations may also reach the market as was highlighted in the case of Agenerase® oral solution (chapter 1.4). For many years, however, solubilizers have been more or less officially accepted as inert. Specific recommendation, for instance, was given by the U.S. Food and Drug Administration (FDA) for the use of a solution of chlorthalidone in polyethylene glycol (PEG) as a bioavailability reference standard for conductance of bioequivalence studies (FDA, 1979). In more recent years, a mixture of VitE-TPGS and propylene glycol has been suggested as a universal oral formulation for the early stages of drug discovery for insoluble compounds (Tong et al., 1998). Drug preparations like Agenerase® oral solution among others employing considerably high amounts of solubilizing agents have been found to result in unexpectedly low bioavailabilities of the active agent. One ingredient, the cosolvent PEG 400, was shown to have a significant accelerating effect on the passage rate of drug preparations through the small intestine when administered at a dose of 10 g, which subsequently caused a decrease in the absorption of the model compound ranitidine (Basit et al., 2001; 2002). The importance of efficiently long residence times of the drug at its absorption site has been demonstrated earlier. The purpose of the thesis was to

investigate and characterize certain types of solubilizing agents with respect to their effects on GI transit and, consecutively, determine the impact of transit alterations on the absorption of model drugs.

The objectives were, therefore, set to be the following:

- Characterization of the employment of PEG 400 in oral solutions with respect to dose-dependency of the effects on GI transit and ranitidine absorption

- Investigation of GI transit accelerating or prolonging effects of other solubilizing agents including propylene glycol, VitE-TPGS and medium chain glycerides

- Characterization of such effects with respect to an impact on the oral bioavailability of various model drugs

- Evaluation and characterization of the use of canines as a model for human subjects regarding GI transit of oral drug solutions, the employment of pharmaceutical excipients and their effects on drug absorption

This study was, therefore, primarily concerned with monitoring GI transit times of oral drug solution in the presence of different types and amounts of solubilizing agents using gamma scintigraphy and pharmacokinetic analysis (pharmacoscintigraphy).

CHAPTER 2

CONCENTRATION-DEPENDENT EFFECTS OF POLYETHYLENE GLYCOL 400 (PEG 400) ON GI TRANSIT AND DRUG ABSORPTION IN HUMANS

2.1. Overview

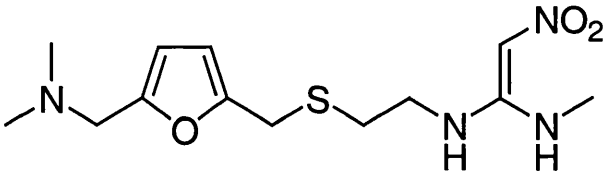
The transit time of a pharmaceutical preparation through the small intestine appears to be a crucial factor in the absorption process of many therapeutic agents, as described in more detail earlier. In the last decade, pharmaceutical excipients employed in effervescent dosage forms, such as sodium acid pyrophosphate (SAPP) and mannitol, have been found to have an accelerating effect on small intestinal transit causing a concomitant reduction in oral drug bioavailability (Koch et al., 1993; Adkin et al., 1995a,b,c). Other excipients that have generally been considered as inert, including the cosolvent polyethylene glycol 400 (PEG 400), have also been observed to affect GI transit of oral liquid formulations. Hansford et al. (1980) discovered in an *in vivo* study in rabbits that griseofulvin, a class II compound, was significantly less bioavailable from an oral solution in PEG 400 than from a powder-filled hard gelatin capsule, and the authors attributed these unexpected results to a transit effect of the polymer rather than precipitation of the drug in the GI fluids. These findings have recently been verified by Basit et al. (2001, 2002), who found a significant acceleration of small intestinal transit of an oral liquid in the presence of PEG 400 when administered at a dose of 10 g. The observed reduction in the mean small intestinal transit time (MSITT) was 35-37 % and resulted in a concomitant 31 % reduction in the oral bioavailability of the model drug ranitidine. 10 g of PEG 400 is generally a high amount of cosolvent to be administered in a single dose of an oral liquid. However, it is contained in Agenerase® oral solution, which is a marketed product for the treatment of HIV and AIDS (acquired immuno-deficiency syndrome). But Agenerase® oral solution, proven to exhibit a 14 % lower bioavailability of the active agent amprenavir than the soft gelatin capsule, is rather exceptional for a marketed drug product with regard to the high content of PEG 400 and other solubilizing agents (propylene glycol 33g, VitE-TPGS 7 g). Such excessive amounts of solubilizing excipients are instead regularly employed in phase I studies or studies of early drug development.

In marketed products, the concentration of solubilizers is often considerably lower. Therefore, it was regarded as worthwhile to investigate the effect of lower amounts of PEG 400 on GI transit and establish a dose dependency. The present study was designed to employ oral solutions containing 0, 1, 2.5 or 5 g PEG 400, which are cosolvent

concentrations more likely to be found in an average single dose of a marketed oral liquid dosage form including soft gelatin capsules. These increasing doses of PEG 400 were also chosen with the objective to find the cut-off concentration above which PEG 400 significantly affects small intestinal transit times as well as to establish a transit pattern of the cosolvent. Furthermore, the dose-dependent effects of PEG 400 on drug absorption were investigated using the H_2-receptor antagonist ranitidine (Fig.2.1). Ranitidine was considered to be the model compound of choice since, as a class III drug, it is poorly absorbed from the GI tract and its use also allowed the comparison of the results with the previous studies.

The *in vivo* performance of the drug solutions within the GI tract was monitored using the non-invasive technique of gamma scintigraphy.

Figure 2.1. Molecular structure of ranitidine.



2.2. Materials

Ranitidine

The drug was provided in the form of ranitidine hydrochloride from GlaxoSmithKline (Ware, Hertfordshire, U.K.), and certified as 99.9 % pure. It is a white to pale yellow, practically odourless, crystalline powder sensitive to light and moisture.

Polyethylene glycol 400

PEG 400 was obtained from Sigma-Aldrich Company (Poole, U.K.). It is a clear, colourless, highly osmotic (2000 mOsm), and viscous liquid with a slight but characteristic odour and a bitter and slightly burning taste.

Technetium-99m

The radionuclide ^{99m}Tc was eluted daily at The Middlesex Hospital (London, U.K.), from a commercial generator (Model 111, Elumatic, Cis, France), and obtained as a sterile pyrogen free solution of sodium pertechnetate ($Na^{+}\ ^{99m}TcO_4^{-}$) in saline.

Diethylenetriaminepentaacetic acid

DTPA (TechneScan®) was purchased from Mallinckrodt Medical B.V. (Petten, Holland). One vial contains 25.0 mg calcium trisodium diethylene-triamine pentaacetate, 0.21 mg tin (II) chloride, gentisic acid, sodium chloride and nitrogen. The sterile pyrogen free powder is sensitive to light and should be stored at 15-25 °C. Once the product is labelled with ^{99m}Tc it is stable for 8 hours and should be stored at 2-8 °C.

2.3. Methods

2.3.1. Dosage forms

2.3.1.1. Preparation of drug solutions

168 mg ranitidine hydrochloride, equivalent to 150 mg ranitidine base, was dissolved in 150 mL of water. Then either 0, 1, 2.5 or 5 g PEG 400 was added and the solutions were stirred to mix.

2.3.1.2. Osmolality of drug solutions

The osmotic pressure of the drug solutions was measured using a 3D3 Advanced Osmometer (Advanced Instruments Inc., Norwood, MA, USA). All drug preparations indicated to be of hypotonic nature: 0 g PEG 400 (4 mOsm kg^{-1}); 1 g PEG 400 (25 mOsm kg^{-1}); 2.5 g PEG 400 (50 mOsm kg^{-1}); 5 g PEG 400 (102 mOsm kg^{-1}).

2.3.1.3. Radiolabelling of drug solutions with technetium-99m

About 2-3 mL of the sodium pertechnetate ($Na^{+}\ ^{99m}TcO_4^{-}$) in saline solution was injected into the DTPA containing vial, which was then thoroughly shaken for the formation of chelate binding. To achieve an average radioactivity of approximately 100 MBq in 5 mL, the solution was appropriately diluted with additional saline. The prepared ^{99m}Tc/DTPA-solution was added drop wise to the drug solutions until a radioactivity of

7 ± 1 MBq was reached. The radioactivity of the solutions was measured using an isotope assay calibrator (model 270, Pitman Instruments, Surrey, UK). After radiolabelling, the drug solutions were stirred to achieve homogeneous mixing.

2.3.1.4. *In vitro* testing of technetium-99m binding to DTPA

For the use of ^{99m}Tc in scintigraphic transit studies, the stability of the chelate complex with DTPA within the GI tract needs to be ensured over an extended period of time. Before and after each study day the extent of chelate binding was investigated using thin layer chromatography (TLC). With this method, two possible instabilities of the ^{99m}Tc/DTPA-complex can be detected, which are disintegration of the complex into free ^{99m}Tc and DTPA and reduction of ^{99m}Tc from pertechnetate ($^{99m}TcO_4^-$) to technetium oxide ($^{99m}TcO_2$).

Two strips of silica impregnated glass fibre sheets (ITLC™, P/N 61886, Gelman Sciences, Ann Arbor, Michigan, USA) were cut to the size of 1 x 5 cm. A small drop of the ^{99m}Tc/DTPA-solution was applied at the start line of the chromatogram, which was 0.5 cm from the bottom of each strip. The strips were then placed into two different glass vials containing either saline or acetone. The chromatograms were run until the solutions reached the level of about 0.5 cm below the top. The length of both strips was cut in half and four pieces of glass fibre strips were obtained, namely the bottom and top part of the strip placed in saline, BS and TS, respectively and the bottom and top part of the strip placed in acetone, BA and TA, respectively. Consecutively, the different pieces were examined for radioactivity using an isotope assay calibrator (model 270, Pitman Instruments, Surrey, UK).

In the case of quantitative chelate binding of ^{99m}Tc and DTPA, radioactivity was expected to be detectable on TS and BA but not on BS and TA. In saline, the formed ^{99m}Tc/DTPA-complex moves with the mobile phase to the top of the chromatogram, whereas in acetone the complex remains at the bottom. In the case of technetium reduction to $^{99m}TcO_2$, the precipitated oxide will maintain position at the start-line of each chromatogram, which means radioactivity can also be found on BS. The other instability, decomposed complex or incomplete ^{99m}Tc-DTPA binding, can be detected in

the TLC run in acetone, where free $^{99m}TcO_4^-$ is able to move with the mobile phase to the top of the chromatogram and radiation can be detected for TA.

The results of the test obtained before and after each study day were the same with no radioactivity detected for BS and TA, whereas radiation was observed for TS and BA. These results suggest that the binding of ^{99m}Tc with DTPA was quantitative and the chelate complex was stable throughout the scintigraphic study.

2.3.2. Gamma scintigraphy

2.3.2.1. The gamma camera

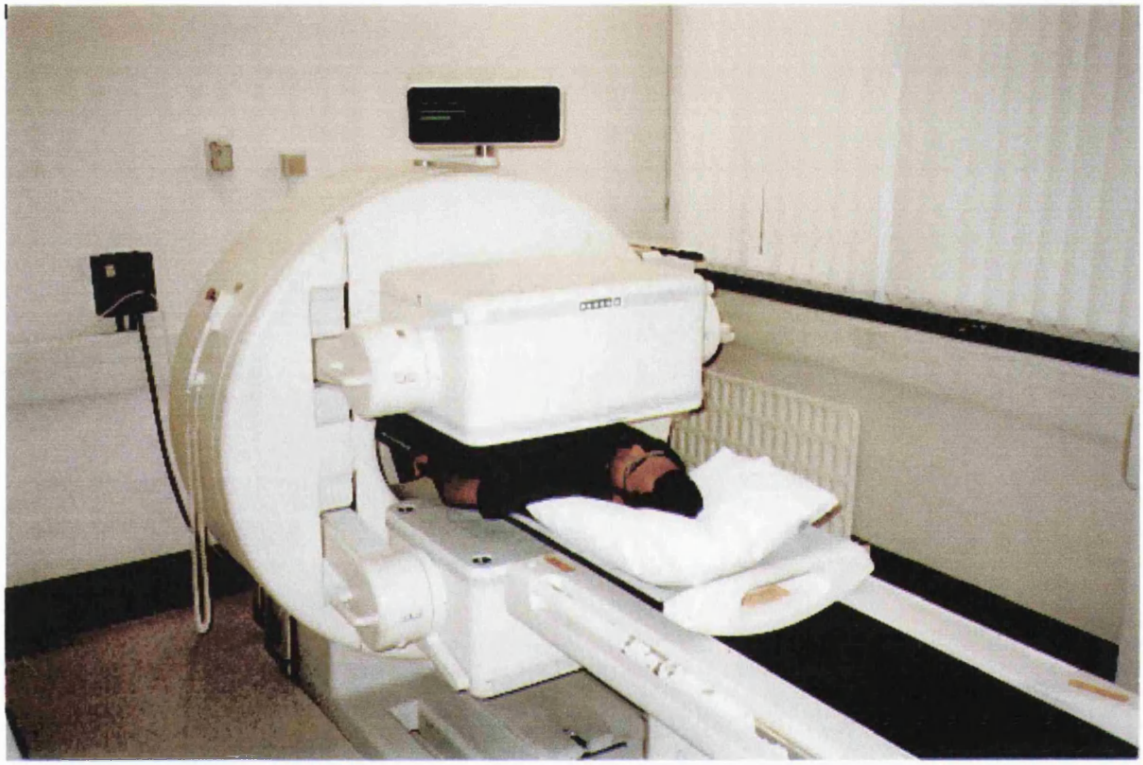
Imaging was conducted using a Maxxus gamma camera (model S8003MM/MN/MP/MR, General Electric Medical Systems, Milwaukee, USA) with two opposed high performance XR detectors, each having a 508 x 368 mm field of view and capable of simultaneous data acquisition (Figure 2.2). Each detector was fitted with a low energy parallel hole collimator suitable for ^{99m}Tc imaging. An on-line computer (Starcam 3200I, General Electric Medical Systems, Milwaukee, USA) was connected to the camera for digital image recording and data were archived onto optical disk for subsequent analysis.

Fitted with two detectors the gamma camera is capable of simultaneously collecting anterior and posterior images, which allows a correction of depth by calculation of the geometric mean. In 1997, Mitchell assessed the geometric mean response of the Maxxus gamma camera to ^{99m}Tc and found it to be satisfactory.

2.3.2.2. Study protocol

Seven male volunteers (age range 21-34 years, median 26 years; weight range 58-98 kg, median 80 kg; height range 1.65 – 1.93 m, median 1.78 m) participated in an open four-way crossover study, after providing written informed consent. All volunteers were non-smokers and declared themselves healthy with the exception of volunteer 7, who was a type-1 diabetic. The volunteers were not taking any medication, apart from the diabetic who was adhering to insulin treatment, and had no history of gastrointestinal disease.

Figure 2.2. The Maxxus gamma camera, showing the position of dual detectors and volunteer during a typical sctintigraphic study.



The Joint UCL/UCLH Committees on The Ethics of Human Research approved the experimental protocol. From the Administration of Radioactive Substances Advisory Committee (ARSAC) at the Department of Health the authority was obtained to administer radio-pharmaceuticals under the responsibility of Professor P.J. Ell, Director of The Institute of Nuclear Medicine, The Middlesex Hospital. The study was conducted in accordance with the provision of the Declaration of Helsinki (1965) and Tokyo (1975) and Venice (1983) revisions.

Each volunteer completed four study-days, which were separated by at least a one-week washout period. On each occasion the type of treatment was varied and given to each volunteer in a randomised order.

The different treatments were:

Control (0 g PEG 400)

- 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 150 mL water; radiolabelled with 7 MBq of ^{99m}Tc-DTPA

1 g PEG 400

- 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 150 mL water and 1 g PEG 400; radiolabelled with 7 MBq of ^{99m}Tc-DTPA

2.5 g PEG 400

- 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 150 mL water and 2.5 g PEG 400; radiolabelled with 7 MBq of ^{99m}Tc-DTPA

5 g PEG 400

- 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 150 mL water and 5 g PEG 400; radiolabelled with 7 MBq of ^{99m}Tc-DTPA

The volunteers were asked to attend to an overnight fast and hereby also refrain from alcohol or spicy food. On the day of the study, the volunteers arrived at The Institute of

Nuclear Medicine, Middlesex Hospital at 8.30 am. They were requested to empty their bladder and provide a 20 mL urine sample in a specimen container for reference purposes, which was stored at –20° C prior to analysis.

Before the administration of the treatments, a small sealed point source of 0.5 MBq ^{99m}Tc was taped to the abdominal skin at the position of the right lower costal margin. This permitted accurate positioning of the volunteer between images, and also acted as an abdominal reference marker.

After oral administration of the appropriate drug solution, the volunteer was positioned supine between the two detector heads of the gamma camera, which acquired an image of 30 seconds duration from both anterior and posterior detectors. This was repeated at 5 minutes intervals until the liquid had emptied from the stomach. In between image acquisitions, the volunteer was free to move away from the camera, but was requested to remain in an upright position. Four hours post dosing the volunteers consumed a standard lunch and water and orange juice was available *ad libitum*. Imaging was continued less frequently after gastric emptying at intervals of about 10-15 minutes duration until the liquid had arrived at the colon.

In addition to imaging, cumulative urine samples were collected throughout the course of the study comprising the collection and measurement of bladder output over the following time periods: 0 (pre-dose), 0 to 2, 2 to 4, 4 to 6, 6 to 12 and 12 to 24 hours. The bladder output was measured with a 1 litre-measuring cylinder and a 20 mL sample was retained and stored at each time point. For the 12 and 24 hours collection the volunteer was provided with a graduated 2 litre container for collection and volume measurement, and two 20 mL sample specimen containers for subsequent sample retention. The sample aliquots were stored at -20° C until further analysis.

2.3.2.3. Analysis of scintigraphic data

On completion of each scintigraphic study, processing of image data was performed using a Hermes image processing workstation (Nuclear Diagnostics, Stockholm, Sweden). Hereby, the series of images acquired for each volunteer was replayed on computer. Firstly, the full sequence of images was viewed to check for movement of the

volunteer. Highlighting the two anatomical markers a region of interest was drawn on the computer screen by cursor. Any volunteer movement was then corrected using a motion correction package (Hermes, Nuclear Diagnostics, Stockholm, Sweden). Following the correction of motion, two further regions of interests were drawn around the stomach and the colon. The counts recorded for each of these regions of interest were calculated for each image by computer. These values were then corrected for different background count rates. This was achieved by subtracting the mean counts per pixel from a region at the edge of the image from each pixel in the region of interest, e.g. stomach or colon. From these net counts the geometric mean was calculated to account for the differential attenuation of the radiation with varying depth of source. Subsequently, the geometric mean counts were corrected for physical decay of ^{99m}Tc as the radionuclide has a very short half-life of 6 hours. Finally, the corrected geometric mean counts for the regions of interest were expressed as percentages of the total counts recorded initially, when all the administered activity was in the stomach and terminally, when all the activity was in the caecum/colon for the values of the curves of gastric emptying and caecum/colon arrival, respectively. The time course of gastric emptying and colon arrival was then estimated from the plot of percentage activity in these regions versus time.

The gastrointestinal transit data were quantitatively assessed via two different approaches. The method of statistical moments was used to calculate the mean gastric residence time (MGRT) and mean caecum/colon arrival time (MCAT) of the drug solutions (Podczeck et al., 1995). The second approach was the determination of t_{50} values, which describe the time, when 50 % of the preparations have emptied from the stomach or reached the caecum/colon. Consecutively, the measures for liquid transit through the small intestine were calculated as the difference between the MGRT and MCAT to obtain the mean small intestinal transit time (MSITT) and as the difference between the t_{50} values for gastric emptying and colon arrival to obtain the t_{50} small intestinal transit time.

2.3.2.4. Analysis of urine samples

After thawing at room temperature and thorough vortex mixing, the urine samples and calibration standards were prepared for ranitidine analysis by diluting 20 µL of sample with 780 µL of a solution consisting of 50 % water adjusted to pH 10 with ammonium

solution (solvent A) and 50 % methanol (solvent B) with sotalol present at 500 μg/mL as an internal standard. Samples requiring dilution to fall into the calibration range were first diluted using blank human urine.

The urine samples were assayed for ranitidine content using a validated HPLC-mass spectrometry (MS) method. The HPLC-MS analysis utilized a Hewlett Packard Series 1100 chromatography system with a CTC PAL autosampler. Aliquots of sample (10 μL) were injected on a 50-mm x 2.1-mm Luna column (2 μm C-18(2); Phenomenex, U.K.) at 40° C. A binary solvent gradient system was used and the flow rate of the mobile phase was set to 0.8 mL/min. The column was initially equilibrated with 95 % solvent A and 5 % solvent B. Immediately after sample injection, the concentration of B was linearly increased over 3 min to a concentration of 60 % and then reduced to the initial concentration of 5 % in the next 0.5 min followed by a 1 min equilibration time before the next sample injection. Subsequent detection of ranitidine was performed via selected reaction monitoring using a Sciex API 3+ mass spectrometer with a turboionspray source at 500° C. Mass detection of ranitidine (*m/z* 315) and the internal standard sotalol (*m/z* 273) was performed in positive single-ion monitoring mode of the corresponding daughter ions (*m/z* 176 and 133, respectively), which showed a characteristic retention time of 2.40 and 1.25 min, respectively.

2.3.2.5. Statistical analysis

A paired Student's t-test was performed on the scintigraphic and pharmacokinetic data to assess the effect of PEG 400 on gastrointestinal transit and ranitidine absorption.

2.4. Results and discussion

2.4.1. Gastrointestinal transit

Figures 2.3 and 2.4 display a series of representative scintigraphic images highlighting the passage of the control formulation and the formulation containing 5 g PEG 400, respectively, through the GI tract of one subject. Upon oral administration the solutions emptied from the stomach rapidly into the small intestine. In the duodenum, the liquids appeared to have a particularly short residence time although the passage of the luminal

fluids was observed to slow down on its way along the small intestine. This pattern of motility is a distinctive feature with the transit rate being fastest in the upper small intestine and gradually decreasing towards the distal parts of the small intestine. Reaching the ileocaecal junction the drug solutions often seemed to accumulate and stagnate before entering the caecum in a bolus. This pattern of caecum entry has been widely reported (Khosla and Davis, 1989; Khosla et al., 1989; Coupe et al., 1991) and despite being poorly understood it is probably related to the rate at which material arrives at the ileocaecal junction (Quigley et al., 1984). However, this pattern of movement was not consistently observed for one specific treatment. Once in the caecum, the movement of the liquid was very slow and the ileocaecal junction prevented retrograde passage into the small intestine.

The profiles of gastrointestinal transit for each individual volunteer are given in Figures 2.5 to 2.11 allowing a good qualitative comparison between subjects and treatments. A single average profile of all volunteers, which is sometimes chosen by other authors (Christensen et al., 1985; Ollerenshaw et al., 1987; Kenyon et al., 1995), was not presented as it may not resemble the shapes and features of the original profiles but rather give a distortion of the overall results (Podczeck and Newton, 1996). The numerical values for gastric emptying, colon arrival and small intestinal transit in each volunteer determined by the method of moments theory are shown in Tables 2.1, 2.3 and 2.5 and the corresponding t_{50} values are listed in Tables 2.2, 2.4 and 2.6.

The fact that volunteer 7 is a type-1 diabetic clearly distinguishes him from the other healthy volunteers. Consequently, his results for gastric emptying, caecum arrival and small intestinal transit as well as ranitidine absorption have not been included in the mean values calculated for the normal subjects. The data for the diabetic are listed in the same Tables (2.1 to 2.7) but have been evaluated separately from the ones obtained for volunteer 1 to 6

Figure 2.2. Scintigraphic images highlighting gastrointestinal transit of an oral solution in the absence of PEG 400 (control).

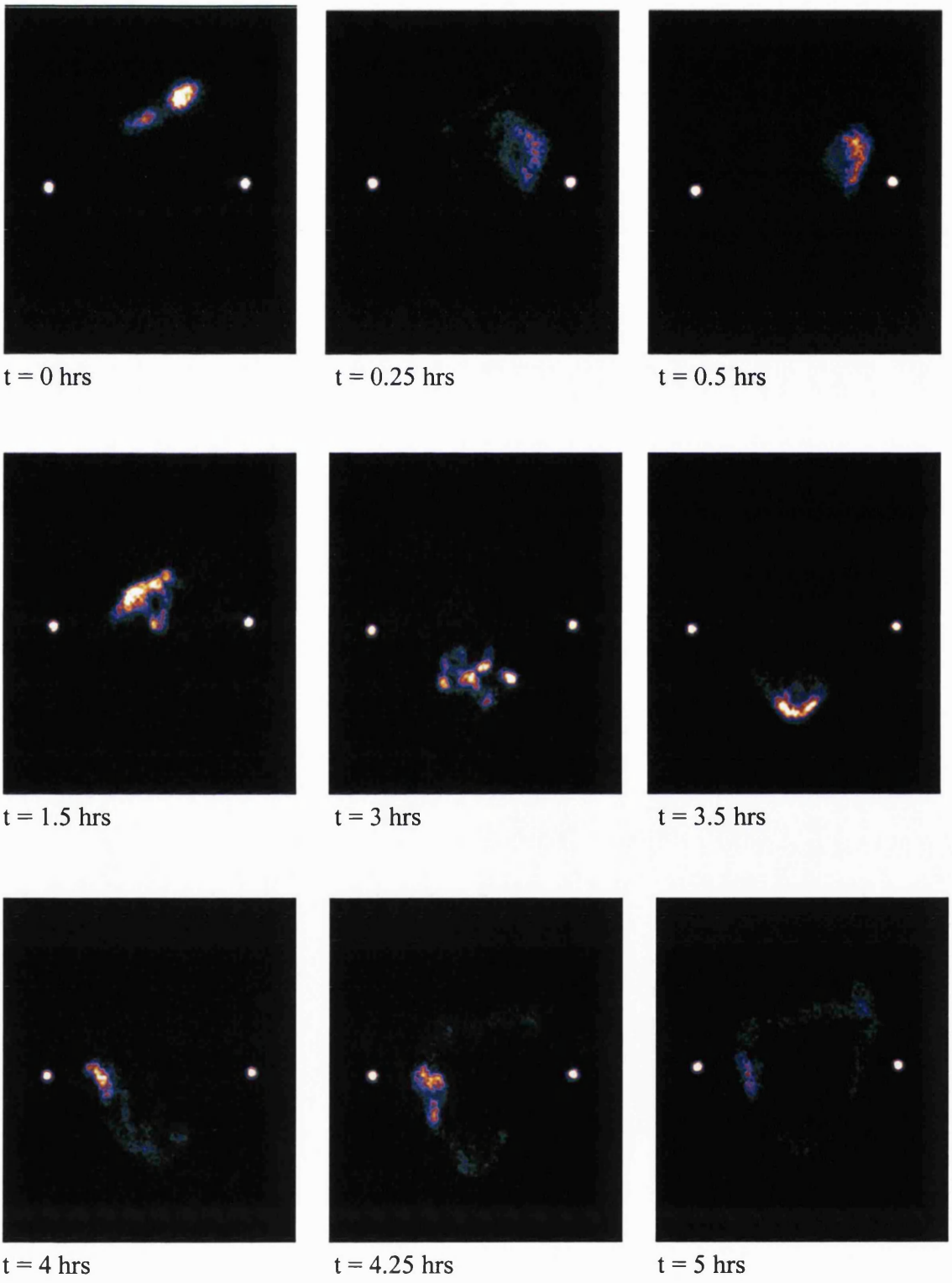


Figure 2.3. Scintigraphic images highlighting gastrointestinal transit of an oral solution in the presence of 5 g PEG 400.

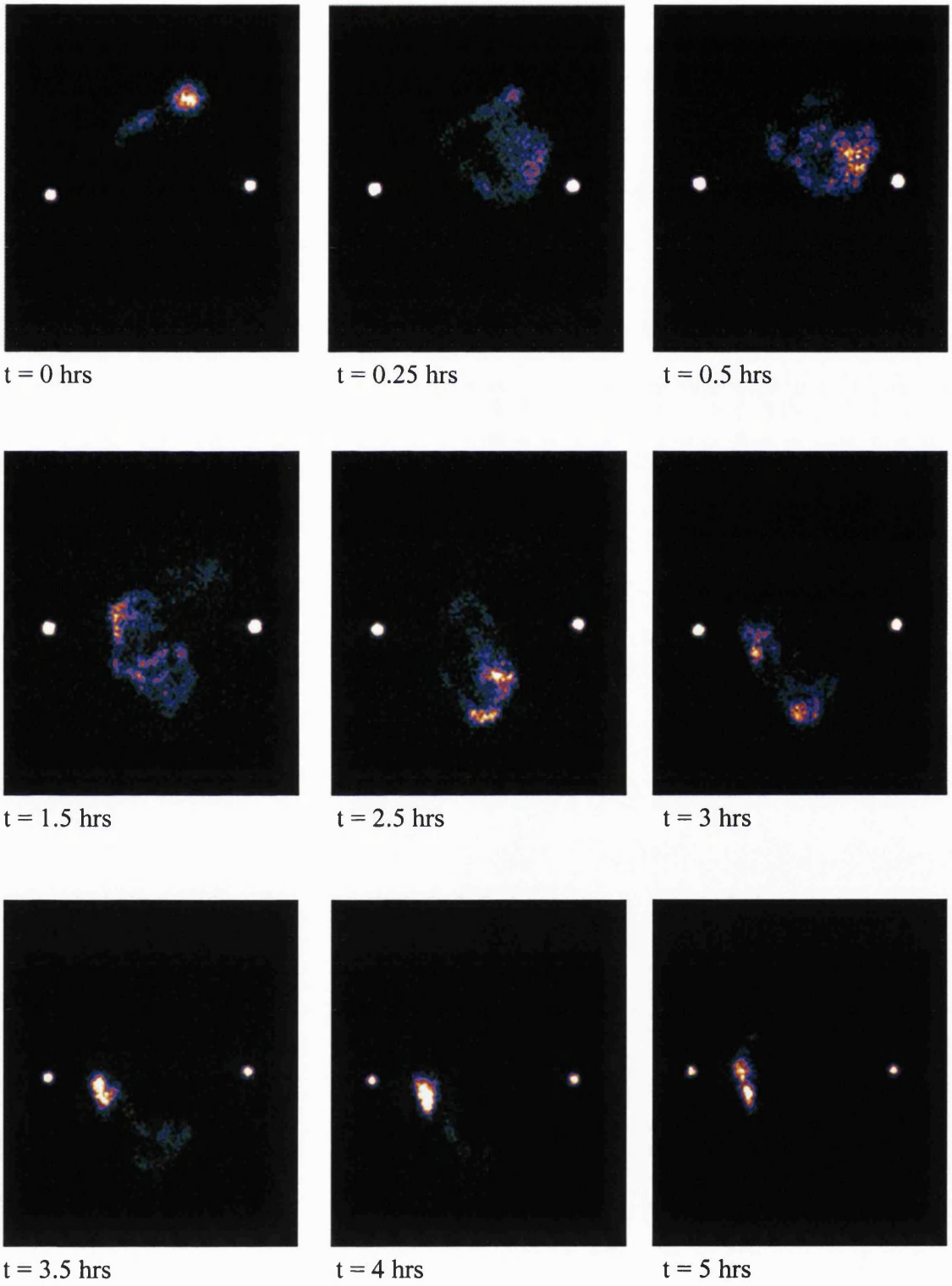


Figure 2.5. Gastrointestinal transit profiles for volunteer 1.

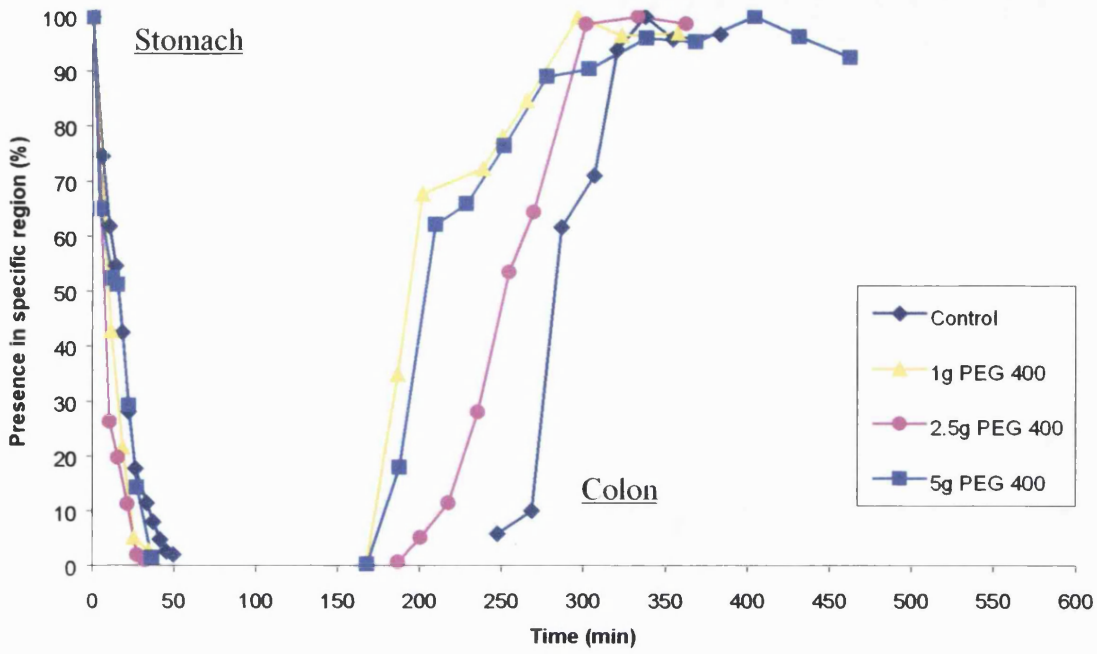


Figure 2.6. Gastrointestinal transit profiles for volunteer 2.

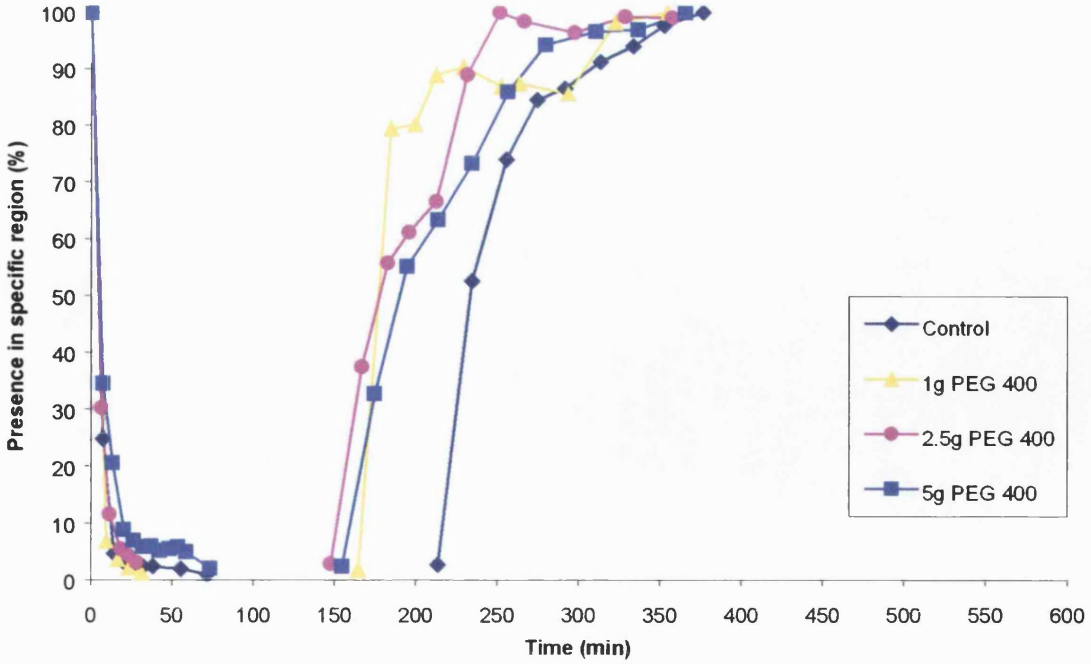


Figure 2.7: Gastrointestinal transit profiles for volunteer 3.

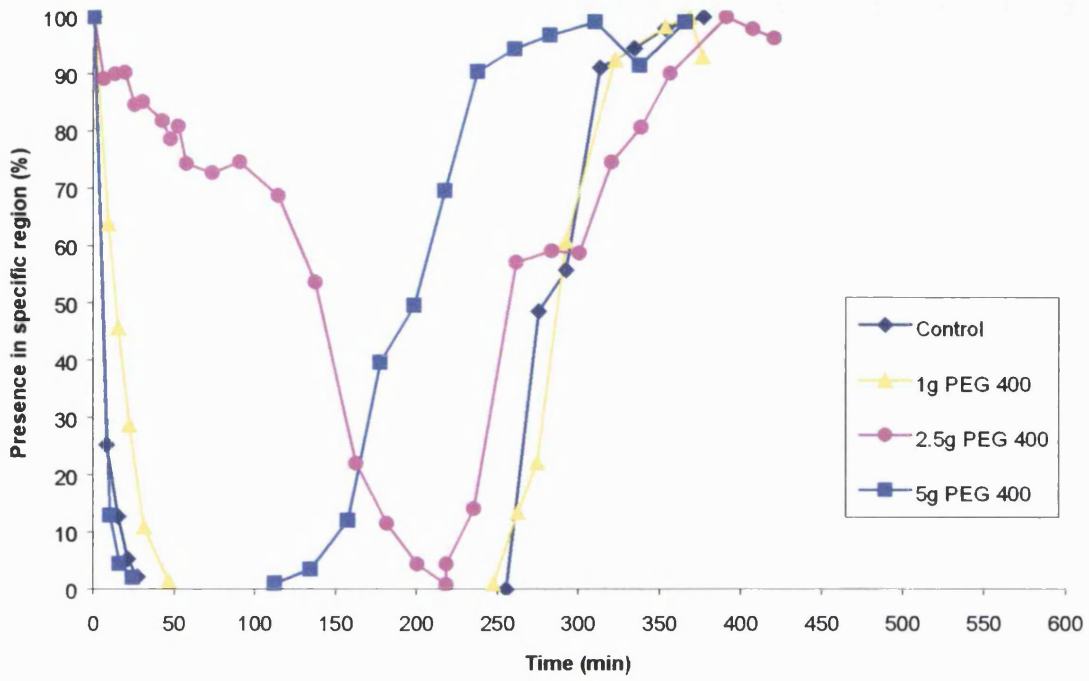


Figure 2.8: Gastrointestinal transit profiles for volunteer 4.

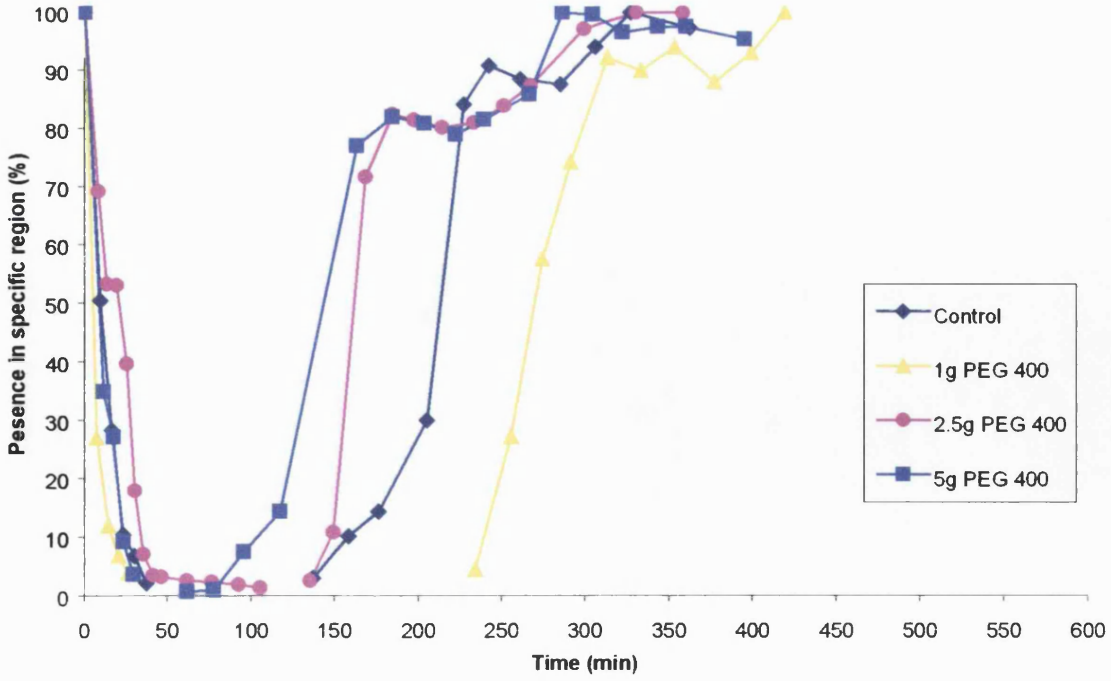


Figure 2.9: Gastrointestinal transit profiles for volunteer 5.

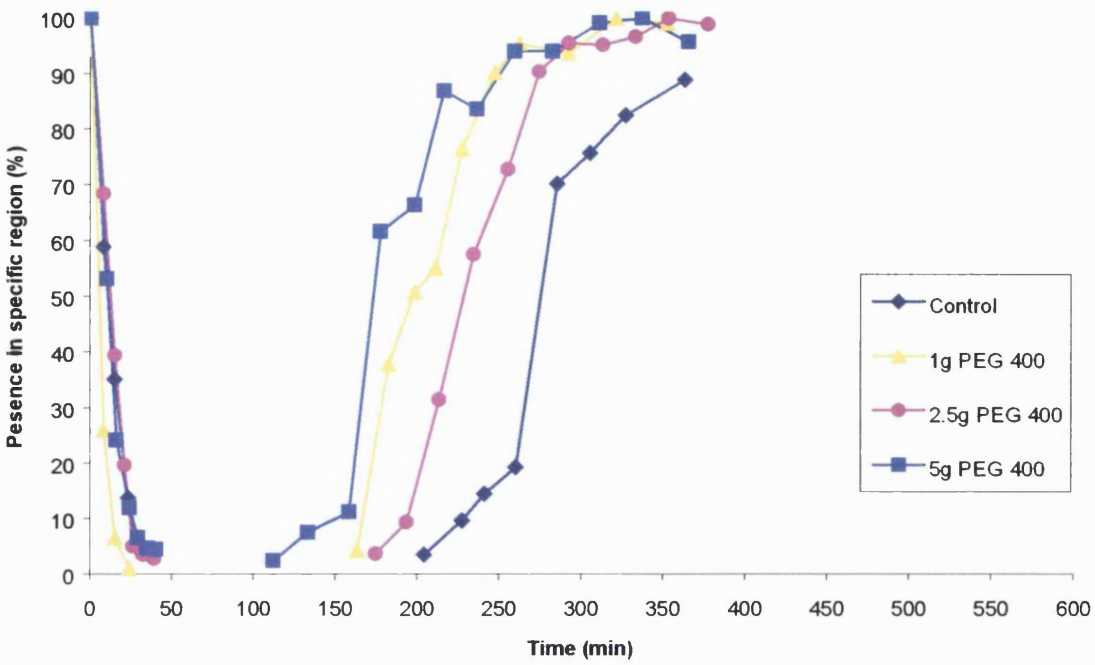


Figure 2.10: Gastrointestinal transit profiles for volunteer 6.

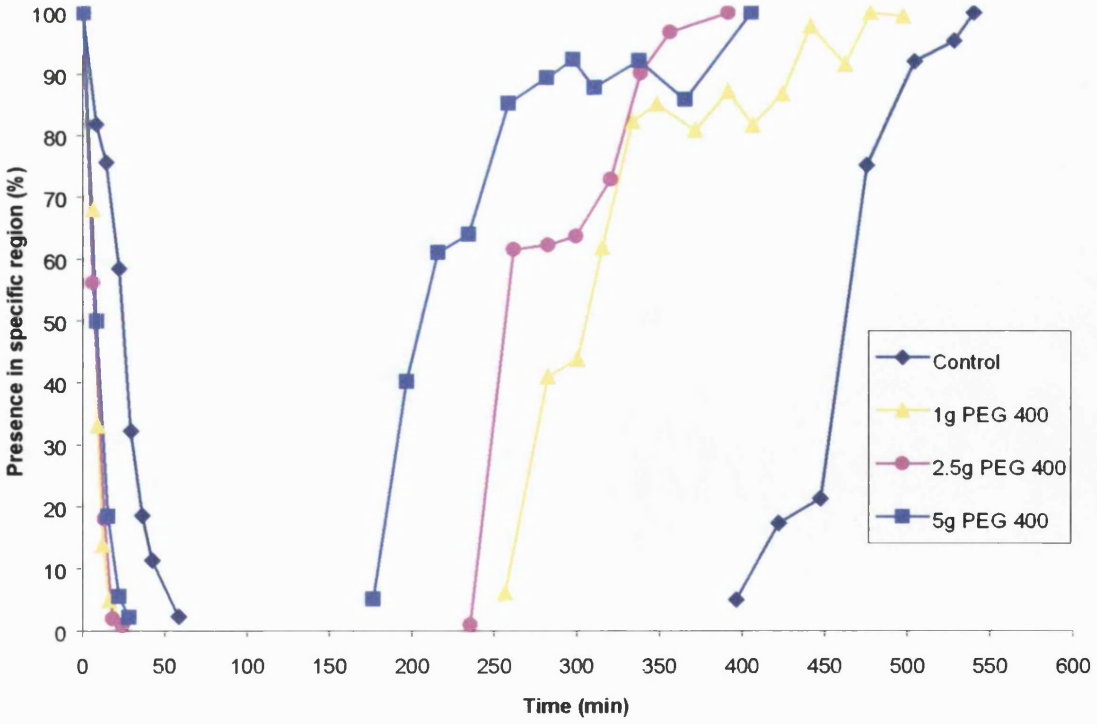
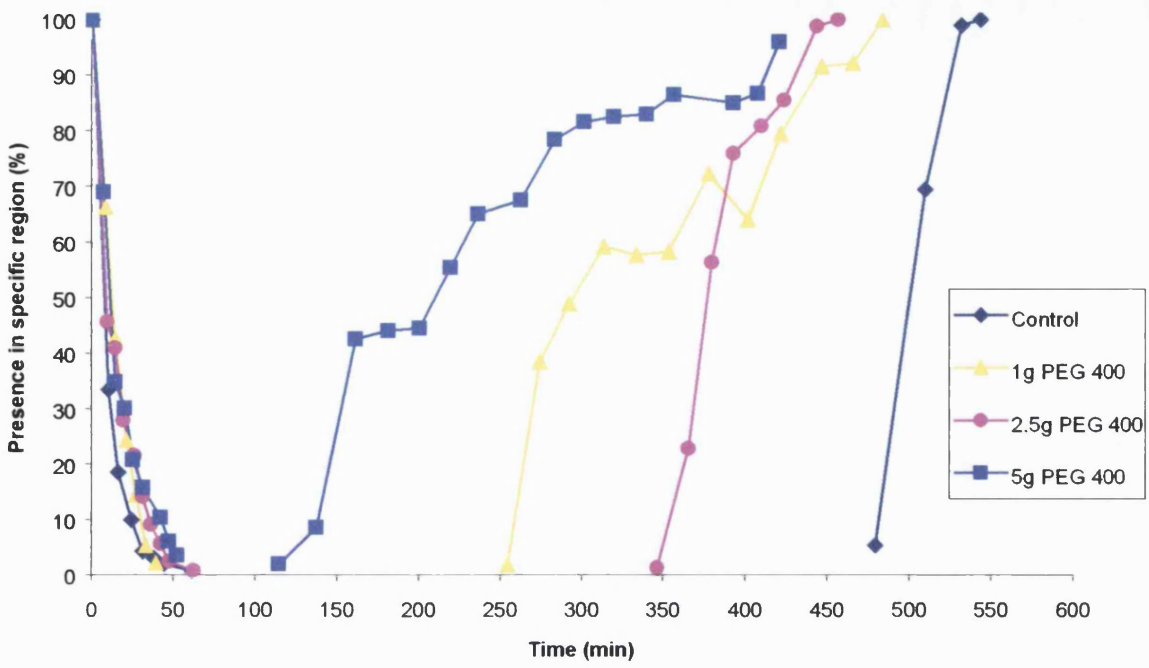


Figure 2.11: Gastrointestinal transit profiles for volunteer 7.



For comparison of the performances of the different treatments the use of numerical values is preferable to the illustrative transit profiles. There exist two major approaches for the numerical description of gastric emptying or caecum/colon arrival: the determination of the t_{50} value or the employment of statistical moments. Both methods have their advantages and limitations.

The t_{50} value is the time taken when 50 % of the oral liquid has emptied from the stomach or arrived at the caecum/colon, respectively. The small intestinal transit time can be calculated as the difference between the two values. The big advantage of the t_{50} value is that it is easily determined and readily visualised in the transit profile. However, its calculation is simply based on two points of the scintigraphic profile. This fact sets the t_{50} approach its limitations, as it does not consider the whole process of emptying or arrival, e.g. it provides no information on the distribution of the data. Additionally, in cases of irregularly shaped profiles, there may be more than one point at the 50 % interval, which makes the determination of the t_{50} value rather difficult and subjective.

Table 2.1. Gastric emptying times calculated using statistical moment theory and represented as MGRT (min) and *VGRT* (min^2).

	Volunteer *								
Treatment	1	2	3	4	5	6	Mean ± S.D.	*P* value	7 (D)
Control	14 *64*	7 *0*	9 *8*	13 *24*	13 *31*	19 *101*	12 ± 4		13 *23*
1 g PEG 400	13 *9*	9 *0*	15 *57*	9 *9*	8 *0*	7 *4*	11 ± 3	*0.400*	13 *41*
2.5 g PEG 400	12 *13*	7 *4*	81 *2508*	16 *58*	12 *23*	8 *11*	22 ± 26	*0.454*	15 *54*
5 g PEG 400	14 *53*	9 *7*	10 *0*	12 *6*	13 *23*	10 *10*	12 ± 3	*0.504*	17 *120*

* volunteer 1 to 6 = healthy; volunteer 7 (D) = diabetic

Table 2.2. Gastric emptying times represented as t_{50} values (min).

	Volunteer								
Treatment	1	2	3	4	5	6	Mean ± S.D.	*P* value	7 (D)
Control	16	5	6	9	11	24	12 ± 7		8
1 g PEG 400	10	5	14	5	6	8	8 ± 4	*0.286*	12
2.5 g PEG 400	7	4	139	20	13	7	32 ± 53	*0.427*	8
5 g PEG 400	16	6	6	8	11	8	9 ± 4	*0.365*	11

Table 2.3. Caecum arrival times calculated using statistical moment theory and represented as MCAT (min) and *VCAT* (min^2).

Treatment	Volunteer 1	2	3	4	5	6	Mean ± S.D.	*P* value	7 (D)
Control	305	249	292	212	301	505	311		509
	220	*93*	*0*	*444*	*700*	*937*	± 102		*0*
1 g PEG 400	238	279	310	280	210	364	280	*0.399*	399
	513	*3035*	*326*	*165*	*253*	*1779*	± 54		*3841*
2.5 g PEG 400	254	206	313	221	239	339	262	*0.133*	403
	293	*471*	*1122*	*1065*	*323*	*1891*	± 53		*306*
5 g PEG 400	263	227	198	156	282	319	241	*0.042*	231
	1131	*669*	*406*	*345*	*3531*	*3949*	± 59		*1697*

Table 2.4. Caecum arrival times represented as t_{50} values (min).

Treatment	Volunteer 1	2	3	4	5	6	Mean ± S.D.	*P* value	7 (D)
Control	282	230	275	214	272	467	290 ± 91		501
1 g PEG 400	194	176	289	264	192	309	237 ± 57	*0.146*	356
2.5 g PEG 400	252	178	259	161	224	258	222 ± 43	*0.065*	374
5 g PEG 400	203	190	185	139	174	221	185 ± 28	*0.016*	202

Table 2.5. Small intestinal transit times calculated using statistical moment theory and represented as the difference between MGRT and MCAT (min).

Treatment	Volunteer						Mean ± S.D.	*P* value	7 (D)
	1	2	3	4	5	6			
Control	291	242	283	199	288	486	298 ± 99		496
1 g PEG 400	225	270	295	271	202	357	270 ± 54	*0.412*	386
2.5 g PEG 400	242	199	232	205	227	330	239 ± 47	*0.042*	388
5 g PEG 400	249	218	188	144	269	309	230 ± 59	*0.037*	215

Table 2.6. Small intestinal transit times represented as the difference in gastric emptying and caecum arrival t_{50} values (min).

Treatment	Volunteer						Mean ± S.D.	*P* value	7 (D)
	1	2	3	4	5	6			
Control	266	225	269	205	261	443	278 ± 85		493
1 g PEG 400	184	171	275	259	186	301	229 ± 56	*0.145*	344
2.5 g PEG 400	245	174	120	141	211	251	190 ± 54	*0.024*	366
5 g PEG 400	187	184	179	131	163	213	176 ± 27	*0.013*	191

An alternative approach to the t_{50} value is the application of statistical moments, which makes use of the entire data set (Podczeck *et al.*, 1995). Hereby, the shapes of the emptying and arrival curves are considered to a full extent providing this method the advantage of great accuracy. The stomach emptying profiles are described by Mean Gastric Residence Time (MGRT) and the caecum/colon arrival data by Mean Caecum Arrival Time (MCAT). The difference between the MGRT and MCAT gives the value of Mean Small Intestinal Transit Time (MSITT). Variance of Gastric Residence Time (*VGRT*) and Variance of Caecum Arrival Time (*VCAT*) are also calculated giving an indication of the spread of the collective data regarding gastric emptying curve and colon arrival curve, respectively.

Besides a comparison of the two methods, a presentation and discussion of both data sets will help a comparison of the present results with those of previously published transit studies, which mainly employed the t_{50} approach.

In general, a correlation can be seen between the values of MGRT and MCAT determined via the statistic moment theory with the corresponding t_{50} values for gastric emptying and caecum/colon arrival. On some study days, however, the correlation is not perfect and a deviation between the values is observed as a result of the differences in the calculation mode of the values. The deviation between the MGRT value and the t_{50} value for gastric emptying seems to be almost negligible. This is due to the fact that the emptying of the solutions from the stomach was rather rapid and appeared to follows an exponential pattern. The results for the MCAT and t_{50} caecum/colon arrival values give a different picture since the deviation of the corresponding values is much greater and appears to follow a certain rule: the MCAT data calculated with the method of statistical moments all give greater values than the t_{50}, a fact, which is also reflected in the values for the small intestinal transit times. The MSITT values, which are determined as the difference between the MGRT and the MCAT values, are generally greater than the corresponding data for t_{50}. According to the characteristics of the two different methods, the values involving statistical moment theory are suggested to have a greater accuracy of the *in vivo* situation of gastrointestinal transit for the reasons mentioned above. This means that the calculation of t_{50} values tends to underestimate the transit time of oral liquids through the small intestine. However, both sets of data are presented here, also

for comparison purposes with literature values as many authors in fact make use of the t_{50} approach. Overall, the data of gastrointestinal transit appeared to give considerable variation both within and in between the individual volunteers. This, however, has to be expected by experiments conducted *in vivo*.

2.4.1.1. Gastric emptying

After oral administration of ranitidine in 150 mL water on the control day, the solution was observed to empty rapidly from the stomach in all volunteers. The average MGRT and emptying half time was noted as 13 ± 4 min and 11 ± 7 min, respectively. The range of the specific values was 7 to 19 min and 5 to 24 min, respectively. These values are in accord with published data from other studies involving healthy volunteers, where the half-emptying time has typically been reported in the 8 to 15 min range (Brener et al., 1983; Hunt et al., 1956). Rees et al. (1979) reported that such short emptying times are to be expected for non-nutrient liquids administered on a fasted stomach, as there is no interruption of the fasting motility pattern. In contrast, Gupta and Robinson (1988) found in dogs that an interruption of the fasted motility pattern caused by fluid volumes greater than 100 mL was responsible for the exponential emptying pattern. Oral nutrient solutions, on the other hand, containing e.g. glucose or fatty acids are known to interrupt the fasting pattern via an activation of duodenal feedback mechanisms and as a consequence can delay liquid gastric emptying to over an hour (Brener et al., 1983). This is reflected in an approximately linear emptying pattern for nutrient liquids. The shape of the transit profiles in Figures 2.4 to 2.10 clearly shows an exponential passage of the solutions into the small intestine and the pattern does not seem to be influenced by the presence of increasing amounts of PEG 400. The proximal stomach is primarily responsible for this emptying pattern (Kelly, 1980). A small pressure gradient between the gastric cavity and the duodenum, which is generated by slow sustained amplitude contractions in the proximal regions of the stomach, cause a continuous flow of liquid into the duodenum (Minami and McCallum, 1984).

On one occasion, however, an unusual emptying pattern was observed for volunteer 3 after administration of the liquid containing 2.5 g PEG 400. The transit profile (Figure 2.6) reveals that the ingested liquid might somehow have been obstructed from entering into the small intestine. The solution did not reach its half-emptying time in the first

2 hours and the stomach was not completely cleared before 3.5 hours had passed. This delayed emptying is also reflected in the MGRT for volunteer 3, which was 81 min. Gastric emptying is known to be highly variable and subject to a variety of factors. Cases of similar abnormal emptying from the stomach, however, have been suggested to be the result of local extra-intestinal structures like the mesenteric artery, for instance, forcing pressure onto the upper duodenum (Schwartz et al., 2001). But such cases, however, are observed only on very rare occasions. The emptying pattern observed for this subject on this particular day appeared to be atypical, since his MGRTs values on earlier and later study days proved to be normal and similar to those observed in the other volunteers. It was therefore assumed that the delayed gastric emptying was not caused by the presence of 2.5 g PEG 400 in the formulation.

Of no particular influence on the mean gastric residence time appeared to be the presence of increasing amounts of PEG 400. The average MGRT values for the healthy volunteers were found to be 10, 23 and 11 min for the formulations containing 1, 2.5 and 5 g PEG 400, respectively. An increase in the concentration of PEG 400 results in increasing osmotic pressure of the administered formulations due to the osmotic nature of the polymer (Schiller et al., 1988). Osmolality of ingested fluids is known to influence gastric emptying in humans. A slowing in the rate of emptying from the stomach was shown for hyperosmolar solutions (Hunt, 1961, 1963). In the present study, however, the osmolality of the test solutions was determined to be 4, 25, 50 and 102 mOsm kg^{-1} for the formulations containing 0, 1, 2.5 and 5 g PEG 400, respectively, and therefore below isotonic conditions. On the other hand, the solutions administered in the study of Basit et al. (2001, 2002), which consisted of plain orange juice with and without 10 g of PEG 400 had an osmolality of 951 and 606 mOsm kg^{-1}, respectively, which means the solutions were hypertonic. The MGRTs of both solutions was found to be 24 and 17 min, respectively, which is slightly longer than the values obtained in the present study and is likely to be the result of the increased osmolality. In general, it is assumed that PEG 400 does not have an impact on gastric emptying by other means since in all cases the emptying from the stomach was rapid with no significant difference noted between the treatments ($P > 0.05$).

2.4.1.2. Colon arrival and small intestinal transit

Considerable differences both between and within the volunteers, however, were found in the caecum arrival times listed as MCAT and t_{50} values in Tables 2.3 and 2.4, respectively. With no changes noted in the gastric emptying times the variation in caecum arrival of the different treatments is the result of differences in the transit times of the formulations through the small intestine. The corresponding values for the small intestinal transit times are given in Tables 2.5 and 2.6 reflecting the moments theory (MSITT) and the t_{50} approach, respectively. Different groups have demonstrated that the small intestinal transit time of ingested material is relatively constant at about 3 to 5 hours (Davis et al., 1986a; Hardy et al., 1985b). In the present study, almost all healthy volunteers had an MSITT slightly longer with an average value of 298 min ranging from 199 to 486 min. However, the values reported by the groups of Davis and Hardy are determined as t_{50} values. In comparison of the t_{50} values obtained in the present study, which were 278 min with a range from 205 to 443 min, the values are in better confinement with the literature values. However, volunteer 6 displayed an unusually long small intestinal transit time between 7 and 8 hours. Although the average value is expected to be in the 3 to 4 hour range, the small intestinal transit time can vary between individuals and longer times are not unusual.

The slowest passage of the control formulation through the small intestine of all participating subjects, however, was obtained for the diabetic volunteer. Depending on the state of the disease a variety of gastrointestinal disorders such as diarrhoea, constipation and delayed gastric emptying is likely to be experienced by about 20 % of patients with diabetes (Feldman and Schiller, 1983; Gwilt et al., 1991). Several studies report prolonged gastrointestinal transit in diabetics compared to healthy volunteers (Lautenbacher et al., 1990; Wegener et al., 1990; El-Salhy, 2001). The gastrointestinal disorders in these patients, especially those suffering from diabetes mellitus type-1, have been suggested to result from reduced propulsive gut motility as a consequence of enteric neuropathy.

Transit of the different formulations through the small intestine appears to follow a distinct trend, with the presence of increasing quantities of PEG 400 resulting in decreasing solution transit times. The mean MSITTs in the healthy subjects for the

solutions containing 0, 1, 2.5 and 5 g PEG 400 were 298, 270, 239 and 230 min, respectively. This corresponds to a reduction in the mean MSITT of 9, 20 and 23 %, respectively, relative to the control (Figure 2.11). Although the decrease in the MSITTs for the 1 g PEG 400 administration did not prove to be statistically significant, the overall trend is indicative of a dose-related effect of the polymer on intestinal transit.

The mechanism behind this effect on intestinal transit is most likely related to the poor absorption of PEG 400 from the gastrointestinal tract. Approximately 50 % of an orally administered dose of PEG 400 has been reported to be absorbed from the intestinal lumen (Chadwick et al., 1977a; Ma et al., 1990). This is mainly due to the low permeability of the PEG molecules as a result of the polymer's hydrophilic nature. In a perfusion study of the human jejunum the effective permeability (P_{eff}) of PEG 400 has been determined to be 0.8×10^{-4} cm/s (Takamatsu et al., 1997). After ingestion, the majority of the PEG 400 dose is therefore expected to remain unabsorbed within the lumen of the gastrointestinal tract and is ultimately excreted unchanged in the faeces. With PEG 400 being osmotically active (Schiller et al., 1988), the polymer will hold back a considerable amount of water in the lumen of the gut (Davis et al., 1980). This retention of water leads to an increase in luminal fluid volume, which in turn stimulates gut motility and, hence, promotes an accelerated passage of the solution through the small intestine.

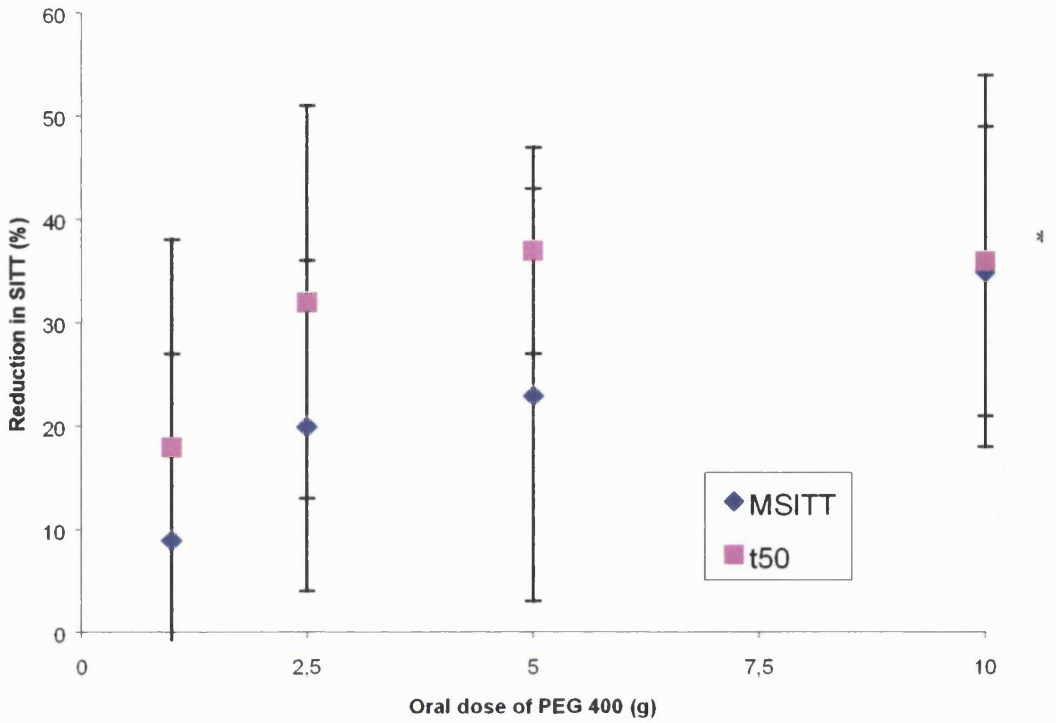
In the diabetic volunteer, the MSITTs values for the small intestinal transit times of the formulations containing 0, 1, 2.5 and 5 g PEG 400 were 496, 386, 388 and 215 min, respectively. Compared to the control formulation, the presence of 5 g PEG 400 was found to result in a relative decrease of the MSITT of 57 %, which is a considerable acceleration of small intestinal transit rate. Another distinctive feature in the transit profile of the diabetic is that PEG 400 appears to affect transit to a greater extent - a phenomenon, which was rather unexpected for the diabetic. Patients with diabetes have been observed to have a "leakier gut" compared to normal subjects and widened tight junctions in the lining epithelium of the intestine are thought to cause an enhancement of paracellular absorption (Carratù et al., 1999). The increased membrane permeability in diabetics has recently been associated with a significant reduction of junctional proteins such as occludin (Antonetti et al., 1998), which is responsible for the barrier function of

the junctions (Kuwabara et al., 2001). Assuming increased paracellular permeability in the diabetic subject it was expected that the presence of PEG 400 would rather exert a lesser effect on the small intestinal transit time in relation to the healthy volunteers. Since the polymer is believed to use mainly paracellular absorption pathways (Ma et al., 1993) PEG 400 will be absorbed from the diabetic gut to a greater extent. This leaves less polymer within the intestinal lumen able to cause an osmotic effect and influence the passage rate of the luminal contents. It is possible, however, that apart from the osmotic activity PEG 400 might exert other effects inside the intestinal lumen. As a polyalcohol with many hydroxyl groups it is likely to interact with the hydrophilic head groups of epithelial phospholipids. PEG solutions of different molecular weight have been examined regarding their effect on the histology of the intestinal mucosa. An isoosmotic PEG 4000 solution perfusing the intestine caused a prompt and specific damage to the enterocytes (Clarke and Kobayashi, 1975), which was also observed with a hyperosmolar PEG 1000 solution (550mosm/l) (Kameda et al., 1968) as well as PEG 2000 solutions at concentrations from 10% to 30% (Bryan et al., 1980). The histological injury to the surface of the epithelium was found in both the jejunum and the ileum (Kameda et al, 1968). However, there seemed to be a rapid reversal of the damage after PEG is removed (Clarke and Kobayashi, 1975) and it was concluded that these effects are not clinically significant. The doses of PEG 400 administered in the present study are considerably lower than those used in the histological experiments. Nevertheless, it is possible that the diabetic gut is more sensitive and irritable towards the presence of the polymer, which might have caused a toxic reaction resulting in the high-accelerated transit in volunteer 7. Unfortunately, no firm conclusions can be drawn from the data of the diabetic, as he was the only subject with this condition participating in the present study (n = 1). Therefore, he may in fact not be representative of the general diabetic population.

2.4.1.3. Correlation of PEG 400 dose and MSITT

The present transit results in healthy volunteers, however, seem to be in general agreement to the previously obtained data for liquid transit in the presence of 10 g PEG 400, which highlighted a 35 to 37 % reduction in the MSITT (Basit et al., 2001; 2002). Comparing the MSITT values of the present study with the values obtained from Basit et al., it appears that the relationship between the amount of PEG 400 present and the observed decrease in the MSITT is not linear (Figure 2.12).

Figure 2.12. Relation between the dose administered of PEG 400 and the reduction in small intestinal transit time relative to the control (%).



* (Values for the oral dose of 10 g PEG 400 are derived from Basit et al., 2001).

A 2-fold increase in PEG 400 concentration would perhaps have been expected to result in a 2-fold reduction in the MSITT. Fitted curves of linear regression were established for both data sets accordingly revealing rather low correlation coefficients (R^2_{MSITT} = 0.888; R^2_{t50} = 0.561). The shape of the curves almost gives the impression that the impact on GI transit reaches a plateau at higher concentrations of PEG 400 - as if the body responds to these higher concentrations with an increased compensation effort. This assumption, however, is to be questioned, as the body is in fact capable of rather extreme fastening of gastrointestinal transit rates exhibiting diarrhoea in the worst cases. Various stimuli of the enteric system such as enterotoxins (Farthing, 1993) or consumption of high amounts of coffee or artificial sweeteners are recognised to potentially cause diarrhoea. The gastrointestinal transit time of patients suffering from chronic diarrhoea was determined to be considerably short with 29 ± 3 min (Lin et al., 2001). However, an accelerating effect on oral liquid passage through the small intestine was established for the excipients SAPP and mannitol, which are mainly employed in the

design of effervescent dosage forms (Koch et al., 1993; Adkin et al., 1995a,b,c). Like PEG 400 both compounds are believed to exert these effects as a result of their osmotic activity within the gastrointestinal lumen. Similar examples are compounds such as PEG 4000, which are used as osmotic laxatives to promote intestinal transit in preparations such as Movicol® and CleanPrep®. In the latter, doses as high as 20 g are employed serving to empty the gut before surgery, which is achieved via the induction of diarrhoea. It is, therefore, very unlikely that both the formulations containing 5 and 10 g PEG 400 ought to have reached a maximum in transit acceleration. Consideration moreover needs to be given to a distinctive difference existing between the experiment of Basit et al. (2001, 2002) and the present study, which might have led to a distortion of the results. The fact that Basit et al. used orange juice instead of water as oral liquid could have been of influence. As mentioned before, a widening of the paracellular pathway might have resulted from the presence of luminal glucose (Nusrat et al., 2000; Sadowski and Meddings; 1993). In this case, one would expect a higher amount of the polymer to be absorbed causing a lesser effect on the gastrointestinal passage rate and, therefore, not leading to a higher reduction as expected. However, in the study of Sadowski and Meddings (1993) conducting an *in vivo* perfusion model of the rat jejunum an increased permeability was found for various dextrans, which are hydrophilic high molecular weight compounds commonly used as paracellular marker molecules. The clearance of PEG 400, however, did not correlate well with those of the dextrans, which was surprising since dextrans have a significantly higher molecular weight than PEG 400. The authors did not observe an enhanced permeability for PEG 400 in the presence of luminal glucose. It was suggested that PEG 400 might use different pathways as an alternative to the paracellular route. However, the discrepancies between the two studies may also have in part been due to natural inter and intra subject variability in gastrointestinal transit, but the results nevertheless demonstrate the existence of a concentration-dependent effect of PEG 400 on small intestinal transit.

2.4.2. Drug absorption

The extent of the absorption of the model drug ranitidine in each individual, as assessed by the cumulative amount of unchanged urine over 24 hours, is presented in Table 2.7. On the control day, the average recovery of ranitidine for the healthy volunteers was 34 ± 16 mg, which corresponds to 23 % (w/w) of the administered dose.

This is in good agreement with values reported in other studies, which found an average amount of 27 % (w/w) of ranitidine eliminated in normal subjects (van Hecken et al., 1982). The variability of the elimination values, however, appeared to be rather high ranging from 15 to 62 mg ranitidine recovered. For ranitidine, such a high variation between individuals has been described with respect to fraction of dose absorbed as well as for other pharmacokinetic parameters (Shim and Hong, 1989), whereas the intra subject variation of this compound is usually very small.

Table 2.7. Concentration-dependent effects of PEG 400 on ranitidine absorption calculated as the cumulative amount of ranitidine excreted in 24 hours (mg).

	Volunteer								
Treatment	1	2	3	4	5	6	Mean ± s.d.	*P* value	7 (D)
Control	29	31	25	41	15	62	34 ± 16		73
1 g PEG 400	46	46	40	52	35	68	48 ± 11	*0.001*	52
2.5 g PEG 400	29	19	14	23	20	18	21 ± 5	*0.116*	42
5 g PEG 400	27	25	17	23	16	18	21 ± 5	*0.117*	20

One could argue that it was to be expected for volunteer 6 to give the highest amount of ranitidine recovered of all normal subjects. He was the volunteer exhibiting the longest small intestinal transit time value for the control formulation giving the drug molecules a long time to be absorbed from the intestinal tract. Nevertheless, an even higher amount of ranitidine, namely 73 mg, which is equivalent to 49 % (w/w) of the administered dose, was recovered in the urine of the diabetic volunteer. This high value is also matched by a particularly slow transit of the oral liquid through the small intestine. In addition, absorption of ranitidine may in part have been enhanced by the "leaky gut" phenomenon,

which is common in patients with diabetes and has been described in more detail earlier. Widened tight junctions have been shown to render the intestinal epithelium more transparent for compounds that primarily use paracellular absorption pathways (Carratù et al., 1999). As a small, hydrophilic molecule with a molecular weight of 315 Da, ranitidine has been demonstrated to mainly traverse the intestinal mucosa via the tight junctions to reach the systemic circulation but not to enter cells to a significant extent (Gan et al., 1993).

In the presence of PEG 400, one would expect the ranitidine absorption results to follow the transit pattern, with increasing concentrations of PEG 400 decreasing not only the small intestinal transit time but also the extent of ranitidine absorption. In healthy volunteers, however, this was only true for the 2.5 and 5 g PEG 400 treatments. Both showed 38 % reduction in the cumulative amount of drug excreted, although it has to be noted that these were not statistically significant reductions due to the large inter subject variability. The lack of a clear relationship between the reduction in the MSITT and drug excretion in some volunteers is suggestive of additional factors being responsible for changes in ranitidine absorption in the presence of PEG 400. A further possibility may be related to the poorly absorbable and osmotically active nature of PEG 400. Inside the intestinal lumen the polymer was found to cause a progressive reduction in water and electrolyte absorption with increasing concentrations (Schiller et al., 1988; Davis et al., 1980). The authors believed this to be the result of the osmotic effect of PEG 400 alone and not some other mechanism such as an inhibition of active absorption or a stimulation of the excretion of water. After oral administration, PEG 400 will therefore retain fluid inside the lumen of the gastrointestinal tract via osmotic effects. This increased fluid load will not only decrease the concentration of the drug present in solution but also the concentration gradient across the intestinal mucosa, which may further hinder ranitidine absorption. Along similar lines, a previous study in humans has shown that the absorption of a series of compounds was adversely influenced by the presence of a non-absorbable osmotic load in the gut (Riley et al., 1992). Therefore, in addition to accelerated transit trans-mucosal fluid fluxes may also play a role.

As another phenomenon of that kind, solvent drag from the intestinal lumen may need to be considered as well. With the oral administration of hypotonic formulations water

absorption is initiated almost immediately to equilibrate the osmolality within the gastrointestinal tract to isotonic conditions. The theory of solvent drag promotes the opinion that small hydrophilic molecules are dragged across the intestinal mucosa in the course of the water stream and as a result show an enhanced absorption (Noach et al., 1994). In the present study, all treatments are hypotonic in nature but increasing in osmolality with increases in PEG 400 concentrations. Since an increase in osmolality is equivalent with a decrease in hypotonicity, the treatments with the lowest amount of PEG 400 would be expected to give the highest solvent drag effect. Since water movement occurs at a very fast pace, the positive effect of solvent drag on drug bioavailability would be greatest in the upper regions of the small intestine. Nevertheless, great disagreement exists in the literature on the general importance of solvent drag for the overall absorption of hydrophilic compounds *in vivo*. Seldom, the positive findings from *in situ*-studies could be verified *in vivo* and some authors doubt that molecules with a molecular weight of more than 200 Da will show any effect of solvent drag (Lennernäs, 1995; Fagerholm et al., 1999). Besides the occurrence of solvent drag in the presence of hypotonic luminal contents, a situation of reverse solvent drag has been observed as a result of the *in situ* perfusion of a hypertonic solution in rabbit intestinal tract (Riad and Sawchuk, 1991). A high concentration of PEG 400 was found to reduce the intestinal permeability of carbamazepine, which the authors partly attributed to the fact that the enhanced osmotic pressure within the lumen initiated the secretion of water into the intestinal lumen. With a water flux occurring against the absorptive direction, the diffusion of the drug molecules was severely hindered. A reduction of the thermodynamic activity of the drug molecules was also believed to have been of negative influence caused by the presence of relatively high amounts of the polymer inside the lumen. Another way of reducing the thermodynamic activity of ranitidine by PEG 400 could be a direct interaction of the polymer with the drug compound. PEG 400 is a polyalcohol with many ether groups allowing the excipient to closely interact with other hydrophilic moieties via hydrogen bonding. Complex formation between the polyethylene glycols (PEGs) and certain drugs has been reported, which resulted in a significantly reduced absorption of the active agent (Singh et al., 1966; Williams et al., 1982). It is, therefore, possible that the relatively high decrease in ranitidine absorption in the presence of 2.5 and 5 g of PEG 400 was caused by complex formation between the drug and the cosolvent having a negative effect on the passive

diffusion of the drug as a result of a decreased thermodynamic activity of ranitidine. However, such effects of the polymer on ranitidine have yet to be established.

The 38 % reduction in the absorption of ranitidine for the formulations containing 2.5 and 5 g PEG 400 appears to be slightly high compared to the data obtained in the study conducted by Basit et al. (2002). Here the authors found a 31 % reduction in ranitidine bioavailability in the presence of 10 g PEG 400 compared to the control with a concomitant transit time reduction of 35-37 %. The outcome of the lower bioavailability reduction in the presence of higher amounts of polymer may have been the result of the differences in study design. Basit and coworkers (2002) administered ranitidine in drug-loaded pellets, which were ingested with 150 mL of orange juice containing 10 g PEG 400. Although the pellets were formulated to immediately release the drug in the stomach it is not unlikely that the dosage form had an influence on the overall absorption of ranitidine. It is possible that some of the PEG 400 solution had emptied from the stomach before the drug had completely dissolved. In addition, the preparations were administered with orange juice instead of water. Albeit fasted volunteers, the presence of ingested glucose inside the lumen will result of a widening of the intestinal tight junctions (Nusrat et al., 2000; Sadowski and Meddings, 1993), hereby opening the absorption route for ranitidine. This in turn resulted in an alteration of the absorption conditions for the model compound. Apart from the differences in the study design, ranitidine is known to exhibit a relatively high inter subject variability (Shim and Hong, 1989), which make a direct comparison of data from different sets of human subjects difficult. It would have been useful, if more pharmacokinetic data were available in the present study, but taking of blood samples always means an invasive procedure for the participating volunteers.

Besides the apparent negative impact of PEG 400 at higher concentrations the presence of 1 g PEG 400, surprisingly, had an effect in the opposite direction, as the mean extent of ranitidine absorption was significantly increased by 41 % in the healthy volunteers. Enhanced absorption was observed in each and every healthy individual but not in the diabetic subject. The presence of low concentrations of PEG 400 may therefore increase, in normal subjects at least, the permeability of the gastrointestinal epithelium, although such effects have not previously been reported for PEG 400. As possible mechanism

behind this permeability enhancing effect one or more of the following might have possibly contributed to the increased permeation of ranitidine. Normally, the intestinal mucosa provides a natural barrier to the absorption of xenobiotics. One such mucosal barrier is afforded by the presence of the efflux transporter P-glycoprotein, which expels absorbed drug back into the lumen of the intestine and the CYP450 class of drug metabolising enzymes, which collaborate with the efflux transporters to eliminate drugs. Interestingly, PEG 300, a lower molecular weight analogue of PEG 400, was found to inhibit P-glycoprotein in Caco-2 cells, albeit at very high concentrations (Hugger et al., 2002). The impact of PEG 400 on P-glycoprotein efflux and P450 3A metabolism was investigated in excised rat intestine (Johnson et al., 2002) and the authors reported a dose-dependent inhibition of both enzymes. An everted gut sac-experiment conducted by Cornaire et al. (2000) investigated the effect of various excipients on P-glycoprotein but did not find a considerable impact of PEG 400. Although ranitidine has been reported to be a substrate for P-glycoprotein (Cook and Hirst, 1994; Collett et al., 1999), such an inhibiting effect with PEG 400 seems unlikely to majorly impact on ranitidine transport, since ranitidine has been shown to be mainly absorbed via paracellular pathways and not to enter cells to a significant extent (Gan et al., 1993). P-glycoprotein has therefore been suggested to play a minor role in the overall absorption of ranitidine, since its intracellular concentration may never reach sufficient levels for an activation of the intestinal efflux mechanism (Lentz et al., 2000). Although ranitidine has been reported to be subjected to a considerable pre-systemic hepatic first-pass metabolism (Roberts, 1984; Lin, 1991), intestinal metabolism comprising of similar enzyme systems as the liver can be expected to not play an important role for the drug. As the metabolising enzymes are located in the wall of the intestinal mucosa, paracellular compounds are expected to predominantly avoid this mechanism of elimination in the intestine (Watkins et al., 1987; Krishna and Klotz, 1994). In a jejunal perfusion study in rats investigating the intestinal clearance of various H_2-receptor antagonists, the metabolism for ranitidine was found to be minimal (Hui et al., 1994). The extent of metabolism, however, was detected as occurring amounts of ranitidine sulfoxide, which is not the main metabolite of ranitidine (Roberts, 1984). Recently, Mountfield and coworkers (2000) demonstrated a potential inhibitory effect of PEG 400 on intestinal cytochrome P450 but considered the inhibition of enzyme activity as insignificant for the *in vivo* situation as relatively high doses of the polymer are required. As an alternative explanation, PEG 400 may be increasing the

permeability of the gastrointestinal epithelium via an affect on the paracellular pathway. Such effects have been shown to occur *in vitro* with certain pharmaceutical excipients for purposes of penetration enhancement (Aungst, 2000). In addition, specific chemicals such as acetaldehyde, glycerol and hydrogen peroxide have recently been shown to interact with tight junctional proteins such as occludin in cell lines and human tissues (Atkinson and Rao, 2001; Wiebe et al., 2000; Kevil et al., 2000). This interaction resulted in a reduction and dislocation of occludin, which is primarily involved in the barrier function of the tight junctions (Kuwabara et al., 2001), leading to a disruption of this barrier and increased paracellular permeability. An interaction of occludin followed by intestinal membrane disruption and has also been demonstrated with the presence of various bacterial enterotoxins, which provoke intense water secretion causing the onset of diarrhoea (Simonovic et al., 2000; Dickman et al., 2000; Wu et al., 2000; McClane, 2001).

It is noteworthy that the diabetic was the only individual not to show an enhanced absorption of ranitidine in the presence of 1 g PEG 400 but instead a 29% reduction. The increased paracellular membrane permeability in diabetics has interestingly been associated with a decrease and redistribution of occludin (Antonetti et al., 1998). PEG 400, like ranitidine, primarily traverses the epithelial membrane via tight junctions (Ma et al., 1993) and in doing so could interact with a component of the tight junction structure to open up the paracellular pathway and hence enhance the absorption of ranitidine in healthy subjects but have no such effect in patients with diabetes. But no firm conclusions ought to be drawn from the data of the diabetic, as he was the only subject with this condition participating in the present study (n = 1) and, therefore, may in fact not be representative of the general diabetic population. A recent study using Caco-2 cell monolayers reported that H_2-receptor antagonists themselves inhibit their own transport across the epithelial lining (Gan et al., 1998). It was suggested that ranitidine causes a tightening of the paracellular pathway by modulating interactions among tight junctional proteins, hence contributing to its poor permeability and limited bioavailability in man. Using this information as a basis, it is possible that the absorption-enhancing effect of PEG 400 could also be via an inhibition of the interaction between ranitidine and the tight junctional proteins, thereby increasing both tight junction and paracellular permeability. Contrary to this, a separate Caco-2 study has

shown that PEG 400 neither alters the integrity of tight junctions nor the transport of ranitidine (Rege et al., 2001). Nevertheless, it should be appreciated that *in vitro* cell culture systems do not always resemble or accurately predict *in vivo* absorption due to the complex and dynamic nature of the human situation.

Although the exact mechanism behind the absorption enhancing effect of low concentrations of PEG 400 remains somewhat unclear, it is postulated that the polymer may directly influence gastrointestinal permeability. The question remains, however, as to whether the absorption-enhancing effect of PEG 400 is specific only to ranitidine or whether it is more generic in nature and equally applicable to other drugs.

2.5. Conclusions

The overall aim of this study was to establish the influence of PEG 400 on the gastrointestinal transit of oral drug solutions. While the presence of PEG 400 did not appear to affect the emptying rate of liquid formulations from the stomach, it was demonstrated that it had a considerable effect on small intestinal transit in a concentration dependent manner. Increasing concentrations of PEG 400 were found to have an increasingly accelerating effect on the passage of liquids through the small intestine. However, the relationship between the amount of cosolvent administered and the reduction in mean small intestinal transit time did not appear to be linear. A concentration-dependent effect of PEG 400 was also demonstrated for the oral absorption of ranitidine, which as a class III compound exhibits poor intestinal permeability. At low concentrations PEG 400 significantly enhances the absorption of ranitidine in normal subjects possibly via modulation of intestinal permeability. At high concentrations PEG 400 was observed to decrease the absorption of ranitidine to a considerable extent. Although this effect was not statistically significant it is believed to be mainly the result of the decreased small intestinal transit time, which might negate the absorption-enhancing effect observed at low amounts of the polymer. Although only one diabetic participated in the present study his results deviated markedly from those obtained in healthy volunteers with respect to transit and drug absorption. Overall, these findings have ramifications for the use of PEG 400 in pharmaceutical formulations.

CHAPTER 3

THE EFFECTS OF SOLUBILIZING AGENTS ON PASSIVE DRUG DIFFUSION ACROSS ARTIFICIAL MEMBRANES *IN VITRO*

3.1. Overview

Drug absorption from the lumen into the systemic circulation is a complex process or more precisely a combination or succession of complicated processes. The most important process is probably that of passive diffusion as every drug molecule is faced with diffusional movement at some stage on its way to its therapeutic destination. This includes, for instance, the release of the drug from its delivery system within the GI tract and diffusion through the luminal fluids across the unstirred water layer, diffusion across the apical phospholipid bilayer of the intestinal mucosa into and through the cytosol and subsequently diffusion across the basolateral membrane of the enterocyte into the blood stream. This transcellular absorption pathway is mainly used by lipophilic drug molecules, which easily partition in and out of the lipophilic mucosa, whereas very hydrophilic compounds make use of the paracellular route diffusing through the tight junctions located in between the epithelial cells. The process of passive diffusion is driven by a concentration gradient, where the drug molecule tries to move from a region of high concentration e.g. the lumen, to a region of lower concentration e.g. the systemic circulation where infinite dilution exists. Drug permeation through the lipid membrane of the GI epithelium as well as drug diffusion along the paracellular route are processes, which are dependent on various actions and interactions taking place inside the lumen, at the surface of the lipid barrier or inside and in between the enterocytes. The magnitude of the concentration gradient, the surface area of the membrane and the physicochemical properties of the molecule are important determinants for the passive diffusion of a molecule (Taylor, 1986). With the coadministration of various pharmaceutical excipients such as cosolvents and surfactants, the diffusion process of the active agent may be influenced in several ways. With the solubilized drug system reaching the site of absorption, drug-solubilizer interactions may occur, as well as solubilizer-membrane interactions. These interactions may impact on the absorption of the drug either in a facilitating and/or aggravating way.

Surfactants, and to an even greater extent cosolvents are known to be very efficient in enhancing the solubility of poorly water-soluble drugs. Quite often an exponential increase in drug solubility is observed with increasing solubilizer concentrations (Yalkowsky and Rubino, 1985; Dumanovic et al., 1992). Such a relation allows the

incorporation of comparatively high amounts of drug in pharmaceutical formulations, which after oral administration may prove problematic *in vivo*. With the formulation reaching the stomach, both the drug and the solubilizer are diluted in the physiological fluids, which can lead to supersaturation of the system resulting in precipitation of the drug in the stomach. If redissolution in the GI tract is impossible to achieve precipitation is likely to result in significantly lower, less reproducible and unpredictable drug bioavailabilities. The risk of drug precipitation upon dilution in the stomach is dependent on the amount of solubilizer administered in the pharmaceutical preparation and appears to be less apparent for surfactants. Here, the system needs to be diluted to surfactant concentrations below the CMC.

Besides the impact on drug solubility and precipitation the presence of solubilizers in the GI lumen has also been shown to influence the octanol/water partition coefficient, log*P* value of a drug, which describes the likeliness of a drug molecule to diffuse through the lipid bilayer (Riad and Sawchuk, 1991). Excipients that are poorly absorbed from the GI tract such as PEGs and sorbitol have been found to impact on water movement across the lining epithelium, which in turn has been shown to influence the absorption of hydrophilic drugs (Riley et al., 1992). Cosolvents are more likely to elicit such effects on fluid fluxes than surfactants, which are able to transverse the lipophilic membrane. Surfactants, on the other hand, are likely to influence drug diffusion processes as a result of the formation of micelles. The presence of high concentrations of micelles has been shown to reduce the permeability of lipophilic and hydrophilic drug compounds due to micellar entrapment of the drug and/or reduced thermodynamic activity in the aqueous continuous phase, respectively (Yoon and Burgess, 1996; Nerurkar et al., 1997).

Other interactions between drug and solubilizing agent have been reported to occur in form of complex formation. Polyethylene glycols (PEGs), in particular, have been suspected of interactions with drug molecules and formation of drug-cosolvent complexes. As polyalcohols with many ether groups per mole, PEGs are likely to interact via hydrogen bonding with other polar groups of various compounds. Such drug-cosolvent complexes can be reversible or irreversible and can sometimes have a positive or negative effect on the solubility and/or permeability of a drug compound (Singh et al., 1966; Chang et al., 1981; Williams et al., 1982).

The aim of the present study was to investigate whether the presence of increasing concentrations of PEG 400 has an influence on the passive diffusion of ranitidine. The results of the previous experiment in healthy volunteers (Chapter 2) demonstrated that an accelerating effect of PEG 400 on small intestinal transit exists. The transit effect was found to be significant at higher doses of PEG 400 and as a result a considerable reduction in the absorption of ranitidine was observed. As a class III compound with a high aqueous solubility but low intestinal permeability ranitidine is characterized by the process of membrane permeation as the rate-limiting step in oral bioavailability. Reduced oral bioavailabilities as a consequence of short small intestinal transit times is likely to occur with class III compounds as they are dependent on an efficiently long residence time at their absorption sites. Considering the results of the individual volunteers, however, a direct correlation between small intestinal transit and ranitidine absorption was not obtained. At times, a considerable reduction in oral absorption was observed although no alteration was found in the MSITT (compare Chapter 2). Therefore, it is not unlikely that PEG 400 might impact on ranitidine absorption in a different way such as influencing the drug's passive diffusion.

The passive diffusion of ranitidine was monitored employing a modified dialysis diffusion technique. Dialysis diffusion has been used by several authors to study the influence of excipients on drug diffusion (Yoon and Burgess, 1996) or for investigating drug release from colloid-disperse dosage forms (Levy and Benita; 1990). The material of the dialysis membrane consists of cellulose derivatives, which are hydrophilic in nature and, therefore, make this type of membrane ideal to study diffusion processes along aqueous drug absorption pathways. The dialysis tubing can be employed as an artificial intestinal membrane using either diffusion cells or dialysis bags. It was decided that the use of dialysis bags with a diameter of 5 cm would be a useful tool in the present study as it allowed the placing of the dialysis bags filled with different drug solutions into a dissolution vessel, which contained the sink medium. This modified set-up of the dialysis diffusion allowed automated monitoring of the ranitidine diffusion via detection of the appearance of the drug in the receiver compartment. In addition to investigating increasing concentrations of PEG 400 and the effects on ranitidine diffusion, the influence of propylene glycol were studied, which like PEG 400 is a commonly employed cosolvent in oral liquid dosage forms. Also, it was considered worthwhile to

look into the effects of micelle formation on the passive diffusion of ranitidine. As a model excipient of surface-active agents D-α-tocopheryl polyethylene glycol 1000-succinate (VitE-TPGS) also known as water-soluble vitamin E was investigated in the present study.

3.2. Materials

Ranitidine was provided in the form of ranitidine hydrochloride from GlaxoSmithKline (Ware, Hertfordshire, U.K.) and certified as 99.9% pure. It is a white to pale yellow, practically odourless, crystalline powder sensitive to light and moisture.

Polyethylene glycol 400 was obtained from Sigma-Aldrich Company (Poole, U.K.). It occurs as a clear, colourless, highly osmotic and viscous liquid with a slight but characteristic odour and a bitter taste. PEG 400 is hygroscopic and completely soluble in water.

Propylene glycol was obtained from Sigma-Aldrich Company (Poole, U.K.). The excipient is a clear, colour- and odourless liquid of medium viscosity that is hygroscopic and completely soluble in water.

D-α-tocopheryl polyethylene glycol 1000 suucinate (VitE-TPGS) was obtained from Eastman Chemical (TN, U.S.A.). It is a pale yellow waxy solid, which is highly miscible with water forming low-viscosity solutions at concentrations < 20 % (w/w).

3.3. Methods

3.3.1. Dialysis Diffusion

The set-up of the dialysis diffusion experiment is pictured in Fig. 3.1. A dialysis bag of 18 cm length was prepared using dialysis tubing with a molecular weight cut-off of 12000-14000 (Visking®, 36/32", Medicell, London, U.K.). One end of the tubing was sealed with a dialysis clip and the open end of the bag was attached to the plastic lid of a dissolution vessel. Initially, the tubing was washed and soaked in water (70° C) for

approximately 30 min to quantitatively free the film from glycerol. The diffusion experiment was set up in an automated dissolution apparatus (PTWS 20, PharmaTest, Hainburg, Germany). The receiver solution consisted of 1000 ml phosphate buffer 6.8 (0.05 M, BP 1993) and was filled into the dissolution vessel after 20 min degassing with helium. The temperature of the waterbath was set to 37 ± 1° C.

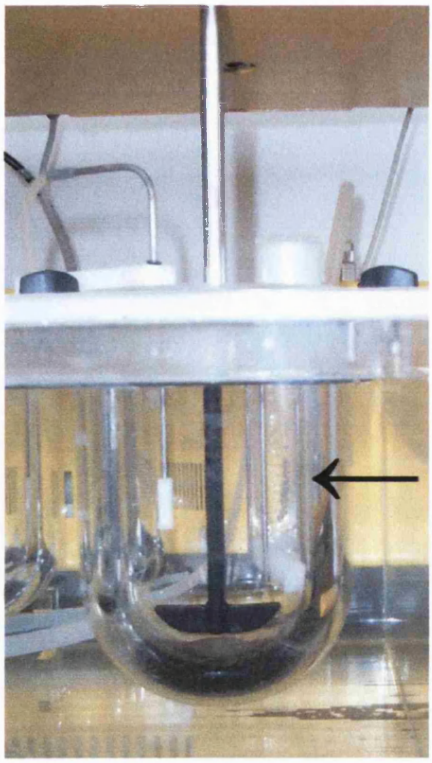
The lid with the attached dialysis bag was put on top of the vessel placing the bag inside the dissolution medium. Four stainless steel rods prevented the bag from touching the glass wall of the vessel or the rotating paddle, which was set to a speed of 50 rpm (Fig. 3.1).

The donor solution was prepared containing 1.25 mg/mL ranitidine in phosphate buffer 6.8 (0.05 M, BP 1993) or solubilizer mixtures with phosphate buffer. For the cosolvent studies PEG 400 concentrations were chosen to be 5, 10 or 30 % (w/w) and those of propylene glycol were 30, 50 or 70 % (w/w). Solutions were prepared by mixing the cosolvent and drug with the aqueous buffer. The concentration for VitE-TPGS was 0.2, 2.0 and 20 % (w/w) and the solutions were prepared by adding the surfactant in liquid form (in oven at 37° C) to the buffer. The mixture was stirred at approximately 60° C until complete dissolution of VitE-TPGS and then cooled down to room temperature before ranitidine was added. Forty mL of the different drug solutions were filled into the dialysis bag. The passive diffusion of ranitidine across the membrane was monitored by assessing the drug concentration in the receiver compartment. Automatically, filtered samples were taken every 60 min over 24 hrs and transferred to the UV spectrometer (Cecil CE 2020, Cecil Instruments, Cambridge, U.K.), where the absorbance of ranitidine was determined at 310 nm. The samples were then replaced into the receiver solution. The experiment was conducted in triplicate.

Figure 3.1. a) The Cecil CE 2020 dissolution apparatus with automatic sampling and UV analysis, and b) a single vessel containing the dialysis bag within the receiver medium.

a)

b)



3.3.2. Mass Spectrometry

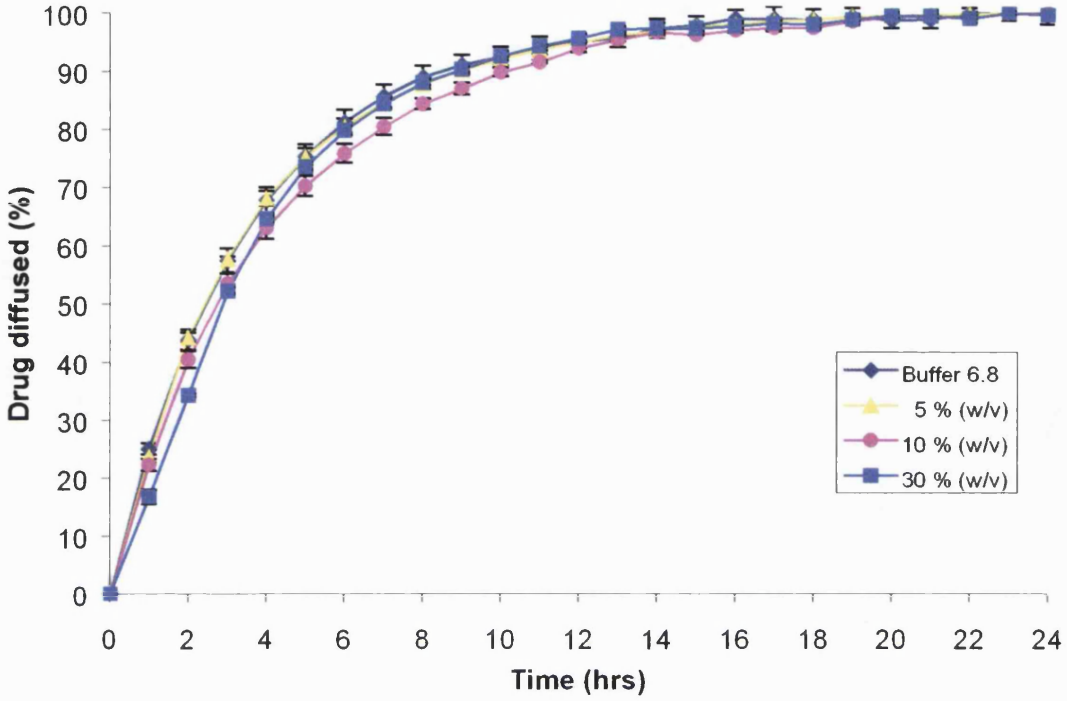
Fast atom bombardment (FAB) mass spectrometry is used for the identification and determination of the molecular weight and structure of organic substances of high molecular weight. The sample, in solution, is bombarded with an atomic argon beam of high velocity, which serves to transform the solution into a gaseous ionic state. These ions are then extracted by means of an electric field and separated according to their mass/charge ratio (m/z). In the mass spectrum obtained by FAB the ion-currents are amplified as peaks and correspond to fragments characteristic for the analyte.

Since neither PEG 400 nor propylene glycol possess a chromophore UV spectroscopy could not be employed and mass spectrometry served to assess the appearance of PEG 400, propylene glycol and VitE-TPGS, respectively in the receiver solution of the dialysis diffusion experiment. FAB mass spectrometry was employed and spectrums were obtained from the respective solubilizing agent in water as a reference, as well as from samples taken from the receiver solution at the end of the experiment.

3.4. Results and Discussion

The passive diffusion of ranitidine across hydrophilic membranes was monitored using a dialysis diffusion technique. To assess the effects of different solubilizers on ranitidine membrane permeation, solutions of the drug in the respective solubilized systems were administered into dialysis bags located in a receiver medium under sink conditions. The concentration of ranitidine were chosen to reflect approximately the amounts used in the previous *in vivo* study (Chapter 2) as were those of PEG 400, which was administered as 5, 10 and 30 % (w/w) cosolvent in water mixtures correlating to amounts present in the donor bag of 2, 4 and 12 g, respectively. The effects of propylene glycol were investigated at considerably higher concentrations of 30, 50 and 70 % (w/w) due to the fact that it is sometimes used at increased amounts e.g. 50 % (w/w) in Agenerase® oral solution. In contrast to the cosolvents PEG 400 and propylene glycol, VitE-TPGS was employed as a surfactant to investigate the influence of micelle formation on ranitidine diffusion. It was, therefore, administered at concentrations of 0.2, 2.0 and 20 % (w/w), which is above the surfactant's CMC of 0.02 % (w/w).

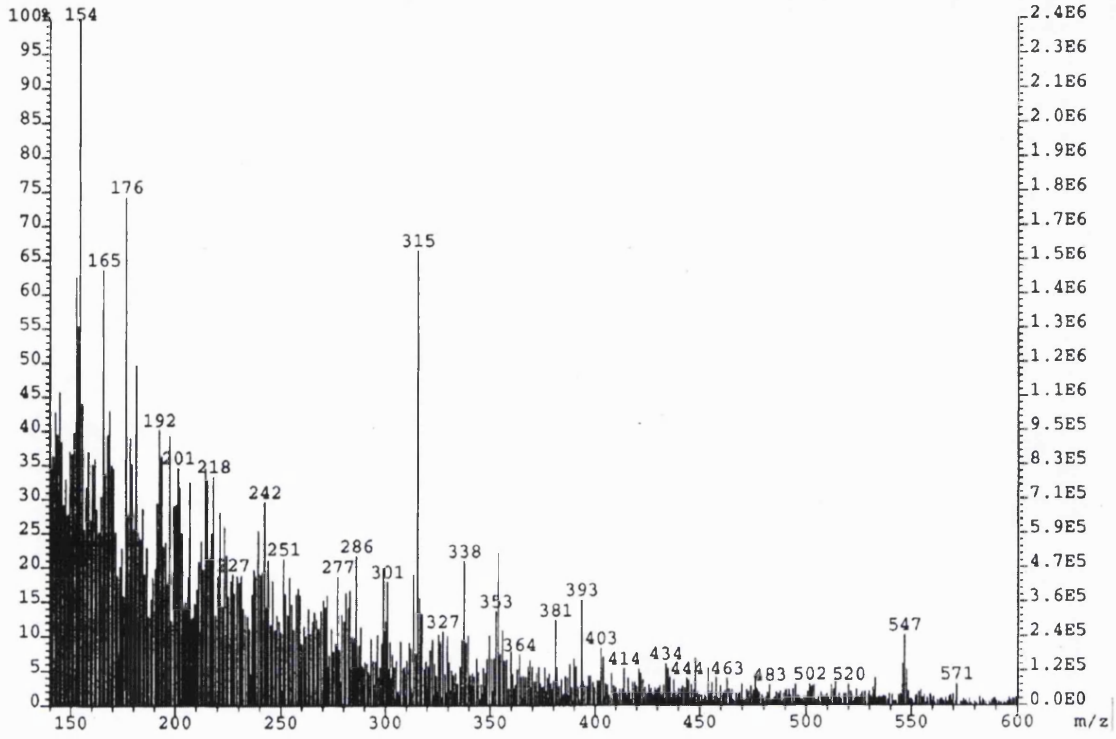
Figure 3.2. The influence of PEG 400 concentration on the passive diffusion of ranitidine using the dialysis diffusion technique.



The results of the passive diffusion of ranitidine across an artificial membrane in the absence and presence of PEG 400 are shown in Figure 3.2. From a solution in phosphate buffer 6.8 ranitidine diffusion reached equilibrium (> 95 %) after the first 12 hrs of the experiment. The presence of PEG 400 appeared to have no significant impact on the passive diffusion of ranitidine. A slight decrease in the drug diffusion rate, however, was observed with the 30 % (w/w) PEG 400 solution in the initial six hours resulting in a short delay of 0.5 to 1 hr in the amount of ranitidine diffused compared to the control formulation.

Figure 3.3 shows the mass spectrum of ranitidine, which was obtained to serve as a reference to identify the drug in the spectra taken from the various samples of the receiver medium. Having a molecular weight of 314 Da ranitidine gives a clear peak in the spectrum with a mass/charge ratio of 315 units.

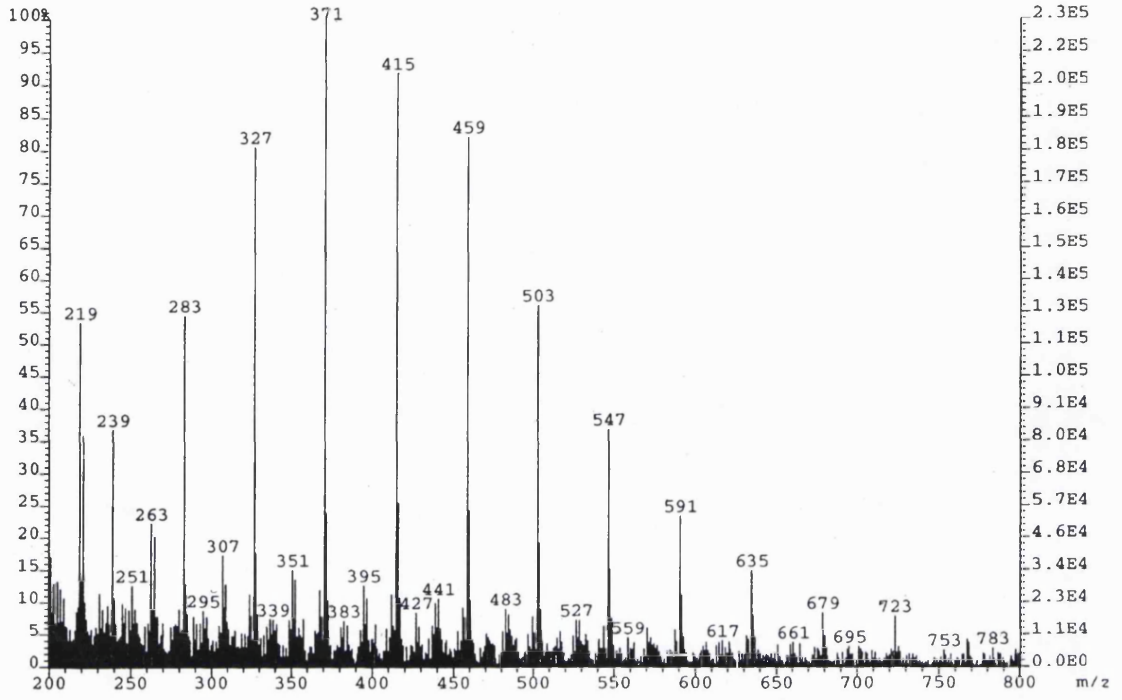
Figure 3.3. Mass spectrum of ranitidine reference.



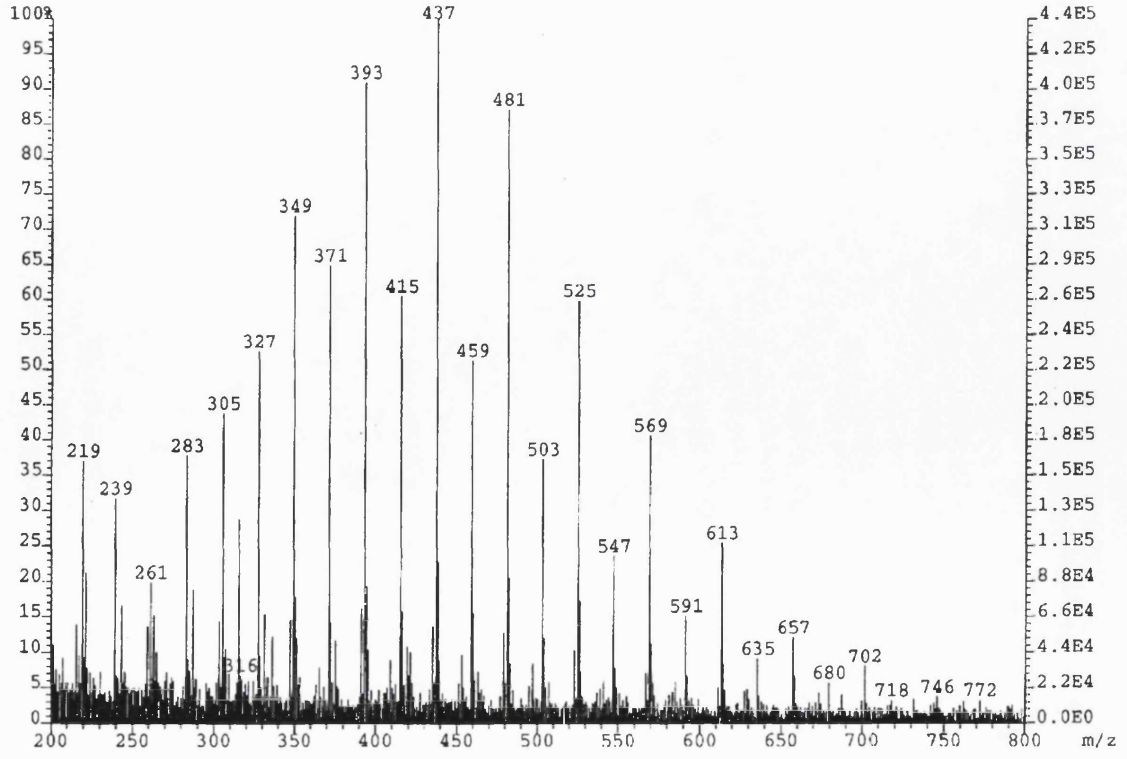
The mass spectra in Figures 3.4a,b represent a PEG 400 reference sample and a 24-hrs sample taken from the receiver solution of the experiment with 30 % (w/w) PEG 400 as the donor solution, respectively. The spectrum of the PEG 400 reference clearly gives the characteristic peaks of the different ethylene glycol polymers comprising the several polymers of PEG 400 with a difference in mass/charge units of 44, which corresponds with the molecular weight of one ethylene glycol unit. The height distribution of the peaks also suggests an average molecular weight of 400 Da. The mass spectrum in Figure 3.4b gives the same peaks but in addition a series of peaks with a height distribution and even spacing of 44 (m/z) units between the peaks similar to the spectrum of the PEG 400 reference. This series of peaks is the result of the sample probe containing phosphate buffer, which adds a 22 (m/z) unit to the PEG 400 peak series as a result of complexation of the polymer with sodium ions.

Figure 3.4. Comparison of the mass spectrum of PEG 400 and samples from the sink solution of the dialysis diffusion study.

a) PEG 400 reference



b) 24 hrs sample from the receiver solution



The presence of ranitidine in the receiver solution was also detected by the mass spectrum of Figure 3.4b, where a small but characteristic peak of the H_2-receptor antagonist shows at 315 (m/z) units. The small size of this peak is the result of the relatively high concentration of the polymer PEG 400, which appears to saturate the spectrum making it difficult to detect other peaks easily. However, Figures 3.4a,b clearly demonstrate that diffusion of PEG 400 across the dialysis membrane has occurred.

The diffusion of ranitidine across the dialysis membrane is driven by the concentration gradient of the drug existing between the donor and the receiver compartment. In an effort to equilibrate drug concentration throughout the system the diffusion rate of molecules is highest in the initial phase of the experiment and lower towards the end when the concentration gradient is minimized. Inside the system a concentration gradient also exists for PEG 400. Similar to the drug compound, the polymer will try to move quickly across the dialysis membrane in an effort to compensate for the difference in concentration. The presence of cosolvents like PEG 400, however, might interfere with the passive diffusion process of a drug compound in many different ways. For instance, in addition to the concentration gradient an osmotic pressure gradient is present created by the osmotic activity of PEG 400. The increased osmotic pressure inside the donor compartment additionally forces the system to equilibrate. On one hand, the enhanced gradient along the artificial membrane might increase the diffusion of PEG 400 and hence ranitidine leading to an increase in drug diffusion rate with increasing PEG 400 concentrations. On the other hand, the osmotic gradient causes water movement in the opposite direction. This means that water flux takes place from the receiver compartment into the donor bag in an attempt to lower the osmotic pressure of the donor solution. Such fluid fluxes have also been recognized *in vivo*. Caused by the luminal presence of unabsorbable macromolecules or compounds like mannitol and sorbitol, which are not properly digested, hyperosmolar conditions are created within the GI lumen. High molecular weight PEGs have been found to exert a substantial osmotic activity in solution despite their molecular size (Bryan et al., 1980; Schiller et al., 1988). In order to lower the osmotic pressure and achieve isotonicity the necessity for water secretion becomes apparent. Some drugs have been shown to transverse the lining epithelium with the absorption flux of water, making use of the so-called "solvent drag"

across the intestinal membrane. In this case, an overall net water efflux from the serosal side into the lumen, which has accordingly been termed "reverse solvent drag", may impair the absorption rate and bioavailability of such drugs considerably (Riley et al., 1992). In the present case, however, the diffusion rate of ranitidine does not appear to be affected by these phenomena to a great extent. Compared to the control in phosphate buffer the diffusion of ranitidine in the presence of increasing concentrations of PEG 400 is probably slightly delayed but not significantly impaired. This may well be the result of PEG 400 being able to cross the dialysis membrane itself, which means that movement across the membrane occurs in both directions. The situation *in vivo*, however, is expected to be more complicated and reverse solvent drag may play a more significant role due to the fact that the extent of PEG 400 absorption is limited as a result of lower pore size. Interestingly, an *in vivo* perfusion study in rats found that the absorption of the polymer itself is affected by induced "reverse solvent drag" (Krugliak et al., 1989). The uptake of PEG 400 was observed to decrease significantly with an elevated osmolality of the perfusate solution, which was achieved with the addition of mannitol.

Besides effects on fluid fluxes across membranes, cosolvents have also been suspected to impact on drug absorption and diffusion through physicochemical interactions with drug molecules. Polyethylene glycols, for instance, are polyalcohols, which possess many hydroxyl-groups per mole and are likely to interact with other polar groups of various compounds via hydrogen bonding, which may lead to the formation of drug-cosolvent complexes. Such complex formation was observed in the case of phenobarbital and PEG 4000, a similar compound to PEG 400 with an average molecular weight of 4000 Da (Singh et al., 1966). The complexation resulted in a highly reduced solubility of the active agent with the consequence of a significant decrease in the absorption of phenobarbital. Surprisingly, the dissolution and absorption of three other barbiturates was not affected by the presence of PEG 4000. As a consequence of complexation the thermodynamic activity of the drug is reduced, which holds the drug back in the lumen and retards the rate of absorption. In an aqueous PEG 400 solution of carbamazepine, drug-cosolvent interaction was believed to be responsible for a reduction of the drug's effective permeability, P_{eff} by decreasing the thermodynamic activity of carbamazepine and hindering it to permeate through the GI membrane (Riad

and Sawchuk, 1991). Complex formation was found to obstruct drug absorption from salicylic acid and chlorthalidone formulations (Williams et al., 1982) containing higher molecular weight PEGs. However, in the present case no evidence was found of complex formation between PEG 400 and ranitidine. The mass spectrum (Figure 3.4b) did not reveal the presence of such a complex, which would have resulted in one or more visible peaks with a mass charge unit in the range of 550 to 1000 ($(m/z)_{complex}$ = 239-657 $(m/z)_{PEG400}$ + 315 $(m/z)_{ranitidine}$).

The diffusion profiles of ranitidine from different concentrated **propylene glycol** solutions are shown in Figure 3.5. Compared to the diffusion profile from the control preparation in phosphate buffer, the presence of 30, 50 and 70 % (w/w) propylene glycol appears to have a slightly delaying effect on the diffusion of ranitidine across the dialysis membrane. The same amount of drug diffused is not detected before 1 to 2 hrs after that in the absence of the cosolvent. In addition, equilibrium of the system (> than 95 % of drug diffused) is not reached before 15 hrs compared to 12 hrs for the solution of ranitidine in buffer.

Figure 3.5: The influence of propylene glycol on the passive diffusion of ranitidine using the dialysis diffusion technique.

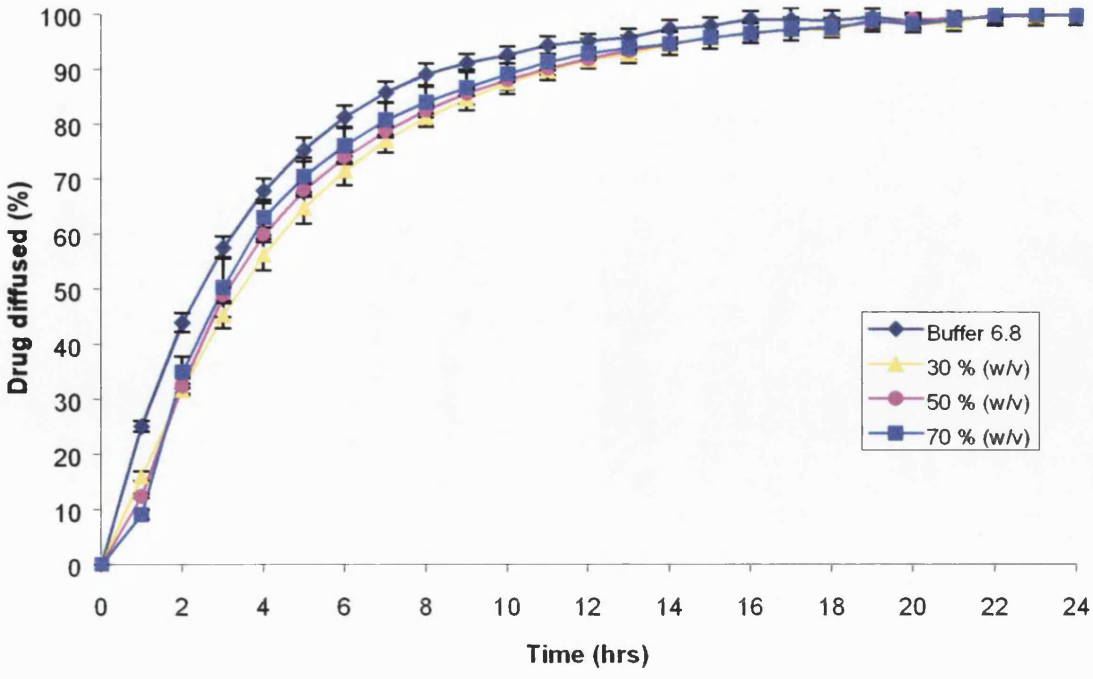
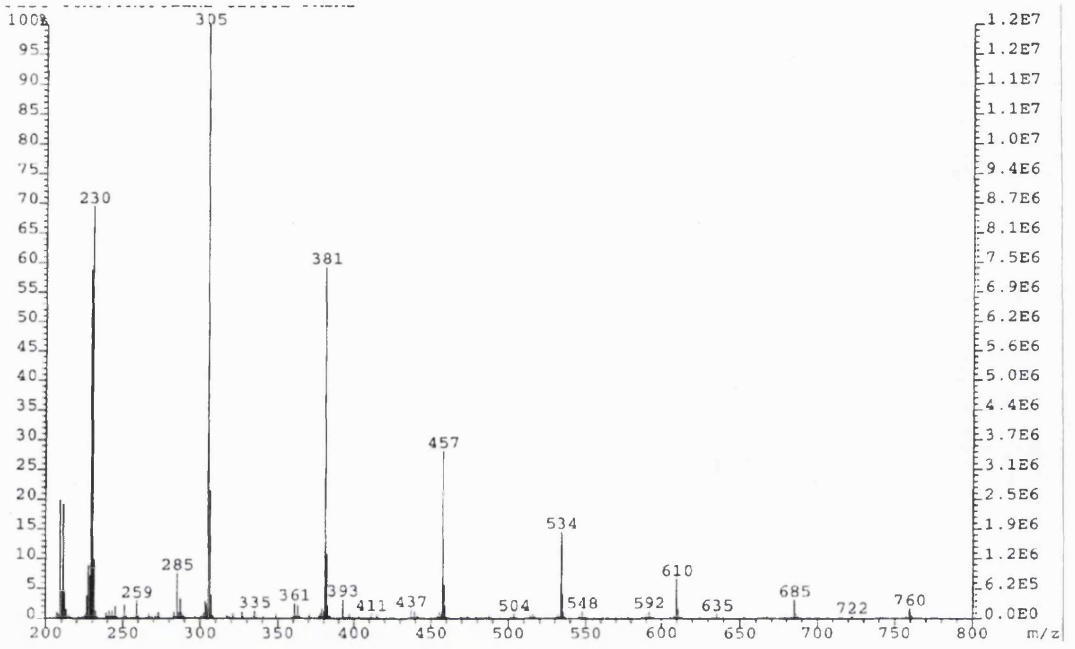
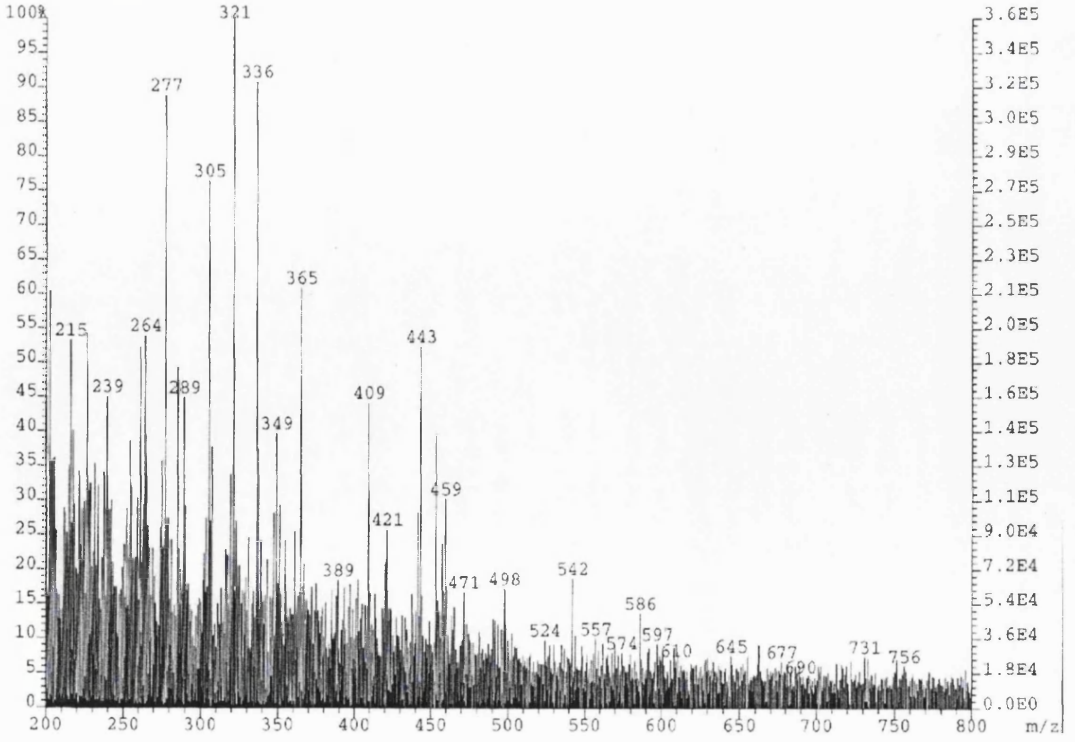


Figure 3.6. Comparison of the mass spectrum of propylene glycol and samples from the sink solution of the dialysis diffusion study.

a) Propylene glycol reference



b) 24 hrs sample.



However, the effect of propylene glycol on the passive diffusion of ranitidine does not seem to be dose-dependent, e.g. increasing cosolvent concentrations did not result in decreasing drug diffusion.

Propylene glycol is a low molecular weight alcohol (Mw = 76 Da), which is expected to rapidly diffuse across the hydrophilic dialysis membrane with a molecular weight cut-off of 12 000-14 000. The results of the mass spectrometry analysis of samples of propylene glycol reference in water and of the receiver solution at the end of the experiment are pictured in Figures 3.6a and 3.6b, respectively. The mass spectra of the cosolvent not only reveal the fact that propylene glycol indeed has diffused across the dialysis membrane but also that self-association of the cosolvent occurred.

In the spectrum of propylene glycol reference an average difference in the mass/charge ratio of the individual peaks of 76 units is apparent, which correlates to the molecular weight of a propylene glycol monomer. Similar peaks can be found in Figure 3.6b in addition to several other peaks that cannot be clearly explained but are likely to belong to fractions of propylene glycol oligomers complexed to sodium or oxygen etc. The peak with a mass/charge ratio of 336 units can be attributed to ranitidine and sodium (314 + 22). It is difficult, however, to clearly detect complex-formation between ranitidine and propylene glycol in the spectrum of Figure 3.6b. Such complexes would have resulted in peaks with an approximate (m/z) unit of 390, 466, 542 etc correlating to 314 + $(76)_n$ units. Relatively small peaks are visible, which have a mass to charge ratio of 389 and 542 units but it is difficult to say whether they are the result of said complexes or are peaks of the matrix. So far, no examples of complex formation of propylene glycol with active agents have been reported in the literature.

It is assumed that the impact of propylene glycol on the diffusion of ranitidine was not caused by physicochemical interactions between the drug and the cosolvent but rather the result of an increased viscosity of the donor solution at such high concentrations of propylene glycol. Increases in viscosity of drug solutions have been shown to result in a decrease in drug absorption due to a reduction in the thermodynamic activity of the active agent (Levy and Jusko, 1965). In addition, the solutions containing propylene glycol are likely to possess elevated osmolalities since the cosolvent being a low

molecular weight alcohol comprises of a considerably high osmotic activity. An impact of adverse fluid fluxes as a result might also have slightly decreased the diffusion rate of ranitidine from propylene glycol solutions. However, the overall effect of propylene glycol on the diffusion of ranitidine did not seem to be of marked extent and it has to be questioned whether the observed delay in diffusion would be of any importance in the absorption of ranitidine *in vivo*.

The results of the diffusion profiles of ranitidine from aqueous solutions containing different concentrations of **VitE-TPGS** are given in Figure 3.7. It can be seen that concentrations of 0.2 and 2.0 % (w/w) of the surfactant did not have any effect on the diffusion of ranitidine, whereas the presence of 20 % (w/w) VitE-TPGS caused a considerable decrease of drug diffusion across the dialysis membrane. Figures 3.8a,b represent the mass spectra of samples of VitE-TPGS in water as reference and of a 24-hrs sample at the end of the diffusion experiment with the 2 % (w/w) VitE-TPGS preparation, respectively. Both spectra give a similar pattern of peaks and it is suggested that VitE-TPGS moved across the membrane throughout the diffusion experiment.

Figure 3.7. The influence of VitE-TPGS on the passive diffusion of ranitidine.

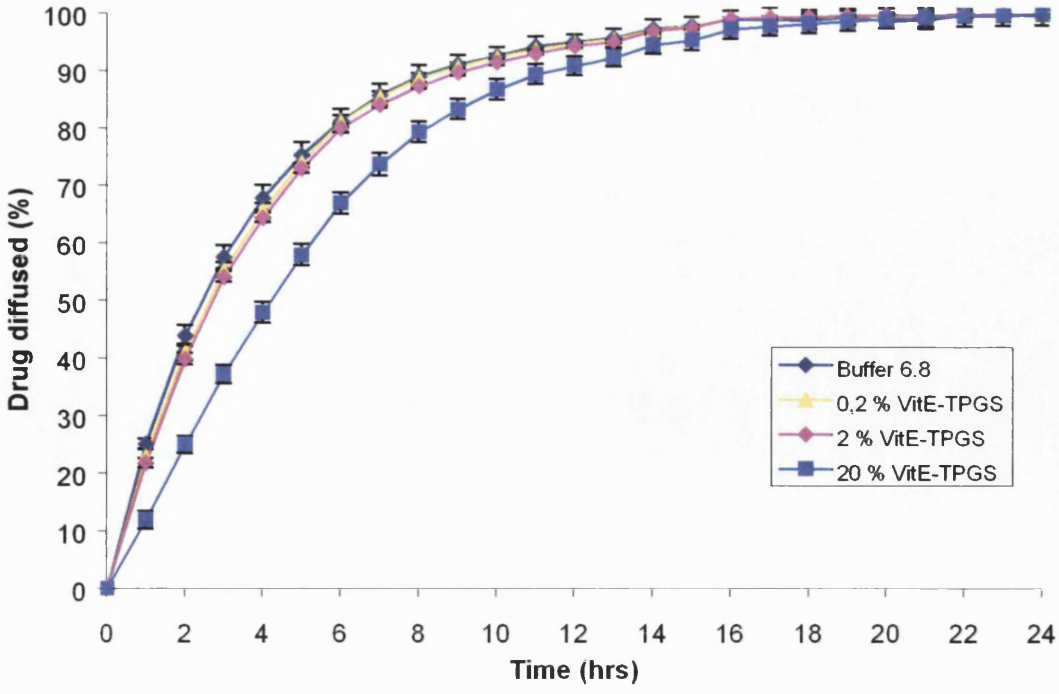
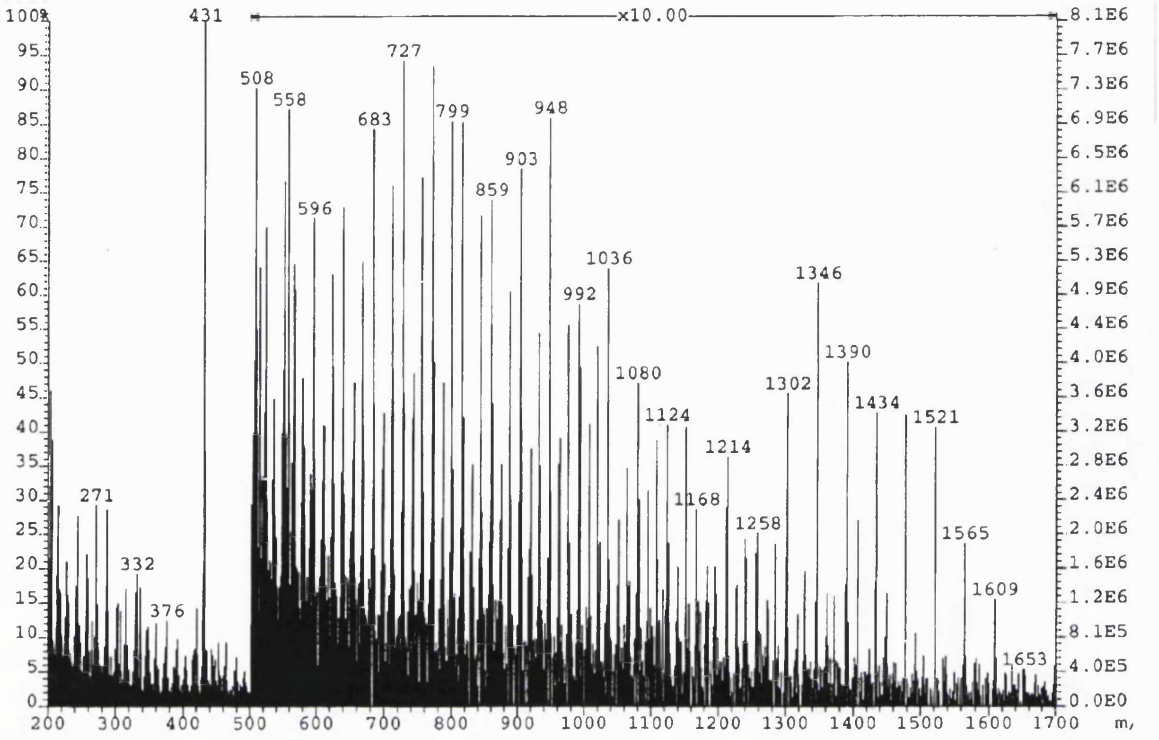
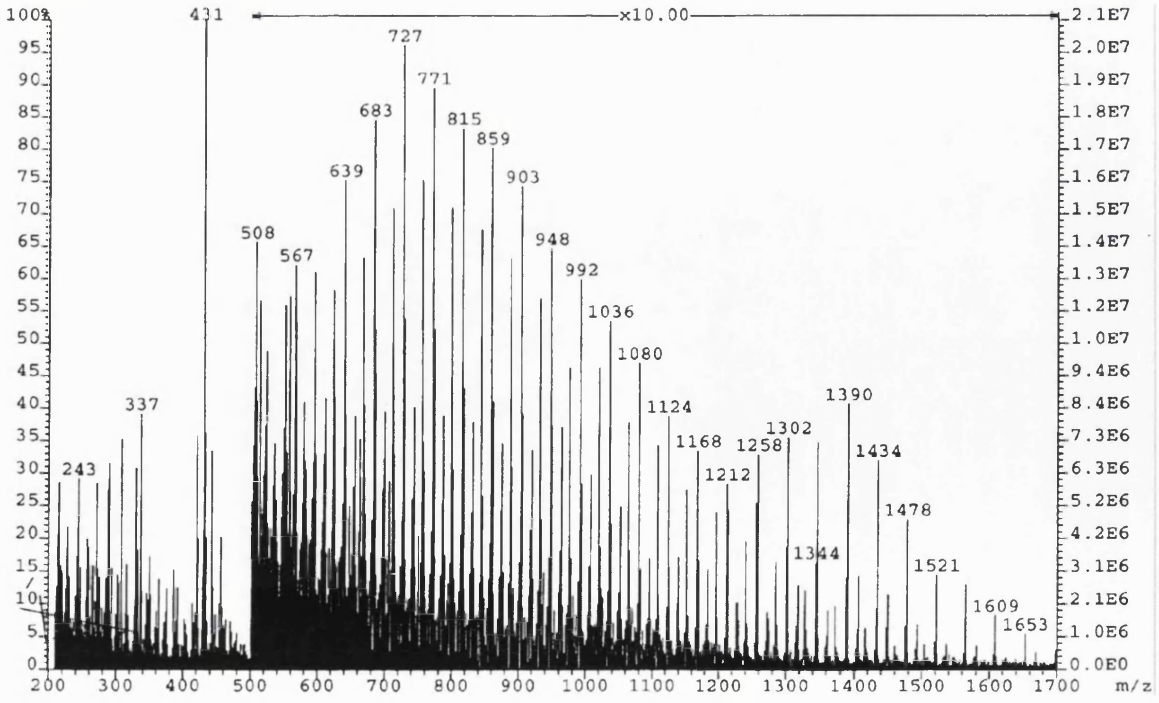


Figure 3.8. Comparison of the mass spectrum of VitE-TPGS and samples from the sink solution of the dialysis diffusion study.

a) VitE-TPGS reference



b) 24 hrs sample



On the right hand side of both spectra a series of peaks is visible, which, in general, differ in their mass to charge ratio by 44 units. This series of peaks belong to the polyethylene glycol part of the surfactant molecule resembling the various polymers of PEG (one ethylene glycol = 44 (m/z) units). Surprisingly, the distribution of peaks does not appear to give an average molecular weight of 1000 Da as thought to be present in the VitE-TPGS (D-α-tocopheryl-PEG 1000-succinate) molecular structure but rather at a lower molecular weight. However, the pattern of polyethylene glycol molecules is apparent. The singular peak present in both spectra with an (m/z)- ratio of 431 units is expected to belong to the tocopherol part of the surfactant molecule (MW = 473). The second single peak in Figure 3.8b at 337 (m/z) ratio units can be attributed to ranitidine-Na (315 + 22) and can therefore not be detected in the reference sample of VitE-TPGS (Figure 3.8a).

VitE-TPGS has a CMC of 0.02 % (w/w), which means that the surfactant molecules will self-associate and form micellar structures at concentrations above the CMC. For dug absorption processes from micellar solutions a pseudo-phase separation model exists comprising the dispersed micellar phase and the continuous aqueous phase surrounding the micelles. The drug is partitioned between both phases at a constant partition ratio. Drug solubilized in micelles, however, is not available for absorption and passive diffusion of the drug is dependent on the thermodynamic activity of the drug in the aqueous phase (Krasowska, 1980). In the present study, the effect of micelle formation on the passive diffusion of ranitidine was investigated. It appeared that VitE-TPGS concentrations of 10 and 100 times that of the CMC, 0.2 and 2 % (w/w) VitE-TPGS, respectively, decreased the diffusion rate of ranitidine only to a small extent. Due to the hydrophilic nature of ranitidine the compound is not likely to be markedly subjected to micellar solubilization but primarily remain in the continuous aqueous phase of the preparation. This is in accordance with the findings of Yoon and Burgess (1996) who investigated the diffusion of different model drugs across hydrophilic and lipophilic membranes *in vitro*. Increasing surfactant concentrations hardly affected the permeability of the hydrophilic compounds, whereas the permeation of lipophilic compounds across hydrophilic dialysis membranes was considerably decreased due to the high extent of micellar solubilization of these drugs.

At high surfactant concentration the shape of the micelles changes from spherical to ellipsoidal, which causes an increase in the continuous phase viscosity. This increased viscosity will result in a reduction of the thermodynamic activity of the drug molecules in the aqueous phase and in turn reduce the diffusion rate of the drug across the membrane (Levy and Jusko, 1965; Krasowska, 1980). The observed significant reduction in passive ranitidine diffusion in the presence of 20 % (w/w) VitE-TPGS is suggested to be the consequence of reduced thermodynamic activity due to an increased continuous phase viscosity. Around a surfactant concentration of 20 % (w/w) VitE-TPGS has been demonstrated to show a rapid increase in solution viscosity as a result of the formation of liquid-crystalline structures (Wu and Hopkins, 1999). In addition to changes in viscosity, adsorption of micelles via dipole interactions of the OH-groups of the cellulose dialysis membrane with surfactant molecules may occur at high concentrations of VitE-TPS having a decreasing effect on drug transport rates. Such interactions, however, are suggested to have played a minor role in the overall diffusion of ranitidine.

3.4.1. Limitations of the study

The present study aimed to find out whether the presence of relatively high amounts of cosolvents or surfactants has a significant impact on the passive diffusion of ranitidine. It needs to be mentioned that the employed technique of dialysis diffusion has certain limitations and can only serve to explain a fraction of the processes taking place at the absorption site of ranitidine *in vivo*. While the employment of the hydrophilic dialysis membrane allowed a satisfying insight into the diffusion of ranitidine along its aqueous absorption pathways the present experiment may not reflect the movement of the drug through the tight junctions in every aspect. Tight junctions, for instance, have been reported to have a rather small pore size in the range of 0.5 to 5 nm (Macheras et al., 1995), which is higher in the small intestine than in the colon (Chadwick et al., 1977b). Larger molecules are, therefore, limited in their extent of absorption, as was shown for PEG 400, which has been described to be absorbed at around 50 % of the administered dose (Chadwick et al., 1977a; Ma et al., 1990). In the present study, however, it is suggested that the polymer diffused quantitatively as a result of the relatively high molecular weight cut-off of the dialysis membrane (12000 to 14000). The permeability of drugs has been shown to be dependent on the pore size of the dialysis membrane, although the impact appeared to increase in significance with increasing lipophilicity of

the compounds investigated (Yoon and Burgess, 1996). Dialysis membranes with a molecular weight cut-off as low as 1000 exist and it is likely that the overall diffusion rate of ranitidine is reduced by a lower membrane pore size. It is, however, assumed that diffusion studies employing such a membrane would not result in contradictory findings to the present study with respect to the influences of solubilizing agents since a molecular weight cut-off of 1000 is expected to also allow quantitative diffusion of the investigated cosolvents PEG 400 and propylene glycol. Nevertheless, it would have been preferably to quantify the diffusion of the administered excipients in addition to the qualitative assessment. Quantitative analysis via FAB mass spectrometry is possible when an internal standard is employed, e.g. ^{13}PEG400. The development of such a method, however, is not straightforward and very time consuming and as a result the input was thought to be out of proportion for the purposes of the present experiment. Alternative methods for quantitative determination of PEG 400, for instance, include refractive index diffraction.

Apart from the limitations of the dialysis technique to assess the in vivo effects of solubilizing agents on ranitidine, it is generally not hydrophilic drugs that are formulated with solubilizers, as they are sufficiently soluble in aqueous media. Poorly water-soluble drugs, e.g. class II drugs, on the other hand, require the addition of these excipients on a regular basis often at relatively high amounts in order to promote their solubility in the physiological fluids. It would have been of interest to also investigate the effects of the employed solubilizers on the passive diffusion of a hydrophobic compound such as amprenavir. The presence of high solubilizer concentrations in Agenerase® oral solution was found to have a significantly negative impact on the oral bioavailability of amprenavir, which might to a certain degree as well be attributable to an effect of the excipients on drug passive diffusion. However, the conduction of an experiment involving a class II drug dissolved in solubilized systems is faced with certain difficulties as a result of the sometimes extremely low aqueous solubility of the drug. In such cases, the solubilized system administered in the dialysis bag will contain comparatively high amounts of drug dissolved, which means drug levels close to saturation. With the start of the experiment water from the sink solution will rapidly penetrate into the dialysis bag concomitantly with efflux of the cosolvent out of the bag. This process results in a rapid decrease in solubilizer concentration, which in turn causes the system to supersaturate

and hence have the active ingredient precipitated. Such an effect was also observed by Levy and Benita (1990), who investigated the *in vitro* release of diazepam from a submicron o/w emulsion employing the dialysis bag technique. Here, the release rate of diazepam was drastically reduced as a result of drug precipitation from a 70 % alcohol solution, which was only obtained when water served as sink medium but not with 70 % alcohol in the receiver vessel. It has been suggested to add non-aqueous solvents or solubilizers to the sink solution e.g. equal solubilizer-water mixtures in donor and receiver department, respectively. With this procedure, however, it is essential to investigate the drug diffusion rate as a function of the concentration of solubilizer in the sink (Washington, 1990). The results, however, are expected to be of little value if the sink additives predominate the effects on diffusion or, as in the present case, the solubilizer themselves are under investigation. It is possible, however, to reduce the concentration of the administered drug to such an extent that dilution of the solubilized system via water influx into the dialysis bag does not lead to supersaturation. This in turn is likely to result in drug concentration levels in the receiver solution that are below the level of detection at least with the set-up of automated UV detection used in the present experiment.

Apart from the mentioned difficulties in conducting the present diffusion study with a poorly water-soluble drug, it is doubtful that such results give valuable information on the absorption behaviour of the drug since hydrophobic molecules do not use aqueous absorption pathways but move across the lipophilic membrane of the enterocytes. It would, therefore, have been more interesting to investigate the effect of solubilizers on the transcellular passive diffusion employing a hydrophobic membrane instead. Membranes of polysiloxanes, for instance, serve as artificial hydrophobic barriers in *in vitro* diffusion cell studies to model intestinal as well as transdermal absorption. The influence of cosolvents and surfactants on passive diffusion of lipophilic drug molecules differs to their effects observed with hydrophilic molecules, which have been mentioned above. Besides their impact on drug solubility and precipitation the presence of solubilizing agents has also been shown to influence the octanol/water partition coefficient, log*P* value of a drug or more precisely the octanol/aqueous phase partition coefficient. Log*P* is a measure for the relative lipophilicity of a drug and its likeliness to diffuse through the lipid bilayer of the epithelium. Increasing concentrations of cosolvent

appear to have a decreasing effect on log*P*, which means that cosolvents reduce the affinity of a hydrophobic drug to leave the aqueous medium of the luminal contents and reach the lipophilic membrane. Significant reductions in log*P* may result in a low permeability of the therapeutic agent. A linear relationship has been described between the logarithm of the effective intestinal permeability, P_{eff}, of carbamazepine and the octanol/water partition coefficient (Riad and Sawchuk, 1991). In this *in situ* perfusion technique on the duodeno-jejunum and ascending colon in the rabbit increasing percentages of PEG 400 decreased the log*P* value of the solution and concomitantly reduced the fraction of drug absorbed (Riad and Sawchuk, 1991). Hamza and Kata (1990) employed a Sartorius absorption simulator in an *in vitro* availability study on allopurinol, which uses artificial lipid barriers consisting of inert membrane filters, the pores of which are filled with a liquid lipid phase. The absorption simulator distinguishes between a stomach wall barrier and an intestinal wall barrier and uptake of drug from simulated gastric or intestinal juice to plasma is monitored. The authors investigated the solubilization effect of various cosolvents on allopurinol diffusion across the lipid barriers and observed a significant decrease in drug diffusion from intestinal juice to plasma with cosolvent solutions of PEG 400, propylene glycol, glycerol and sorbitol (Hamza and Kata, 1990). In contrast, an enhancement of allopurinol diffusion was observed with the addition of a surfactant (Tween® 80). Effects of the non-ionic surfactant Brij 97 on the effective permeability, P_{eff}, of various model drugs administered as emulsions was investigated by Yoon and Burgess (1996) in an *in vitro* diffusion study using polysiloxane membranes. Permeation of the hydrophobic membrane by lipophilic drugs was found to increase with increasing surfactant concentrations up to 1.0 % (w/w) and then decrease with further increase in micellar concentration. At the same time increasing Brij 97 concentrations reduced the oil/surfactant-in-water partition coefficient of the drugs. Since only drug molecules present in the continuous aqueous phase of a micellar solution are available for absorption the presence of high amounts of micelles will result in entrapment of the drug molecules within the core of the micelles. In addition to entrapment inside the micelles, drug molecules traversing the continuous aqueous phase to reach the lipophilic absorptive membrane face reduced thermodynamic activity as a result of high micelle concentrations. The benefit of micellar solubilization regarding increased drug solubility at lower surfactant concentrations has, therefore, been found to have a negative impact on drug permeability at higher concentrations

(Yamada et al., 1966; Saski, 1968; Yoon and Burgess, 1996). In contrast, decreases in the drug's thermodynamic activity as a result of complex formation between drug and solubilizer is not likely to affect lipophilic drugs to a significant extent since complexation primarily occurs via hydrogen bonding between hydrophilic moieties.

3.4.2. Alternative approaches

As much as the simplicity of the dialysis diffusion technique was an advantage to meet the objectives of the present study it is in several ways inferior to methods, which more closely resemble the properties of the intestinal barrier *in vivo*. Such methods include *in vitro, in situ* and *in vivo* models, which have recently been reviewed in detail by Le Ferrec et al. (2001) and will briefly be discussed in the following. *In vitro* methods for drug diffusion experiments can be divided into cell culture and tissue culture studies. Various cell culture models exist deriving cells from different tissues such as canine kidney (MDCK), human colorectal adenocarcinoma (Caco-2) or others. In culture, they differentiate spontaneously into intestinal epithelial cells possessing an apical brush border as well as tight junctions between adjacent cells and also express certain metabolic enzymes and typical microvillar transporters. Tissue culture studies such as everted gut sac and Ussing chamber technique, on the other hand, involve the use of sheets of membrane tissue stripped of rat intestine. In comparison to cell culture studies they offer the advantage for measuring the absorption at different sites in the small intestine. *In situ* methods including open or closed loop perfusion studies of rat small intestine resemble the *in vivo* conditions even more closely than *in vitro* methods as here the uptake of drug compounds into the systemic circulation can be monitored. A disadvantage of these techniques is, however, that the animal is anaesthetised, which sometimes may have a significant effect on intestinal drug absorption (Yuasa et al., 1993). *In vivo* methods for investigating drug diffusion include perfusion experiments such as the Loc-I-gut technique, which involves the isolation of a specified part of the small intestine with inflatable balloons. The advantage of such experiments is that it allows the assessment of drug permeation in humans and it has been stated, "the only real model for man is man" (Davis and Wilding, 2000). All of the discussed methods also have limitations. The more closely a model reflects the *in vivo* situation the more complex it becomes, which makes it rather difficult to separate the variables involved in the process of drug absorption, i.e. it does not allow the identification of the individual

rate-limiting factors. In addition to an increasing complexity of the various methods in the order of *in vitro*, *in situ* and *in vivo* experiments is the increasing expenditure of cost and time involved.

3.5. Conclusions

The primary objective of the present study was to investigate the influence of different solubilizing agents on the passive diffusion of ranitidine. The presence of PEG 400 at concentrations of 5, 10 and 30 % (w/w) was found to have no major effect on the diffusion rate of ranitidine. A slight delay in drug diffusion, however, was observed at PEG 400 concentrations of 30 % (w/w) in the initial 6 hrs of the experiment. Solutions containing relatively high amounts of propylene glycol, 30, 50 and 70 % (w/w) also resulted in a slight delay in the diffusion of ranitidine across the dialysis membrane. The effect, however, did not appear to be dependent on the dose of propylene glycol present in the donor solution. It is suggested that these findings for both cosolvents are partly the consequence of an increased osmotic pressure within the donor compartment eliciting an adverse flux of water, which is more apparent in the case of propylene glycol having a considerable osmotic activity. The main impact of a delaying effect of the presence of high cosolvent concentrations is expected to be an increased viscosity of the solution, which results in a decrease in the thermodynamic activity of ranitidine. PEG 400 and propylene glycol were both shown to move across the artificial hydrophilic membrane. Therefore, an increased osmolarity and/or viscosity of the donor preparations is expected to be decreased during the course of the experiment as a result of the diffusion of the cosolvents into the sink solution. No interaction or complex formation between the drug and either cosolvent was observed. For the passive diffusion of the hydrophilic compound ranitidine in the presence of increasing concentrations of non-ionic surfactant it can be concluded that drug diffusion was not compromised through micelle formation in solutions containing 0.2 and 2 % (w/w) VitE-TPGS. At concentrations as high as 20 % (w/w), VitE-TPGS had a marked negative effect on drug diffusion showing a considerable decrease in the diffusion rate of ranitidine. This effect is thought to be primarily the consequence of the increased viscosity of the continuous aqueous phase in the presence of high micellar concentrations, which leads to a reduction in the

thermodynamic activity of ranitidine and hence a decrease in the passive diffusion of the drug.

The results obtained from the present diffusion experiments using artificial hydrophilic membranes lead to the assumption that the considerable reduction in ranitidine bioavailability, which was observed after the oral administration of an oral solution containing relatively high amounts of PEG 400 in healthy volunteers *in vivo* (Chapter 2) was not caused by physicochemical interactions of the polymer with the active agent.

CHAPTER 4

THE EFFECTS OF DIFFERENT TYPES OF SOLUBILIZING AGENTS ON GI TRANSIT AND DRUG ABSORPTION IN BEAGLE DOGS

4.1. Overview

The dog is widely used as an animal model for humans because of its similarity in GI anatomy and motility, as well as in drug pharmacokinetics (Anderson, 1970; Dressman, 1986). In addition, the canine model comprises the advantage of being less expensive and more easily available as well as less time consuming, whereas *in vivo* studies with healthy volunteers often demand a high degree of organisation. Several studies investigating GI transit have been conducted in dogs concerning gastric emptying of meals and transit of different dosage forms through the GI tract. One primary objective of the present study was, however, to find out whether the canine model may be a useful tool for investigating excipient effects in the human GI tract.

The results presented in Chapter 2 demonstrated that the apparent accelerating effect of PEG 400 on gastrointestinal transit in humans is dose-dependent. As solubility-enhancing excipients, cosolvents like PEG 400 are extensively employed in liquid formulations of poorly water-soluble drugs, very often in combination with other solubilizing agents – be it another cosolvent or surfactant. In the case of Agenerase® oral solution, a single dose contains 7 g VitE-TPGS, 10 g PEG 400 and 33 g propylene glycol. The active agent amprenavir was found to have a 14 % lower bioavailability with this particular formulation than with the soft gelatine capsule preparation, which contains considerably lower amounts of these excipients. The reduced bioavailability of amprenavir might perhaps be the result of an accelerated passage through the GI tract, which has been shown in Chapter 2. So far, PEG 400 is the only compound of the class of solubilizers, which has been investigated with respect to an influence on GI transit. It is likely that PEG 400 is responsible for the observed decrease in amprenavir absorption as it was shown to have a significant impact on GI transit and drug absorption at such doses (Basit et al., 2002). Nevertheless, the solution formulation also contains high amounts of the co-administered solubilizers VitE-TPGS and propylene glycol, which might exhibit an effect on GI transit themselves. Recently, a mixture of VitE-TPGS and propylene glycol has been suggested as a universal oral formulation for solubility enhancement of insoluble compounds in the early stages of drug discovery to increase oral drug bioavailability (Tong et al., 1998). VitE-TGS, however, cannot be regarded as pharmaceutically inert in general as it also possesses absorption enhancing properties.

Apart from identifying excipients that accelerate GI transit, it would also be desirable to find a way to prolong the residence time of drug compounds in the small intestine for purposes of bioavailability enhancement. Mixtures of medium chain glycerides such as Labrasol® have recently been introduced as new pharmaceutical excipients. They might prove suitable for retarding GI transit via activation of the ileal-brake similar to other fat digestion products such as oleic acid (Dobson et al., 1999; 2000). This braking mechanism has not only been demonstrated to function in humans but also in dogs (Dreznik et al., 1994).

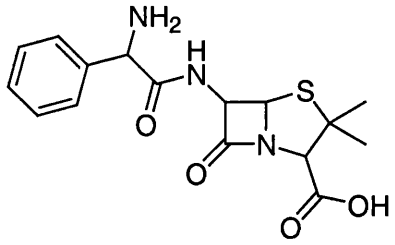
The primary aim of the present study was, therefore, to identify the potential of different commonly used solubilizing agents with regard to their influence on GI transit. The excipients chosen for investigation were propylene glycol, as well as VitE-TPGS and Labrasol®. Water and PEG 400 were also administered on two separate occasions to serve as reference.

Also, the effect of gastrointestinal transit and luminal presence of excipient on the absorption of different drugs was investigated with the simultaneous administration of two model compounds, ampicillin and antipyrine (Fig. 4.1). Ampicillin, a β-lactam aminopenicillin derivative, is mainly transcellularly absorbed using an active dipeptide transporter in the upper small intestine (Lee, 2000). The drug comprises of a poor oral bioavailability suggested to be the consequence of its poor absorption from the GI tract since it is not subjected to significant hepatic or intestinal first-pass metabolism (Yano et al., 1989; Haruta et al., 1998). Being poorly permeable, ampicillin exhibits a GI transit-dependent bioavailability, which was demonstrated in rats with a drug-induced prolongation of small intestinal transit (Haruta et al., 1998). In contrast, antipyrine is the classic model compound for absorption via the passive transcellular route transversing the lipophilic membrane through pores and is well absorbed throughout the entire gastrointestinal tract (Ungell et al., 1998; Gui and Carraway, 2001). Beside its relatively high bioavailability, antipyrine is subjected to hepatic first-pass metabolism to a certain extent, which led to its regular use as a model compound for changes in liver enzyme activity (Branch, 1982). The distinct differences in the absorption profiles of both ampicillin and antipyrine were decisive for their use as model drugs in the present study.

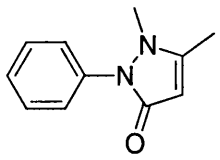
Ranitidine, which was employed in the earlier transit study in humans, was considered to be unsuitable since its bioavailability in dogs has been shown to be 100 % (Chiou et al., 2000) as a result of significantly wider tight junctions in the dog compared to human (He et al., 1998).

Figure 4.1. Molecular structure of a) ampicillin, and b) antipyrine.

a)



b)



The present study was conducted at the animal research facility of GlaxoSmithKline at Research Triangle Park in North Carolina (U.S.A.) using four beagle dogs. The treatments consisting of model drug, solubilizer and water were administered to the dogs in capsules for natural swallowing. The use of a gavage for intra-gastric administration was expected to be very strenuous for the animals and as a result might impact gastrointestinal transit times and distort the overall result. For instance, intubation in humans has been found to affect the passage of solids through the GI tract (Read et al., 1983). The transit of the oral liquids through the gastrointestinal tract was monitored using gamma scintigraphy and the absorption of the model drugs were assessed via frequent blood sampling. One study day involved the intravenous administration of both model compounds to complete the pharmacokinetic data set. The plasma samples were analysed on site using HPLC after a suitable analysis method was developed.

4.2. Materials

Ampicillin was obtained in the form of ampicillin sodium from Sigma Chemical (MO, U.S.A.). It is a white, practically odourless, crystalline or amorphous powder that is hygroscopic and very soluble in water or other aqueous solutions.

Antipyrine was obtained from Sigma Chemical (MO, U.S.A.). It is a white, practically odourless crystalline powder with a high aqueous solubility.

Polyethylene glycol 400 was obtained from Spectrum Chemical (NJ, U.S.A.). It occurs as a clear, colourless, highly osmotic and viscous liquid with a slight but characteristic odour and a bitter taste. PEG 400 is hygroscopic and completely soluble in water.

Propylene glycol was obtained from Dow Chemical (TX, U.S.A.). The excipient is a clear, colour- and odourless liquid of medium viscosity that is hygroscopic and completely soluble in water.

D-α-tocopheryl polyethylene glycol 1000 succinate (VitE-TPGS) was obtained from Eastman Chemical (TN, U.S.A.). It is a pale yellow waxy solid, which is highly miscible with water forming low-viscosity solutions at concentrations < 20 % (w/w).

Labrasol® was obtained from Gattefossé (NJ, U.S.A.). It is a clear, viscous liquid of pale yellow colour of oily consistency.

Technetium-99m

The radionuclide ^{99m}Tc was obtained daily from Photon Imaging (NC, U.S.A.) in the form of the complex ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) dissolved in saline. The complex was declared to be stable throughout the study day.

4.3. Methods

4.3.1. Dosage forms

4.3.1.1. Preparation of drug solutions

For the oral preparations, 212 mg ampicillin sodium, equivalent to 200 mg ampicillin acid, and 100 mg antipyrine were dissolved in 8 g of solvent. The solvent consisted of water (control) or a mixture of either 12.5 % (w/w) PEG 400, 25 % (w/w) propylene glycol, 12.5 % (w/w) VitE-TPGS or 25 % (w/w) Labrasol® in water. These concentrations equate to 1, 2, 1 and 2 g, respectively, of solubilizer present in the

preparations. The PEG 400, propylene glycol and Labrasol® solutions were obtained by simply mixing the respective cosolvent or surfactant with water. For the VitE-TPGS solution, the waxy surfactant was heated in an oven (37° C) to liquefy prior to mixing with water. The mixture was stirred at approximately 60° C until complete dissolution of VitE-TPGS and then cooled down to room temperature before the drug compounds were added. The drug solutions were filled in two 5 mL Torpac Lock Ring capsules and radiolabelled by adding a few drops of the ^{99m}Tc-DTPA in saline-solution. The radioactivity of the solutions was measured using an isotope assay calibrator (model 270, Pitman Instruments, Surrey, UK) and determined to be 15 ± 3 MBq. For the intravenous preparations, a stock-solution was prepared containing 212 mg ampicillin sodium, equivalent to 200 mg ampicillin acid, and 100 mg antipyrine dissolved in 4 mL sterile saline solution. The single intravenous dose administered was 4 mL of the stock-solution, which was freshly prepared each morning.

4.3.1.2. Osmolality of drug solutions

The osmotic pressure of the drug solutions was measured using a freezing-point osmometer (Model 3D3, Advanced Instruments, MA, U.S.A.). The preparations were found to have the following osmolalities: Control (192 mOsm kg^{-1}); PEG 400 (766 mOsm kg^{-1}); propylene glycol (3784 mOsm kg^{-1}); VitE-TPGS (266 mOsm kg^{-1}); Labrasol® (631 mOsm kg^{-1}). For propylene glycol and Labrasol® solutions a dilution with water (1:3 and 1:1, respectively) was necessary to achieve freezing. These osmolalities were determined for the 8 mL drug solutions administered in capsules regardless of the 50 mL of water coadministered with the capsules since it is often difficult in dogs to achieve the quantitative ingestion of liquids.

4.3.1.3. pH properties of drug solutions

The pH values of the different preparations were determined (pH-meter 345, Corning Life Sciences, NY, U.S.A.) and found to be: Control (8.06); PEG 400 (8.15); propylene glycol (7.94); VitE-TPGS (7.97); Labrasol® (7.91).

4.3.2. Gamma scintigraphy

4.3.2.1. The gamma camera

An e.Cam Fixed 180 dual head SPECT camera (Siemens Medical Solutions, PA, U.S.A.) was used for scintigraphic imaging (Fig. 4.2). The two opposed detectors, each having a 533 x 387 mm field of view were fitted with low energy parallel hole collimators suitable for ^{99m}Tc imaging. An on-line computer was connected to the camera and digital image recording was performed using an e.Soft programme (Siemens Medical Solutions). For subsequent analysis data were archived onto CD and hard drive.

4.3.2.2. Study Protocol

Each dog completed six study-days, which were separated by a washout period of at least two days. The type of treatment was varied on each occasion and given to each animal in a randomised order.

The different treatments were:

Control

- 212 mg ampicillin sodium (corresponds to 200 mg ampicillin) and 100 mg antipyrine dissolved in 8 g water; radiolabelled with 18 MBq of ^{99m}Tc-DTPA; dosed via two 5 mL Torpac Lock Ring capsules; administered with 40 ml of water.

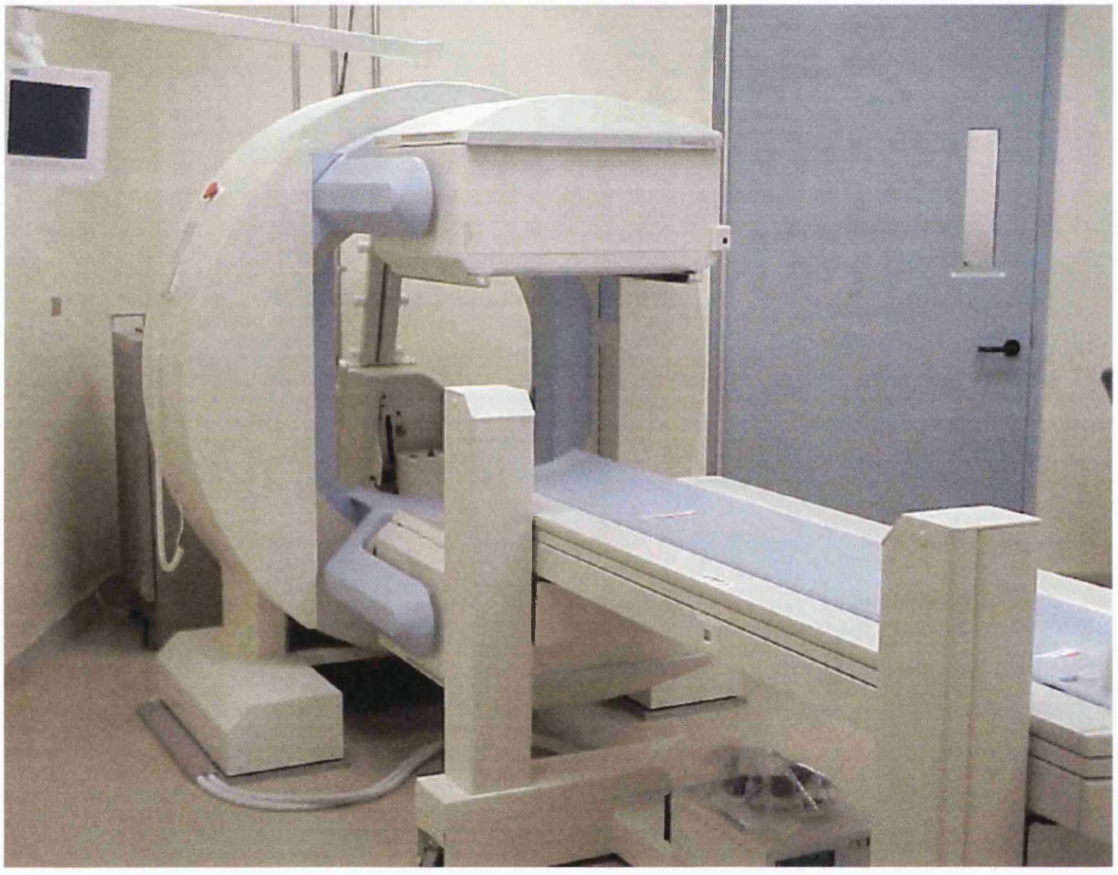
PEG 400

- 212 mg ampicillin sodium (corresponds to 200 mg ampicillin) and 100 mg antipyrine dissolved in 8 g of 12.5 % (w/w) PEG 400 in water (corresponds to 1 g PEG 400); radiolabelled with 18 MBq of ^{99m}Tc-DTPA; dosed via two 5 mL Torpac Lock Ring capsules; administered with 40 ml of water.

Propylene glycol

- 212 mg ampicillin sodium (corresponds to 200 mg ampicillin) and 100 mg antipyrine dissolved in 8 mL of 25.0 % (w/w) propylene glycol in water (corresponds to 2 g propylene glycol); radiolabelled with 18 MBq of ^{99m}Tc-DTPA; dosed via two 5 mL Torpac Lock Ring capsules; administered with 40 ml of water.

Figure 4.2. The e.Cam Fixed 180 SPECT gamma camera, showing the position of dual detectors. The dog was seated on the bed between the detectors during imaging procedure.



VitE-TPGS

- 212 mg ampicillin sodium (corresponds to 200 mg ampicillin) and 100 mg antipyrine dissolved in 8 g of 12.5 % (w/w) VitE-TPGS in water (corresponds to 1 g Vitamin E-TPGS); radiolabelled with 18 MBq of ^{99m}Tc-DTPA; dosed via two 5 mL Torpac Lock Ring capsules; administered with 40 ml of water.

Labrasol®

- 212 mg ampicillin sodium (corresponds to 200 mg ampicillin) and 100 mg antipyrine dissolved in 8 g of 25.0 % (w/w) Labrasol® in water (corresponds to 2 g Labrasol®); radiolabelled with 18 MBq of ^{99m}Tc-DTPA; dosed via two 5 mL Torpac Lock Ring capsules; administered with 40 ml of water.

i.v. injection

- 212 mg ampicillin sodium (corresponds to 200 mg ampicillin) and 100 mg antipyrine dissolved in 4 mL sterile saline; dosed via injection into the cephalic vein.

On the day prior to each study day, the beagle dogs underwent a 24 hrs-fast. On the morning of the day of study, one fiducial labelled with 1 MBq ^{99m}Tc was placed at the axillary line on either the left or right front shoulder of the dog to serve as an anatomical reference marker for the stomach. The study commenced at 08.00 hrs. A cannula was inserted into the cephalic vein of the right or left front leg allowing regular blood sampling. Samples of 5 mL blood were taken at specific times. The times were set to be at 0 (pre-dose), 5, 15, 30, 45 min, 1 hr, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 hrs on the days of oral administration and 3, 7, 10, 15, 30, 45 min, 1 hr, 1.5, 2, 3, 4 and 6 hrs after intravenous administration. The blood samples were transferred into heparin vials (Vacutainers®, BD, NJ, U.S.A.) and centrifuged at 10 000 rpm for 5 min (Jouan GT 422, VA, U.S.A.). The separated plasma was transferred into plasma vials and immediately stored at –20° C prior to analysis.

Dosing of the treatments was performed placing one capsule with approximately 20 mL of water in the mouth, holding the jaws closed until the dog swallowed. The

procedure was repeated for the second capsule. The dogs were seated comfortably in a sling under the camera and scintigraphic images of 30 seconds duration were simultaneously acquired from both anterior and posterior detectors. During the first 30 min images were acquired in a continuous manner until the liquid had emptied from the stomach. Imaging was continued less frequently after gastric emptying at intervals of about 15-20 minutes duration until the liquid had arrived at the colon. Between image acquisitions, the dogs were allowed to move freely in the room or were brought back to their cages. Water was made available *ad libitum* once the drug solution had emptied from the stomach and the dogs were fed their daily standard food (Canine food 5006, LabDiet®, IA, U.S.A.) four hours after dosing.

4.3.2.3. Analysis of scintigraphic data

On completion of each scintigraphic study, processing of image data was performed using a DICOM image processing software programme (Siemens Medical Solutions). The series of images acquired for each dog was replayed on computer. According to the previous scintigraphic study in healthy volunteers (Chapter 2) the images were firstly corrected for motion. Following the correction of motion, two regions of interests were drawn around the stomach and the colon and radioactivity was quantitated within these regions for each image. These values were then corrected for different background count rates and for physical decay of ^{99m}Tc for both anterior and posterior data sets. From these net counts the geometric mean was calculated to account for the differential attenuation of the radiation with varying depth of source. Finally, the corrected geometric mean counts for the regions of interest were expressed as percentages of the total counts recorded initially, when all the administered activity was in the stomach and terminally, when all the activity was in the caecum/colon for the values of the curves of gastric emptying and caecum/colon arrival, respectively. The time course of gastric emptying and colon arrival was then estimated from the plot of percentage activity in these regions versus time.

The gastrointestinal transit data were quantitatively assessed using statistical moments to calculate the mean gastric residence time (MGRT) and mean caecum arrival time (MCAT) (Podczeck et al., 1995). In addition, the t50 values were determined, which describe the time at which 50 % of the radioactive counts have left the stomach,

gastric emptying time (GET), or reached the colon, colon arrival time (CAT). Although the method of moments has been repeatedly shown to give superior information, the t50 value is still widely used and is therefore described in the results of the following section for comparing the values assessed to those reported in the literature. As measures of small intestinal transit time the MSITT and t_{50}-SITT were calculated as the difference between the MGRT and MCAT and the GET and CAT, respectively.

4.3.2.4. Analysis of plasma samples

After thawing at room temperature and thorough vortex mixing, the plasma samples were pre-treated for protein precipitation according to Akhtar et al. (1993). Here, 70 µL of buffered TCA consisting of 1:3 (v/v) citrate-phosphate buffer (pH 5.5) and 70 % (w/v) trichloroacetic acid (TCA) was added to 0.7 mL of plasma. The mixture was vortex mixed for 30 s and centrifuged at 4 000 rpm for 5 min (5415C, Eppendorf, Germany). Of the supernatant 0.4 mL was transferred into an HPLC vial containing 10 µL of 5 M sodium hydroxide, which was then sonicated in an ultrasonic water bath for 10 s.

The HPLC analysis utilized a Hewlett Packard Series 1100 chromatography system with a CTC PAL autosampler. Aliquots of sample (150 µL) were injected on a 100-mm x 4.6-mm Luna column (3 µm C-18(2); Phenomenex, CA, U.S.A.) at 40° C. A binary solvent gradient system was used and the flow rate of the mobile phase was set to 1.0 mL/min. The mobile phase comprised of an aqueous and an organic solvent, which were 0.05 % (v/v) trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B), respectively. The column was initially equilibrated with 95 % solvent A and 5 % solvent B. Immediately after sample injection, the concentration of B was linearly increased over 15 min to a concentration of 30 % and then reduced to the initial concentration of 5 % in the next 3 min followed by a 1 min equilibration time before the next sample injection. Two standards containing 2 µg/mL ampicillin and antipyrine in plain plasma (treated as mentioned above) were measured as reference samples to determine the concentration of the model drugs. The retention times of ampicillin and antipyrine were found to be 8.8 and 9.5 min, respectively.

4.3.2.5. Statistical analysis

A paired Student's t-test was performed on the scintigraphic and pharmacokinetic data to assess the effect of solubilizing agent on gastrointestinal transit and drug absorption.

4.4. Results and discussion

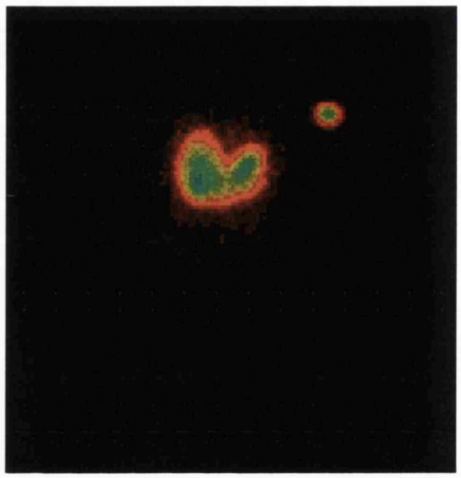
4.4.1. Gastrointestinal transit

A series of scintigraphic images are given in Figure 4.3 highlighting the passage of the control treatment in one of the dogs as an example. The profiles of the GI transit for each individual dog are given in Figures 4.4 to 4.7 and the results of the times for gastric emptying, colon arrival and small intestinal transit are presented in Tables 4.1, 4.3 and 4.5, respectively, using statistical moments, whereas Tables 4.2, 4.4 and 4.6, respectively, list the corresponding t_{50} values.

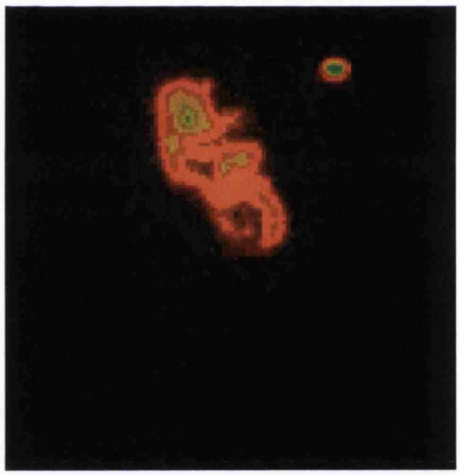
The gastrointestinal anatomy in the dog has close similarities to that of the human as can be seen from the series of images presented in Figure 4.2. Both species have an average stomach volume of approximately 1 L (Dressman and Yamada, 1991). The small intestine, on the other hand, is shorter in the dog. The length of the beagle dog small intestine was given as 225 to 290 cm in total, of which the first 25 cm is the duodenum and the last 15 cm is the ileum (Anderson, 1970). In contrast, the human small intestine consists of 25 cm duodenum, 300 cm jejunum and 300 cm ileum giving it an average total length of 625 cm (Ritschel, 1991). In the series of images it becomes apparent that the liquid-filled hard gelatin capsules disintegrated almost immediately in the stomach after oral administration (Figure 4.3).

Figure 4.2. Scintigraphic images highlighting the passage of an oral solution through the gastrointestinal tract in the beagle dog; a) stomach, b) emptying into the small intestine and c) caecum and colon.

a)



b)



c)

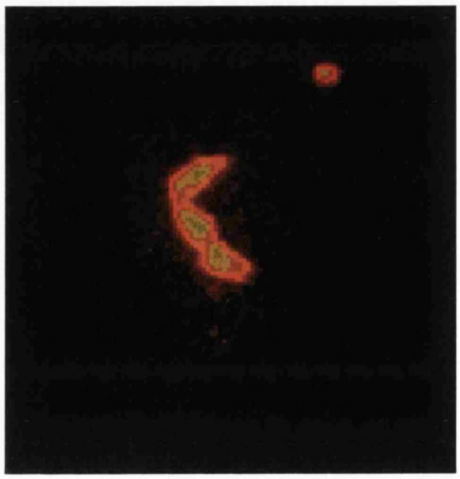


Figure 4.4. Gastrointestinal transit profiles for dog 1.

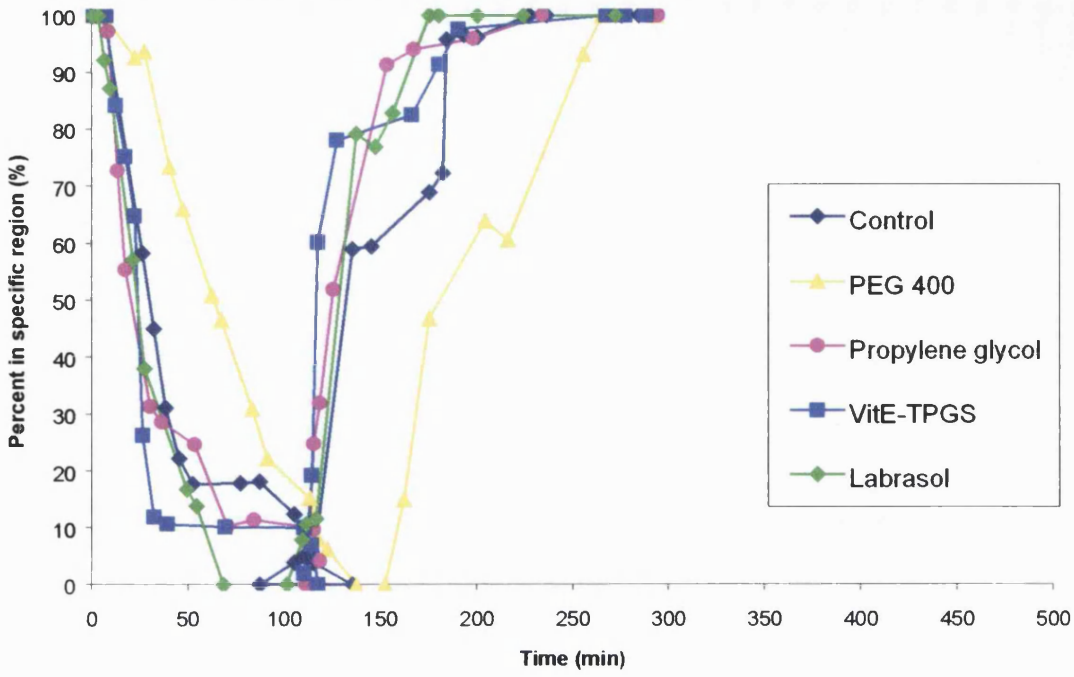


Figure 4.5. Gastrointestinal transit profiles for dog 2.

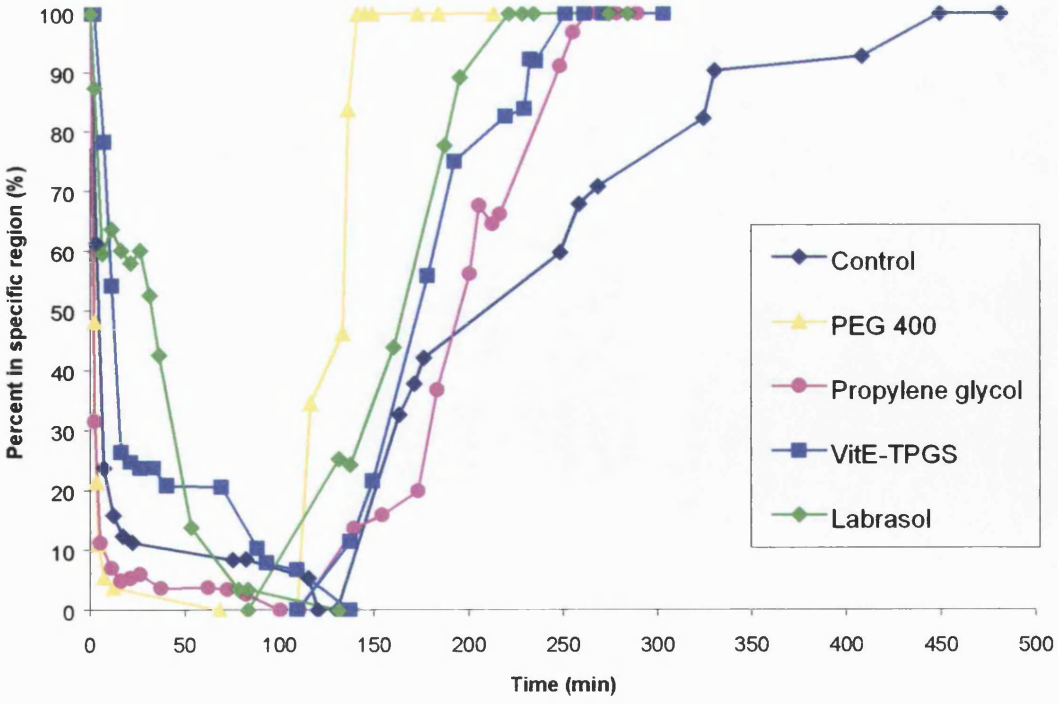


Figure 4.6. Gastrointestinal transit profiles for dog 3.

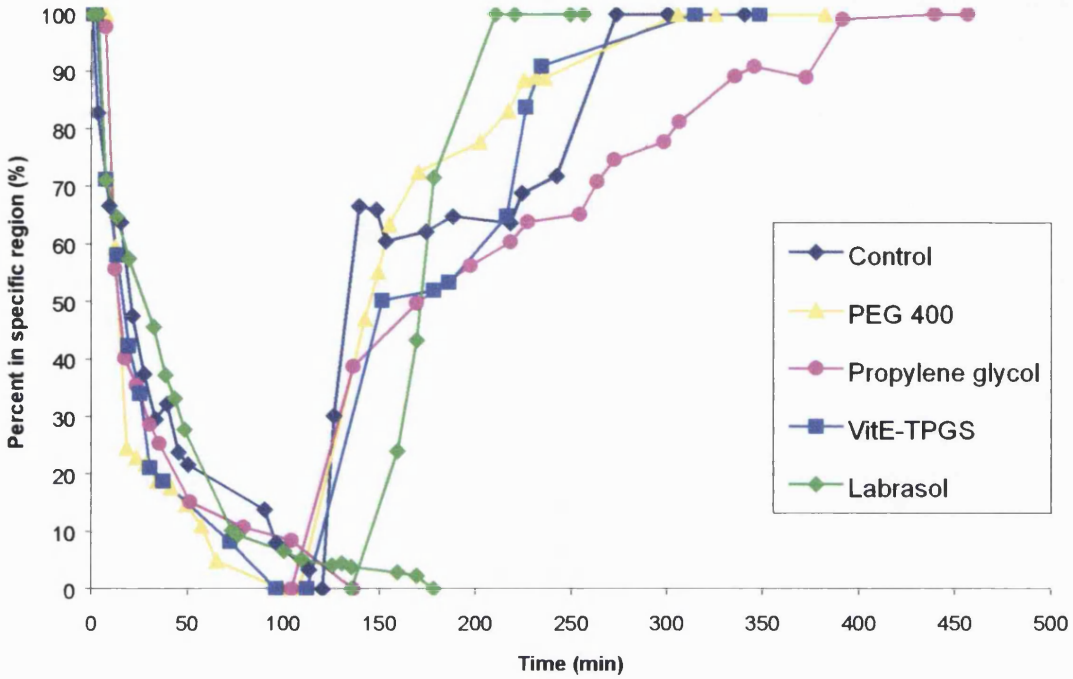


Figure 4.7. Gastrointestinal transit profiles for dog 4.

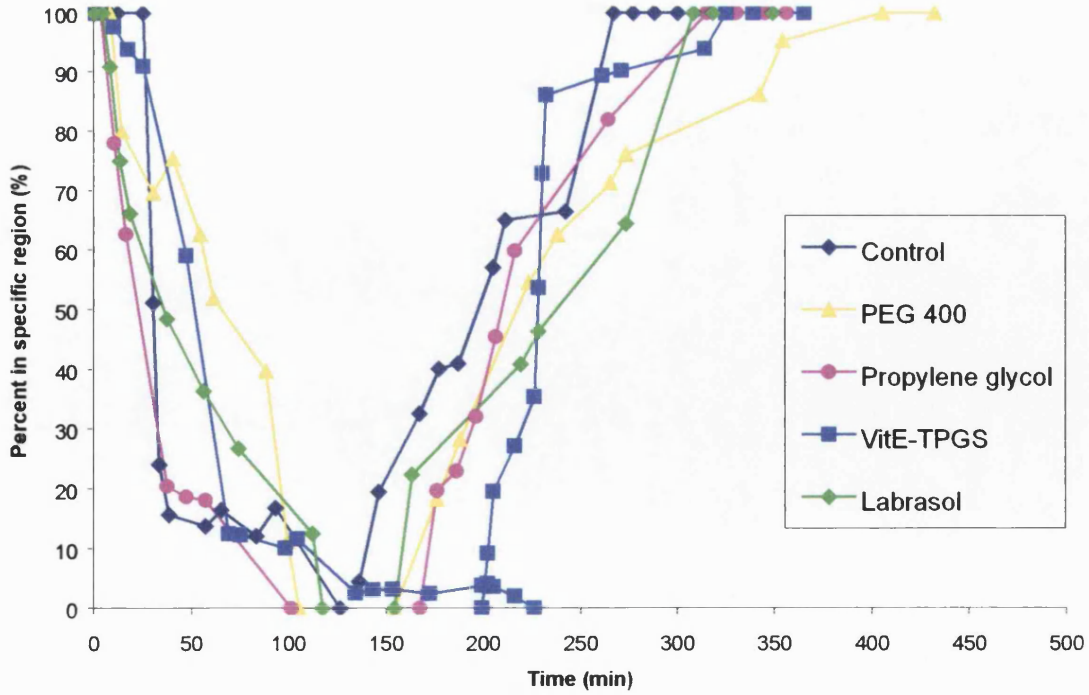


Table 4.1. Gastric emptying times calculated using statistical moment theory and represented as MGRT (min) and *VGRT* (min^2).

Treatment	Dog 1	Dog 2	Dog 3	Dog 4	Mean ± S.D.	*P* value
Control	41 *881*	9 *39*	39 *1022*	31 *585*	30 ± 15	
PEG 400	47 *908*	3 *2*	21 *302*	47 *854*	30 ± 22	*0.950*
Propylene glycol	38 *1083*	5 *13*	35 *1009*	25 *280*	26 ± 15	*0.007*
VitE-TPGS	34 *1123*	40 *1101*	28 *595*	49 *672*	38 ± 9	*0.497*
Labrasol®	24 *276*	25 *264*	36 *804*	48 *1226*	33 ± 11	*0.717*

Table 4.2. Gastric emptying times represented as t_{50} values (min).

Treatment	Dog 1	Dog 2	Dog 3	Dog 4	Mean ± S.D.	*P* value
Control	30	4	20	30	21 ± 12	
PEG 400	63	2	14	65	36 ± 33	*0.276*
Propylene glycol	20	1	14	22	14 ± 9	*0.020*
VitE-TPGS	24	12	16	53	26 ± 18	*0.511*
Labrasol®	23	32	27	35	30 ± 5	*0.325*

Table 4.3. Caecum arrival times calculated using statistical moment theory and represented as MCAT (min) and *VCAT* (min^2).

Treatment	Dog 1	Dog 2	Dog 3	Dog 4	Mean ± S.D.	*P* value
Control	164 *341*	280 *1840*	196 *1177*	212 *891*	213 ± 49	
PEG 400	203 *751*	134 *2*	201 *632*	296 *2626*	209 ± 67	*0.934*
Propylene glycol	155 *156*	221 *867*	259 *4258*	247 *760*	221 ± 46	*0.797*
VitE-TPGS	163 *313*	207 *507*	203 *452*	251 *288*	206 ± 36	*0.786*
Labrasol®	145 *87*	179 *276*	174 *19*	249 *688*	187 ± 44	*0.423*

Table 4.4. Caecum arrival times represented as t_{50} values (min).

Treatment	Dog 1	Dog 2	Dog 3	Dog 4	Mean ± S.D.	*P* value
Control	132	208	133	197	168 ± 41	
PEG 400	180	133	144	217	169 ± 38	*0.972*
Propylene glycol	124	195	170	209	175 ± 37	*0.582*
VitE-TPGS	116	173	151	228	167 ± 47	*0.976*
Labrasol®	128	165	171	237	175 ± 45	*0.721*

Table 4.5. Small intestinal transit times calculated using statistical moment theory and represented as the difference between MGRT and MCAT (min).

Treatment	Dog				Mean ± S.D.	*P* value
	1	2	3	4		
Control	123	271	157	181	183 ± 63	
PEG 400	154	131	180	249	179 ± 51	*0.929*
Propylene glycol	117	216	224	222	195 ± 52	*0.692*
VitE-TPGS	129	167	175	202	168 ± 30	*0.656*
Labrasol®	121	154	138	201	154 ± 34	*0.401*

Table 4.6. Small intestinal transit times represented as the difference in gastric emptying and caecum arrival t_{50} values (min).

Treatment	Dog				Mean ± S.D.	*P* value
	1	2	3	4		
Control	102	204	113	167	147 ± 48	
PEG 400	117	131	130	152	133 ± 14	*0.552*
Propylene glycol	104	194	156	187	161 ± 41	*0.319*
VitE-TPGS	92	161	135	175	141 ± 36	*0.710*
Labrasol®	105	133	144	202	146 ± 41	*0.985*

This was observed in each dog independent of the type of treatment administered. All liquids were observed to empty from the stomach into the small intestine in a continuous manner. A few times it was noted that the solutions slowed down at the ileo-caecal junction and emptied into the large intestine as a bulk but this phenomenon was not consistently observed for a specific treatment.

In general, the values obtained for gastrointestinal transit were greater when the moments theory approach was used compared to the t_{50} approach, which had also been found to be the case in the previous transit study in humans (Chapter 2). In the present chapter, the discussion of the results will primarily focus on the values determined with the method of moments theory.

4.4.1.1. Gastric emptying

The average mean gastric emptying time (mean MGRT) for the control formulation was 30 min. In comparison, this value is considerably longer than the mean MGRT obtained for gastric emptying of oral liquids in human subjects (Chapter 2), which was 12 min. An explanation can be found in the slight anatomical differences between both species. In dogs, the pyloric valve seems to be tighter than in humans. Multiparticulate dosage forms have been found to have a significantly smaller cut-off size for gastric emptying, which was 2-7 mm in dogs compared to 11-13 mm in humans (Coupe et al., 1991). A tighter pyloric valve limiting the particle size of material entering the small intestine does not necessarily need to affect the emptying of fluid from the stomach. But to some extent it might be likely to delay the emptying of larger amounts of fluid. In the literature, however, some studies have demonstrated similar gastric emptying times in canines and humans (Stephens et al., 1975; Ehrlein and Prove, 1982; Dressman, 1986) and in the present study, the gastric emptying in the dogs seemed to be highly variable ranging from 9 to 41 min. In the case of dog 2, the gastric emptying time appeared to be markedly shorter compared to the other dogs. Gupta and Robinson (1988), however, gave a more plausible explanation for the high variation in gastric emptying times in canines demonstrating that in dogs the time and pattern of stomach emptying is dependent on the volume of the oral liquid administered. The authors reported an exponential gastric emptying pattern with short emptying times only for solutions administered at amounts higher than 100 mL as a result of an interruption of the fasting stomach motility. Since in the present study the overall amount of fluid administered

was approximately 50 mL, an interruption of the stomach motility in the subjects is not to be expected. This means the emptying time is strongly dependent on the state of the fasting cycle. If the oral dosing of the treatment solutions takes place during Phase I or II of the myoelectric motor complex (MMC), the emptying from the stomach would be rather slow and continuous and more or less follow a relatively linear pattern. In contrast, an oral administration during Phase III of the MMC would result in an abrupt emptying of the stomach reflecting in an exponential pattern of the gastric emptying curve in the transit profiles. Since the emptying in dog 2 was much quicker compared to the other dogs it is suggested that the treatment administration occurred during Phase III. This phenomenon would also account for the longer values observed for this particular dog with the treatments VitE-TPGS and Labrasol®, which might have occurred during a different motility state of the fasted stomach. With such a high variation in gastric emptying as a result of the high dependency on the state of stomach motility at the time of oral administration it seems difficult to draw any firm conclusions with respect to a possible effect of the presence of solubilizing agents in the administered formulation.

The mean MGRT values for the gastric emptying time of the formulations containing PEG 400, propylene glycol, VitE-TPGS and Labrasol® were 30, 26, 38 and 33 min, respectively. There was no significant difference in the presence of the solubilizers tested compared to the control preparation with the exception of propylene glycol, which exhibited slightly but significantly faster gastric emptying times ($P < 0.05$). The average acceleration was a mere 4 min, which does not appear to be of great influence passage of oral liquids through the stomach in general. A shorter MGRT compared to the control, however, was observed in each individual dog in the presence of the cosolvent. The comparatively faster gastric emptying of the propylene glycol formulation was rather surprising since this preparation in particular possesses a considerably high osmotic pressure (3784 mOsm kg^{-1}). Generally, an increased osmolality in the stomach is expected to cause a prolonged residence time of gastric contents, as the body will first try to compensate the unisotonic conditions with the secretion of water before the contents are released into the small intestine (Hunt et al., 1951; Hunt, 1963). The observed faster emptying of this particular formulation from the stomach could result from an irritation of the stomach wall upon disintegration of the ingested capsules and local release of the oral liquid. This irritation might in turn elicit a distinct propulsive

reaction propagating the gastric contents into the small intestine. However, the observation may also have been coincidental and simply been the result of the fact that only four dogs were used in the present study.

As mentioned earlier, no significant alterations in gastric residence times were observed for the formulations containing PEG 400, VitE-TPGS or Labrasol®. With the co-administration of about 40 mL of water the preparations will immediately be diluted upon release in the stomach, which has a decreasing effect on the osmolarity of the treatments. As a result, the osmotic pressure of the 1 g PEG 400 treatment (766 mOsm kg^{-1}) and the 2 g Labrasol® treatment (631 mOsm kg^{-1}) will be reduced to approximately isotonic conditions, whereas the preparation with 1 g VitE-TPGS (266 mOsm kg^{-1}) is hypotonic. Besides considerable deviation from isotonicity gastric presence of nutrients is well known to influence the MGRT. Lipid delivery systems, for instance, cause a prolongation of gastric emptying and gastrointestinal motility (Bates and Sequeira, 1975; Chakrabarti and Belpaire, 1978). High calorific load will slow the transit in order to ensure complete digestion and absorption of the nutrients. A delay in gastric emptying and small intestinal motility is generally favoured for poorly soluble and poorly permeable drugs, as it allows more time for time-limited absorption processes but also for drugs that have a narrow absorption window, as it provides a steady source of drug to the absorption site, which is usually the small intestine.

The pH-values of the administered formulations are not expected to have played a role in gastric emptying since all preparations comprised of an approximate pH of 8, which will face an immediate decrease by the acidic conditions of the gastric juice.

4.4.1.2. Colon arrival and small intestinal transit

Arrival of the formulations in the colon was determined to be 213, 209, 221, 206 and 187 min (MCAT-values; Table 4.3) for the control, PEG 400, propylene glycol, VitE-TPGS and Labrasol® treatment, respectively. None of the observed differences in the presence of co-administered solubilizer appeared to be significant ($P > 0.05$).

The results obtained for the small intestinal transit times in form of MSITTs are listed in Table 4.5. On the control day, the mean MSITT of all four dogs was observed to be 183 ± 63 min ranging from 123 to 271 min. In comparison to the values obtained for the

control solution in the previous transit study in healthy volunteers (Chapter 2), which were determined to be 298 ± 99 min, the canine small intestinal transit appears to be about half as long as in humans. This observation is confirmed by the results of previous transit studies comparing human and canine data (Dressman, 1986). The shorter transit times in dogs are primarily the result of differences in gastrointestinal anatomy of both species since the length of the small intestine in humans (625 cm total length; Ritschel, 1991) is about 2 to 2.5 times that in the beagle dog (225-290 cm total length; Anderson, 1970). Orally administered solutions were measured to pass through the small intestine at a flow rate of about 26 mL/min in the fasted state, compared to 10.4 mL/min in the fed state in dogs (Scholz et al., 2002). The shorter transit time in dogs compared to humans has also been attributed to the longer villi present in the dog intestinal mucosa, which promote the propagation of luminal contents (Kararli, 1995).

Dressman (1986) reported a higher variability on a percentage basis in the dog small intestinal transit times and concluded a more variable and less complete drug absorption in dogs compared to humans as a result. The present data do not agree with these findings with respect to variability as on a percentage basis the variability in small intestinal transit times (MSITT) was calculated to be 34 and 33 % in dogs and humans, respectively. Also, the present results of the t_{50} values, which is the approach used by the authors, do not give a greater variability, as the percentage was found to be 33 % in dogs and 31 % in humans. While, in general, passage through the small intestine is considered to be relatively stable and not significantly influenced by various factors within one individual, variability between subjects is likely to vary more considerably due to natural differences in anatomy, physiology and biochemistry. In dogs, other factors such as renal disease (Lefebvre et al., 2001) and age (Weber et al., 2002) have been shown to be of impact, whereas body size did not influence the orocaecal transit time. In addition, it was found that laboratory dogs that are regularly subjected to radioactivity show a decrease in the duration and frequency of GI contractions (Otterson et al., 1992).

In the presence of different solubilizing agents the passage of the drug solutions through the small intestine was observed to give a certain degree of variation, although the observed effects were found to be of no statistical significance with any of the treatments ($P > 0.05$).

For the formulation containing **PEG 400** a mean MSITT of 179 ± 51 min ranging from 131 to 249 min was determined. Interestingly, considerably longer MSITTs with the PEG 400 formulation were found for dog 1, 3 and 4 and the shorter mean MSITT was merely the consequence of a marked acceleration in dog 2, which was more than 2-fold compared to the control. However, the results appear to correlate well with those observed in the transit study in humans investigating the effect of different concentrations of PEG 400 (Chapter 2). In healthy volunteers the presence of 1 g PEG 400 was found to accelerate the passage through the small intestine slightly in the mean MSITT value but the effect was not significant. The results with 1 g PEG 400 from the dog study correlate more closely to the ones obtained in human subjects with the 1 g rather than the 5 g PEG 400 formulation. Since the bodyweight of an average beagle dog (12 kg) is approximately one fifth of that of an average human (70 kg) active agents are regularly scaled down for use in canine studies. However, the amounts of excipients co-administered in the formulation are often equivalent to those employed in humans, which in the present case of PEG 400 resulted in a good correlation. In order to generalize the obtained results for other solubilizing agents the specific properties of the polymer need to be considered. In humans, the accelerating effect of higher amounts of PEG 400 on small intestinal transit is thought to be the result of the polymer's poor absorption from the human small intestine. Exerting its osmotic activity within the gastrointestinal lumen, PEG 400 increases the luminal fluid volume via the retention of water causing in turn an enhancement of intestinal motility. In comparison to the human gut the intestinal epithelium in the dog has been shown to be more permeable to hydrophilic compounds due to larger pore size and larger frequency of pores in the canine paracellular route (He et al., 1998). Respective authors investigated the permeability of polyethylene glycols of various molecular weights and found quantitative absorption for oligomers up to 600 Da. PEG 400, comprising of ethylene glycol oligomers with a molecular weight range from 238 to 594 Da would therefore be expected to be completely absorbed from the dog small intestine. This means that PEG 400 is not available inside the GI lumen of the dog to exhibit its osmotic nature. As a result PEG 400 is suggested not to impact on small intestinal transit of oral liquids in the dog to the same extent as in humans even when administered at higher concentrations.

The small intestinal transit results for the formulation containing **propylene glycol** indicated a mean MSITT of 195 ± 52 min ranging from 117 to 224 min, which is to be compared to 183 min of the control formulation. Although no significant effect on small intestinal transit was observed, the average MSITT of the luminal contents was slightly prolonged in the presence of 2 g propylene glycol. As a result of the highly hypertonic nature of the cosolvent preparation (3784 mOsm kg^{-1}), the formulation, once released in the stomach, is diluted in the gastric fluids and subsequently emptied into the small intestine. Here, the body's efforts continue to compensate for the hyperosmotic load with the secretion of water, while at the same time the extraction of propylene glycol from the luminal fluids is enhanced to reduce the osmotic pressure in the lumen. As a low molecular weight alcohol propylene glycol is generally expected to be rapidly and thoroughly absorbed from the upper small intestine - a fact leading to the assumption that the cosolvent would not be present in the lumen to impact on small intestinal transit. The slight prolongation in the transit time might also be the result of the body's reaction to complete the absorption of the osmotic active agent as well as the re-absorption of water. Prolonged transit times, however, were only observed for dog 3 and 4, whereas dog 1 and 2 did not show an alteration in the transit time compared to the control. In four beagle dogs, the presence of 2 g propylene glycol appeared to have no effect on small intestinal liquid transit ($P > 0.05$). The administration of higher amounts of the cosolvent, as in the case of a single dose of Agenerase® oral solution (33 g) might, however, give a different picture.

The oral solution containing the solubilizer **VitE-TPGS** had an average MSITT of 171 ± 34 min, which was in the range of 129 to 202 min. In comparison to the passage rate of the control formulation through the small intestine, the presence of 1 g VitE-TPGS showed a slight acceleration in the mean value. The reduction in small intestinal transit time was, however, of no statistical significance ($P > 0.05$) due to the fact that accelerated transit times were only observed for dog 2, which exhibited a markedly faster transit in the presence of VitE-TPGS, whereas all three other dogs did not show an effect or had a slightly prolonged transit. The chemical structure of VitE-TPGS comprises a linkage of vitamin E via succinate bridge to polyethylene glycol 1000 (PEG 1000), which is a polymer similar to PEG 400 with an average molecular weight of 1000 Da. The surfactant was, therefore, suspected to bear the potential of influencing the small intestinal transit of oral liquids according to PEG 400. In this respect PEG 1000

might cause a promotion of intestinal motility via the exertion of its osmotic activity inside the lumen leading to retention of water. The osmolality of administered formulations will become important when the osmotic agent is poorly absorbed from the gastrointestinal tract and as a result will remain inside the lumen preventing the absorption of water. The way and extent of absorption of VitE-TPGS is not yet fully understood. Having amphiphilic properties, VitE-TPGS forms micelles in aqueous media, which enables the surfactant to cross from the intestinal lumen through the unstirred water layer to the enterocytes. An *in vitro* study using Caco-2 cells revealed that VitE-TPGS enters the cells intact and intracellularly will undergo hydrolysis of the ester bond between vitamin E and PEG 1000 (Traber et al., 1988). It is, however, unclear whether this process occurs quantitatively or whether ester hydrolysis may already take place inside the lumen of the upper small intestine. Sokol et al. (1987), for instance, only found a small amount (< 5%) of PEG 1000 to be absorbed and secreted in the urine. Overall, an oral dose of 1 g VitE-TPGS does not provide a large portion of PEG 1000 (0.6 g). An osmotic effect on gastrointestinal transit with released PEG 1000 is, therefore, not very likely at the doses administered in the present study but might be possible at higher concentrations in a similar way to PEG 400.

In the presence of 2 g **Labrasol®** the oral liquid was found to have a mean MSITT of 154 ± 34 min ranging form 121 to 201 min. Although not significant, the mean MSITT was slightly reduced compared to the average control formulation. This was rather surprising, as Labrasol® would have been expected to slow down the passage of luminal contents rather than causing acceleration. Labrasol®, which are caprylcaproyl macrogol glycerides, comprises of medium chain mono-, di- and trigycerides as well as PEG 400. In general, medium chain glycerides are rapidly hydrolysed in the gastrointestinal fluids by lingual and gastric lipase and most importantly by the pancreatic lipase/colipase complex (Sek et al., 2002). The digestion of the mono- and diglyceride is more rapid than the corresponding triglyceride but conversion to monoglyceride and fatty acid is expected to be complete after 30 min digestion (Sek et al., 2002). The presence of fatty acids inside the intestinal lumen has been shown to activate the duodenal or ileal braking mechanism, which resulted in a slowing in the passage of the luminal contents (Dobson et al., 1999). Another explanation might be that the impact of ileal brake activators on small intestinal transit only works as a feedback mechanism, which means that a slowing

effect of the oral preparations might have been observed with a pre-dosing of Labrasol® followed by a delayed administration of the drug solution. However, it is also likely that the administered amount of Labrasol® was not sufficient enough to elicit a slowing effect on the passage of the administered solution.

4.4.2. Drug absorption

In order to determine the impact of the presence of solubilizing agents and gastrointestinal transit on drug absorption ampicillin and antipyrine were administered as model compounds. Both drugs were chosen as a result of their differences in gastrointestinal absorption but also for their high aqueous solubility, which is 1 in 2 parts of water for ampicillin-Na and 1 in 1 part of water for antipyrine. Adequate solubility in the physiological fluids guaranteed that changes in drug bioavailability were solely related to changes in transit time and drug permeability but not to drug dissolution. The total dose of ampicillin and antipyrine was readily soluble in the administered fluid volume of approximately 50 mL of water, which means that precipitation from one of the solubilized systems was not expected to occur upon dilution in the stomach.

The individual and mean plasma concentration versus time-profiles obtained with the oral formulations are given in Figures 4.8 to 4.12 for ampicillin and Figures 4.13 to 4.17 for antipyrine, respectively. Figure 4.18 presents the plasma levels of both drugs observed after intravenous administration. The oral and intravenous pharmacokinetic data are listed in Tables 4.7 and 4.9 for ampicillin and Tables 4.8 and 4.10 for antipyrine, respectively.

The absolute oral drug bioavailability was determined as the dose ratio of the i.v. and oral treatment multiplied by the ratio of the infinite areas under the plasma concentration time curve (*AUC* inf) obtained for the orally administered treatment (Table 4.7 and 4.9) and the *AUC* inf obtained after intravenous administration (Table 4.8 and 4.10).

Figure 4.8. Plasma ampicillin concentration-time profiles for dog 1.

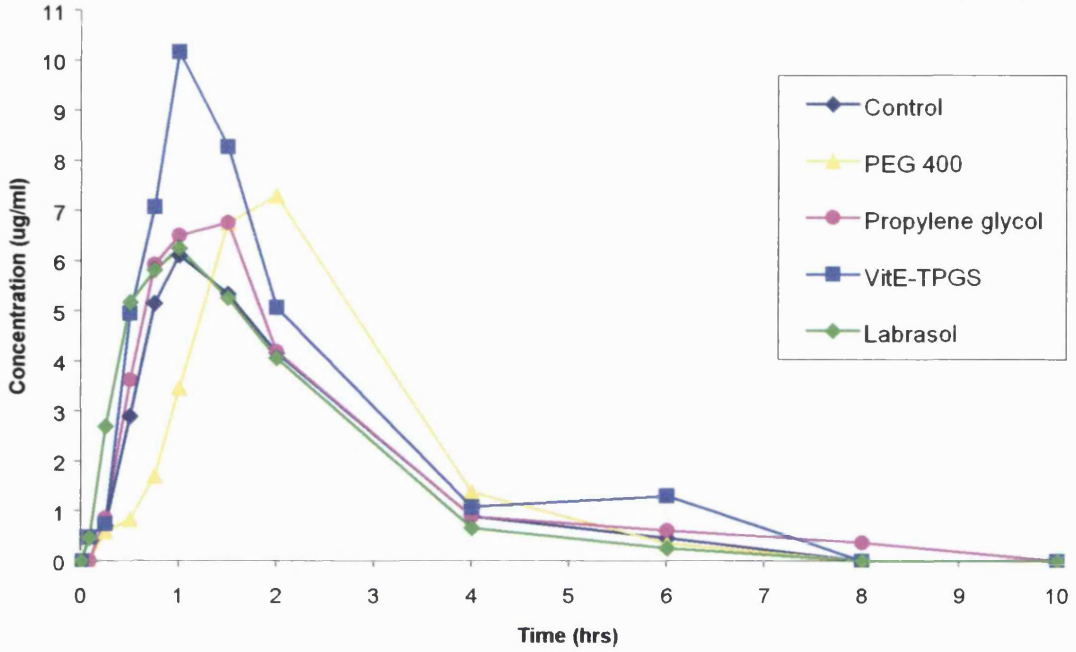


Figure 4.9. Plasma ampicillin concentration-time profiles for dog 2.

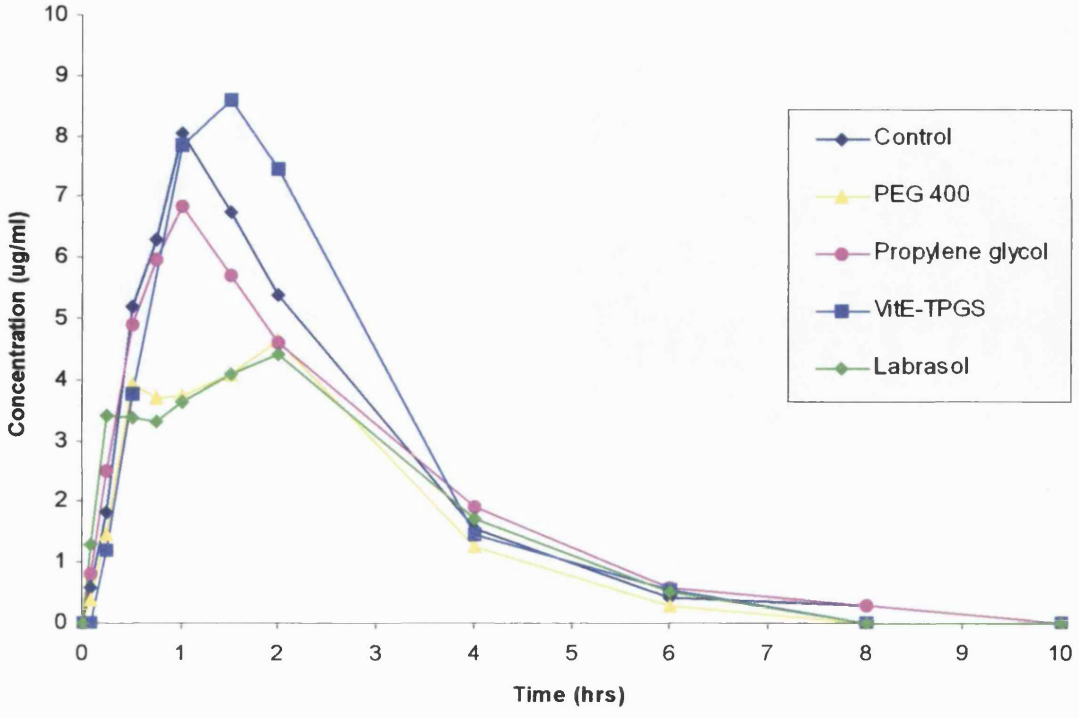


Figure 4.10. Plasma ampicillin concentration-time profiles for dog 3.

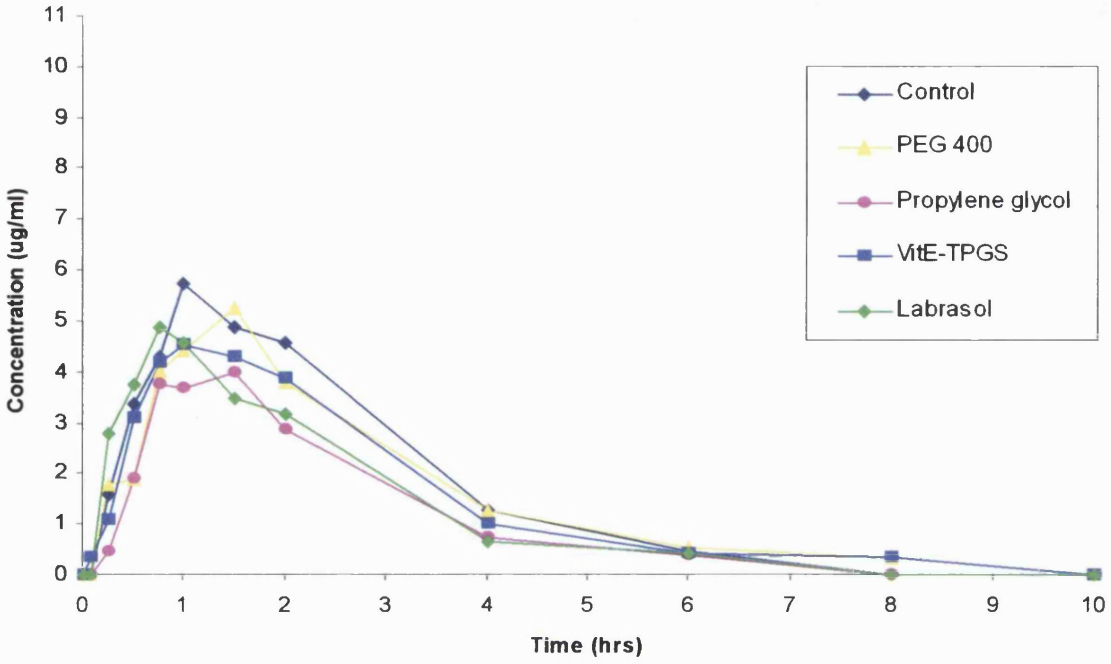


Figure 4.11. Plasma ampicillin concentration-time profiles for dog 4.

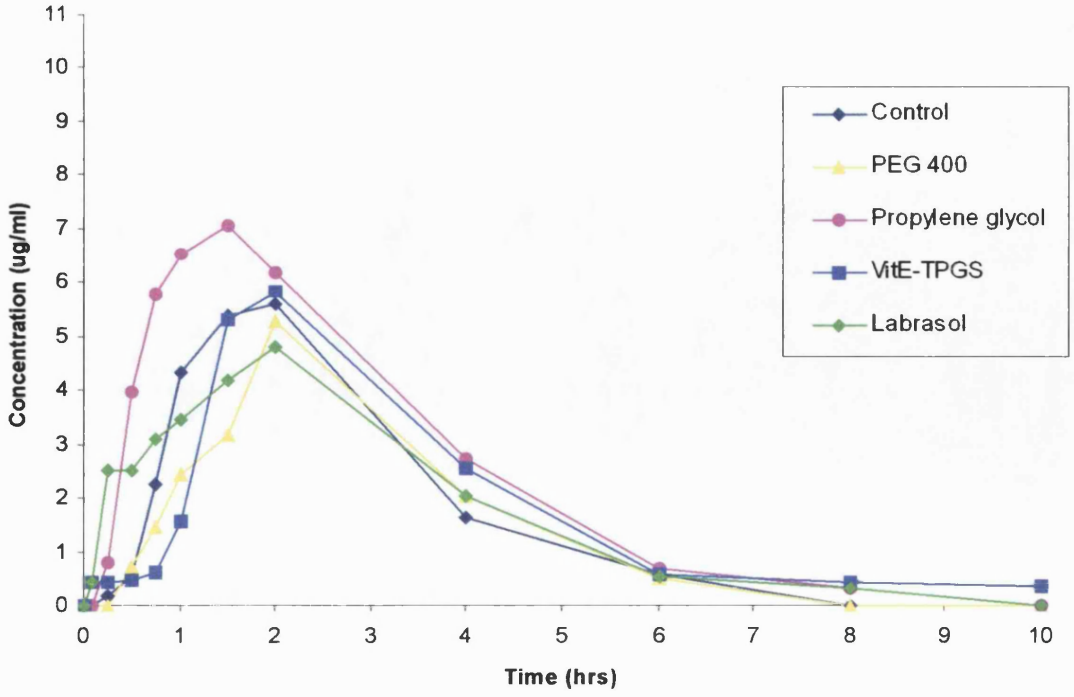


Figure 4.12. Mean (± S.E.) plasma ampicillin concentration-time profiles.

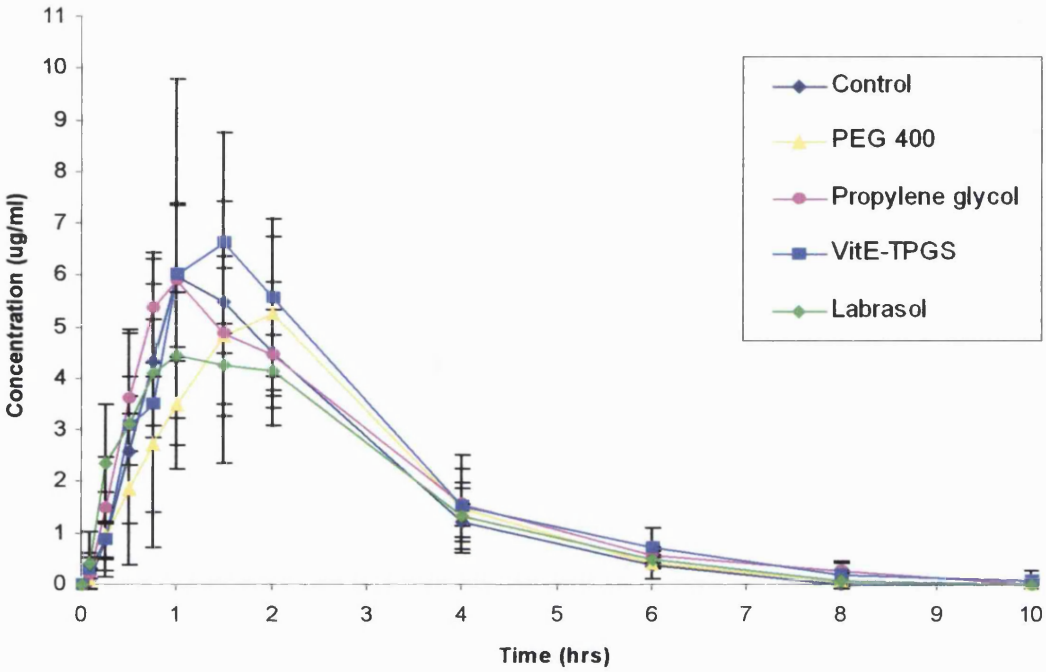


Figure 4.13. Plasma antipyrine concentration-time profiles for dog 1.

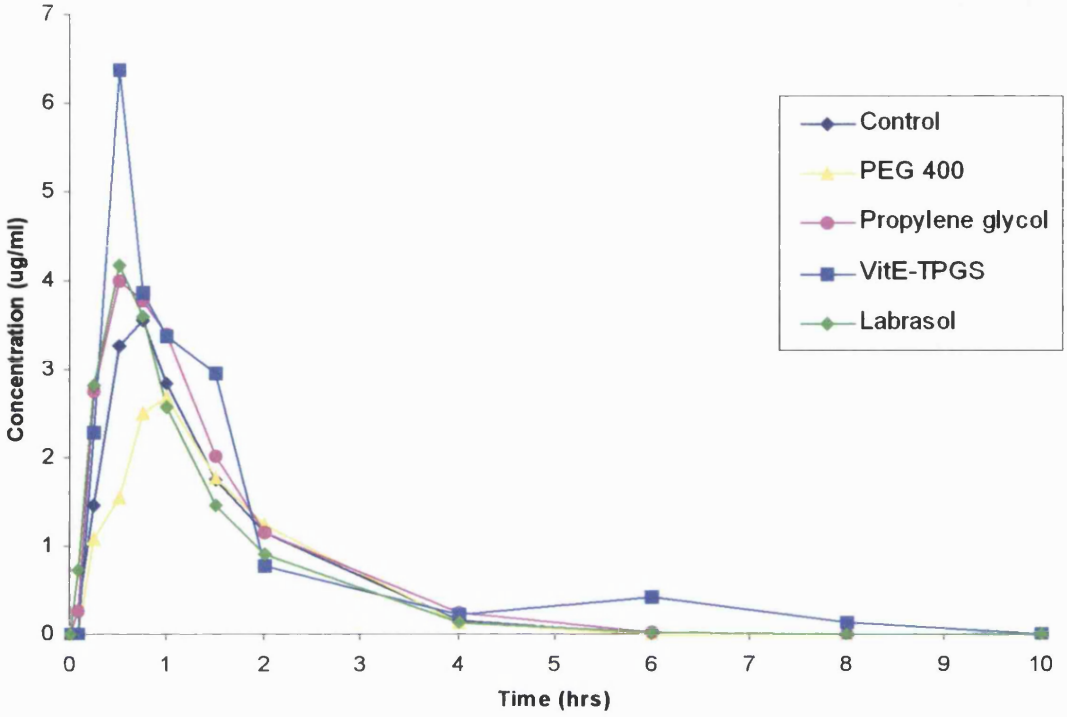


Figure 4.14. Plasma antipyrine concentration-time profiles for dog 2.

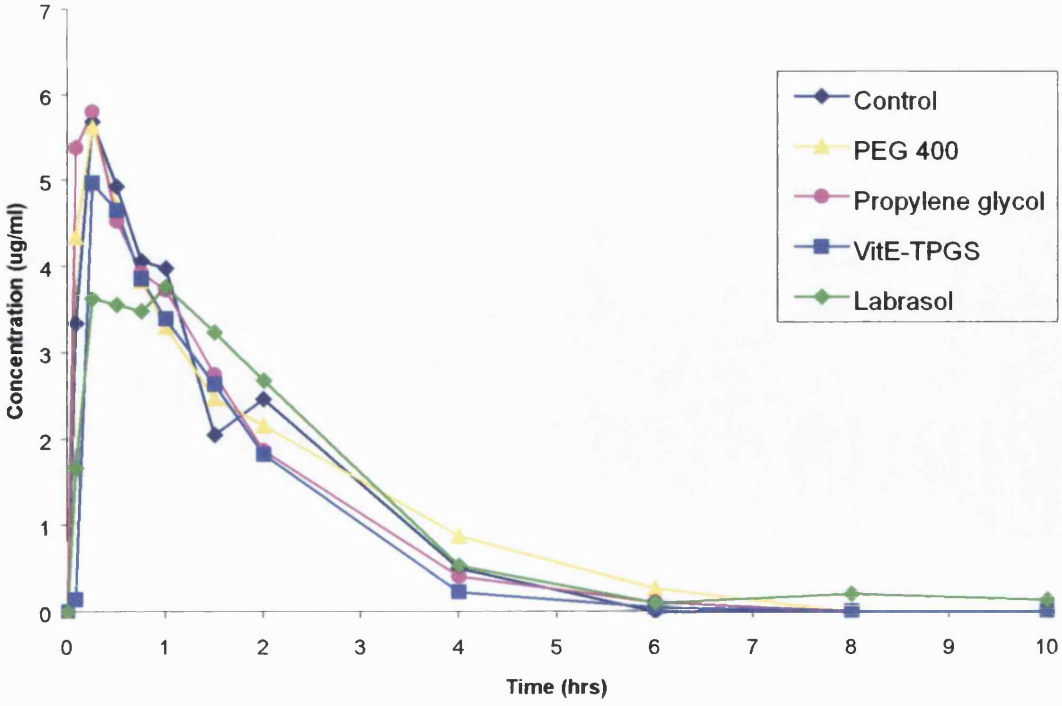


Figure 4.15. Plasma antipyrine concentration-time profiles for dog 3.

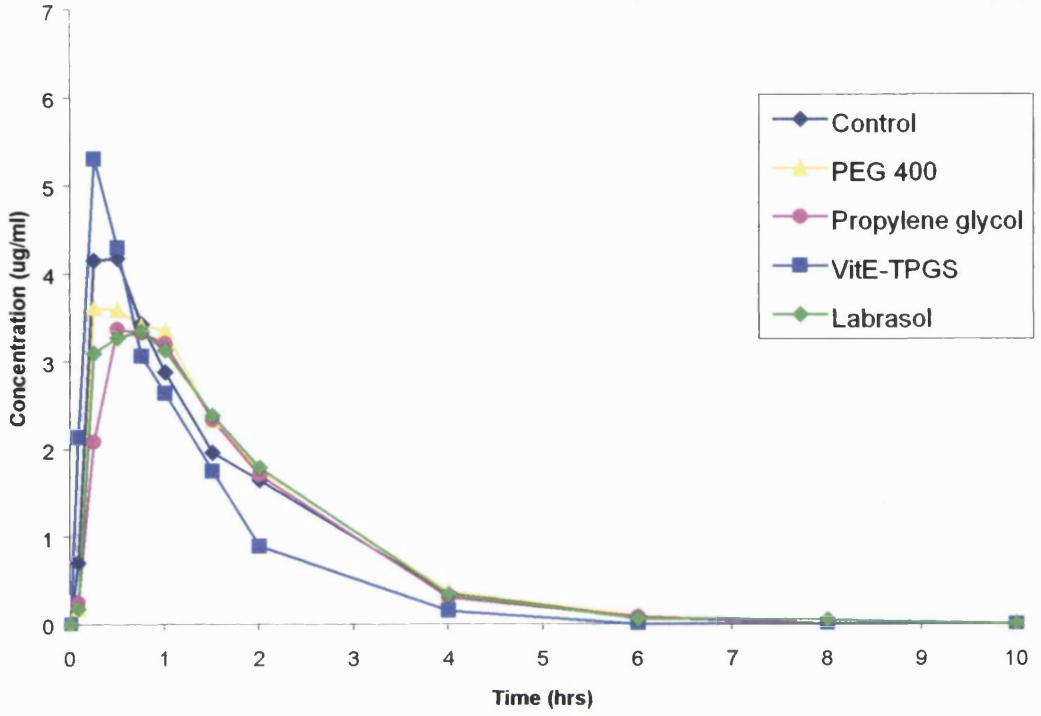


Figure 4.16. Plasma antipyrine concentration-time profiles for dog 4.

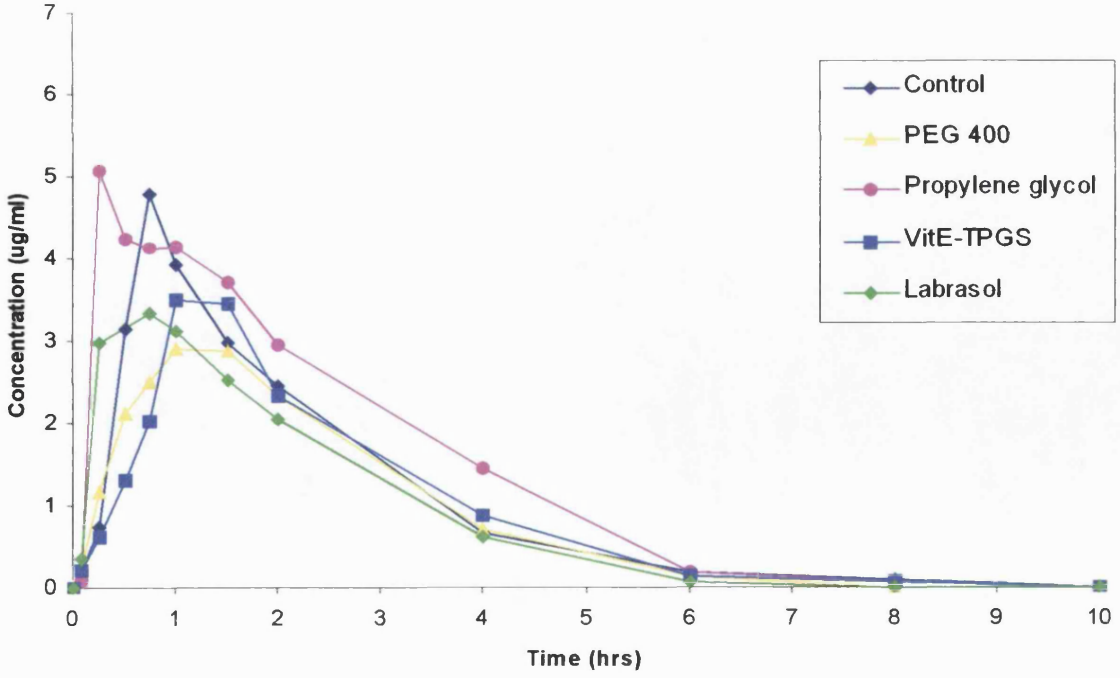


Figure 4.17. Mean (± S.E.) plasma antipyrine concentration-time profiles.

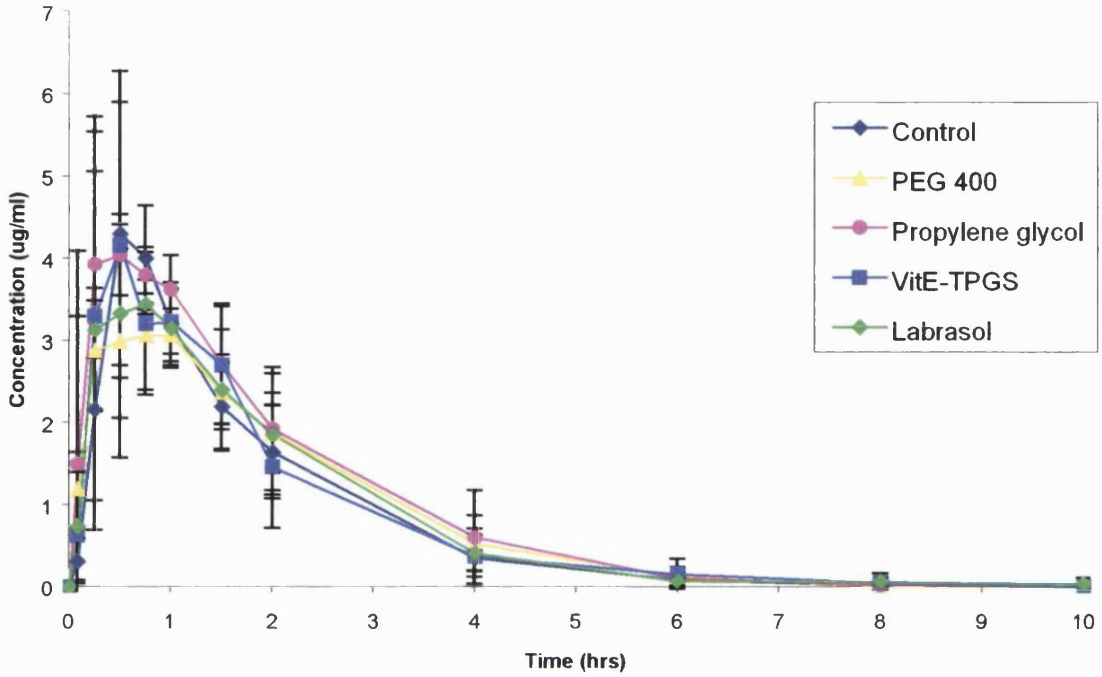


Figure 4.18. Mean (± S.E.) plasma concentration-time profiles after i.v. administration.

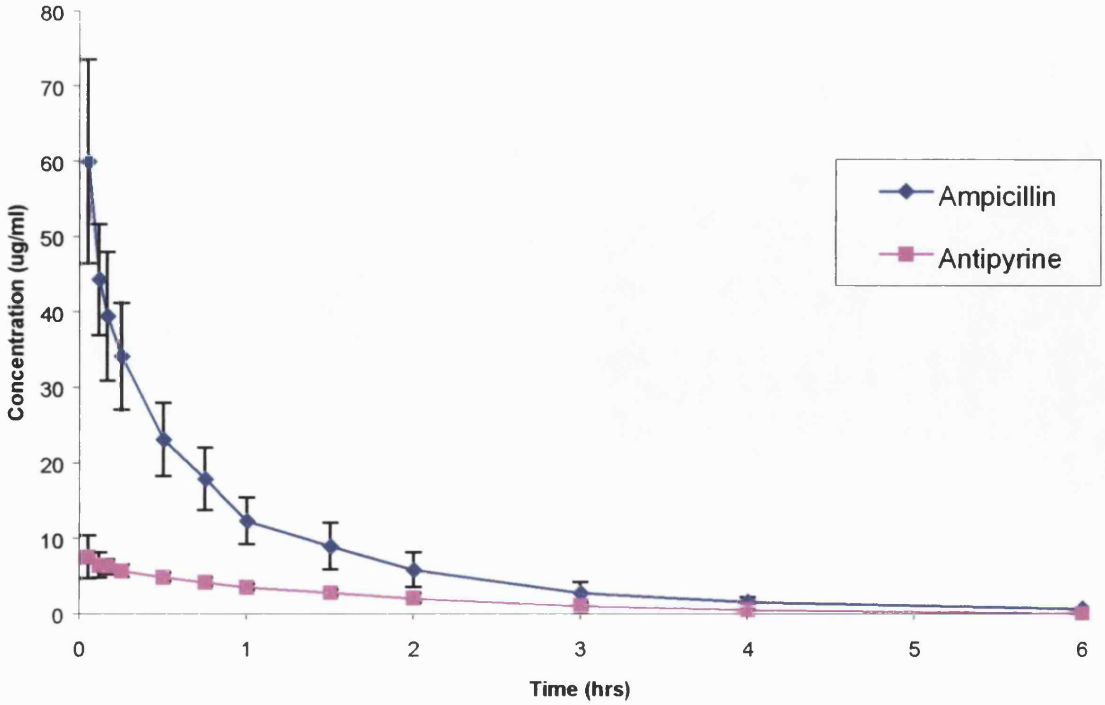


Table 4.7. Pharmacokinetic data for ampicillin after oral administration.

Treatment	Dog	Cmax (μg/mL)	Tmax (hrs)	AUC last (hr* μg/mL)	AUC inf (hr* μg/mL)	Bioavail. (%)
Control	1	6.90	1.0	15.44	16.36	31.1
	2	9.07	1.0	22.19	22.82	32.8
	3	6.48	1.0	16.67	17.62	40.6
	4	6.33	2.0	16.84	17.96	42.5
	Mean	**7.19**	**1.25**	**17.79**	**18.69**	**36.0**
	(± s.d.)	(± 1.27)	(± 0.50)	(± 3.00)	(± 2.84)	(± 5.65)
PEG 400	1	8.23	2.0	17.92	18.46	35.1
	2	5.23	2.0	15.15	15.65	22.5
	3	5.95	1.5	16.15	17.05	39.3
	4	5.96	2.0	15.10	16.06	38.0
	Mean	**6.34**	**1.88**	**16.08**	**16.81**	**32.3**
	(± s.d.)	(± 1.30)	(± 0.25)	(± 1.32)	(± 1.25)	(± 7.68)
	P-value					*0.385*
Propylene glycol	1	7.62	1.5	18.14	19.96	37.9
	2	7.72	1.0	21.55	22.28	32.0
	3	4.50	1.5	11.00	11.78	27.1
	4	7.97	1.5	25.48	26.23	62.1
	Mean	**6.95**	**1.38**	**19.04**	**20.06**	**38.6**
	(± s.d.)	(± 1.64)	(± 0.25)	(± 6.14)	(± 6.10)	(± 15.53)
	P-value					*0.689*
VitE-TPGS	1	11.47	1.0	22.43	25.63	48.7
	2	9.70	1.5	22.62	23.62	34.0
	3	5.10	1.0	15.05	16.06	37.0
	4	6.57	2.0	19.81	23.65	56.0
	Mean	**8.21**	**1.38**	**19.98**	**22.24**	**42.0**
	(± s.d.)	(± 2.90)	(± 0.48)	(± 3.53)	(± 4.23)	(±10.27)
	P-value					*0.248*
Labrasol®	1	7.04	1.0	15.71	16.13	30.6
	2	4.98	2.0	16.65	17.77	25.6
	3	5.48	0.75	12.73	13.66	31.5
	4	5.43	2.0	18.28	19.07	45.2
	Mean	**5.73**	**1.44**	**15.84**	**16.65**	**32.1**
	(± s.d.)	(± 0.90)	(± 0.66)	(± 2.33)	(± 2.33)	(± 8.40)
	P-value					*0.292*

Table 4.8. Pharmacokinetic data for antipyrine after oral administration.

Treatment	Dog	Cmax (μg/mL)	Tmax (hrs)	AUC last (hr* μg/mL)	AUC inf (hr* μg/mL)	Bioavail. (%)
Control	1	4.00	0.75	5.92	6.10	64.2
	2	6.41	0.25	10.59	11.48	76.7
	3	4.71	0.50	8.27	8.39	77.4
	4	5.41	0.75	10.70	10.87	83.8
	Mean	**5.13**	**0.56**	**8.87**	**9.21**	**76.3**
	(± s.d.)	(± 1.03)	(± 0.24)	(± 2.26)	(± 2.47)	(± 8.20)
PEG 400	1	3.03	1.0	5.00	5.15	54.2
	2	6.35	0.25	12.09	12.70	84.9
	3	4.08	0.25	8.67	8.71	80.4
	4	3.28	1.0	9.01	9.22	71.1
	Mean	**4.19**	**0.63**	**8.69**	**8.94**	**74.1**
	(± s.d.)	(± 1.51)	(± 0.43)	(± 2.90)	(± 3.09)	(± 13.56)
	P-value					*0.604*
Propylene glycol	1	4.52	0.5	7.27	7.30	76.8
	2	6.55	0.25	10.79	10.96	73.3
	3	3.80	0.5	7.77	7.89	72.8
	4	5.71	0.25	14.70	14.84	114.4
	Mean	**5.15**	**0.38**	**10.13**	**10.25**	**84.9**
	(± s.d.)	(± 1.22)	(± 0.14)	(± 3.42)	(± 3.46)	(± 20.14)
	P-value					*0.366*
VitE-TPGS	1	7.20	0.5	8.82	9.12	96.0
	2	5.61	0.25	8.98	9.05	60.5
	3	5.99	0.25	6.87	7.05	65.0
	4	3.95	1.0	9.68	9.79	75.5
	Mean	**5.69**	**0.50**	**8.59**	**8.75**	**72.5**
	(± s.d.)	(± 1.34)	(± 0.35)	(± 1.20)	(± 1.18)	(± 15.80)
	P-value					*0.915*
Labrasol®	1	4.72	0.5	6.14	6.18	65.1
	2	4.24	1.0	11.55	11.95	79.9
	3	3.77	0.75	8.26	8.35	77.0
	4	3.77	0.75	8.94	9.06	69.9
	Mean	**4.13**	**0.75**	**8.72**	**8.88**	**73.6**
	(± s.d.)	(± 0.45)	(± 0.20)	(± 2.23)	(± 2.38)	(± 6.75)
	P-value					*0.551*

Table 4.9. Pharmacokinetic data for ampicillin after intravenous administration of a 200 mg dose.

Treatment	Dog	Cmax (calculated) (μg/mL)	Cmax (observed) (μg/mL)	AUC last (hr* μg/mL)	AUC inf (hr* μg/mL)	AUC inf % extrapolated	t ½ (hours)	Cl (mL/min)	Vd (Litres)
IV Ampicillin	1	98.66	72.54	51.53	52.65	2.13	0.91	64.8	4.46
	2	94.36	78.15	68.01	69.53	2.18	1.12	49.0	4.10
	3	100.43	74.72	41.77	43.43	3.83	1.81	78.5	6.28
	4	48.57	44.98	40.33	42.21	4.45	1.68	80.8	7.50
	Mean	**85.50**	**67.60**	**50.41**	**51.95**	**3.15**	**1.38**	**68.3**	**5.59**
	(± s.d.)	(± 24.76)	(± 15.26)	(± 12.74)	(± 12.61)	(± 1.17)	(± 0.43)	(± 14.66)	(± 1.59)

Table 4.10. Pharmacokinetic data for antipyrine after intravenous administration of a 100 mg dose.

Treatment	Dog	Cmax (calculated) (μg/mL)	Cmax (observed) (μg/mL)	AUC last (hr* μg/mL)	AUC inf (hr* μg/mL)	AUC inf % extrapolated	t ½ (hours)	Cl (mL/min)	Vd (Litres)
IV Antipyrine	1	6.37	6.32	9.40	9.50	1.06	0.55	180.9	11.4
	2	12.09	10.72	14.87	14.96	0.61	0.69	114.6	10.4
	3	14.80	11.77	10.77	10.84	0.60	0.79	145.3	11.8
	4	6.03	6.03	12.61	12.97	2.72	1.11	124.0	13.7
	Mean	**9.83**	**8.71**	**11.92**	**12.07**	**1.25**	**0.79**	**141.2**	**11.8**
	(± s.d.)	(± 4.33)	(± 2.96)	(± 2.37)	(± 2.40)	(± 1.0)	(± 0.24)	(± 29.42)	(± 1.38)

4.4.2.1. Control

On the control day the mean oral bioavailability for ampicillin was found to be 36 %, which is in good agreement with literature values. Kimura and Higaki (2002) reported a 32 % oral bioavailability of ampicillin when administered as an oral solution in humans. The low bioavailability of ampicillin is the result of the drug's poor absorption from the gastrointestinal tract, as it does not undergo pre-systemic first-pass elimination (Yano et al., 1989). Ampicillin is primarily absorbed from the gastrointestinal tract using the dipeptide transporter in the upper small intestine (Oh et al., 1992). This explains the decreasing absorption rate constants *ka* of this compound towards the lower small intestine, which is less than 1/10 in the ileum compared to that in the duodenum and jejunum (Sawamoto et al., 1997). Being relatively hydrophilic ampicillin may to a small extent also make use of paracellular absorption pathways in the dog. The canine small intestine is known to be more permeable for hydrophilic compounds as a result of larger pore size and greater pore frequency in the lining epithelium. This phenomenon could account for the slightly higher bioavailability in the present study, especially for Dog 3 and 4, who had an oral bioavailability greater than 40 % (Table 3.7) compared to the data obtained in human studies. In contrast, a study conducted by Ushida et al. (1986) observed a lower oral bioavailability of ampicillin in beagle dogs compared to humans. This study, however, investigated different sustained release systems using ethyl cellulose microcapsules loaded with ampicillin. The authors attributed the lower bioavailabilities found in dogs to the overall shorter transit time in canines compared to human subjects as a result of the shorter length of the dog ileum. In the present study, maximum plasma levels of ampicillin were 7.2 µg/mL in average, which were reached after 1.25 hours. The volume of distribution for ampicillin has been reported to be 0.2 to 0.5 L/kg in humans and dogs, which correlates well with the values presented here of 5.59 L at an average bodyweight of 12.3 kg.

Antipyrine, on the other hand, gave a higher oral bioavailability compared to ampicillin. The absolute bioavailability of the oral solution on the control day was 76 % on average ranging from 64 to 84 %. The mean maximum plasma levels, which were determined to be 5.1 µg/mL, were reached at 0.57 hrs. These values correlate well with those demonstrated in a similar study in beagle dogs (Vickers et al., 1989), where an oral antipyrine solution (5 mg/kg) gave an absolute bioavailability of 78 %.

The mean antipyrine plasma half-life and volume of distribution were determined to be 0.79 hrs and 1.0 litres/kg, respectively and the drug was estimated to have a clearance of 11.5 ± 2.4 ml/min/kg for the intravenous route and 15.1 ± 3.1 ml/min/kg for the oral route. Antipyrine administered as an oral solution is rapidly and completely absorbed from the intestinal tract in all species thus far examined. It is therefore suggested that pre-systemic first-pass metabolism is responsible for the loss of 24 % of the oral dose. In comparison, Tmax was observed to be considerably shorter in antipyrine than in ampicillin, which may be explained by antipyrine's physicochemical properties. Despite its high solubility in aqueous media, antipyrine comprises of an octanol/water partition coefficient of 0.4, which means that the compound also comprises of some lipophilic characteristics. Therefore, it is not unlikely that antipyrine absorption might already occur to some degree in the stomach. The mean MGRT was determined to be 30 min (Table 3.1), which is approximately the Tmax of antipyrine. Additionally, once the drug is in the small intestine, it will be rapidly absorbed across the lipophilic membranes of the lining epithelium.

The employment of various solubilizers was found to have no effect of statistical significance on the bioavailability and pharmacokinetics of either ampicillin or antipyrine ($P > 0.05$). Although no significant alteration in gastrointestinal transit was observed with any of the treatments, some changes in the absorption profile of the model drugs would have been expected, as some of the utilized excipients have also been reported to comprise of absorption enhancing properties. In the following this will be discussed in more detail for each of the solubilizing agents.

4.4.2.2. PEG 400 and propylene glycol

The presence of the two cosolvents investigated, PEG 400 and propylene glycol, was found to affect the average bioavailability of both compounds in opposite directions although not to a significant extent. The treatment containing 1 g PEG 400 was determined to have an oral bioavailability for ampicillin and antipyrine of 32 ± 8 % and 74 ± 14 %, respectively. The obtained mean bioavailability values were slightly reduced in comparison to the control formulation. The employment of 2 g propylene glycol, on the other hand, resulted in a slight average increase in oral bioavailability of ampicillin and antipyrine since an absolute bioavailability of 39 and 85 % was

determined, respectively. Cosolvents are usually employed to increase the bioavailability of lipophilic drugs by enhancing their aqueous solubility. The mechanism is a decrease in the strong dipole characteristics of pure water due to penetration of the cosolvent molecules into the rigid cluster structures of water (compare Chapter 1). In the present study, however, both compounds comprised of excellent solubility in aqueous media, which means that no extra positive effect on drug bioavailability will be achieved with the employment of cosolvents as a result of their solubilizing qualities. On the contrary, the release and distribution of the hydrophilic drugs from the administered capsule formulation might even be hindered sterically in the presence of PEG 400. Reduced bioavailability as a result of a reduced thermodynamic activity of the active agent has been reported for carbamazepine in the presence of PEG 400 in an *in situ* perfusion study in rabbits (Riad and Sawchuk, 1991). In addition, it was also suggested that the reduction of the octanol/water partition coefficient of carbamazepine in the presence of PEG 400 was responsible for the reduced fraction of drug absorbed. The latter, however, will only be true for lipophilic compounds but will not come into account in the present case. The impact of limited thermodynamic activity in the small intestine, on the other hand, would be expected to be greater for the poorly permeable compound ampicillin, whose absorption is highly dependent on efficient interaction with its carrier than for antipyrine, which is well absorbed throughout the gastrointestinal tract. In comparison with the transit study conducted earlier in healthy volunteers (Chapter 2), it was interesting to find no increase in drug bioavailability in the presence of 1 g PEG 400 as was observed in humans with an oral solution of ranitidine. In humans the increase in drug absorption was determined to be 41 % and significant ($P < 0.01$). The mechanism behind this effect is not yet fully understood but it is suggested to be the result of either an inhibition of the intestinal efflux transporter P-glycoprotein or an interaction of PEG 400 with tight junctional proteins since ranitidine is mainly paracellularly absorbed. In contrast to ranitidine, neither ampicillin nor antipyrine have been reported to be a substrate of P-glycoprotein and an inhibition of the carrier system would, therefore, not result in an enhancement of oral bioavailability. At the same time, ampicillin and antipyrine both cross the epithelial membrane transcellularly via a transport-carrier and passive diffusion, respectively. A widening of the tight junctions would, therefore, most likely not majorly impact their overall absorption. However, both compounds are relatively small and hydrophilic and to a

certain extent could benefit from an opening of an additional absorption route suited for small hydrophilic compounds. An enhancing effect, however, was not seen for any of the drugs in the presence of 1 g PEG 400 in dogs. But canines are known to comprise of more frequent and larger pores in the paracellular route compared to humans (He et al., 1998) and an additional widening might not occur in this species. In the same study it was also observed that oligomers of PEG with a molecular weight up to 600 Da were quantitatively absorbed from the canine small intestine. It is therefore possible that the amount of PEG 400 remaining unabsorbed inside the dog intestinal lumen was negligible or non-existent and no permeation-enhancing effect was obtained due to the lack of polymer present at the apical side of the epithelial membrane. On the other hand, the permeability enhancement found in the human study might have been solely true for ranitidine, which has been shown to comprise of certain characteristic absorption pattern. It will, therefore, still need to be established whether the difference in drug absorption observed between the humans and dogs in the presence of 1 g PEG 400 is due solely to interspecies differences.

With the treatment containing propylene glycol, on the other hand, a slight increase in ampicillin and antipyrine bioavailability was observed compared to the control formulation. Coincidently, this preparation had the longest mean MSITT, which might account for the observed increase in drug absorption. Besides a longer small intestinal transit time, the presence of 2 g propylene glycol also resulted in shorter gastric residence time. After release from the disintegrating hard gelatin capsules the two drugs are quickly emptied into the small intestine. The small intestine is the primary site of absorption especially for passive transcellularly absorbed compounds such as antipyrine due to its superiority in absorptive surface area. As a result of faster emptying from the stomach antipyrine was observed to have a decreased Tmax value (Tmax = 0.375 hrs), which was not the same for ampicillin (Tmax = 1.375 hrs). The propylene glycol preparation comprises of a considerably high osmolality (3784 mOsm kg^{-1}), which is likely to cause alterations in GI water movement. With disintegration of the capsules in the stomach, the hypertonic solution is released and diluted in the gastric fluids until emptying into the small intestine. Water secretion occurs in order to lower the high osmotic pressure inside the lumen. In addition, propylene glycol is rapidly absorbed until isotonic conditions in the GI lumen are met. These fluid fluxes across the lining epithelium caused by the presence of an

osmotic absorbable load might affect the overall absorption of the hydrophilic compounds ampicillin and antipyrine. Propylene glycol/water-systems have, for instance, been demonstrated to increase percutaneous drug flux across a model membrane in a concentration dependent manner (Pellett et al., 1994). It is, however, not known whether propylene glycol might be capable of exerting such effects also on intestinal membranes. As presented earlier (Chapter 3), in an *in vitro* diffusion study using hydrophilic membranes the presence of high concentrations of propylene glycol did not have an effect on the passive diffusion of the hydrophilic model compound ranitidine. The *in vitro* study, however, is suggested to have limitations in resembling the *in vivo* situation of solvent drag as a result of greater pore size of the artificial membrane.

Another way of enhancing the bioavailability of drugs might be via the inhibition of drug metabolism. In an *in vivo* experiment involving rabbits Walters et al. (1993) found a reduction in drug clearance after intramuscular administration of dimenhydrinate solution containing propylene glycol. Subsequent *in vitro* studies with isolated rabbit liver microsomes revealed significant inactivation of microsomal drug metabolism by propylene glycol, which was directly proportional to the amount added. However, such an effect of the cosolvent on gastrointestinal metabolising enzymes has not yet been established. In addition, a reduction of intestinal metabolism would only result in an enhancement of the bioavailability of antipyrine but not ampicillin. Ampicillin is not subjected to any considerable first-pass metabolism (Yano et al., 1989), whereas antipyrine's bioavailability is directly impacted by pre-systemic elimination (Branch, 1982).

4.4.2.3. VitE-TPGS and Labrasol®

The treatments containing surfactants as solubilizing agent were also found not to have a significant impact on the bioavailability of the model drugs. However, slight differences were observed in the presence of VitE-TPGS and Labrasol® on ampicillin or antipyrine absorption. The oral bioavailability of ampicillin was 42 % in the presence of VitE-TPGS, which means it was slightly increased compared to the control formulation. Antipyrine, on the other hand, showed an absolute bioavailability of 72.5 % meaning a slight reduction relative to the control. An increase in ampicillin absorption was obtained in three of the dogs but the increase did not coincide with the

MSITTs observed in these subjects. This is rather surprising, since ampicillin bioavailability has been shown to be dependent on its residence time in the small intestine (Haruta et al., 1998), where an induced prolongation of gastrointestinal transit with propantheline resulted in a significant increase in drug absorbed. However, only four dogs were used in the present study and the variability was found to be considerable. The enhancement of ampicillin absorption could be the result of the surfactant's surface-active properties, which allow a greater spreadability of the oral solution across the intestinal surface membrane bringing the drug more closely into contact with its transport-carrier. VitE-TPGS is an amphiphatic molecule with a relatively high HLB of 13. In aqueous solution VitE-TPGS forms micelles above a concentration of 0.02 % (w/w) (CMC). Micellar solubilization by VitE-TPGS, however, is expected to play a minor role in ampicillin uptake. As a hydrophilic molecule the drug remains mainly in the continuous aqueous phase rather than being included into micelles (Poelma et al., 1991; Nerurkar et al., 1996). Based on the starting concentration of VitE-TPGS administered in 50 mL fluid the surfactant concentration in the stomach is estimated to be 0.5 to 1 % (w/w) maximum, considering the fasted state. The *in vitro* diffusion studies presented in chapter 3 demonstrate that concentrations of VitE-TPGS of up to 2 % (w/w) have no negative effect on the passive diffusion of hydrophilic drugs. According to the BCS antipyrine is classified as a class I compound as it has a good aqueous solubility as well as high membrane permeability. It comprises of an octanol/water partition coefficient of 0.4, which is rather weak due to its good solubility in aqueous media but states a certain degree of lipophilic properties. In the presence of micelles it is, therefore, possible that antipyrine will be incorporated into micellar structures to a certain extent, which decreases the fraction of free drug in solution. Drug molecules entrapped in micelles face reduced thermodynamic activity and reduced contact with the absorptive surface area, which is expected to affect the normally high permeability of a drug like antipyrine. This could explain the reduced oral bioavailabilities observed for antipyrine with both surfactants, which were 72.5 % with VitE-TPGS and 73.6 % with Labrasol® meaning a slight reduction compared to the control formulation albeit not statistically significant ($P > 0.05$). In contrast to VitE-TPGS, the presence of Labrasol® also resulted in a slight decrease in the mean absorption of ampicillin with an oral bioavailability of 32.1 %. Labrasol® is a surfactant (HLB = 14) comprising of polyglycolized medium chain mono- and diglycerides, which has been utilised as

lipid-based oral drug delivery systems for enhancing the solubility of lipophilic drugs. Upon release in the stomach and emptying into the small intestine, Labrasol® will be hydrolysed by lipases releasing free fatty acids, caprylic (C_8) and capric acids (C_{10}), predominantly. This process has been shown to occur quantitatively for medium chain mono- and diglycerides in the first 30 min independent of the presence of bile salts (Sek et al., 2002). The lipolytic products will form mixed micellar systems with bile salts and lecithin, which will incorporate lipophilic drugs and hence increase their solubility within the gastrointestinal lumen. Surfactants including medium chain glycerides have been shown to exhibit permeability-enhancing properties as a result of interactions with the phospholipid bilayer of the lining epithelium (Muranushi et al., 1981; Swenson and Curatolo, 1992). In cell and tissue culture studies medium chain fatty acids have been demonstrated to significantly increase the permeation of paracellular compounds via an intracellular regulation of tight junction permeability (Yeh et al., 1994; Lindmark et al., 1998). Recently, *in vivo* studies conducted in rats described a significantly enhanced absorption for the poorly absorbable drugs gentamicin (Hu et al., 2001) and insulin (Eaimtrakarn et al., 2002) in the presence of Labrasol®. Similar results were found for vancomycin in the presence of Labrasol® and VitE-TPGS when administered to the rat ileum in an *in situ* experiment (Prasad et al., 2003). Labrasol® was also observed to enhance the permeability of cephalexin (Koga et al., 2002), a β-lactam antibiotic which makes use of the intestinal dipeptide transporter similar to ampicillin. Koga et al. (2002) demonstrated in *in vitro* and *in situ* permeability experiments that Labrasol increased both the active and passive transport of the drug, whereas no effect was observed in the presence of another medium chain glyceride surfactant Gelucire 44/14. Yet another possible way of absorption enhancement is the inhibition of intestinal efflux carriers such as P-glycoprotein, which reduce the bioavailability of drugs by expelling already absorbed drug molecules from the cytosol back into the lumen. Both VitE-TPGS (Dintaman and Silverman, 1999) and Labrasol® (Hu et al., 2001) have been reported to inhibit P-glycoprotein in cell culture studies. There are no reports in the literature identifying ampicillin or antipyrine as substrates of intestinal efflux transporters making it doubtful whether an inhibition of P-glycoprotein would indeed result in an enhanced drug bioavailability. In addition, cell cultures such as Caco-2 cells tend to over-express P-glycoprotein and significant findings in decreased efflux *in vitro* may not reach significance *in vivo*. In an *in situ* study in rats, for instance, VitE-TPGS was

found not to have a significant effect on P-glycoprotein efflux or enterocyte-based metabolism (Johnson et al., 2002). Because of their amphiphilic structure, surfactants are able to interrupt the activity of enzymes, e.g. metabolising enzymes such as CYP450 3A (Mountfield et al., 2000). The effect, however, is highly dependent on the uptake of the excipient across the intestinal wall, which in many cases is unlikely to occur at significant amounts (Johnson et al., 2002; Mountfield et al., 2000). In the present study, the absorption-enhancing effect of either surfactant has proven not to be significant *in vivo* in dogs. It is possible that the excipient concentrations employed in the present study were not sufficient to give a significant positive effect.

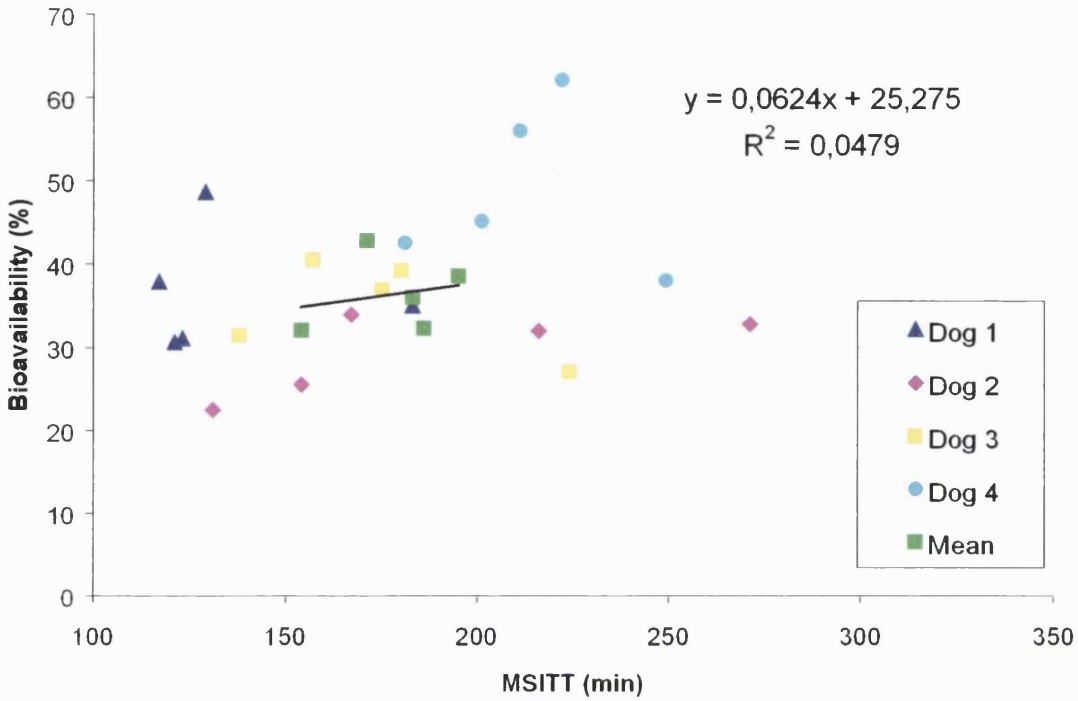
4.4.3. Correlation between GI transit and drug absorption

The main objective of the present study was to identify potential effects of solubilizers on the transit of oral liquids through the small intestine and investigate whether such effects directly impact on the oral bioavailability of poorly absorbable drug compounds and to what extent. In Figures 4.19a,b and 4.20a,b the relationship between the mean small intestinal transit times (MSITTs) and the oral bioavailability of ampicillin and antipyrine, respectively, is shown.

Considering the relatively high intra- and intersubject variability, the data are presented for each individual dog in Figures 4.19a and 4.20a for ampicillin and antipyrine, respectively. A linear line of regression was derived for the mean values of all four dogs. For ampicillin, the regression coefficient, R^2, was 0.0479, which means that in the present study no direct correlation between the small intestinal transit time and the oral bioavailability of ampicillin was found in dogs. An increase in the MSITT does not necessarily lead to an enhanced absorption of the amino-penicillin. Different observations, however, were obtained in rats, where a direct dependency was demonstrated of the oral bioavailability of ampicillin on the residence time of the drug at its site of absorption (Haruta et al., 1998). The authors prolonged the small intestinal transit time of the drug solution with the administration of propantheline and as a result the absorption of the β-lactam antibiotic was significantly enhanced. Such a relation, however, does not appear to exist for ampicillin in canines.

Figure 4.19. Correlation between the MSITT and the oral bioavailability of ampicillin considering:

a) Intra- and interindividual variation



b) Effect of treatment

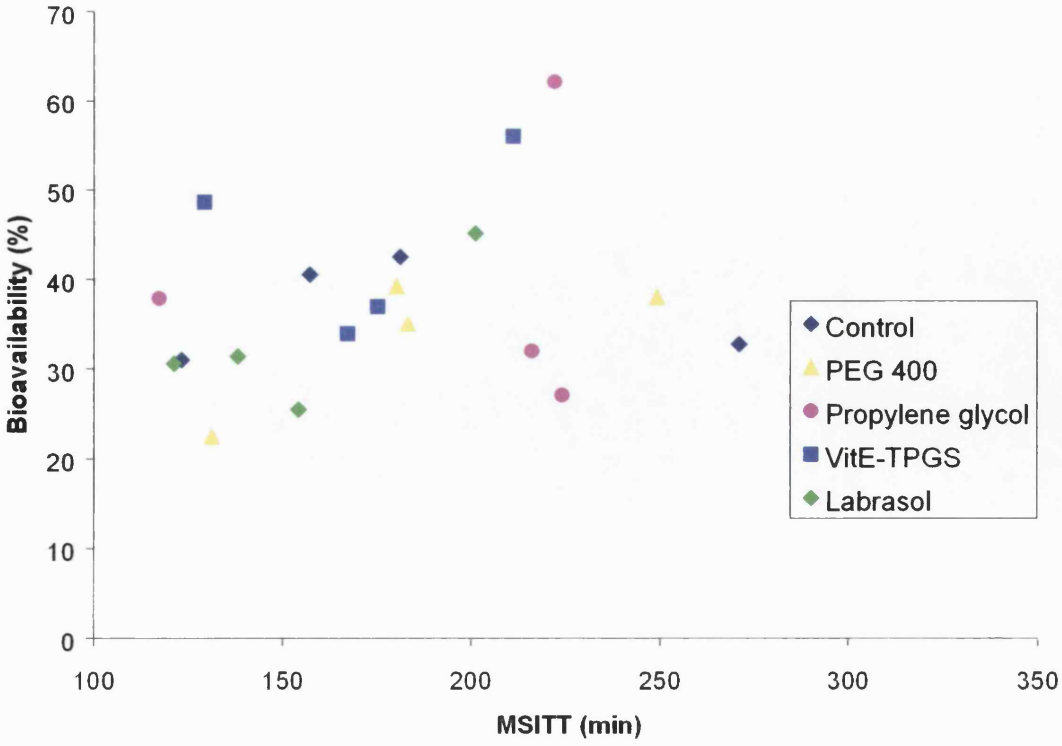
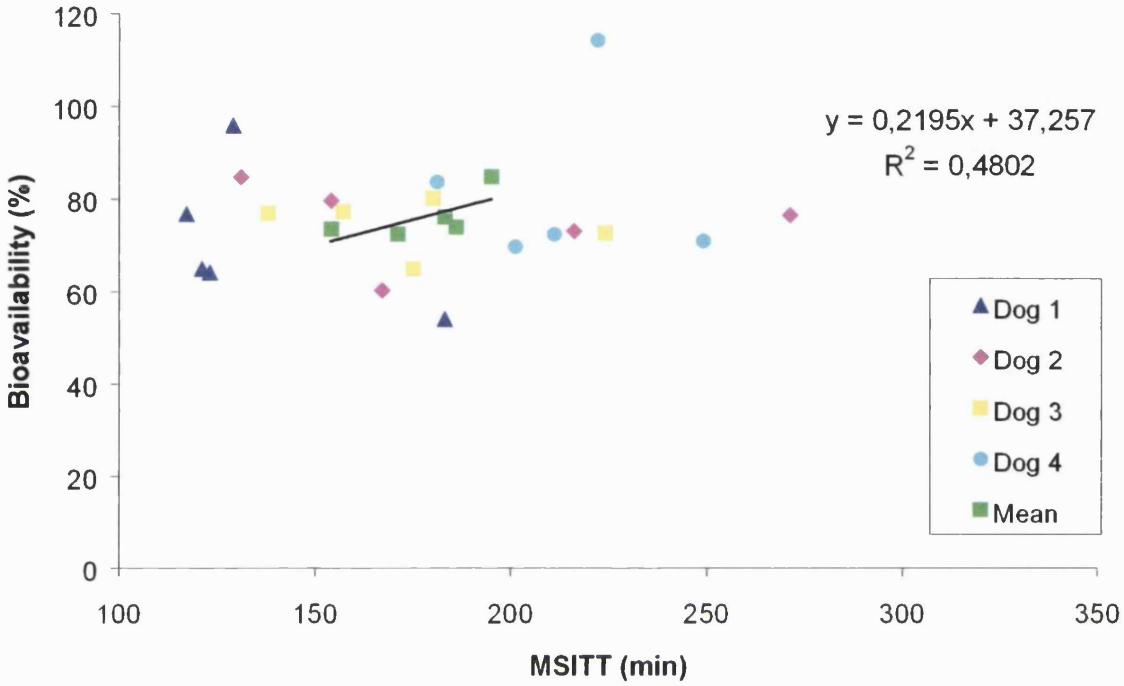
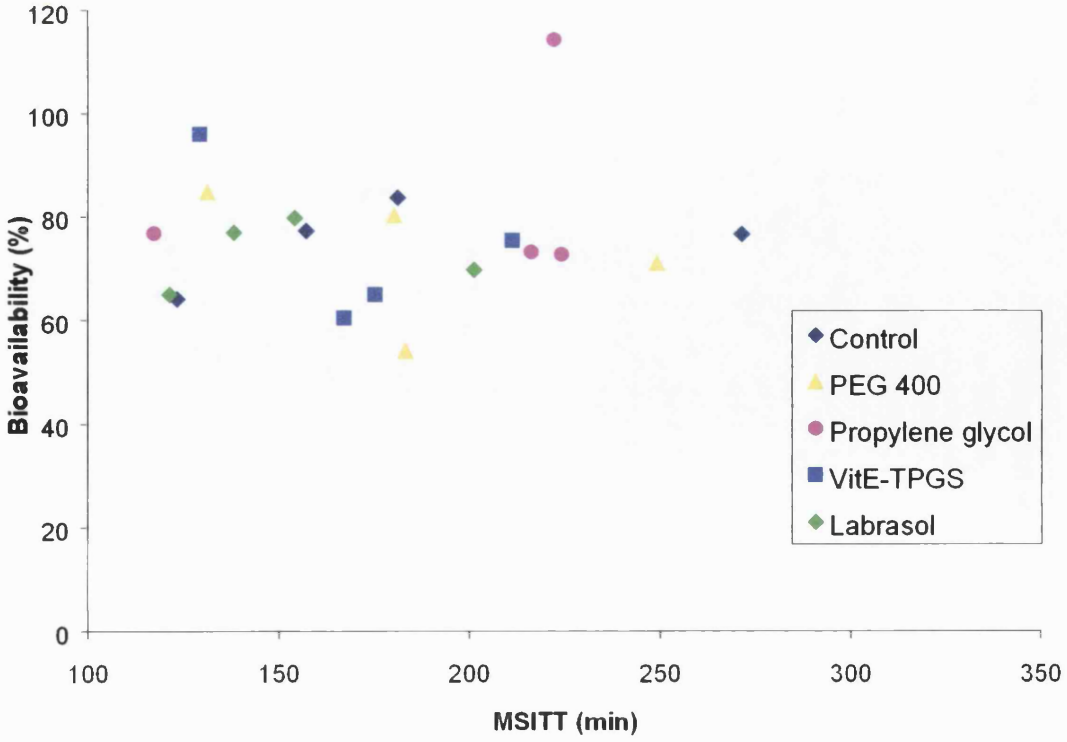


Figure 4.20. Correlation between the MSITT and the oral bioavailability of antipyrine considering:

a) Intra- and interindividual variation



b) Effect of treatment



It is, however, possible that this poor correlation is the result of the influence of the different solubilizers present in the administered solution with the excipient affecting drug bioavailability directly via an enhancement or a reduction of ampicillin absorption.

Figure 4.19b shows the relation of the data in dependence of the type of solubilizer present in the formulation. Effects of the specific excipient such as transit acceleration or prolongation and/or an increase or decrease in the absorption of ampicillin was expected to show in the distribution of the data in the graph. In contrast, the non-significance of the effects of the administered solubilizers becomes apparent as the distribution of values does not seem to follow a specific order.

Similar results were obtained for the model compound antipyrine. Figure 4.20a presents the correlation of the mean small intestinal transit times and oral antipyrine bioavailability for the individual dogs, whereas Figure 4.20b on the other hand, shows the relationship between the two with respect to the influence of different solubilizing agents. Although the correlation between the MSITT and the absorption of antipyrine appears to be better than that observed for ampicillin, the proportionality is not perfect as the correlation coefficient, R^2, is only 0.4802. Also, no direct influence on the correlation of GI transit and oral drug bioavailability was observed for any of the administered solubilizers.

These findings demonstrate that in the present study no direct correlation was observed between the small intestinal transit time and the oral bioavailabilities of ampicillin and antipyrine and the effects of coadministered excipients were found not to be significant. It needs to be established whether the dog is a good model for investigating GI transit and drug absorption in the presence of solubilizing agents.

4.5. Conclusions

One aim of the present *in vivo* study was to investigate different types of excipients, which are commonly employed to increase the aqueous solubility of therapeutic agents, with respect to their impact on gastrointestinal transit of oral drug solutions. In four beagle dogs no significant effect on liquid gastric emptying times was

observed in the presence of 1 g PEG 400, 1 g VitE-TPGS or 2 g Labrasol®. The administration of 2 g propylene glycol, however, resulted in a slight reduction of the mean MGRT, which was most likely caused by the high osmolality of the preparation eliciting a propulsive reaction of the fasted dog stomach. Regarding the passage of the preparations through the small intestine, none of the solubilizing agents appeared to have either an accelerating or slowing effect in dogs at the concentrations administered in the present study. Mean reductions in the MSITT of 2, 7 and 16 % were, however, observed in the presence of PEG 400, VitE-TPGS and Labrasol®, respectively, as well as a mean increase in the MSITT with the propylene glycol formulation of 7 % but all effects were determined to be statistically not significant.

The presence of the different solubilizing agents on drug absorption was investigated using ampicillin and antipyrine as model compounds. No significant effect on the oral bioavailability of either of the drugs was observed with any of the treatments compared to the control. However, the treatments containing a cosolvent or surfactant exhibited a decrease rather than an increase in drug bioavailability. Both drugs are highly water-soluble. Unlike lipophilic drugs an enhancement of drug bioavailability in the presence of solubilizer does therefore not occur via an enhancement in solubility or dissolution of the compounds. For ampicillin being actively absorbed via the dipeptide transporter in the upper small intestine, an efficient and easy access to the carrier is crucial for its uptake. Antipyrine, on the other hand, traverses the lining epithelium transcellularly via passive diffusion and is considered a highly permeable compound. In this respect, the luminal presence of macromolecules such as PEG 400 or micelle-forming surfactants may instead be a hindrance to the uptake of the drugs from the lumen. Propylene glycol, however, appeared to have a slight, although not significant, increasing effect on the oral bioavailability of both drugs. Intestinal fluid fluxes caused by the osmotically active yet highly absorbable cosolvent are suggested to be involved.

Another objective of the present study was to establish whether the dog might be a good model for investigating GI transit and drug absorption in the presence of solubilizing agents in oral liquids. The results obtained for PEG 400 correlate well with those observed in the previously conducted transit study in humans since no significant effect on either gastric emptying or small intestinal transit was found in

both studies. Differences between the two studies, however, were observed regarding the effect of PEG 400 on drug absorption since the absorption enhancing effect of the polymer in humans was not obtained in dogs. These findings might be the result of interspecies differences like the leakier absorptive membrane in canines quantitatively removing PEG 400 from the intestinal lumen, but it is also possible that the enhancing effect of the polymer is specific for the uptake of paracellular drugs.

Considering the overall correlation of GI transit and drug absorption in the dog no direct relationship was found between the two parameters. The poor correlation in the present study is most likely the result of high intra- and intersubject variability. However, the presence of solubilizing agents was found not to affect small intestinal transit and oral bioavailability of both ampicillin and antipyrine in the dog. It is possible that the doses administered were not sufficient but an impact may be observed with higher doses. The administration of low doses of PEG 400 revealed the existence of considerable differences between the canine and the human with respect to the polymer effect on drug absorption. It is questionable whether these differences are generally true and also exist for other excipients and model drugs. More investigations are necessary to characterize the use of the dog as a model for *in vivo* effects of pharmaceutical excipients in humans.

CHAPTER 5

THE EFFECTS OF DIFFERENT TYPES OF SOLUBILIZING AGENTS ON GI TRANSIT AND DRUG ABSORPTION IN HUMANS

5.1. Overview

On investigating the influence of certain cosolvents and surfactants on gastrointestinal transit and drug absorption in the beagle dog it was shown that none of the administered solubilizers had a significant effect in comparison with the performance of the control formulation (Chapter 4). In general, the dog is widely used as an animal model as a result of its similarities to man regarding drug pharmacokinetics, anatomy and motility (Anderson, 1970; Dressman, 1986). Considerable differences between the two species, however, were observed for the absorption of model drugs from oral solutions containing low doses of PEG 400. Such cases show that the dog can only be a model for the *in vivo* situation in the human despite the close similarities between the two species. One of the main objectives of the present study in healthy volunteers was, therefore, to investigate the performance of the solubilizers used previously in canines and verify the beagle dog as a suitable model for the investigation of excipient effects as well as influences on GI transit and drug absorption.

From the similar results found between the 1 g PEG 400 treatment in dogs and the same dose in humans it is suggested that the dose of co-administered excipient in dogs should be equivalent to those employed in humans. As mentioned earlier, the results in the dog study investigating different types of solubilizers were observed to be of no statistical significance. This could be the consequence of the small group of animals used, which enhances high inter-individual variation. On the other hand, it is also possible that the amount of solubilizer administered was too low to have an effect on transit (1 g for PEG 400 and VitE-TPGS, 2 g for propylene glycol and Labrasol®). A dose-dependent effect on GI transit has previously been described for PEG 400 (Chapter 2). Although the canine study did not reveal any transit effect of statistical significance in the presence of any of the solubilizing agents, trends in alterations in the mean small intestinal transit times (MSITTs) were noted. It was decided that it would be of interest to see whether these trends become significant at higher concentrations or merely reflect inter-individual variation. The amount of solubilizer administered to the healthy subjects in the present study was, therefore, increased to 5 g.

The types of excipients under investigation were chosen to be propylene glycol, VitE-TPGS and Capmul® MCM. Capmul® MCM comprises of a mixture of mono-, di- and triglycerides of medium chain fatty acids, mainly caprylic and capric acid and it is therefore often referred to as medium chain glycerides. A more detailed description of this surfactant is given in Chapter 1. Capmul® MCM was employed in the present study to substitute the previously used Labrasol®, which failed to meet the expectations to slow down the passage of luminal contents through the small intestine. As model drugs, ampicillin sodium salt and ranitidine hydrochloride were used to enable a direct comparison to the results observed in the previous two studies in canines and humans, respectively (Chapter 4 and 2, respectively).

5.2. Materials

Ampicillin was obtained in the form of ampicillin sodium from Sigma-Aldrich (Poole, U.K.). It is a white, practically odourless, crystalline or amorphous powder that is hygroscopic and very soluble in water or other aqueous solutions.

Ranitidine was provided in the form of ranitidine hydrochloride from GlaxoSmithKline (Ware, Hertfordshire, U.K.) and certified as 99.9% pure. It is a white to pale yellow, practically odourless, crystalline powder sensitive to light and moisture.

Polyethylene glycol 400 was obtained from Sigma-Aldrich (Poole, U.K.). It is a clear, colourless, highly osmotic and viscous liquid with a slight but characteristic odour and a bitter and slightly burning taste. PEG 400 is hygroscopic and completely soluble in water.

Propylene glycol was obtained from Sigma-Aldrich (Poole, U.K.). The excipient is a clear, colour- and odourless liquid of medium viscosity that is hygroscopic and completely soluble in water.

D-α-tocopheryl polyethylene glycol 1000 succinate (VitE-TPGS) was obtained from Eastman Chemical (TN, U.S.A.). It is a pale yellow waxy solid, which is highly miscible with water forming low-viscosity solutions at concentrations < 20 % (w/w).

Capmul® MCM was obtained from Abitec Corp. (WI, U.S.A.). Capmul® MCM is a clear, viscous liquid of oily consistency with a pale yellow colour.

Technetium-99m

The radionuclide ^{99m}Tc was obtained daily from Amersham International (Amersham, U.K.) in the form of the complex ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) dissolved in saline. The complex was declared to be stable throughout the study day.

5.3. Methods

5.3.1. Dosage forms

5.3.1.1. Preparation of drug solutions

For the oral preparations, 551 mg ampicillin sodium, equivalent to 500 mg ampicillin acid, and 168 mg ranitidine hydrochloride, equivalent to 150 mg ranitidine base, were dissolved in 50 mL of solvent. The solvent consisted of water (for Control and Capmul® MCM treatment) or a mixture of either 10 % (w/w) propylene glycol or 10 % (w/w) VitE-TPGS in water. The propylene glycol solution was obtained by simply mixing 5 g of the cosolvent with 45 mL of water. For the VitE-TPGS solution, the waxy surfactant was heated in an oven (37° C) to liquefy prior to adding 5 g of the surfactant to water. The mixture was stirred at approximately 60° C until complete dissolution of VitE-TPGS and then cooled down to room temperature. For reasons of taste masking, 5 g Capmul® MCM was administered separately filled in four "000" hard gelatin capsules. The drug solutions were radiolabelled with the addition of a few drops of the ^{99m}Tc-DTPA in saline-solution. The radioactivity of the solutions was measured using a radionuclide dose calibrator (Biodex, Atomlab 100 Dose Calibrator, NY, U.S.A.) and determined to be 7 ± 0.5 MBq.

5.3.1.2. Osmolality of drug solutions

The osmotic pressure of the drug solutions was measured using a freezing-point osmometer (Type 5R, Roebling, Germany). The preparations were found to have the following osmolalities: Control (65 mOsm kg^{-1}); propylene glycol (1550 mOsm kg^{-1}); VitE-TPGS (90 mOsm kg^{-1}) and Capmul® MCM (116 mOsm kg^{-1}). For propylene glycol the solution was diluted (1:10) to achieve freezing. Considering the co-administration of these preparations with 100 mL of water, the overall osmolalities of ingested fluid was determined as: Control (21 mOsm kg^{-1}); propylene glycol (512 mOsm kg^{-1}); VitE-TPGS (26 mOsm kg^{-1}) and Capmul® MCM (43 mOsm kg^{-1}).

5.3.2. Gamma scintigraphy

5.3.2.1. The gamma camera

Imaging was conducted using a single headed General Electric Maxicamera 400AC (400T; Milwaukee, U.S.A.) (Figure 5.1). The detector comprising of a 40 cm diameter field of view and was fitted with a low energy parallel hole collimator suitable for ^{99m}Tc imaging. An on-line computer (Starcam 3200I, General Electric Medical Systems, Milwaukee, USA) was connected to the camera for digital image recording. For subsequent analysis data were archived onto CD and hard drive.

5.3.2.2. Study protocol

Seven male volunteers (age range 24-48 years, median 28 years; weight range 61-89 kg, median 76 kg; height range 1.68 – 1.86 m, median 1.78 m) participated in an open four-way crossover study, after providing written informed consent. All volunteers were non-smokers, declared themselves healthy and had no history of gastrointestinal disease.

The East London & The City Health Authority Research Ethics Committee approved the experimental protocol. From the Administration of Radioactive Substances Advisory Committee (ARSAC) at the Department of Health the authority was obtained to administer radiopharmaceuticals under the responsibility of Dr Neil Garvie, Consultant in Nuclear Medicine and Consultant Radiologist of The Royal London Hospital, Whitechapel. The study was conducted in accordance with the

provision of the Declaration of Helsinki (1965) and Tokyo (1975) and Venice (1983) revisions.

Each volunteer completed four study-days, which were separated by at least a two-day washout period. On each occasion the type of treatment was varied and given to each volunteer in a randomised order.

The different treatments were:

Control

- 551 mg ampicillin sodium (corresponds to 500 mg ampicillin acid) and 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 50 mL water; radiolabelled with 7 MBq of ^{99m}Tc-DTPA; co-administered with 100 mL water.

Propylene glycol

- 551 mg ampicillin sodium (corresponds to 500 mg ampicillin acid), 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) and 5 g propylene glycol dissolved in 45 mL water; radiolabelled with 7 MBq of ^{99m}Tc-DTPA; co-administered with 100 mL water.

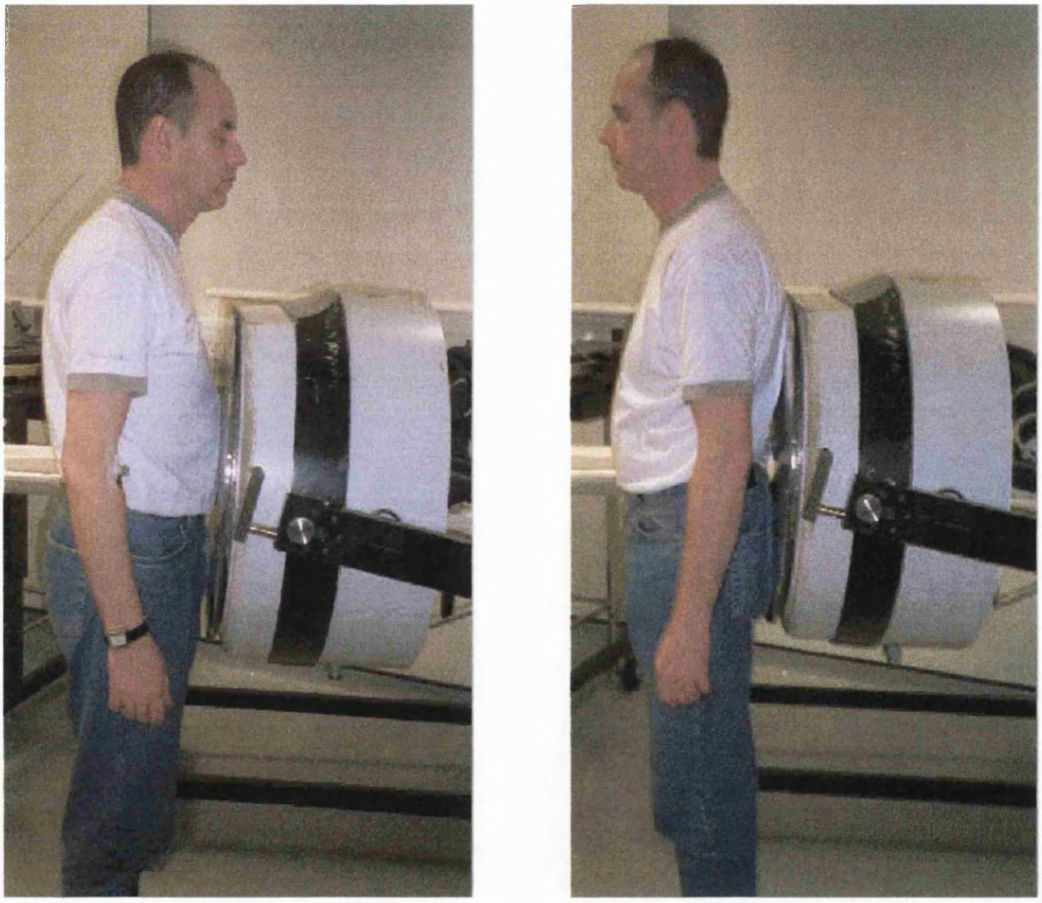
VitE-TPGS

- 551 mg ampicillin sodium (corresponds to 500 mg ampicillin acid) and 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 50 mL 10 % (w/w) VitE-TPGS in water; radiolabelled with 7 MBq of ^{99m}Tc-DTPA; co-administered with 100 mL water.

Capmul® MCM

- 551 mg ampicillin sodium (corresponds to 500 mg ampicillin acid) and 168 mg ranitidine hydrochloride (corresponds to 150 mg ranitidine base) dissolved in 50 mL water; radiolabelled with 7 MBq of ^{99m}Tc-DTPA; four "000" hard gelatin capsules filled with a total of 5 g Capmul® MCM; co-administered with 100 mL water.

Figure 5.1. The single headed GE Maxicamera, showing the position of the detector and volunteer for anterior and posterior imaging, respectively.



The volunteers were asked to attend to an overnight fast and also refrain from alcohol or spicy food. On the day of the study, the volunteers arrived at The Wingate Institute of Neurogastroenterology, Whitechapel at 8.30 am. A canula was inserted into the right forearm and a pre-dose blood sample was taken at 0 hours. Before the administration of the treatments, a small sealed point source of 0.5 MBq ^{99m}Tc was taped to the abdominal skin at the position of the right lower costal margin. This permitted accurate positioning of the volunteer between images, and also acted as an abdominal reference marker.

After oral administration of the appropriate drug solution, the volunteer was positioned upright standing in front of the detector head facing the gamma camera for the anterior image. The posterior image was taken in the same manner immediately in connection with the anterior image, which required the volunteer to turn by 180 degrees. This was repeated at 5-10 minutes intervals until the liquid had emptied from the stomach. Image acquisition time was 30 s. In between image acquisition, the volunteer was free to move away from the camera, but was requested to remain in an upright position. Four hours post dosing the volunteers consumed a standard lunch and water and orange juice was available ad libitum. Imaging was continued less frequently after gastric emptying at intervals of about 20 minutes duration until the liquid had arrived at the colon.

In addition to imaging, 5 mL blood samples were collected throughout the course of the study at the following times: 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 hours. The blood samples were transferred into heparin vials (LH PST™, BD Vacutainers, U.K.) and centrifuged at 5 000 rpm for 5 min (GS-6R Centrifuge, Beckman, U.K.). The separated plasma was transferred into plasma vials and immediately stored at –20° C until further analysis.

5.3.2.3. Analysis of scintigraphic data

On completion of each scintigraphic study, processing of image data was performed using customized computer software (MicasV Nucmed™ software, Park Medical Systems, Farnborough, U.K.). Regions of interest (ROI) of the stomach and the colon were drawn around computer-generated images of the anterior and posterior scans for each volunteer. After correcting for movement of the volunteers the counts of

radioactivity in each ROI was calculated at each time point and corrected for background radiation. From these net counts the geometric mean was calculated to account for the differential attenuation of the radiation with varying depth of source. Subsequently, the geometric mean counts were corrected for physical decay of ^{99m}Tc. Finally, the corrected geometric mean counts for the regions of interest were expressed as percentages of the total counts recorded initially, when all the administered activity was in the stomach and terminally, when all the activity was in the caecum/colon. The time course of gastric emptying and colon arrival was then estimated from the plot of percentage activity in these regions versus time.

The gastrointestinal transit data were quantitatively assessed via two different approaches. The method of statistical moments was used to calculate the mean gastric residence time (MGRT) and mean caecum/colon arrival time (MCAT) of the drug solutions (Podczeck et al., 1995). The second approach was the determination of t_{50} values, which describe the time, when 50 % of the preparations have emptied from the stomach or reached the caecum/colon. Consecutively, the measures for liquid transit through the small intestine were calculated as the difference between the MGRT and MCAT to obtain the mean small intestinal transit time (MSITT) and as the difference between the t_{50} values for gastric emptying and colon arrival to obtain the t_{50} small intestinal transit time.

5.3.2.4. Analysis of plasma samples

The plasma samples were analysed for ampicillin and ranitidine content via HPLC after undergoing solid-phase extraction using a SPE vacuum manifold (Phenomenex, Cheshire, U.K.). The samples were thawed at room temperature, thoroughly vortex mixed and centrifuged at 10 000 rpm for 5 min (Micromax 230, IEC, MA, U.S.A.). Consecutively, 1.0 mL of each sample was mixed with 1.0 mL of water and loaded onto a Strata-X solid phase extraction tube (30 mg/mL, Phenomenex, Cheshire, U.K.), which had been equilibrated with acetonitrile and water. The SPE tubes were washed with 1 mL 1 % (v/v) methanol (HPLC-grade, Sigma, U.K.) in water to eliminate plasma proteins. Ampicillin and ranitidine were eluted with 1.0 mL 90 % (v/v) acetonitrile in water and after thorough vortex mixing transferred into HPLC vials using 250 µL inserts.

The samples were assayed for ampicillin and ranitidine content using the following HPLC-method. The HPLC analysis utilized a Hewlett Packard Series 1100 chromatography system with a CTC PAL autosampler. Aliquots of sample (200 µL) were injected on a 50-mm x 2.1-mm Luna® column (5 µm C-18; Phenomenex, Cheshire, U.K.). A binary solvent gradient system was employed using an aqueous and an organic solvent, which were 0.1 % (v/v) trifluoro acetic acid in water (solvent A) and 0.1 % (v/v) trifluoro acetic acid in 90 % (v/v) acetonitrile in water (solvent B), respectively. The flow rate of the mobile phase was set to 1.2 mL/min. The column was initially equilibrated with 100 % solvent A. Immediately after sample injection, the concentration of B was linearly increased over 12 min to a concentration of 60 % and then exponentially reduced to the initial concentration of 0 % in the next 3 min before the next sample injection. Calibration curves of ampicillin and ranitidine in plain plasma were determined in the concentration range of 0.125 to 4 µg/mL. The retention time of ampicillin and ranitidine were obtained to be 6.5 and 8.1 min, respectively.

5.3.2.5. Statistical analysis

A paired Student's t-test was performed on the scintigraphic and pharmacokinetic data to assess the effects of the different solubilizing agents on gastrointestinal transit and drug absorption.

5.4. Results and discussion

5.4.1. Gastrointestinal transit

In Figures 5.2 to 5.8 the profiles of gastrointestinal transit are displayed for each individual volunteer. The numerical values for the gastric emptying, colon arrival and small intestinal transit times are listed for each volunteer in Tables 5.1, 5.3 and 5.5, respectively, having been calculated by the method of moments theory. In Tables 5.2, 5.4 and 5.6 the corresponding t_{50} values are given, respectively, to complete the set of data but these results will not be discussed in detail.

Figure 5.2. Gastrointestinal transit profiles for volunteer 1.

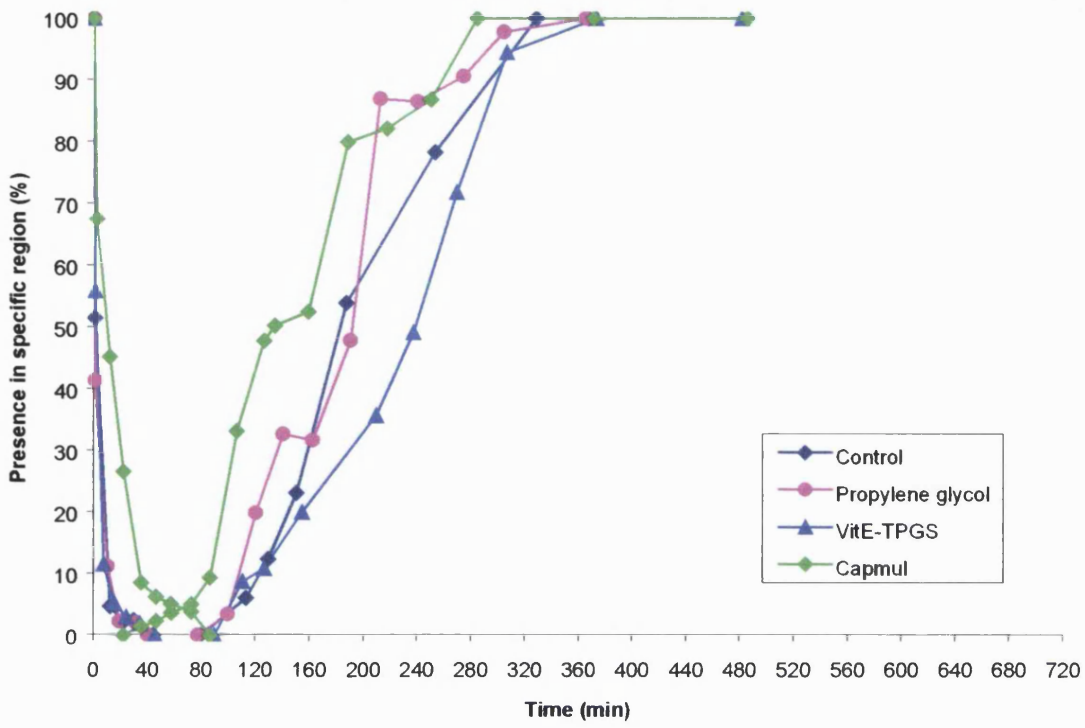


Figure 5.3. Gastrointestinal transit profiles for volunteer 2.

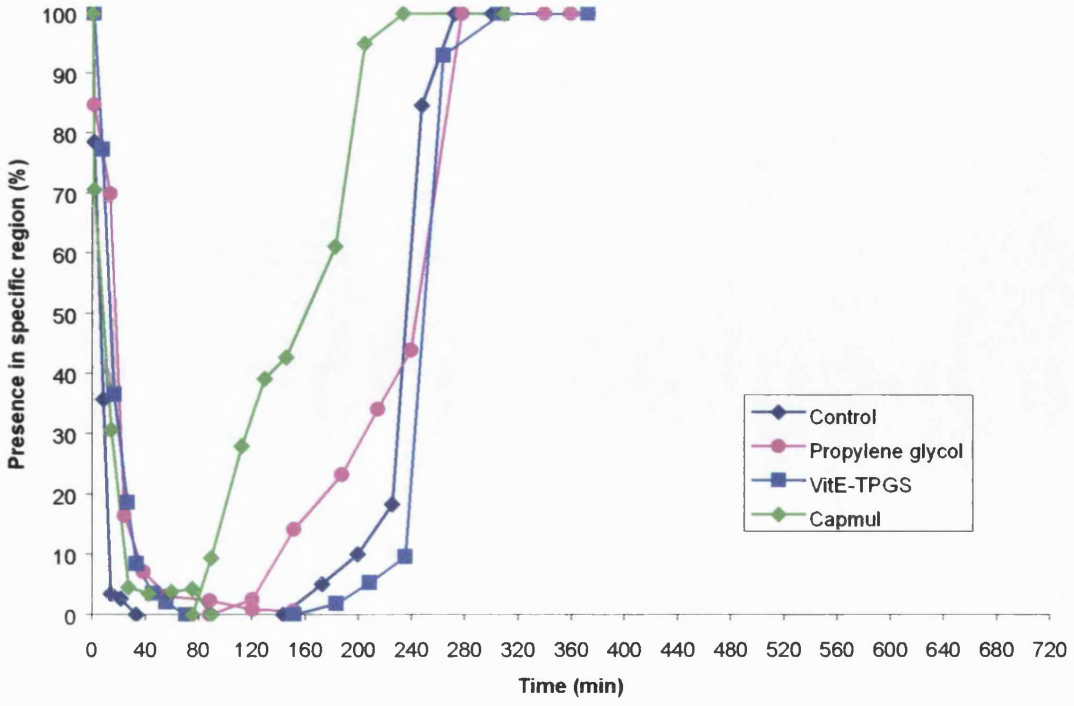


Figure 5.4. Gastrointestinal transit profiles for volunteer 3.

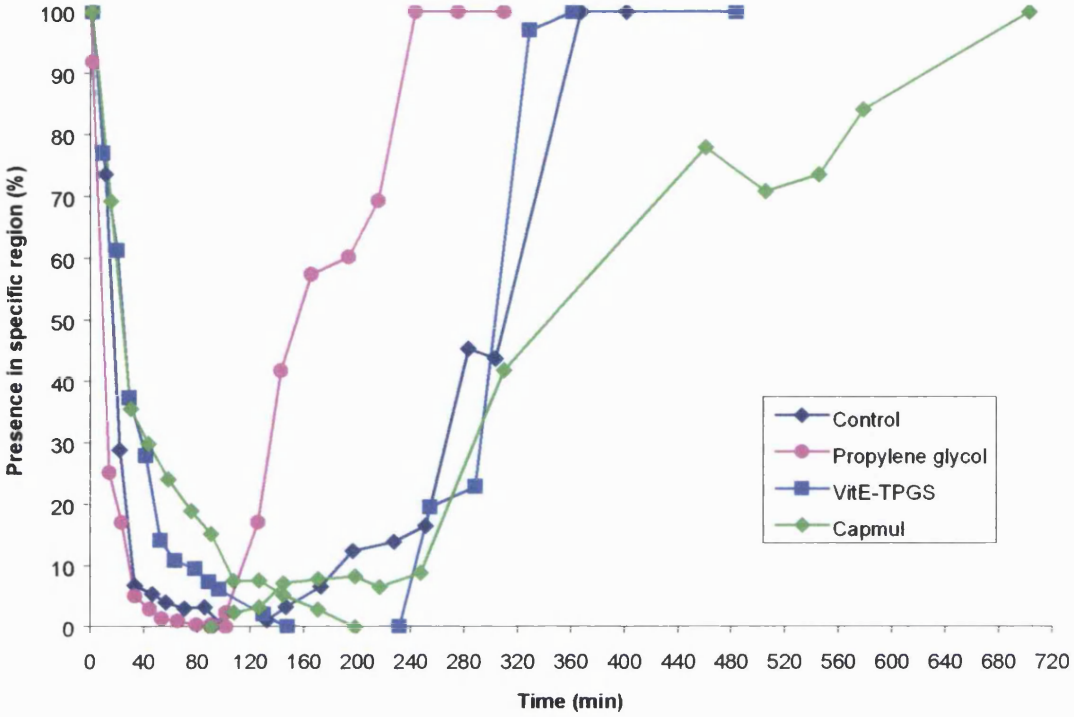


Figure 5.5. Gastrointestinal transit profiles for volunteer 4.

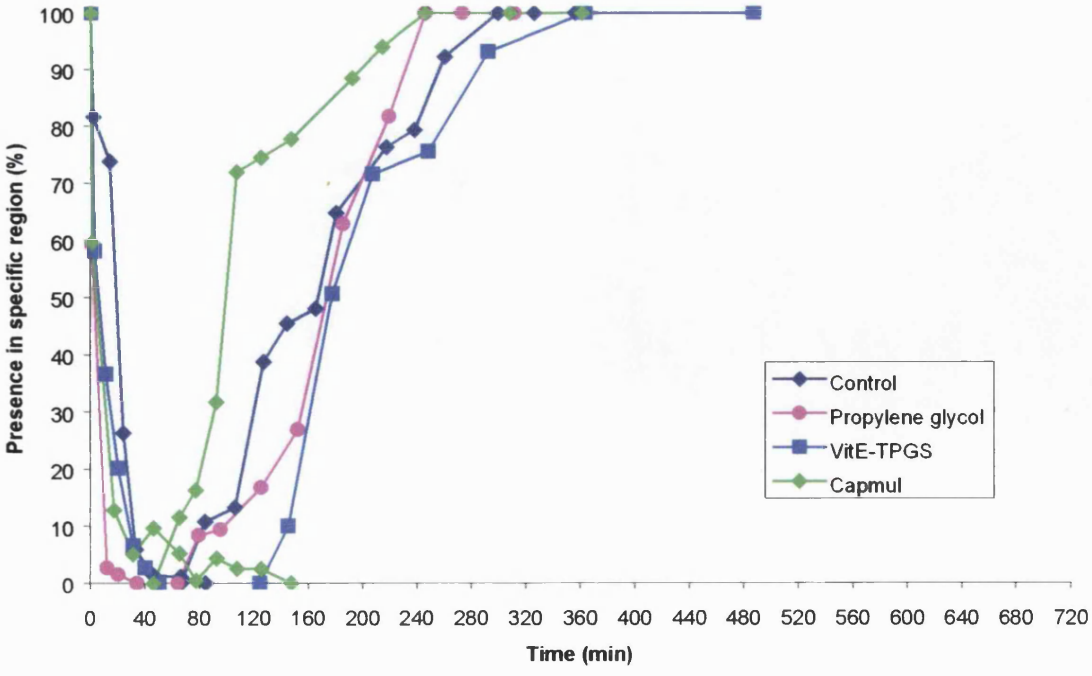


Figure 5.6. Gastrointestinal transit profiles for volunteer 5.

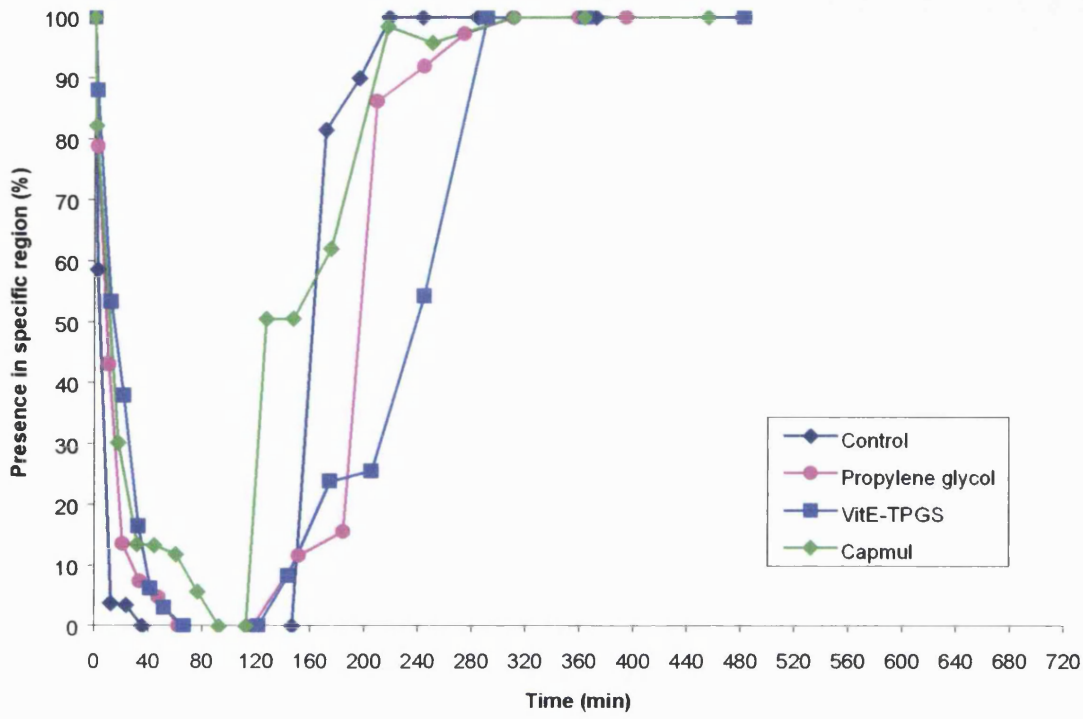


Figure 5.7. Gastrointestinal transit profiles for volunteer 6.

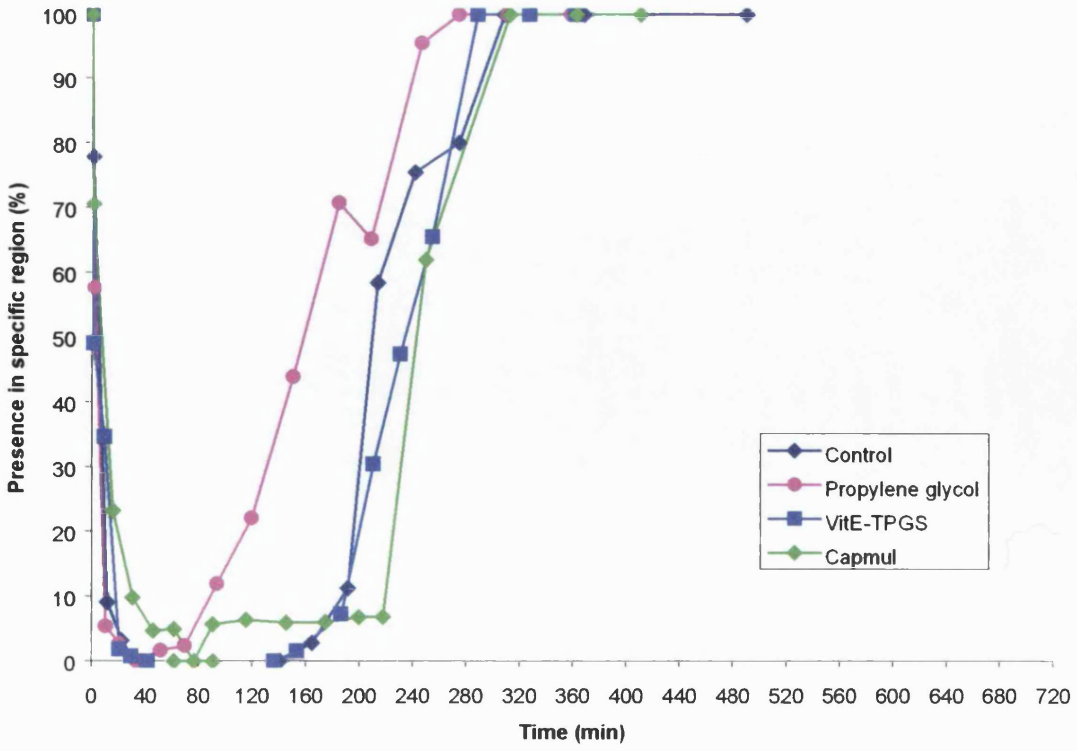


Figure 5.8. Gastrointestinal transit profiles for volunteer 7.

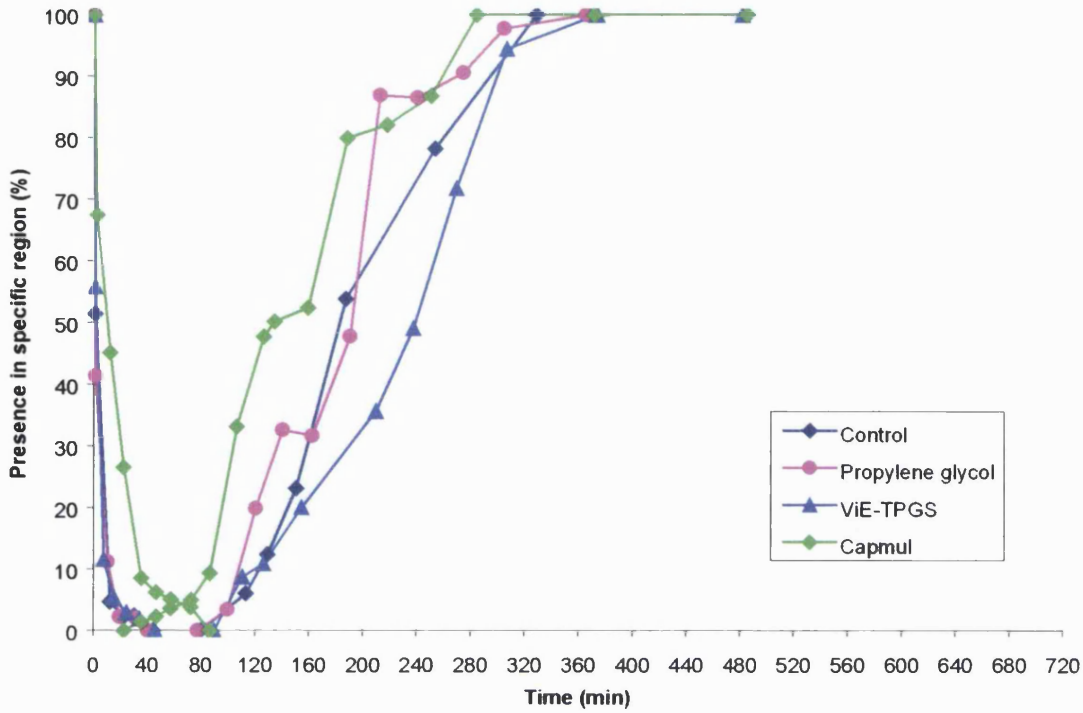


Table 5.1. Gastric emptying times calculated using statistical moment theory and represented as MGRT (min) and *VGRT* (min^2).

Treatment	Volunteer 1	2	3	4	5	6	7	Mean ± S.D.	*P* value
Control	23 *290*	7 *7*	18 *103*	17 *63*	2 *0*	9 *14*	1 *0*	11 ± 8	
Propylene glycol	11 *16*	18 *80*	16 *57*	1 *0*	14 *86*	7 *15*	8 *14*	11 ± 6	*0.947*
VitE-TPGS	7 *11*	15 *78*	31 *537*	14 *82*	18 *111*	8 *10*	7 *20*	14 ± 9	*0.456*
Capmul® MCM	18 *99*	13 *17*	48 *1293*	30 *375*	30 *408*	18 *67*	21 *194*	25 ± 12	*0.022*

Table 5.2. Gastric emptying times represented as t_{50} values (min).

Treatment	Volunteer 1	2	3	4	5	6	7	Mean ± S.D.	*P* value
Control	14	6	17	19	4	5	1	9 ± 7	
Propylene glycol	7	17	9	3	8	3	1	7 ± 5	*0.468*
VitE-TPGS	5	13	24	6	14	1	2	9 ± 8	*0.967*
Capmul® MCM	18	8	24	4	11	7	10	12 ± 7	*0.482*

Table 5.3. Caecum arrival times calculated using statistical moment theory and represented as MCAT (min) and *VCAT* (min^2).

Treatment	Volunteer 1	2	3	4	5	6	7	Mean ± S.D.	*P* value
Control	279 *139*	239 *257*	299 *2244*	207 *1950*	187 *197*	256 *878*	245 *2207*	245 ± 39	
Propylene glycol	270 *141*	228 *1047*	190 *821*	194 *1238*	227 *535*	187 *1544*	225 *2011*	217 ± 30	*0.183*
VitE-TPGS	249 *282*	251 *189*	304 *163*	244 *1604*	238 *1084*	247 *578*	251 *2249*	255 ± 22	*0.355*
Capmul® MCM	223 *474*	173 *950*	453 *9105*	168 *1609*	173 *556*	258 *1804*	194 *2343*	235 ± 102	*0.740*

Table 5.4. Caecum arrival times represented as t_{50} values (min).

Treatment	Volunteer 1	2	3	4	5	6	7	Mean ± S.D.	*P* value
Control	267	236	310	167	162	209	182	219 ± 55	
Propylene glycol	266	243	154	173	196	158	191	197 ± 43	*0.407*
VitE-TPGS	250	249	303	176	238	233	238	241 ± 37	*0.132*
Capmul® MCM	190	160	343	99	127	242	133	185 ± 84	*0.110*

Table 5.5. Small intestinal transit times calculated using statistical moment theory and represented as the difference between MGRT and MCAT (min).

Treatment	Volunteer							Mean ± S.D.	*P* value
	1	2	3	4	5	6	7		
Control	256	232	281	190	185	247	244	234 ± 35	
Propylene glycol	259	210	174	193	213	180	217	207 ± 28	*0.174*
VitE-TPGS	242	236	273	230	220	239	244	241 ± 16	*0.426*
Capmul® MCM	205	160	405	138	143	240	173	209 ± 94	*0.385*

Table 5.6. Small intestinal transit times represented as the difference in gastric emptying and caecum arrival t_{50} values (min).

Treatment	Volunteer							Mean ± S.D.	*P* value
	1	2	3	4	5	6	7		
Control	253	230	293	148	158	204	181	210 ± 53	
Propylene glycol	259	226	145	170	188	155	190	190 ± 40	*0.447*
VitE-TPGS	245	236	279	170	224	232	236	232 ± 32	*0.101*
Capmul® MCM	190	152	319	95	116	235	123	173 ± 79	*0.083*

5.4.1.1. Gastric emptying

The mean gastric emptying time for the **control** formulation was 11 min on average and the individual values ranged from 1 to 23 min (Table 5.1). This is in good agreement with the findings from our previous transit study in healthy volunteers, where an average emptying time of 12 min was obtained on the control day (Chapter 2). Here, the control treatment also consisted of 150 mL of water with the model compound in solution. Comparing the transit profiles of the individual volunteers (Figures 5.2 to 5.8) emptying of the solutions from the stomach was observed to be rapid and followed an exponential pattern – a phenomenon that has been commonly described for orally administered liquids. As explained earlier the gastric emptying pattern is dependent on the amount of fluid administered (Gupta and Robinson, 1988). The ingestion of a fluid volume of 100 mL or more is likely to cause an interruption of the gastric motility present in the fasted stomach resulting in the exponential emptying pattern of the solution.

In the presence of **propylene glycol** the mean gastric emptying time (MGRT) was 11 min with a range of 1 to 18 min meaning that there was no difference compared to the control formulation. In the *in vivo* study conducted earlier in beagle dogs, however, propylene glycol appeared to have a propagating effect on stomach emptying since the gastric residence time was determined to be significantly shorter (13 %) than that of the control (Chapter 4). In the present study the amount cosolvent administered was even higher with 5 g compared to 2 g in dogs. For the observed differences several reasons may be possible. It was suggested that the accelerated emptying time in the dog occurred as the result of an irritation of the gastric mucosa by local concentration of highly osmotic propylene glycol (3784 mOsm kg^{-1}) upon disintegration of the ingested hard gelatin capsules causing a toxic reaction of the stomach. The study in humans, for instance, employed the dose of propylene glycol dissolved in 50 mL of water taken with an extra amount of 100 mL water, which means the osmolality of the preparation was less concentrated (512 mOsm kg^{-1}). In general, hypertonic solutions have been found to empty more slowly from the stomach (Hunt et al., 1951; Hunt, 1963) since the body will first try to compensate for deviations from isotonicity before releasing the contents into the small intestine. Another aspect worth considering is the amount of fluid administered in both studies. With only 50 mL given to the dogs, the fluid volume was thought to be too low to

cause an interruption of the fasted motility pattern. An interruption of this motility pattern, however, might have been initiated by a toxic reaction of the stomach to the release of concentrated propylene glycol resulting in an exponential gastric emptying. In humans such an effect of propylene glycol would have gone unnoticed since an interruption of the fasted pattern was already given by the ingestion of a larger fluid volume. As a consequence of the differences in the administration of fluid volumes in both studies, a comparison of the effects of the solubilizing agents on gastric emptying is difficult and might lead to erroneous conclusions. However, in humans it was shown that propylene glycol does not affect the residence time of oral solutions in the stomach compared to the control formulation when administered at an amount of 5 g.

Upon ingestion, the formulation containing D-α-tocopheryl polyethylene glycol 1000 succinate (**VitE-TPGS**) is expected to distribute evenly in the stomach and spread across the gastric wall due to the surface-active properties of the excipient. The emptying from the stomach was observed to follow an exponential pattern similar to that of the control preparation as can be seen in the graphic transit profiles (Fig.5.2 to 5.8). One exception of the rapid exponential emptying was found in volunteer 3, who gave a visibly delayed release of the formulation into the small intestine following a similar pattern to the one in the presence of Capmul® MCM. In general, however, the treatments containing 5 g VitE-TPGS were found to have an average MGRT of 14 min ranging from 7 to 31 min. The slight increase of 3 min compared to the mean MGRT of the control formulation meaning a 27 % longer gastric residence time was, however, not significant ($P > 0.05$). The results correlate well with the findings in dogs where the treatment containing 1 g VitE-TPGS also gave a 27 % longer gastric emptying time (38 min compared to 30 min) of no statistical significance. The observed increase of 3 min is thought to have been the result of inter- and intra-individual variations rather than being a trend to a prolonging effect of the surfactant towards longer MGRTs. It is assumed that VitE-TPGS does not influence the residence time of oral solutions in the stomach to any extent, even when administered at amounts as high as 5 g.

In the presence of **Capmul® MCM** the oral solution was found to give an average MGRT of 25 min ranging from 13 to 48 min. In comparison to the control

formulation this means a significant increase in the gastric emptying time of 127 % ($P < 0.05$). In contrast, the approach determining the average half emptying time from the stomach (t_{50}) gives a markedly different result as the delay in emptying is only 33 % compared to the control (12 and 9 min, respectively) and the effect is not significant. Taking into account the graphic transit profiles of the volunteers (Figures 5.2 to 5.8) the reason for the differences in the results of the two evaluation methods becomes apparent stating an example of the greater accuracy of the approach using the method of moments theory. In nearly every individual the gastric emptying pattern for the formulation containing Capmul® MCM appears to follow an exponential order similar to the other treatments at the beginning until most of the solution has entered the small intestine. Then the pattern seems to change into a more linear order delaying the completion of gastric emptying considerably. In many cases this delay was observed to last until some of the preparation entered the colon. The method determining t_{50} values, however, will not take this kind of emptying pattern into consideration but will determine the gastric emptying time at 50 %, which is in the exponential part of the curve. An explanation for this specific emptying pattern in the presence of Capmul® MCM could be that upon release of the surfactant into the small intestine digestion of the glycerides commences freeing some of the medium chain fatty acids causing a delayed emptying from the stomach. This delaying phenomenon has been shown for orally administered nutrient solutions containing fatty acids and an approximately linear emptying pattern was observed (Rees et al., 1979; Brener et al., 1983) Another factor may be the administration of the Capmul® MCM treatment in form of hard gelatin capsules, while the model compounds were given separately in 50 mL of water containing most of the radiolabel ^{99m}Tc-DTPA. After disintegration of the capsules Capmul® MCM is expected not to mix easily with aqueous fluids in the stomach, as it is lipophilic in structure comprising of an HLB value of 5 and of viscous consistency. It requires the presence of auxiliary surfactants, which are more hydrophilic in nature to form an emulsion inside the GI lumen (Shah et al., 1994). The emulsifying process is likely to occur once Capmul® MCM has emptied from the stomach forming mixed micelles with bile salts present in the small intestine. The initial exponential emptying pattern observed with the treatment could therefore be the result of emptying of the coadministered aqueous drug solutions, which have a larger volume and empty more rapidly, whereas the viscous lipophilic Capmul® MCM is retained in the stomach for longer and released slowly in a more

linear emptying pattern. This explanation also correlates well with the findings in dogs, where such a significant delay in gastric emptying was not observed with the medium chain glyceride-mixture Labrasol®. Here, the Labrasol® preparation was prolonged the mean MGRT by only 10 % compared to the control (33 min and 30 min, respectively), which was not significant ($P > 0.05$). In the canine study, less fluid was administered and the drug solution was given as a mixture with the surfactant filled in capsules. As a result the emptying pattern was not interrupted and more time was given for the capsules to disintegrate and mix with the gastric fluids. However, the amount of Labrasol® administered was lower with only 2 g, which might not have been high enough to release an efficient amount of fatty acids to have a effect on gastric emptying. In case that digestion of the glyceride surfactant plays an important role in delaying emptying from the stomach, differences between the species with respect to fat digestion may also need to be considered. The results of the present study nevertheless suggest that a 5 g dose of Capmul® MCM delays gastric emptying of oral solutions by more than 2-fold in humans.

5.4.1.2. Colon arrival and small intestinal transit

Comparing the graphic GI transit profiles of all volunteers regarding colon arrival of the various treatments it appears that colonic arrival is quantitatively completed after 360 min for all preparations. One exception is volunteer 3, who exhibited a considerably slow transit in the presence of Capmul® MCM and colon arrival was not quantitative until over 10 hours. On average, arrival of the formulations in the colon was 245, 217, 255 and 235 min for the control, propylene glycol, VitE-TPGS and Capmul® MCM treatments, respectively using methods of moments (Table 5.3). The differences in the MCAT values were found to be of no statistical significance ($P > 0.05$). Trends and changes as a consequence of solubilizer present in the gastrointestinal tract are discussed with the results of the small intestinal transit times as the MCAT values are directly linked to the SITTs.

Table 5.5 lists the results obtained for the small intestinal transit times calculated using statistical moments as the difference between the MGRT and MCAT. The **control** formulation was found to have an average small intestinal passage time of 234 min in a range of 185 to 281 min. In our earlier scintigraphic study investigating polyethylene glycol 400 (PEG 400) in healthy male volunteers an average value of

298 min was determined for the control solution (Chapter 2). In general, the small intestinal transit time has been reported to be constant between 3 to 5 hours (Davis et al., 1986a; Hardy et al., 1985b) and the present results agree well with these findings.

The presence of the various solubilizers was found to have no statistically significant effect on the passage of the oral solutions through the small intestine ($P > 0.05$). Some variations between the different treatments, however, were observed and the specific trends will be discussed in the following for each excipient investigated and compared with the findings of the previous study in beagle dogs (Chapter 4).

For the formulation containing 5 g **propylene glycol** a mean MSITT of 207 min was observed with the individual values ranging from 174 to 259 min. Compared to the control treatment this means a slight average reduction in the MSITT albeit not significant. Following the trend observed in the previous canine study in the presence of 2 g propylene glycol where the average MSITT was found to be slightly delayed compared to the control formulation, a prolongation rather than an acceleration would have been expected. An accelerated transit was observed in four of the volunteers (subjects 2, 3, 6 and 7), whereas others did not show any effect (subjects 1 and 4) or gave a considerably prolonged transit (subject 5). The propylene glycol treatment administered had an osmolality of 512 mOsm kg^{-1}, which was the highest of all treatments employed in the present study. Upon oral ingestion the body will try to compensate for the hyperosmolar gastric load with an increased secretion of water, which continues to occur in the small intestine once the contents have been released from the stomach. In the small intestine the low molecular weight alcohol propylene glycol is rapidly absorbed and the extraction of the cosolvent from the luminal fluids is expected to be enhanced as an additional means to reduce the osmotic pressure inside the lumen. The observed acceleration of MSITT might be explained with the initially marked increase in luminal fluid volume promoting GI motor activity, until the absorption process of both water and propylene glycol commences. However, comparing the present results with those obtained in beagle dogs with 2 g propylene glycol, which gave a slight prolonging effect on small intestinal transit it appears that trends obtained from statistically insignificant results may mislead the reader into drawing erroneous conclusions. Although the dose of excipient chosen in the present study was increased from 2 g in dogs to 5 g in humans, no firm conclusions can be

drawn about a likely propylene glycol effect on small intestinal transit. Furthermore, an extrapolation of the observed effects from 5 g to 33 g, which are administered in a single dose of Agenerase® oral solution, is even more difficult.

In the presence of 5 g **VitE-TPGS** the oral liquid was determined to have an average MSITT of 241 min with a range of 220 to 273 min. Compared to the control formulation this means that the passage through the small intestine was on average 3 % slower for the VitE-TPGS preparation. In beagle dogs, on the other hand, the preparation containing 1 g VitE-TPGS showed a slight acceleration of the small intestinal transit time of 7 % compared to the control. Besides the observed trends going in opposite directions the results of both studies proved to be of no statistical significance ($P > 0.05$). It is, therefore, believed that the presence of VitE-TPGS does not impact on the passage of oral liquids through the small intestine. With respect to the MSITTs of the individual subjects deviations from the transit times of the control treatment were negligible for most of the volunteers with the exception of subjects 4 and 5, who exhibited a respectively 40 and 35 min longer transit in the presence of VitE-TPGS (Table 5.5). The fate of the surfactant inside the intestinal lumen is still not fully understood. After the administration of 5 g VitE-TPGS as a 10 % (w/w) oral solution the luminal concentration of the surfactant is expected to be well above the CMC (0.02 % (w/w)), which means that micelles are formed by the amphiphilic molecules. The dispersed micelles are able to easily pass through the unstirred water layer on the surface of the enterocytes. Findings by Traber et al. (1988) from *in vitro* cell culture studies described the intact uptake of the entire VitE-TPGS molecule across the membrane of the enterocytes. Inside the cytosol of the absorptive cells hydrolysis of the ester bond will take place and free tocopherol will be absorbed into the systemic circulation. As a result of the PEG 1000 content of VitE-TPGS the surfactant was initially suspected to exert an osmotic effect on GI transit in a similar way as PEG 400. With the present administration of a 5 g dose of the surfactant the portion of PEG 1000 would be 3 g. Although 3 g of PEG 1000 comprise of a lower osmotic pressure than the same amount of PEG 400 as a result of the higher molecular weight of the polymer, a transit effect of PEG 1000 would be likely since the polymer is not as well absorbed as PEG 400 due to the overall longer chain length of the molecules. According to Traber et al. (1988), however, the entire VitE-TPGS molecule is absorbed across the intestinal mucosa, facilitated by its amphiphilic

nature, removing the PEG 1000 part of the molecule from the intestinal lumen in the process and circumventing any potential influence on small intestinal transit.

The oral liquid containing the surfactant **Capmul® MCM** was observed to have an average small intestinal transit time of 209 min with a range from 138 to 405 min giving a slight reduction of the mean MSITT value in comparison to the control formulation. As observed with the other treatments this effect appeared to be of no statistical significance ($P > 0.05$). The trend, however, was surprising since the presence of medium chain glycerides was expected to have a rather delaying impact on small intestinal transit times via initiation of the ileal brake response in the gastrointestinal tract. Similar results were observed in dogs with the preparation containing 2 g of the medium chain glycerides Labrasol®. Although also no statistical significance was obtained a slight decrease in the average MSITT was found compared to the control. In the present study in humans, almost every individual subject showed a considerable reduction in the MSITT (58 ± 13 min in volunteer 1, 2, 4, 5 and 7), whereas one subject showed a negligible reduction of 7 min (volunteer 6) and volunteer 3 gave a surprisingly high increase in the MSITT of more than 2 hours. It appears as if subject 3 is exceptional and such an extreme difference in the results obtained for the respective individual may not have been caused by the treatment. As a consequence, the results reflected a considerably enhanced variability, which was found to be 3 times as high as with the control treatment (variability of 45 % and 15 %, respectively). However, similar observations have been reported in the presence of other agents, which were expected to retard small intestinal transit via an activation of the duodenal or ileal brake mechanism. Dobson et al. (2002) found considerable variation between volunteers with the administration of modified release tablets loaded with the proven ileal brake activators oleic acid and DMG-04, which is a proprietary mixture of monoglycerides. The small intestinal transit times determined for the treatments containing the brake activators were found to range from 50 % to 200 % of the transit time obtained for the control formulation. The authors attributed the unexpectedly shortened transit times, which occurred to a significant extent in the presence of the relatively high dose of administered oleic acid, to a physical irritation of the intestinal mucosa as a result of the large localized quantity of irritant fatty acid causing a purgative effect. Such an effect is also known to occur with triglycerides of ricinoleic acid (present in castor oil) forming by-products in the small intestine, which

potentially lead to an irritation of the intestinal mucosa and subsequent acceleration of bowel content movement (Davis et al., 1988). An irritation of the GI mucosa is possible to also have occurred in the present study with the administration of 5 g Capmul® MCM. Some of the volunteers reported a slightly burning sensation shortly after ingestion of the capsules, which might have been caused by an irritation of the stomach mucosa as a reaction to the high local concentration of the excipient. In this respect, a high variation between individuals can also be expected as a result of general differences in daily diet. Various ethnic groups such as Africans and Indians, for instance, use spices in cooking that are potential irritants of the GI mucosa. The GI tract of a volunteer regularly exposed to such irritants is likely to react differently in comparison to that of an individual used to a European diet.

5.4.2. Drug absorption

The effects of the various types of solubilizers on drug absorption were investigated with the administration of ampicillin and ranitidine. Both drugs are hydrophilic in nature providing them with a high solubility in aqueous media. In general, such drugs would not normally be formulated in an oral dosage form together with solubilizing agents since they already possess sufficient solubility in the physiological fluids. However, their employment in the present study was justified by two means. On one hand, their good aqueous solubility guaranteed that oral ingestion of the solubilized systems did not result in precipitation of the model compound upon dilution in the stomach, as might be the case with preparations of hydrophobic drugs. Therefore, alterations in the oral bioavailability of the model drugs could be more easily compared to changes in GI transit in the presence or absence of the pharmaceutical excipients under investigation. On the other hand, both drug compounds exhibit a relatively poor permeability of the intestinal mucosa, which makes them particularly dependent on sufficiently long residence times in the small intestine. Ampicillin absorption is carrier-mediated and as a result it is more or less limited to the upper small intestine while ranitidine is primarily paracellularly absorbed via tight junctions. Some of the solubilizers administered in the present study have been found to enhance the permeability of poorly absorbable drugs in various ways e.g. interactions with absorptive membrane, tight junctions or efflux transporters. The present drug absorption results will be compared and correlated to the GI transit of

the treatments and the effects of the administered excipients on the permeability of the two model compounds will be discussed in the following.

The individual and mean plasma concentration versus time profiles are given in Figures 5.9 to 5.16 for ampicillin and Figures 5.17 and 5.24 for ranitidine, respectively. The pharmacokinetic data calculated for ampicillin and ranitidine are presented in Tables 5.7 and 5.8, respectively. No intravenous data were collected in the present study, which means that no data are available on the absolute oral bioavailability of the oral preparations but relative bioavailability variations in the different treatments are assessable from comparison of the determined AUC values.

5.4.2.1. Control

For ampicillin the AUC was 10.93 (± 1.89) hr*μg/mL with the formulation administered on the control day. The mean maximum plasma level was 5.5 μg/mL ranging from 4.21 to 7.07 μg/mL, which was reached in average at 1.43 hours. Findings from Eshelman and Spyker (1978) reported similar pharmacokinetic results after the administration of 500 mg ampicillin to healthy volunteers. Ranitidine, on the other hand, follows a different absorption pattern. In the present study, the mean AUC was 2.90 (± 0.49) hr*μg/mL and maximum plasma concentration of 0.516 (± 0.130) μg/mL were reached after 4.29 hours, which is in agreement with pharmacokinetic data found by other authors (van Hecken et al., 1982; Shim and Hong, 1989). Comparing the plasma concentration time curves of ranitidine with those determined for ampicillin, it becomes apparent that ranitidine absorption follows a different pattern. Following oral administration ranitidine absorption is rapid showing an initial peak ranging from 0.239 to 0.506 μg/mL at 1.5 to 2.5 hours after dosing. In most cases, the initial peak is followed by a second one ranging from 0.393 to 0.694 μg/mL appearing around 4 hours after dose administration.

Figure 5.9. Plasma ampicillin concentration-time profiles for volunteer 1.

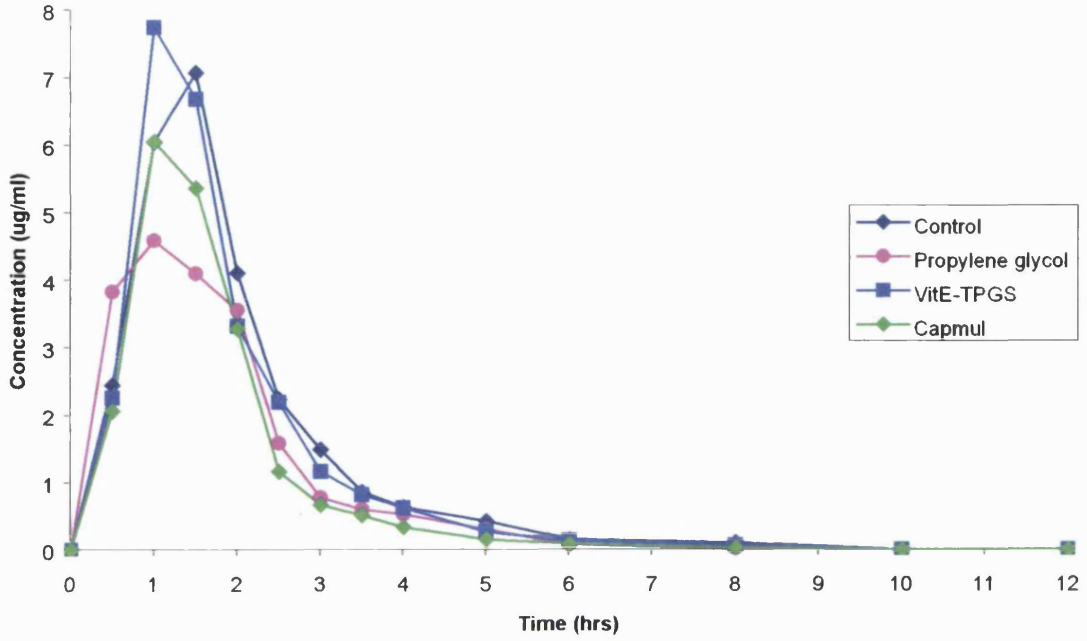


Figure 5.10. Plasma ampicillin concentration-time profiles for volunteer 2.

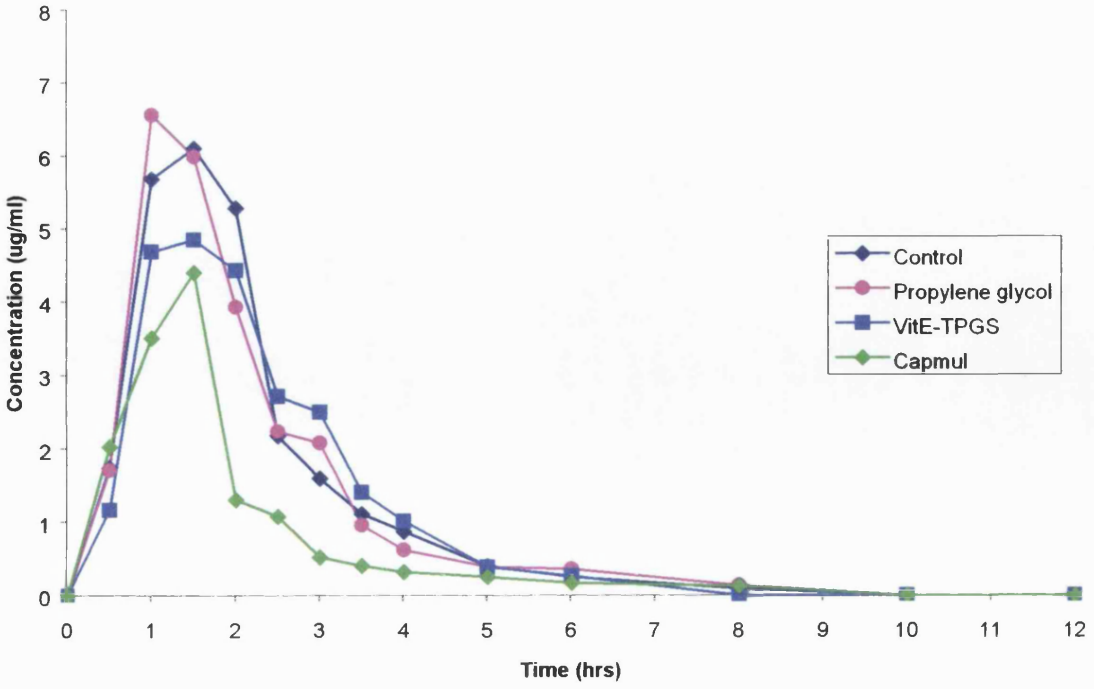


Figure 5.11. Plasma ampicillin concentration-time profiles for volunteer 3.

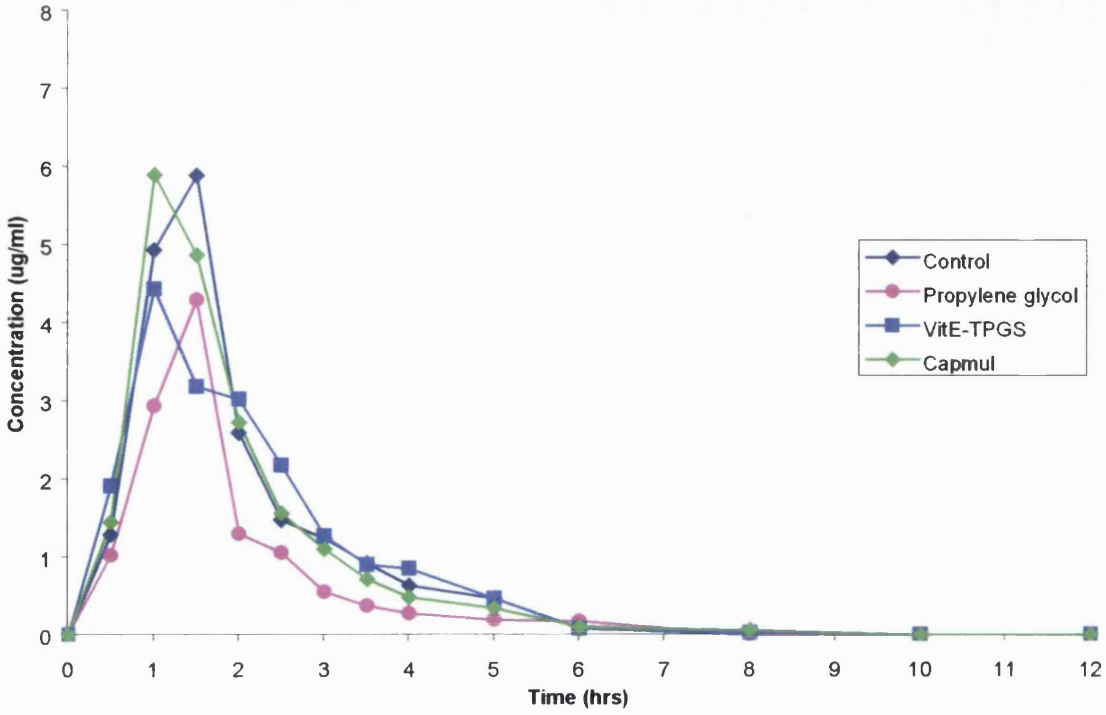


Figure 5.12. Plasma ampicillin concentration-time profiles for volunteer 4.

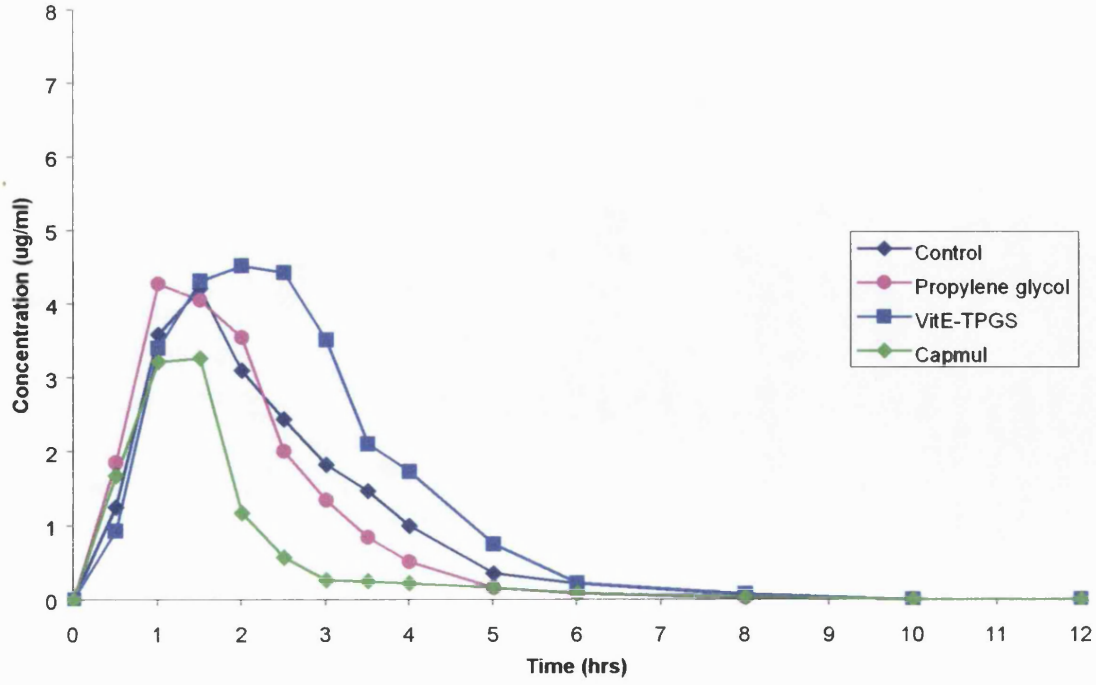


Figure 5.13. Plasma ampicillin concentration-time profiles for volunteer 5.

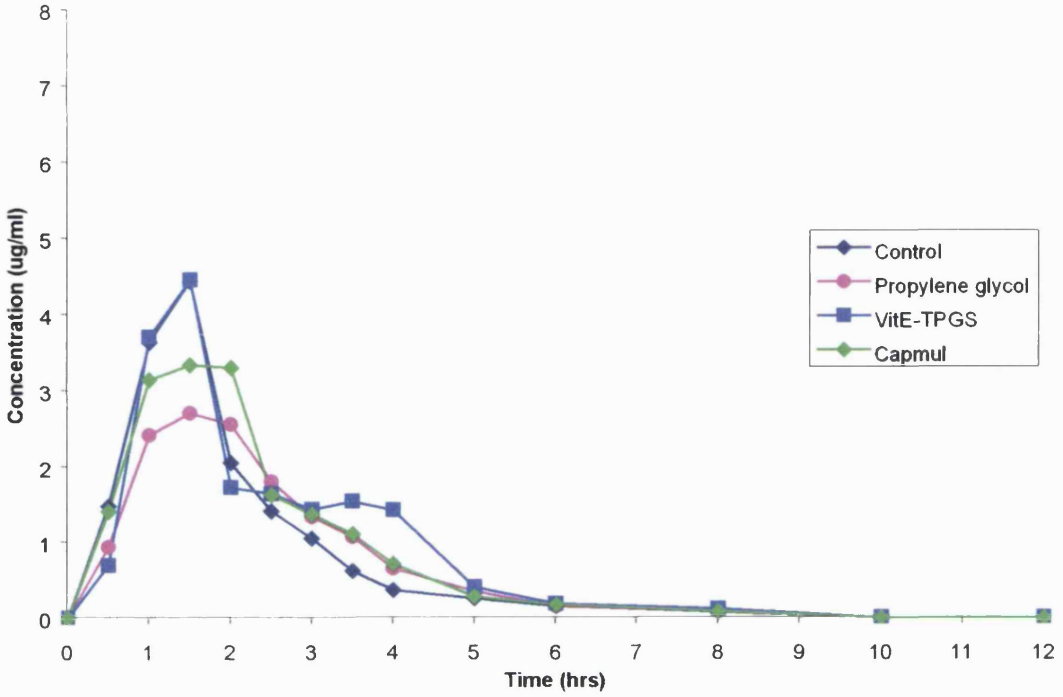


Figure 5.14. Plasma ampicillin concentration-time profiles for volunteer 6.

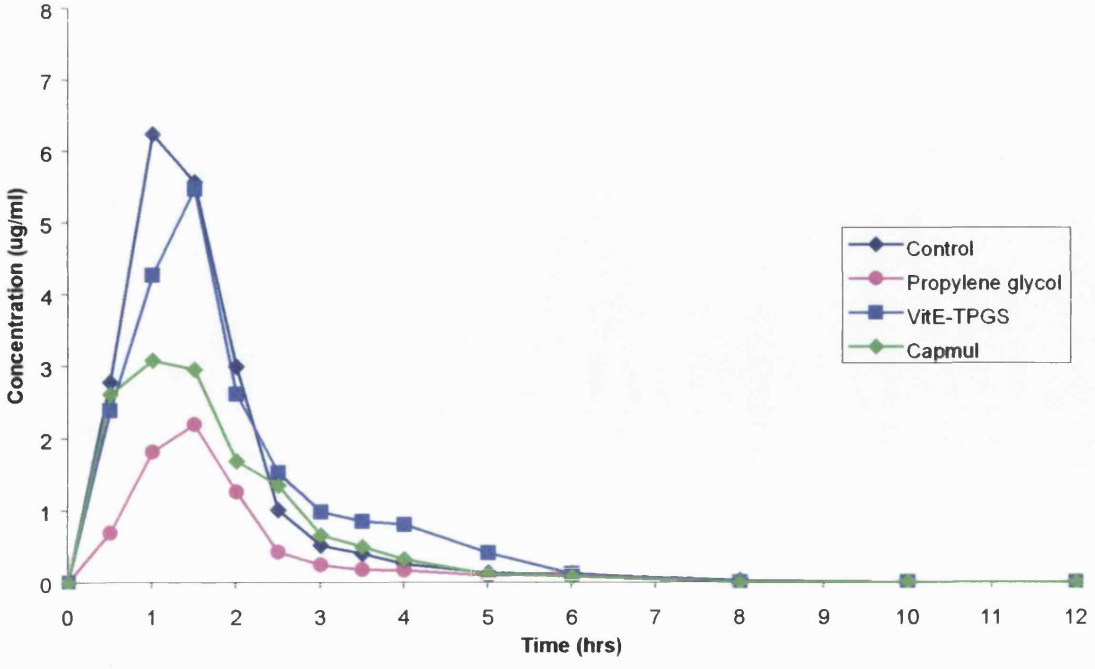


Figure 5.15. Plasma ampicillin concentration-time profiles for volunteer 7.

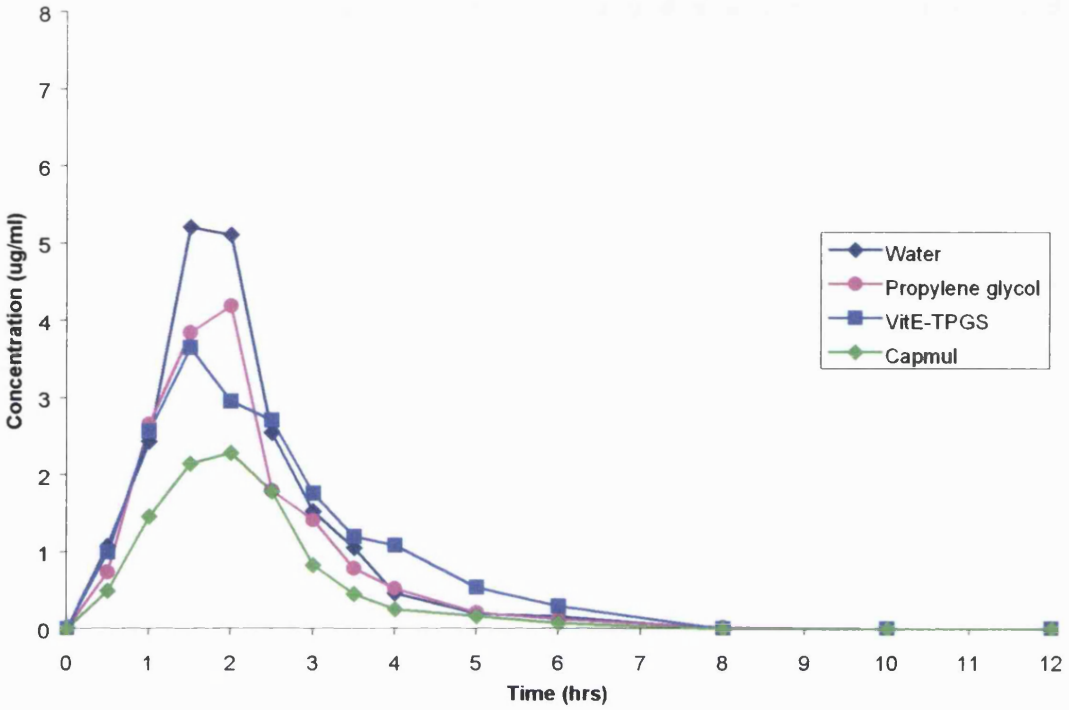


Figure 5.16. Mean (± S.E.) plasma ampicillin concentration-time profiles.

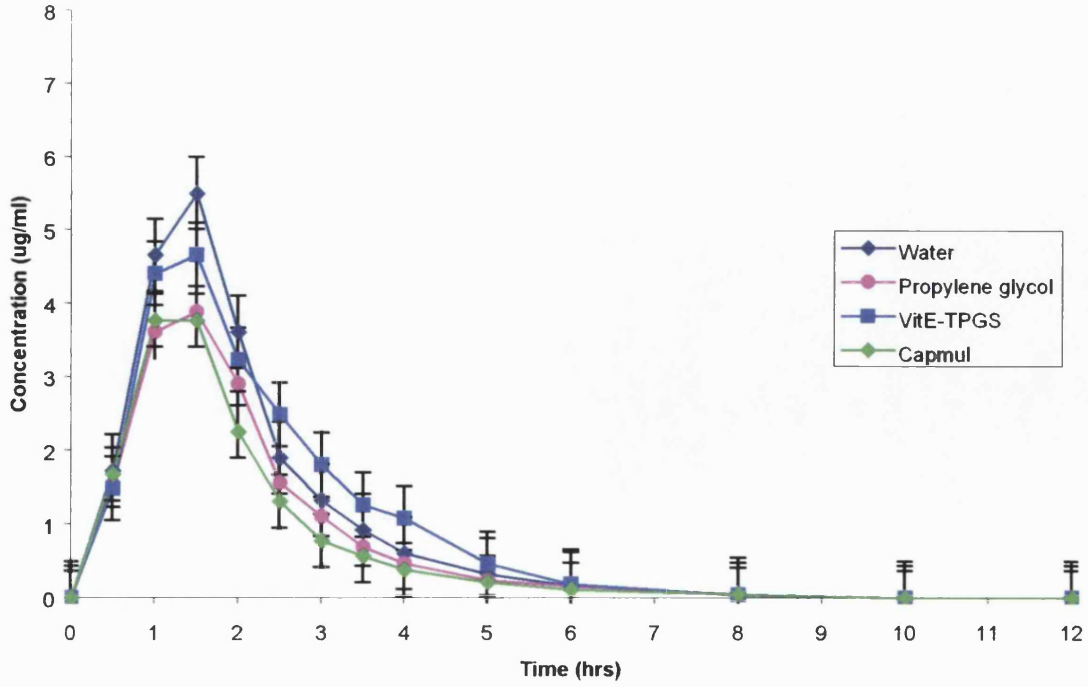


Figure 5.17. Plasma ranitidine concentration-time profiles for volunteer 1.

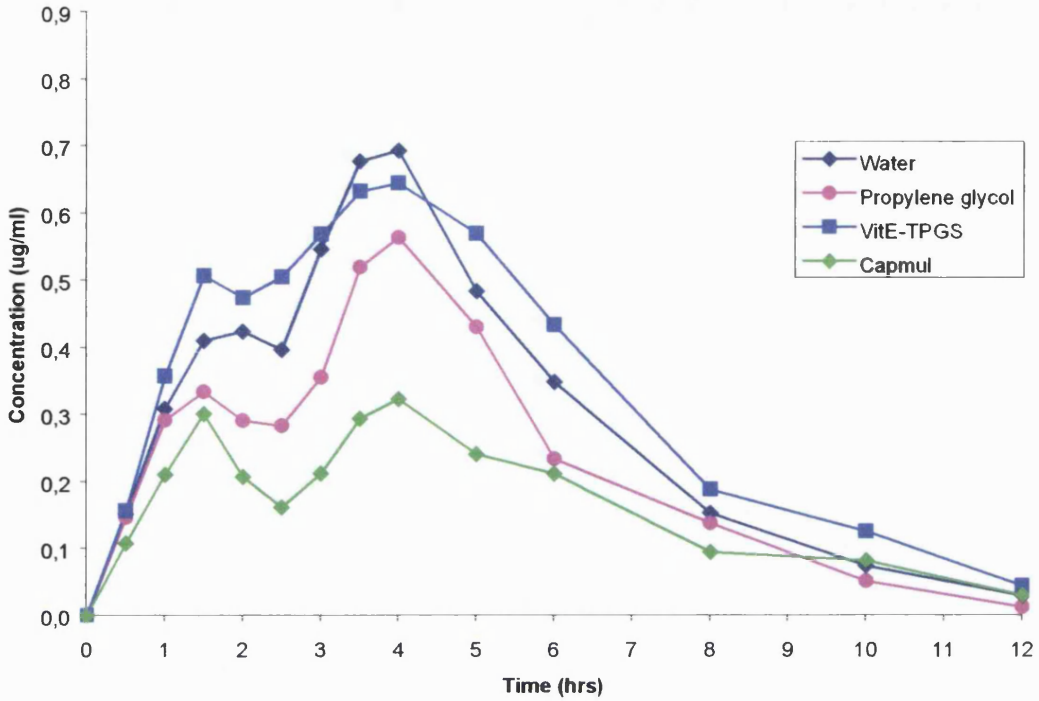


Figure 5.18. Plasma ranitidine concentration-time profiles for volunteer 2.

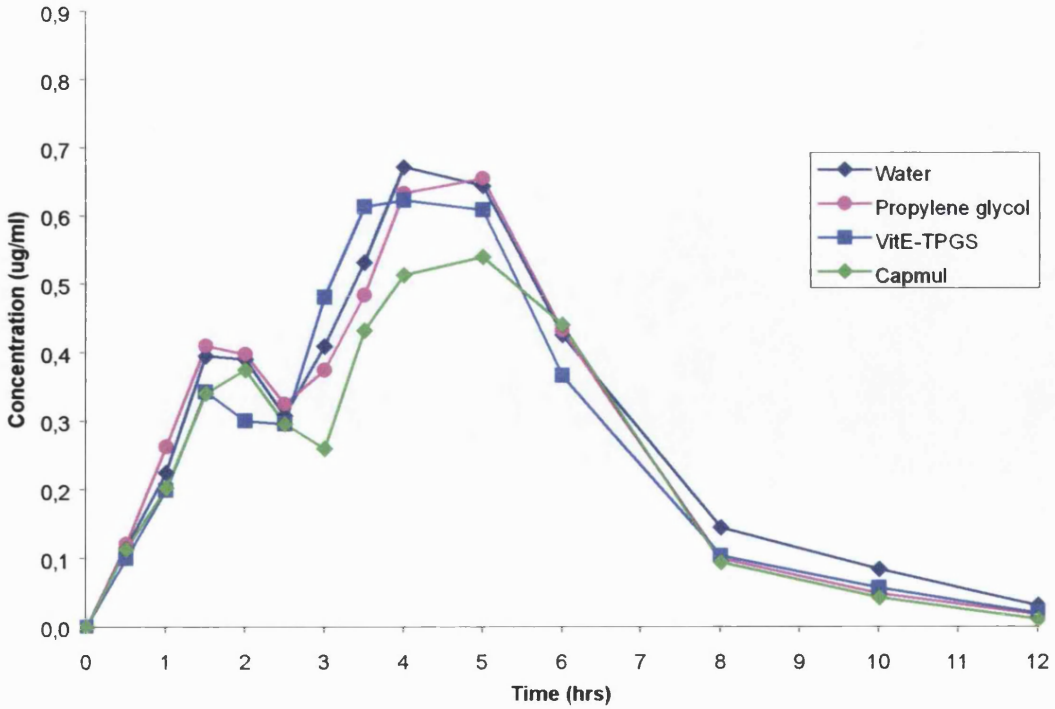


Figure 5.19. Plasma ranitidine concentration-time profiles for volunteer 3.

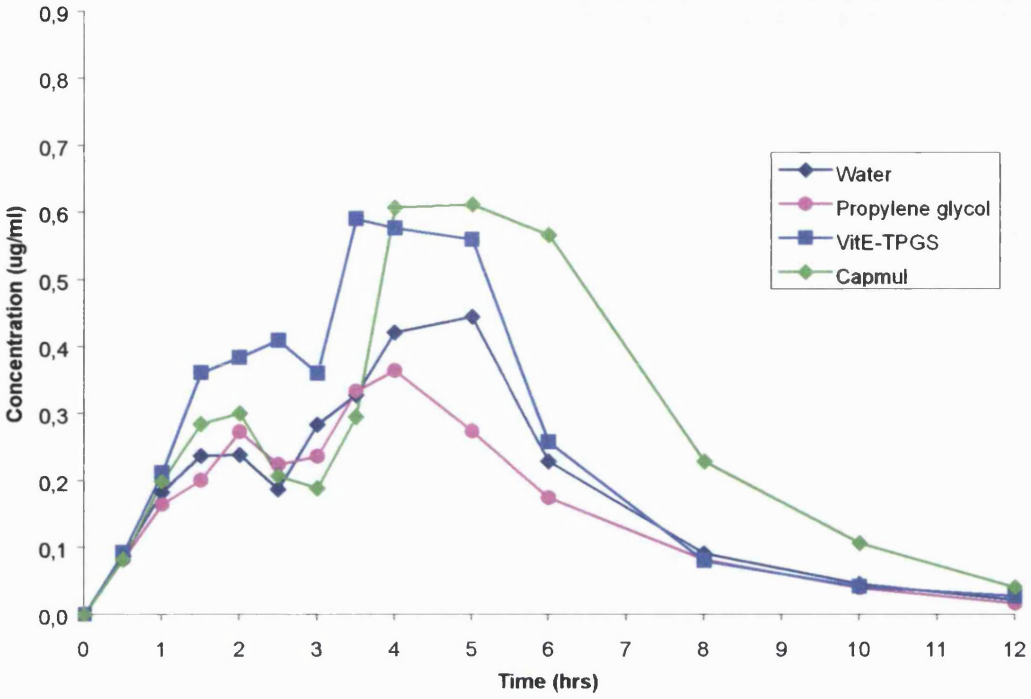


Figure 5.20. Plasma ranitidine concentration-time profiles for volunteer 4.

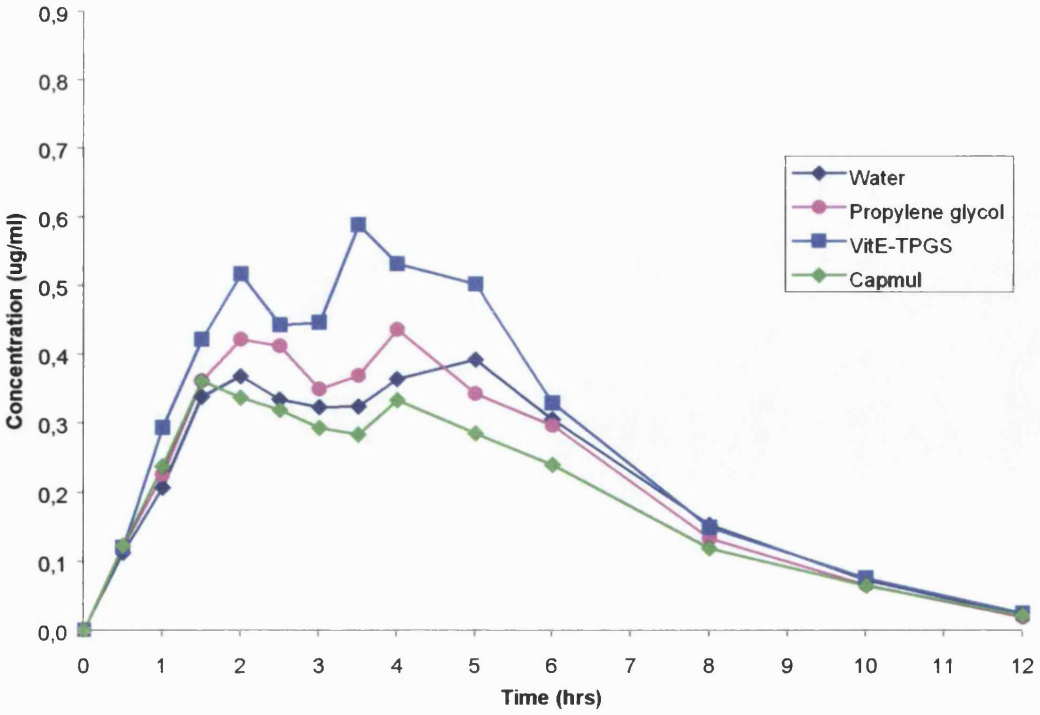


Figure 5.21. Plasma ranitidine concentration-time profiles for volunteer 5.

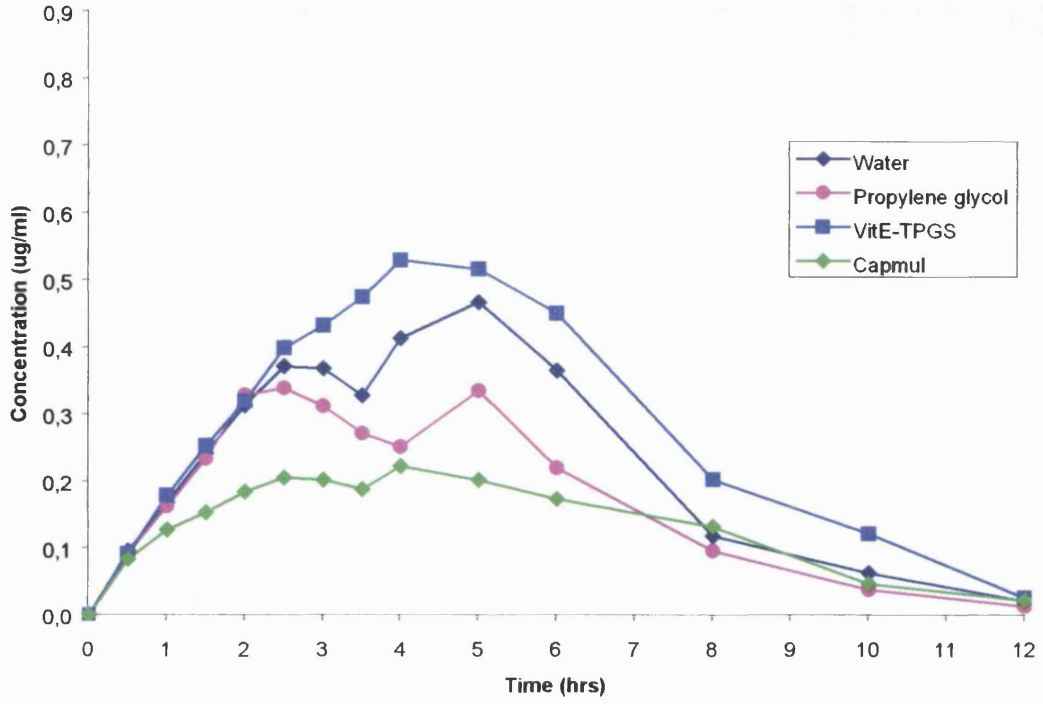


Figure 5.22. Plasma ranitidine concentration-time profiles for volunteer 6.

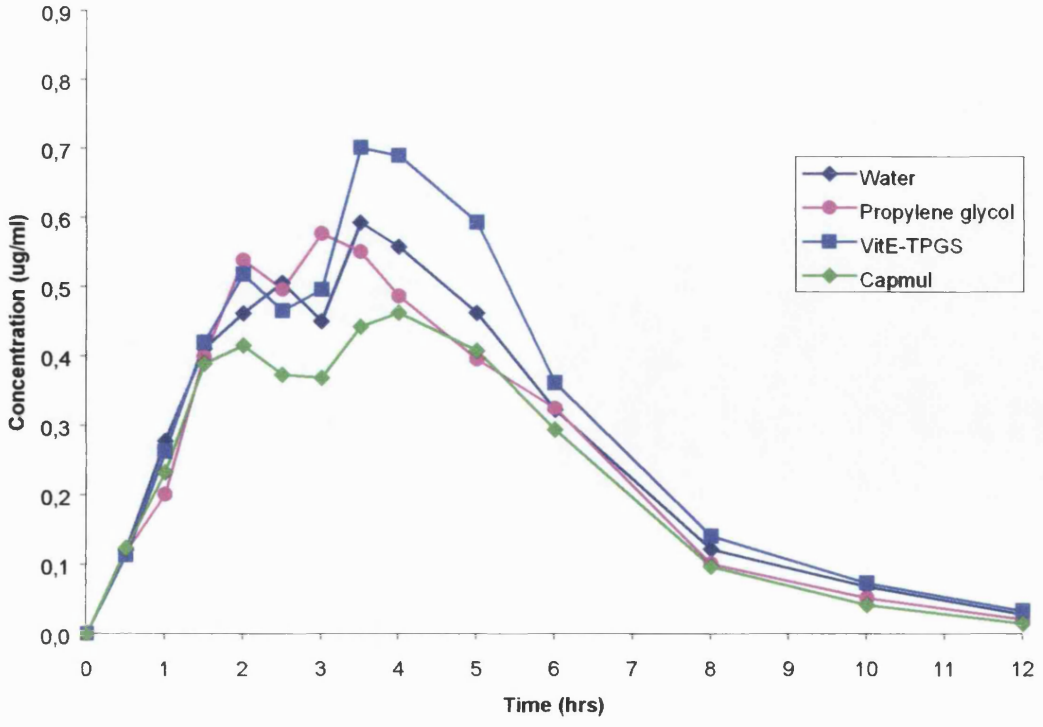


Figure 5.23. Plasma ranitidine concentration-time profiles for volunteer 7.

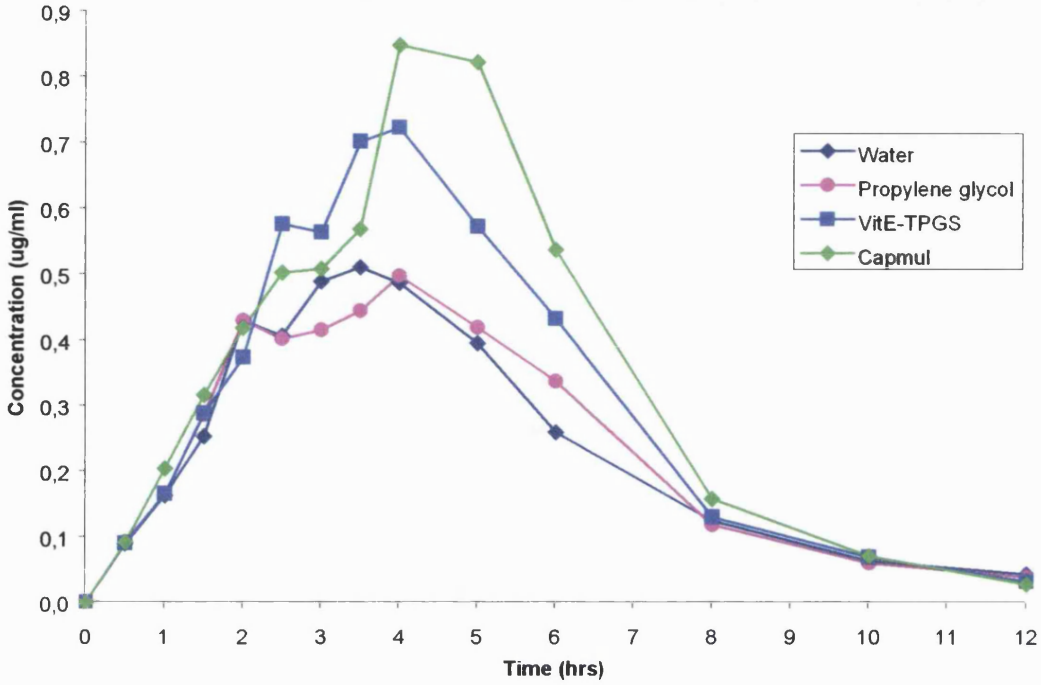


Figure 5.24. Mean (± S.E.) plasma ranitidine concentration-time profiles.

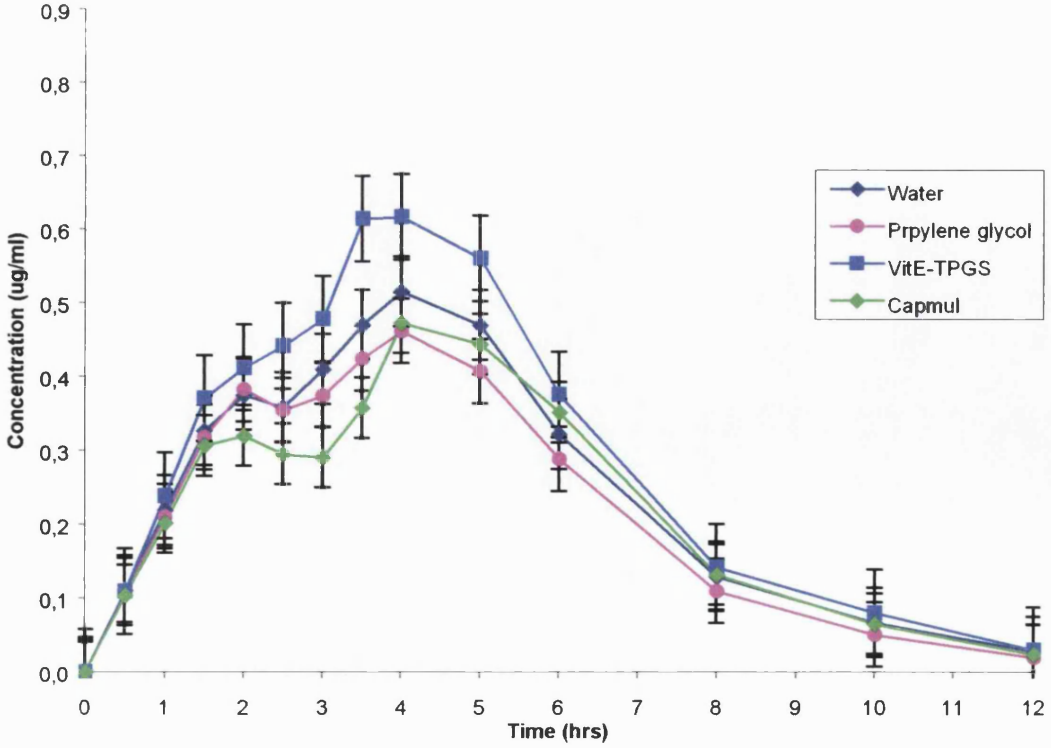


Table 5.7. Pharmacokinetic data for ampicillin.

	Treatment											
	Control			Propylene glycol			VitE-TPGS			Capmul® MCM		
Vol.	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)
1	7.07	1.5	13.47	4.59	1.0	10.38	7.75	1.0	13.15	6.05	1.0	10.16
2	6.11	1.5	13.45	6.56	1.0	13.38	4.86	1.5	12.43	4.40	1.5	7.61
3	5.89	1.5	10.27	4.30	1.5	6.45	4.44	1.0	9.76	5.90	1.0	10.17
4	4.21	1.5	10.48	4.28	1.0	9.65	4.52	2.0	14.14	3.26	1.5	5.72
5	4.43	1.5	8.26	2.70	1.5	7,65	4.45	1.5	9.59	3.33	1.5	8.89
6	6.25	1.0	10.30	2.19	1.5	3.81	5.48	1.5	10.24	3.09	1.0	6.90
7	5.21	1.5	10.28	4.19	2.0	8.56	3.65	1.5	9.73	2.29	2.0	5.21
Mean	5.50	1.43	10.93	3.88	1.36	8.55	4.66	1.43	11.29	3.77	1.36	7.81
± s.d.	± 0.99	± 0.19	± 1.89	± 1.22	± 0.38	± 3.05	± 1.17	± 0.35	± 1.90	± 1.65	± 0.38	± 2.01
P-value						*0.032*			*0.579*			*0.016*

Table 5.8. Pharmacokinetic data for ranitidine.

	Treatment											
	Control			Propylene glycol			VitE-TPGS			Capmul® MCM		
Vol.	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)	Cmax (μg/mL)	Tmax (hrs)	AUC_{last} (hr*μg/mL)
1	0.694	4.0	3.47	0.564	4.0	2.71	0.645	4.0	3.98	0.323	4.0	1.82
2	0.672	4.0	3.46	0.655	5.0	3.28	0.623	4.0	3.13	0.540	5.0	2.83
3	0.445	5.0	2.18	0.365	4.0	1.83	0.590	3.5	2.86	0.612	5.0	3.26
4	0.393	5.0	2.61	0.437	4.0	2.67	0.589	3.5	3.29	0.338	2.0	2.18
5	0.467	5.0	2.65	0.339	2.5	2.01	0.529	4.0	3.34	0.223	4.0	1.51
6	0.593	3.5	3.19	0.578	3.0	3.02	0.702	3.5	3.61	0.463	4.0	2.61
7	0.511	3.5	2.74	0.497	4.0	2.81	0.722	4.0	3.57	0.848	4.0	3.96
Mean	0.516	4.29	2.90	0.462	3.79	2.62	0.617	3.79	3.40	0.473	4.00	2.60
± s.d.	± 0.130	± 0.70	± 0.49	± 0.126	± 0.81	± 0.52	± 0.075	± 0.27	± 0.36	± 0.210	± 1.00	± 0.85
P-value						*0.062*			*0.015*			*0.484*

This bimodal pattern of the plasma concentration versus time curve is known as the double-peak phenomenon and has also been shown for other H_2-receptor antagonists (Mummaneni et al., 1995). For ranitidine, this phenomenon has been widely observed (van Hecken et al., 1982; Roberts, 1984; Williams et al., 1992; Basit et al., 2002) and different explanation theories exist. Two of the most commonly proposed mechanisms involve an enterohepatic recycling of the drug on one hand and the existence of two absorption windows for ranitidine on the other. According to the first theory, suggested by Roberts (1984), a ranitidine metabolite is excreted from the bile into the intestinal lumen, where it is reconverted to the parent compound and subsequently reabsorbed into the systemic circulation. The second explanation of the double peak was promoted by Gramatte et al. (1994). After infusing ranitidine solutions into different regions of the human small intestine the absorption of the H_2-receptor antagonist was found to be highest from the proximal duodenal-jejunum and distal jejunum-ileum regions, whereas no absorption occurred from the mid-jejunum. In general, the absorption of ranitidine has been demonstrated to comprise of a relatively high inter-subject variability (Shim and Hong, 1989).

In contrast to the findings obtained in the previous study using beagle dogs, the co-administration of solubilizing agents in healthy volunteers appeared in part to have a significant impact on the absorption of the model compounds, which will be discussed in detail in the following.

5.4.2.2. Propylene glycol

In the presence of 5 g propylene glycol the average AUCs of ampicillin and ranitidine were 8.55 (± 3.05) and 2.62 (± 0.52) hr*μg/mL, respectively. Compared to the AUC value found on the control day, these results correspond to a decrease in oral bioavailability for the propylene glycol formulation, which was seen for both drugs. The AUC of ampicillin appeared to be significantly reduced by 21.7 % ($P < 0.05$), whereas ranitidine absorption was affected to a lesser extent. The reduction in AUC relative to the control was calculated to be 9.7 % but statistically not significant ($P = 0.062$). The reasons for the observed effects might be several fold. Firstly, the small intestinal transit times of the preparation containing propylene glycol need to be considered. Here, on average a 12 % reduction of the MSITT was observed in the presence of the cosolvent. Considering the MSITTs and AUC values of the individual

volunteers the influence of shorter small intestinal transit times becomes apparent especially in the case of ampicillin. The individuals, who experienced the greatest acceleration of small intestinal transit (subjects 3 and 6), also give the greatest reduction in AUC in the presence of cosolvent compared to the control. Although a slight reduction of ranitidine absorption also appears to have occurred in the two mentioned volunteers, the greatest negative effect on AUC was observed in subjects 1 and 5, who had the same MSITT with either preparation or even a longer passage time in the presence of propylene glycol. For ranitidine, therefore, this direct relation between MSITT and AUC does not appear to be perfect in the presence of propylene glycol. However, in several volunteers the AUC of ampicillin was found to be reduced although the MSITT of the propylene glycol preparation is the same as for the control formulation (subjects 1 and 4) or even prolonged as found for subject 5. Therefore, the presence of 5 g of cosolvent appears to have a negative impact on drug absorption in addition to the transit effect and this effect is more considerable with ampicillin than with ranitidine.

One explanation is likely to be found in the hypertonic nature of the propylene glycol formulation. The presence of a hypertonic load inside the intestinal lumen has been shown to have a negative impact on the absorption of hydrophilic compounds (Riley et al., 1992). High osmolality of the luminal fluids causes secretion of water into the lumen as the body's response to compensate for the enhanced osmotic pressure. Small hydrophilic molecules that use aqueous pathways for absorption will be faced with fluid fluxes in the opposite direction and as a result are likely to suffer a reduction in uptake ("reverse solvent drag"). In addition, secretorial fluxes of large amounts of water lead to a dilution of the therapeutic agent at its site of absorption adversely affecting oral drug bioavailability. This dilution, in turn, reduces the concentration gradient between the mucosal and serosal side of the lining epithelium, which is generally the driving force for passive drug diffusion. Water secretion will be highest in the upper small intestine, which might explain why ampicillin absorption is affected to a greater extent than ranitidine comprising of a second absorption window in the lower small intestine. However, propylene glycol up-take is rapid in order to reduce the osmotic pressure inside the lumen and once isotonic conditions are achieved water reabsorption occurs. It is difficult to estimate the overall importance of such fluid fluxes in the complexity of the absorption process.

According to the dilution theory and occurrence of reverse solvent drag it would have been expected that the pharmacokinetic data of ranitidine comprise of a shift towards slightly delayed Tmax values, as well as a smaller initial peak and an enhanced second peak. This was found not to be the case in any of the volunteers (Figures 5.18 to 5.25). Also ampicillin Tmax values were found to be slightly shorter (Tmax 1.36 and 1.43 hours, respectively). For both model drugs, however, the propylene glycol formulation did not appear to change the shape of the plasma level versus time curves compared to the control.

Another aspect worth mentioning is a possible reduction of the thermodynamic activity of the model compounds within the lumen in the presence of propylene glycol. Especially for ampicillin, which is dependent on quick and easy access to its active carrier a hindrance in free diffusion across the aqueous boundary layer could prove crucial in its overall absorption. Besides simply reducing the drug's thermodynamic activity the cosolvent might interfere with the transport carrier itself or other components of the epithelial membrane compromising sufficient functioning of the carrier system. In *in vitro* diffusion studies, however, it was shown earlier that propylene glycol did not affect the passive diffusion of ranitidine to a significant extent albeit when employed at concentrations as high as 70 % (w/w). In the present study, the administered solution contains propylene glycol at a concentration of 3.3 % (w/w), which will be further diluted in the physiological fluids. Interactions of the drug and the cosolvent were also shown not to occur for ranitidine and are not very likely to take place with ampicillin.

5.4.2.3. VitE-TPGS and Capmul® MCM

The drug absorption results obtained with the preparations containing surfactants revealed opposite effects depending on the type of surfactant. It also seemed that these effects had different impact on either the amino-penicillin or the H_2-receptor antagonist. In general, VitE-TPGS appeared to enhance drug absorption while Capmul® MCM appeared to give a negative effect.

In the presence of 5 g VitE-TPGS the pharmacokinetic data were 11.29 (± 1.90) hr*μg/mL AUC, 4.66 (± 1.17) μg/mL Cmax and 1.43 (± 0.35) hours Tmax for ampicillin (Table 5.7) as well as 3.40 (± 0.36) hr*μg/mL AUC, 0.617 (± 0.08) μg/mL

Cmax and 3.79 (± 0.27) hours Tmax for ranitidine, respectively. Comparing the AUC values of the VitE-TPGS formulation with those obtained on the control day, the presence of the surfactant resulted in an average increase of 3.3 % for ampicillin, which appeared to not be of statistical significance ($P > 0.05$). The AUC observed for ranitidine, however, was enhanced by a significant 17.2 % ($P < 0.05$). This is likely to be the result of a permeation enhancing effect of the excipient. For ampicillin the extent of the increased drug absorption correlates well with the observed prolongation in small intestinal transit time, which was found to be 3 %. Although an average increase of 3 % seems to be negligible and in addition has not proven to be statistically significant ampicillin absorption is known to be highly dependent on sufficiently long residence times at its site of absorption. Considering the results for the individual volunteers a marked increase in the AUC of both drugs was observed for subjects 4 and 5, who showed a prolonged MSITT of 40 and 35 min, respectively, in the presence of VitE-TPGS. While all other volunteers except subject 2 appeared to have an enhanced absorption of ranitidine independent of variations in MSITT ampicillin absorption seemed to be reduced in each of the other individuals with the surfactant preparation. A decreasing effect of drug absorption in the presence of VitE-TPGS might be explained by a reduction of drug thermodynamic activity due to micelle formation of the surfactant. VitE-TPGS is a surface-active agent with a relatively low CMC of 0.02 % (w/w). With 5 g VitE-TPGS dissolved in 150 mL of water the administered formulation of the VitE-TPGS treatment reaches a concentration of 3.3 % (w/w), which once ingested will face certain dilution in the physiological fluids. In an *in vitro* dialysis diffusion experiment it was demonstrated earlier (Chapter 3) that VitE-TPGS does not affect the passive diffusion of ranitidine at surfactant concentration of 2 % (w/w). Hydrophilic molecules are not likely to be subjected to micellar solubilization to a significant degree, as they will remain primarily in the aqueous continuous phase. The luminal concentration of micelles was expected to be very high after the ingestion of the VitE-TPGS preparation since the surfactant concentration was more than 100 times its CMC. A high concentration of micelles, will in turn decrease the thermodynamic activity of drug molecules in the aqueous phase and thus also their absorption (Levy and Jusko, 1965; Krasowska, 1980).

On the other hand, high interfacial tension between the aqueous phase on the luminal side of the epithelium and the lipophilic absorptive membrane will be reduced in the presence of surfactants resulting in an improved contact between the formulation and intestinal epithelium. The surface activity of a drug molecule has been found in an *in situ* experiment in rats (Lindahl et al., 1999) to be an important physicochemical factor in its effective intestinal permeability. In addition, it has been suggested that one of the absorption-enhancing properties of VitE-TPGS might be the result of the surfactant protecting the therapeutic agent from pre-systemic metabolism (Chang et al., 1996; Bittner et al., 2002). Although ranitidine appears to be subjected to a considerable pre-systemic hepatic first-pass metabolism (Roberts, 1984; Lin, 1991), intestinal metabolism comprising of similar enzyme systems as the liver can be expected not to play an important role for the drug. As the metabolising enzymes are located in the wall of the intestinal mucosa, paracellular compounds are expected to predominantly avoid this mechanism of elimination in the intestine (Watkins et al., 1987; Krishna and Klotz, 1994). In a rat jejunal perfusion study investigating the intestinal clearance of various H_2-receptor antagonists, the metabolism of ranitidine was found to be minimal (Hui et al., 1994) and more recently, an *in vitro* experiment using excised rat intestine found no effect of VitE-TPGS on CYP450 3A metabolism with a solution of 0.01 % (w/w) VitE-TPGS (Johnson et al., 2002). Chang et al. (1996) reported an enhanced bioavailability of cyclosporine A in the presence of VitE-TPGS but suggested a metabolism avoidance rather than a direct inhibition of metabolizing enzymes as no changes in metabolite ratios were observed.

Surfactant molecules are known to interact with phospholipids of the lining epithelium. Monomers are capable of partitioning into the cell membrane where they can form polar defects in the lipid bilayer and alter the fluidity of the membrane. At high enough surfactant concentrations in the cell membrane surfactant-surfactant contact occurs and the membrane can be dissolved into surfactant-membrane mixed micelles (Swenson and Curatolo, 1992). In cell culture studies, such changes in membrane fluidity in the presence of surfactants resulted in an inhibition of the apical efflux transporter P-glycoprotein (Woodcock et al., 1992; Dudeja et al., 1995; Nerurkar et al., 1997). VitE-TPGS has also been found to have an inhibitory effect on P-glycoprotein, significantly decreasing the serosal to mucosal flux of several transporter substrates in different cell models (Dintaman and Silverman, 1999; Yu et

al., 1999). These efflux-inhibiting effects were observed at VitE-TPGS concentrations below its CMC suggesting that monomers of the surfactant are responsible. Ranitidine has been reported to be a substrate of P-glycoprotein (Cook and Hirst, 1994; Collett et al., 1999). Although no reports in the literature exist about ampicillin being subjected to intestinal efflux secretory transport is generally believed to have a significant role on compounds with low passive permeability such as more hydrophilic compounds, peptides and peptidomimetics (Aungst and Saitoh, 1996). However, ranitidine mainly uses paracellular absorption pathways and as a result does not enter cells to a significant extent (Gan et al., 1993). P-glycoprotein has therefore been suggested to play a minor role in the overall absorption of the H_2-receptor antagonist since its intracellular concentration may never reach sufficient levels for an activation of the intestinal efflux mechanism (Lentz et al., 2000). Troutman and Thakker (2003) have very recently supported these findings classifying ranitidine as a "substrate largely unaffected by P-glycoprotein mediated efflux activity". In contrast, ampicillin absorption is primarily transcellular via the dipeptides transporter in the upper small intestine (Oh et al., 1992). Towards the lower small intestine the absorption rate constant of ampicillin was significantly decreased with k_a being 1/10 in the ileum compared to that in the duodenum and jejunum (Sawamoto et al., 1997). These observations have been related to the decreasing presence of the active carrier system, but may partly be enhanced by a greater presence of P-glycoprotein further down the small intestine. In this respect, a P-glycoprotein inhibiting effect of VitE-TPGS would be more pronounced with ampicillin than ranitidine, but the absorption of the amino-penicillin was hardly enhanced with the surfactant formulation. It is possible that P-glycoprotein inhibition occurs by alterations in intestinal membrane fluidity in the presence of VitE-TPGS. Such alterations, however, might not only affect the functioning of efflux transporters but influx transporters as well such as the dipeptide carrier located at the apical side of the lining epithelium. The average maximum plasma level for ampicillin, for instance, was markedly decreased in the presence of VitE-TPGS compared to the control (Cmax 5.50 and 4.66 μg/mL, respectively). However, other *in vitro* studies found that Solutol HS, which fluidises membranes and effectively inhibits P-glycoprotein in epidermoid carcinoma cells, has no impact on the active carrier-mediated uptake of alanine and glucose (Coon et al., 1991). The inhibitory effect of surfactants on efflux transporters may, therefore, not be the result of a non-specific mechanism of membrane disruption but a relatively

specific surfactant mediated impact on the microenvironment of the transport protein in the plasma membrane of cells. In a recent *in vitro* study by Johnson et al. (2002) with excised rat intestine, the effects of VitE-TPGS on P-glycoprotein and enterocyte-based metabolism were found to be insignificant at a concentration of 0.01 % (w/w). Regarding the overall significant increase of ranitidine absorption in the presence of VitE-TPGS, an important factor may be that the fluidization of the phospholipid membrane with concomitant disruption of the epithelial barrier led to an increase in drug permeability via the paracellular route. Lindmark et al. (1998) showed such an effect of enhanced absorption of hydrophilic molecules with medium chain fatty acids, which caused a modification of the tight-junctional proteins. In addition, it has been speculated that surfactants as a class establish their permeation enhancing properties via the significant damaging effect on the epithelial barrier by the solubilization of membranal lipids. The extent of damage is dependent on the type and amount of surfactant and the time of exposure. However, the epithelium is also subjected to regular dietary induced damage (i.e. bile salts, fatty acids and monoglycerides). These damages are rapidly reversible once the irritant compound is removed and mechanisms have evolved for immediate repair (Swenson and Curatolo, 1992; Erickson, 1988; Moore et al., 1989). Immediate repair involves villus shortening for a reduction of the injured surface area and migration of epithelial cells to cover the injured area. Local toxicity of surfactants has been shown to decrease with increasing surfactant polarity (Bryan et al., 1980).

In the present study a less polar surfactant was employed in the form of Capmul® MCM, which has an HLB value of 5. The presence of 5 g Capmul® MCM was found to affect the pharmacokinetic parameters of the model drugs in the following way. Ampicillin reached maximum plasma concentrations of 3.77 (± 1.65) μg/mL at 1.36 hours and an average AUC of 7.81 (± 2.01) hr*μg/mL. In the case of ranitidine Cmax, Tmax and AUC were 0.473 (± 0.210) μg/mL, 4.00 hours and 2.60 (± 0.85) hr*μg/mL, respectively. In comparison with the control treatment the preparation containing Capmul® MCM appeared to have a negative effect on the absorption of both model compounds. The AUC values of ampicillin and ranitidine were reduced by 28.5 % and 10.5 %, respectively but only the decrease in the absorption of ampicillin was of statistical significance ($P < 0.05$). As mentioned earlier in this chapter, the mean passage of the administered Capmul® MCM formulations through

the small intestine was slightly accellerated. Although a good correlation does seem to exist also for the Capmul® MCM preparation between the MSITT and the AUC of the individual subjects the extent of drug bioavailability reduction was rather unexpected and suggests that other factors might have been involved. It was also surprising that the absorption-reducing effect on ampicillin was more pronounced than that on ranitidine. The treatment containing Capmul® MCM was demonstrated to significantly delay gastric emptying by more than 2-fold compared to the control formulation. This effect was expected to be beneficial for the absorption of the amino-penicillin since longer gastric emptying times provide a slow but steady source of the drug to its dipeptide carrier in the upper small intestine. Fast emptying from the stomach, on the other hand, results in a high drug concentration present at the carrier for only a short period of time likely to cause a saturation of its transport capacity. Therefore, a slower entering of ampicillin into the small intestine was believed to have a positive impact on its absorption and not as observed a negative effect. The dosing of the Capmul® MCM preparation was done in form of liquid-filled hard gelatin capsules for reasons of taste masking while the drug solution was administered separately. As explained earlier in this chapter, it is possible that the aqueous drug solution emptied from the stomach ahead of the excipient, which was not released before the capsules disintegrate. Although capsule disintegration is rapid in the fasted stomach, Capmul® MCM is rather viscous and lipophilic in nature (HLB 5), which means that mixing of the surfactant with the aqueous fluid contents in the stomach is limited. Entering into the small intestine Capmul® MCM will form mixed micelles with present bile salts and pancreatic lipase/co-lipase will commence the digestion processes to release the medium chain fatty acids from the mono-, di- and triglycerides. Medium chain glycerides have been shown to have penetration enhancing qualities in addition to enhanced solubilization (Swenson and Curatolo, 1992; Aungst, 2000). Beskid et al. (1988) reported that a formulation incorporating Capmul 8210 (a mixture of glyceryl mono- and dicaprylate) enhanced enteral, rectal and oral (from enteric-coated capsules) absorption of sodium ceftriaxone in rats, rabbits and squirrel monkeys. It has been suggested that the absorption enhancing effect of medium chain glycerides is due to their effects on the phospholipid bilayer structure (Muranushi et al., 1981) in a similar way as that discussed above for surfactant interactions. In an *in vitro* experiment with intestinal rabbit epithelium of the colon and the ileum, Yeh et al. (1994) found a significant absorption enhancement

of the paracellular marker mannitol in the presence of Capmul® MCM, where the colon appeared to be more sensitive to the glycerides than the ileum. In the same study, the authors demonstrated that the mucosal to serosal transport of cephalexin, which uses an active carrier system in the ileum, was reduced by 40 % in the presence of 1 % Capmul® MCM, whereas at the same time mucosal to serosal transport was 2.5 fold enhanced. These findings support the suggested effects of the surfactant on the epithelial membrane that the transcellular crossing of drug molecules through the defective lipid bilayer is enhanced while at the same time the function of active transporters might be compromised. It is possible that the active carrier system for ampicillin was affected by the presence of Capmul® MCM, which might explain the more enhanced reduction in absorption compared to ranitidine. In addition, the concentration of Capmul® MCM was rather high in the upper small intestine, whereas further down the GI tract the amount of medium chain glycerides would have been reduced as a result of dilution as well as glyceride digestion and uptake of the digestive products. One of the digestive products of Capmul® MCM is the C_{10} fatty acid sodium caprate, which has been shown to cause a widening of the tight junctions in Caco-2 cells and hence enhancing the absorption by the paracellular route (Anderberg et al., 1993; Lindmark et al., 1998). More recent cell culture studies also found an inhibition of the efflux transporter P-glycoprotein by sodium caprate (Lo and Huang, 2000). Such an effect could be similar to that observed with VitE-TPGS but the impact on the present model compounds is doubtful.

Generally, however, Capmul® MCM was expected to have permeation enhancing qualities similar to VitE-TPGS or Labrasol®. The extreme differences observed between the treatments, increase and decrease in drug absorption in the presence of VitE-TPG and Capmul® MCM, respectively are not clear. One reason could have been the difference in HLB value. Besides the fact that surfactants with a low polarity are known to cause an increased local toxicity (Bryan et al., 1980), a recent study in Caco-2 cells found that an intermediate HLB of 10 to 17 is optimal for an absorption enhancing and intestinal efflux inhibiting effect (Lo, 2003). In agreement with these observations, Crison and Amidon (1999) have reported a trend in the improvement of the bioavailability of nifedipine in dogs using a high HLB surfactant (Labrasol, HLB 14) over that of a surfactant with a low HLB value (lauroglycol, HLB 4), even though both formulations appeared to solubilize the drug to the same extent. Dudeja et al.

(1995) suggested earlier that all surfactants with multi drug resistance reversing effects contain a polyoxyethylene hydrophilic side chain attached to hydrophobic moieties, which is the case for VitE-TPGS. Another reason for the observed differences in the presence of VitE-TPGS and Capmul® MCM might be the administration mode of the latter. Good permeation-enhancing qualities of excipients can only have an effect when the excipient and the drug are present at the absorption site at the same time. Due to the fact that Capmul® MCM was administered in hard gelatin capsules and also is a viscous lipid that does not mix well with aqueous fluids, it is likely that the drug solution emptied from the stomach ahead of the excipient. In addition, the MSITTs of the Capmul® MCM treatment was found to be decreased to a considerable extent in five of the seven volunteers. It is therefore not unlikely, that the reduction in the absorption of ampicillin and ranitidine was the result of an accelerated passage of the formulations through the GI tract.

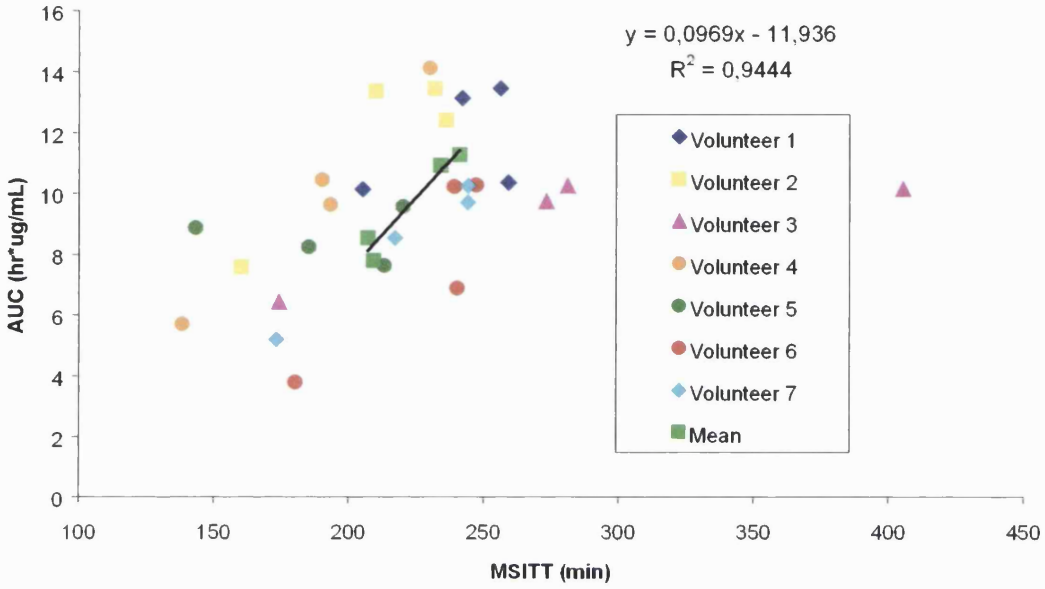
5.4.3. Correlation between GI transit and drug absorption

In the preceding paragraphs the effects of different solubilizers on GI transit and drug absorption in healthy volunteers have been identified and discussed in detail. The correlation between the two parameters represented by the MSITT and AUC, respectively, is illustrated in Figures 5.25a,b for ampicillin and Figures 5.26a,b for antipyrine.

Considering the relatively high intra- and intersubject variability, the ampicillin data are presented for each individual subject in Figure 5.25a and a linear line of regression was derived for the mean values of all volunteers. The correlation coefficient, R^2, was calculated to be 0.9444 stating a direct proportionality of ampicillin absorption on the time spent at its absorption site. These findings are in good agreement with Haruta et al. (1998), who demonstrated this dependency of ampicillin bioavailability on small intestinal transit times in rats. Animals pretreated with propantheline, a muscarinic receptor antagonist, which prolongs GI transit showed a significantly increased absorption of the β-lactam antibiotic.

Figure 5.25. Correlation between the MSITT and the AUC of ampicillin considering:

a) Intra- and interindividual variation



b) Effect of treatment

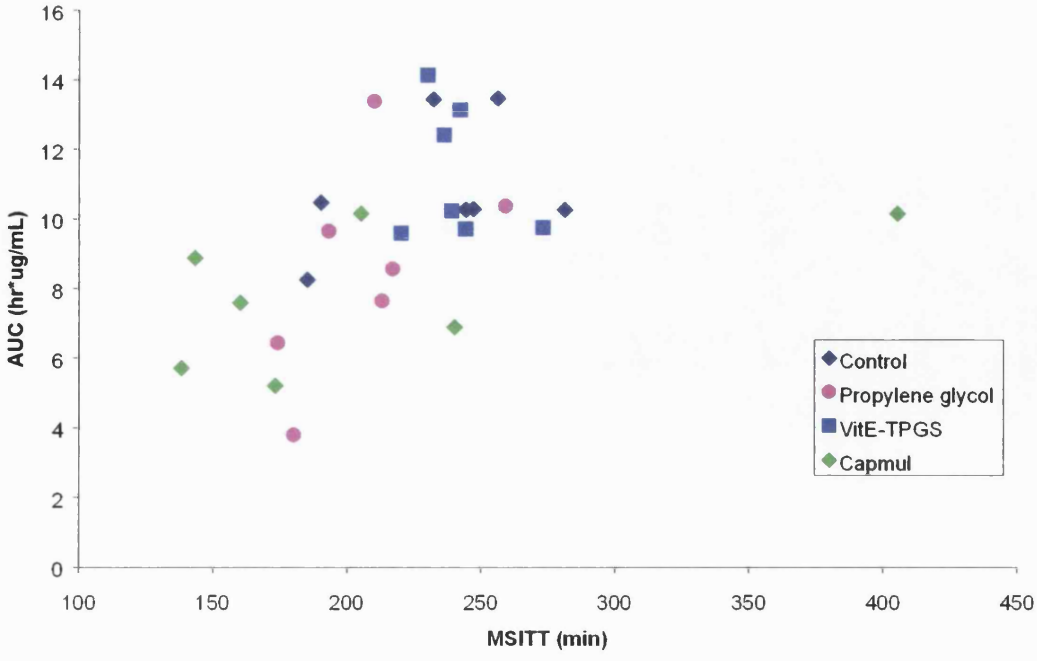
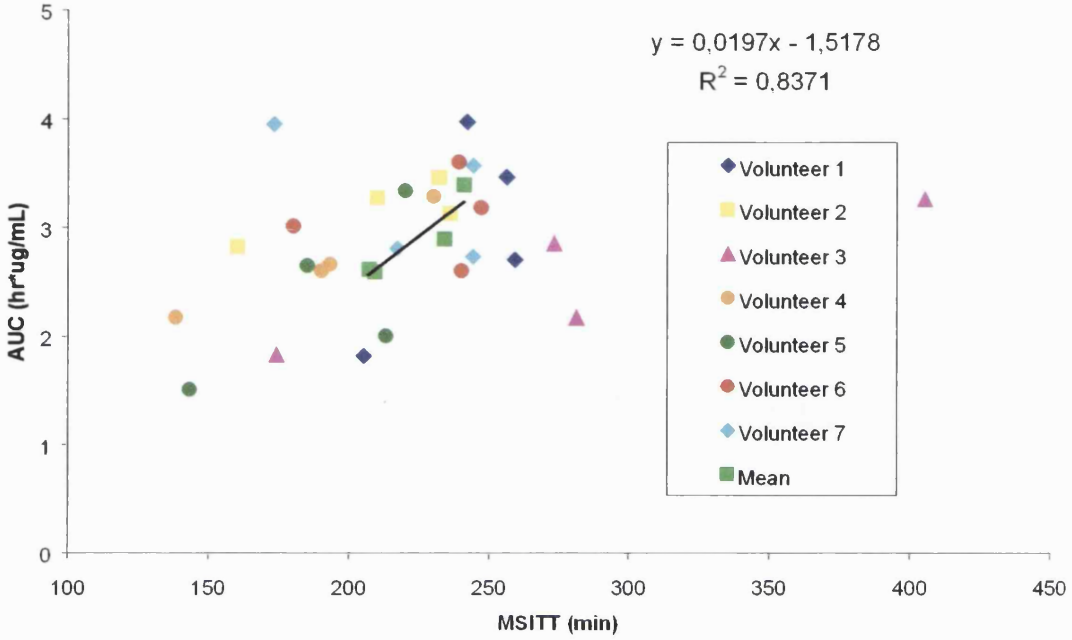
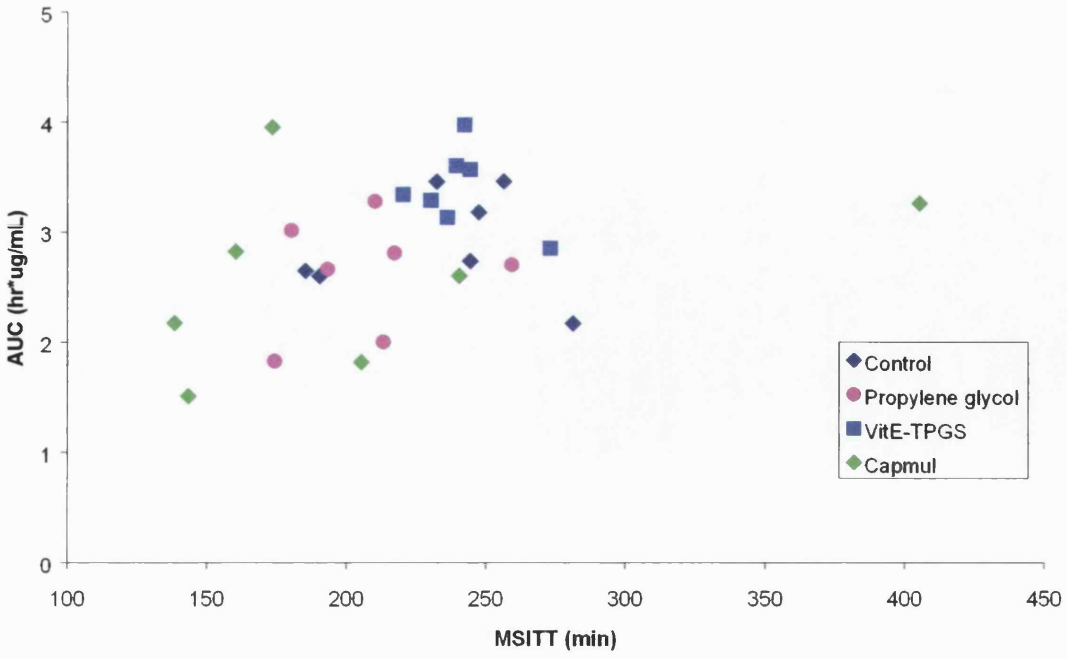


Figure 5.26. Correlation between the MSITT and the AUC of ranitidine considering:

a) Intra- and interindividual variation



b) Effect of treatment



In contrast, however, are the results obtained previously in four beagle dogs (Chapter 4), which did not give a good correlation between GI transit and the bioavailability of ampicillin (R^2 = 0.0479). These considerable differences in correlation of GI transit and drug absorption data between the dog and the human questions the arbitrary use of the dog in GI transit studies investigating pharmaceutical excipients. The presence of solubilizers in the administered treatments appeared to have no effect on either transit or absorption in the canine model. In the present human study, however, certain trends can be derived from Figure 5.25b for the influence of the different excipients.

While the values of the control formulation are rather evenly distributed in average MSITT and AUC, the data obtained for the propylene glycol and Capmul® MCM formulations appear to spread towards the bottom left quarter of the graph. The data of VitE-TPGS preparation on the other hand tend to concentrate around average transit times and greater AUCs.

Similar results were observed for ranitidine, which are shown in Figures 5.26a,b. For the overall absorption of ranitidine, the transit time through the small intestine also seems to be of great importance as a direct relationship was established between the MSITT and AUC. The linear regression of the mean values of the individual volunteers, however, shows that the correlation between the two parameters is not as perfect as that observed for ampicillin (R^2 = 0.8371). This may be the result of the differences of both compounds with respect to their absorption site. It is possible that the acceleration of intestinal transit does not occur at an even pace throughout the entire small intestine but might be greater in the duodenum and upper jejunum and considerably less in the ileum. Here, ranitidine has a second absorption window, which may make it more independent of a fast passage through the upper small intestine. The effects of the different solubilizers present in the ranitidine formulations give a similar distribution of the values according to ampicillin (Figure 5.26b) with the data of propylene glycol and Capmul® MCM spreading towards the bottom left quarter and the data of VitE-TPGS concentrating in the area of average transit times but slightly increased drug absorption.

Such tendencies in data distribution, however were not observed for either ampicillin nor antipyrine in the previous study conducted in dogs (Chapter 4). In addition, the correlation coefficient for both drugs was calculated to be < 0.5 for a linear relationship between small intestinal transit time and drug bioavailability. Although the canine does not appear to be a good model for investigating the effects of solubilizing agents on transit and drug absorption in the present case differences in the design of both studies need to be considered. On one hand, the preparations administered to the beagle dogs consisted of a total fluid volume of 50 mL compared to 150 mL in humans, which is likely to have influenced the transit of the formulations through the GI tract. A low fluid volume does interrupt the fasting cycle of the gastric motility, which means that differences in the motility pattern of the GI tract were present in the two models. On the other hand, physiological differences exist between the species as, for instance, the leakier epithelium of the dog. These differences become particularly important in cases when the transit accelerating and/or absorption enhancing effect is dependent on the poor absorption of the excipient from the intestinal lumen, e.g. PEG 400.

5.5. Conclusions

The objective of the present *in vivo* study was to determine the effect of different solubilizing agents on GI transit and drug absorption in humans and to correlate the findings to the previous study conducted in canines. The emptying of the solutions from the stomach was rapid and not affected by the presence of solubilizers with exception of the formulation containing Capmul® MCM, which delayed the mean gastric residence time (MGRT) by more than two fold. None of the investigated solubilizers, propylene glycol, VitE-TPGS and Capmul® MCM were found to have a significant delaying or accelerating impact on the passage of the treatments through the small intestine when administered at a dose of 5 g. These results appear to be in agreement with those obtained from the transit studies in beagle dogs. In the canine model, employing a lower dose of solubilizer, no significant alterations in the MSITTs were detected in the presence of propylene glycol, VitE-TPGS or Labrasol®. With propylene glycol the MGRT was slightly reduced in dogs but not in humans. The results of gastric emptying, however, are difficult to compare between the two studies due to the differences in administered fluid volume.

In the present study, differences between solubilizing agents were found regarding an effect on the absorption of the model compounds ampicillin and ranitidine. A considerable decrease in ampicillin absorption was observed for the formulation containing propylene glycol whereas ranitidine uptake appeared unchanged compared to the control. This effect is most likely due to induced secretion of water into the lumen as a reaction to the hypertonic nature of the propylene glycol treatment. The adverse fluid fluxes are more apparent in the upper small intestine decreasing towards the ileum as a result of absorption of the cosolvent, which explains the significant impact of propylene glycol on ampicillin but not ranitidine having a second absorption window in the ileum. In the dog, ampicillin absorption was not affected by propylene glycol most likely due to the widened tight junctions in canines compensating for a reduced absorption in the upper small intestine by opening the paracellular route. VitE-TPGS, on the other hand, was found to increase the absorption of ranitidine while no significant effect was observed for ampicillin. The enhanced bioavailability of the H_2-receptor antagonist by VitE-TPGS is most likely the result of surfactant interactions with the intestinal membrane modulating its permeability, which proved to be beneficial for the paracellular compound but not for ampicillin dependent on carrier-mediated uptake. The absorption of the β-lactam antibiotic, however, was decreased to a considerable extent in the presence of Capmul® MCM possibly as a result of high local concentrations of the surfactants in the upper small intestine having a negative effect on membrane integrity and the function of the transport carrier. In general, the formulations of Capmul® MCM showed a high variability between the individual subjects.

In general, a good direct correlation was obtained for the residence time of the formulations in the small intestine and the relative bioavailability of the model drugs, which is in contrast to findings of the previous study conducted in canines. Although lower doses of excipients were employed in the dog study, these results seem to confirm, "the best model for man is man" (Davis and Wilding, 2000).

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

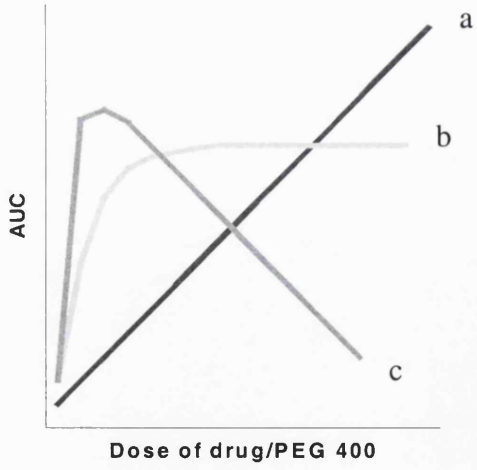
The overall goal of the project was to identify and characterize the *in vivo* effects of pharmaceutical excipients commonly employed as solubility enhancing agents on GI transit and drug absorption. For many years, these excipients have been more or less accepted as pharmaceutically inert although they are regularly used at excessive amounts in early drug development and phase I studies allowing the formulation of poorly water-soluble drugs in oral solutions for the assessment of bioavailability. Some of the preparations containing high doses of solubilizers may also reach the market as in the case of Agenerase® oral solution. Here, however, a reduced oral bioavailability is obtained in comparison to the corresponding soft gelatin capsule preparation having a markedly lower content of these excipients.

One of the solubilizers contained in Agenerase® is PEG 400, which has previously been shown to have a considerable accelerating effect on the transit of liquid preparations through the GI tract causing a concomitant reduction in the bioavailability of the active agent. In the present study this transit effect of the cosolvent was established to be dose-dependent decreasing the mean residence time of oral liquids in the small intestine even at concentrations as low as 1 g, although this effect was only statistically significant at 2.5 g. A dose-dependency of PEG 400 was also demonstrated to exist regarding the absorption of the administered model compound ranitidine. At higher concentrations the bioavailability of ranitidine was markedly reduced most likely as a consequence of the accelerated passage through the small intestine since PEG 400 was found not to affect passive drug diffusion. When the polymer, however, was administered at an amount of 1 g ranitidine absorption was enhanced significantly suggesting that low doses of PEG 400 modulate intestinal drug permeation. The mechanism behind this effect is not yet established and it is unclear whether this permeability enhancement is only true for ranitidine.

These findings on the dose-dependent effects of PEG 400 with respect to GI transit and drug absorption are expected to have considerable implications for its use in pharmaceutical preparations. Since it is regularly used in preparations of new chemical entities (NCE) erroneous interpretation of pharmacokinetic drug data is possible, contributing observed effects to properties of the therapeutic agent when in fact they result from *in vivo* actions of the co-administered excipient. To illustrate the

likeliness of such misinterpretations of pharmacokinetic data, a case scenario is displayed in Figure 6.1. An oral solution of a NCE in PEG 400, for instance, is expected to result in increasing oral bioavailabilities of the NCE with the administration of increasing doses of the formulation (*a*). Instead, a relationship between the AUC and the administered dose of solution is found according to (*b*) or (*c*). With the drug bioavailability reaching a plateau at higher doses (*b*) the investigator will be tempted to assume that active transport processes are involved in the absorption of the NCE, which become saturated at higher drug concentrations due to limited capacities of the transport carrier. In fact, this effect is the result of reduced small intestinal transit times due to the presence of high doses of PEG 400 thought to be an inert solvent. In the case of an obtained profile of the AUC/dose relation of (*c*) it is possible to assume a high intestinal permeability for the NCE when it is initially administered at low doses. This might lead to classify the drug as a highly permeable compound when in reality its absorption is enhanced by the presence of PEG 400. It is suggested that the solubilizing agent is employed more thoughtfully.

Figure 6.1. Examples of PEG 400 effects on the absorption of NCEs.



A further issue worth considering in the employment of excessive amounts of solubilizers is the risk of inducing drug-drug interactions. Agenerase® oral solution, containing 10 g PEG 400, 33 g propylene glycol and 7 g VitE-TPGS in a single dose, is used in the treatment of HIV and AIDS. In this disease the majority of patients also suffer from various complementary diseases, which require additional medication. In such cases the presence of high solubilizer concentrations in Agenerase® may have

the potential to adversely affect the absorption of coadministered drugs, leading to sub-therapeutic or toxic drug levels.

Other solubilizers investigated including propylene glycol, VitE-TPGS and the medium chain glycerides Labrasol® and Capmul® MCM were found not to affect small intestinal transit of oral liquid treatments but influenced the absorption of different coadministered model drugs. Compared to the control formulation ampicillin absorption was considerably reduced in the presence of propylene glycol, which was most likely the result of the osmotic activity of the cosolvent inducing changes in fluid movement across the intestinal epithelium, which are most apparent in the upper small intestine. The surfactant VitE-TPGS, on the other hand, was observed to increase the oral bioavailability of ranitidine but not ampicillin. Alterations in membrane fluidity and possible modulation of tight junctions are suggested to be responsible for the enhanced permeation of the paracellular compound through the absorptive membrane. Medium chain glycerides are the newcomers among pharmaceutical excipients employed for increasing the solubilities of lipophilic drugs as well as for enhancing the absorption of hydrophilic macromolecules. Comprising of fat digestion products Labrasol® and Capmul® MCM were both expected to have a slowing effect on small intestinal transit via activation of the body's braking mechanisms. Both excipients, however, did not meet these expectations. A high variability between subjects was observed and the majority of subjects showed markedly shorter transit times and reduced absorption of ampicillin and ranitidine, the cause of which requires further investigation.

As another primary objective, this work aimed to establish whether the dog might be a good model to investigate oral liquid transit and the effects of solubilizing agents on drug absorption. The results obtained in an *in vivo* scintigraphic study in four beagle dogs correlated well regarding gastrointestinal transit since no significant effects were observed. On this basis, however, it is difficult to draw firm conclusions whether both models would give a good correlation regarding the sensitivity to detect significant effects. Differences were observed in drug absorption most likely as a result of interspecies differences in the permeability of the intestinal mucosa confirming that "the best model for man is man" (Davis and Wilding, 2000).

CHAPTER 7

FUTURE WORK

Although the overall objective of this project, identification and characterization of the effects of solubilizing agents with respect to gastrointestinal transit and drug absorption, was achieved, some outstanding questions still remain. In order to understand more fully the *in vivo* behaviour of solubilizing agents, the following are suggested as possibilities for further areas of investigation:

- *In vitro* and *in vivo* investigations into the absorption enhancing effects of low doses PEG 400 would be of particular pharmaceutical interest. This would help understand the mechanism behind this effect and establish whether it is more generic in nature or only applies for certain drug compounds.

- Oral liquid dosage forms of poorly soluble drugs in solubilized systems are often likely to have the therapeutic agent precipitated out in the stomach leading to reduced oral bioavailabilities. It would be of great importance to establish *in vitro* and *in vivo* methods to investigate the precipitation of drugs in different solubilized systems.

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Schulze J.D., Waddington W.A., Ell P.J., Parsons G.E., Coffin M.D. and Basit A.W. (2003) Concentration-dependent effects of polyethylene glycol 400 on gastrointestinal transit and drug absorption. Pharm. Res. 20, 1984-1988.

ABSTRACTS:

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