PARTICLE FORMULATION FOR THE ENHANCEMENT OF ORAL BIOAVAILABILITY OF POORLY WATER-SOLUBLE DRUGS



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Abstract

The oral route is the most common route of drug administration for its ease of administration and convenience, which in turn, leads to a relatively high patient compliance. However, problems arise when trying to administer poorly water-soluble drug through the oral route. These drugs usually show a variable and unpredictable bioavailability *in vivo* with the dissolution rate acting as the rate-limiting step. Increasing the saturation solubility and/or reducing the particle size of these poorly water-soluble drugs are techniques which can be used to increase dissolution rate and subsequently oral bioavailability.

The first part of this study was focused on producing novel micro- and nanoparticles of a model hydrophobic drug, which show a better dissolution rate than the control (the unprocessed model drug). Spray drying was one of the techniques used to produce particles. Once suitable spray dried particles were produced, the next step was to spray dry with a hydrophilic surfactant. The aim of using surfactant was to increase the wetting ability of the drug to prevent or reduce aggregation as well as possibly increasing the saturation solubility of the drug. The second technique chosen to form particles was a modified solvent-diffusion method. The particles produced were then characterised using a wide range of pharmaceutical techniques including scanning electron microscopy (SEM), powder X-ray diffraction (PXDR), dynamic contact angle (DCA) and *in vitro* dissolution testing (USP II apparatus).

Once characterisation work was complete, the particles produced were used in *in vivo* absorption studies in rats. The drug concentration in the plasma was assayed using high performance liquid chromatography (HPLC) and used to obtain pharmacokinetic data including area under the curve (AUC) and bioavailability. The results obtained showed that the particles produced by spray drying had a bioavailability (6.9%) which was significantly higher than those obtained from spray drying without surfactant (3.5%), solvent-diffusion (3.4%) and the control (3.9%). No significant differences were seen in the bioavailability of the control and particles spray dried without surfactant. Although the solvent-diffusion particles had a similar bioavailability to the control, they showed highly variable and unpredictable plasma concentrations.

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Finally, I would like to thank my family for their support and patience throughout the course.

Plagarism Statement

This thesis describes research conducted in the School of Pharmacy, University of London between 2001 and 2004 under the supervision of Professor I. W. Kellaway and Dr. S. Murdan. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature

24th MG 2005

Date

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Abbreviations

θ	Contact angle
ΔH	Enthalpy of heat
Α	Effective surface area
ΑΟΤ	Bis(2-ethylhexyl) sodium
ASES	Aerosol solvent extraction system
ATP	Adenosine triphosphate
AUC	Area under the curve
BCS	Biopharmaceutics Classification System
BET	Brunauer, Emmett and Teller
CAB	Cholesteryl 4-(2-anthryloxy)butanoate
CMC	Critical micelle concentration
D ₅₀	50% undersize distribution
DCA	Dynamic contact angle
DDOA	2,3-di(dodecyloxy)anthracene
DSC	Differential scanning calorimetry
EMEA	European Medicines Evaluation Agency
EPAS	Evaporative precipitation into liquid solution
F	Bioavailability
FDA	Food and Drug Administration
FTIR	Fouriers Transform Infrared
GAS	Gas anti-solvent
GI	Gastrointestinal
HHR	Hydrophilic/hydrophobic ratio
HLB	Hydrophobic-lipophilic balance
HPC	Hydroxypropylcellulose
HPLC	High performance liquid chromatography
iv	Intravenous
J	Flux (diffusion)
LALLS	Low angle laser light scattering
LMOG	Low molecular weight organogelators
ММС	Migrating myoelectric cycle
NSAID	Non-steroidal anti-inflammatory drug
PABA	Paraaminobenzoic acid

PCL	Poly(C-caprolactone)
PCS	Photon correlation spectroscopy
PEG	Polyethylene glycol
PEO	Polyethylene oxide
PLA	Polylactic acid
PLGA	Poly(lactide-co-glycolide)
PPO	Polypropylene oxide
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
PXRD	Powder x-ray diffraction
SAA	Supercritical assisted atomisation
SCF	Supercritical fluid
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEDS	Solution enhanced dispersion by supercritical fluids
SEM	Scanning electron microscopy
SFL	Spray freezing into liquid
Tg	Glass transition temperature
ТЕМ	Transmission electron microscopy
USP	United States Pharmacopoeia
XPS	X-ray photoelectron spectroscopy

Chapter 1: General Introduction

1.1 Introduction

Therapeutic agents can be administered via various routes, such as oral, transdermal and intravenous. However, the oral route of drug delivery remains the most desirable and preferred means of administering therapeutic agents for their systemic effects. In the pharmaceutical industry, oral medication is generally considered to be the first avenue investigated when discovering and developing new drug entities as well as pharmaceutical formulations (Kim and Singh, 2002). This is due to the fact that oral dosage forms such as tablets, capsules, solutions, emulsions and suspensions allow easy and convenient self-medication leading to patient acceptance which in turn is reflected in overall good compliance. Oral dosage forms are also more cost effective to manufacture as it does not require the sterile conditions that intravenous and ocular formulations need.

However, the development of oral dosage forms becomes challenging when the therapeutic agent is poorly absorbed from the gastrointestinal (GI) tract or is unstable in the presence of various enzymes present within the GI tract, such as the proteolytic enzymes. The absorption of a drug compound from the GI tract requires the compound to be completely dissolved in the GI fluids and the dissolved compound to then permeate the membrane of the GI tract to enter the systemic circulation. Therefore, dissolution is one of the major factors controlling the absorption rate of drugs from the GI tract (Katchen and Symchowicz, 1967). Hydrophobic drug compounds which possess a low solubility in aqueous medium often exhibit poor and highly unpredictable bioavailability when administered orally.

Poor solubility is one of the major challenges to drug development today as an estimated 40% of all newly developed drugs are poorly soluble or insoluble in water (Naseem et al, 2004) while up to 50% of orally administered drug compounds suffer from formulation problems related to their high lipophilicity (Gursoy and Benita, 2004). Subsequently, much research has been conducted into developing strategies to improve the solubility and dissolution rates in order to enhance the oral bioavailability of these hydrophobic drug compounds.

These strategies which include the formation of prodrugs (Chaumeil, 1998; Choi and Jo, 2004; Hu, 2004; Cundy et al, 2004), complexation with cyclodextrins (Thompson, 1997; Stella and Rajewski, 1997), milling (Chaumeil, 1998) and micro- and nanoparticle formation (Trotta et al, 2003; Rasenack et al, 2004) are briefly reviewed in Section 1.7.

1.2 Anatomy and Physiology of the Gastrointestinal (GI) Tract

The gastrointestinal (GI) tract (Figure 1-1) which is composed of the oral cavity, oesophagus, stomach, small and large intestine and rectum, together with the accessory organs (salivary glands, liver, gallbladder and pancreas) make up the GI system (Vander et al, 2001; Mayersohn, 2002). The GI tract is a highly specialised region of the body with its primary functions being the ingestion and digestion of food, the absorption of nutrients and the elimination of waste products. Besides food, orally administered drugs are also absorbed into the systemic circulation from the GI tract.



Figure 1-1: Schematic diagram of the GI tract (adapted from Cull, 1989)





The basic structure of the GI tract can be described as a hollow, muscular tube which runs through the body starting at the mouth and ending at the anus. Although the overall structure of the GI tract is very similar along its entire length, each part of the GI tract exhibits distinguishing physiological and anatomical characteristics related to the specific roles of those particular regions. These characteristics which cover surface structure, pH and enzymes are summarised in Table 1-1.

The walls of the GI tract have the same basic organisation from midoesophagus onwards whereby it is made up of four concentric layers of tissue – mucosa, submucosa, muscularis externa and serosa (Figure 1-2). The structure of the outer three layers (submucosa, muscularis externa and serosa) remains relatively unchanged throughout the GI tract. However, the mucosa which is located by the luminal surface of the GI tract shows unique structural

-Region	Length (m)	Absorbing surface area (m²)	Transit time of solids (hr)	рН	Enzymes
Stomach	0.2 (variable)	0.1 – 3.5 (variable)	1 – 3 (variable)	1.0 – 2.5	Proteases and lipases
Small intestine	0.3	1 0		50-60	Polysaccharidases,
Jejunum	2.8	184	3 – 5	6.0 - 7.0	oligosaccharidases, peptidases, lipases, nucleases, nucloetides
Ileum	4.2	276		7.0 – 7.5	
Large intestine					
 Caecum 	0.2	0.05	20 – 60 (variable)	6.4 - 7.0	Broad spectrum of bacterial enzymes
 Colon 	1.5	0.25		7.0 – 7.5	

 Table 1-1: Anatomical and physiological characteristics of the human GI tract (adapted from Evans et al, 1988; Rouge et al, 1996;

 Daugherty and Mrsny, 1999)

and physiological differences which are specially adapted to the functions of each particular region of the GI tract.

As shown in Figure 1-2, the mucosa can be sub-divided into a further three layers - epithelium, lamina propria and muscularis mucosa. The innermost epithelium layer consists of a single layer of columnar epithelial cells. These cells are involved in the absorption of nutrients as well as drugs and the secretion of various enzymes. They also act as a physiological barrier against various pathogens. The epithelial cells are interconnected by direct contact via specific proteins located across the intercellular space, called the tight junctions. These tight junctions seal the neighbouring cells together thus preventing the uncontrolled diffusion of fluids and compounds from the apical to the basolateral sides of the cells and vice versa. The next layer, the lamina propria is composed of connective tissue which acts to support the epithelium and contains the blood and lymph vessels and the nerve fibres. In order to be absorbed into the bloodstream, ingested substances must penetrate into this region. The outermost mucosal layer, the muscularis mucosa is the layer that separates the mucosa from the submucosa. It is made up of two thin muscular layers, 3-10 cells thick and is where the nerve plexus is located.

The stomach can be divided into three regions – fundus, body and antrum. Although there are no clear divisions between the three regions, the proximal stomach (fundus and body) acts as a reservoir for the storage of ingested material while the distal section is the major site where mixing of the luminal contents occurs. The antrum also acts as a pump for gastric emptying to occur. The layer of epithelial cells that line the mucosal surface of the stomach is unique in that it also contains tubular invaginations known as gastric pits. At the base of these gastric pits are specialised secretory cells which produce and secrete about 2 l of gastric fluid daily. The luminal surface of the epithelial cells is covered with a layer of mucus which is around 1.0 - 1.5 mm thick. This mucus layer protects the mucosa from the hydrochloric acid secreted from the gastric pits. Mucus also aids in the lubrication of ingested material. Generally, the stomach is not thought to be a major site for drug absorption due to the low number of absorptive cells and relatively low surface area when compared to the small intestine. The stomach is believed to function more as a reception

area for food and drugs. However, in some cases, drug absorption does occur, especially with drugs such as weak acids which are unionised in the stomach and may profit from the sometimes relatively long residence time in the stomach of up to 12 hr (Davis et al, 1984a).

The small intestine consists of the duodenum, jejunum and ileum and accounts for around 60% of the length of the GI tract. Besides its length, it has a unique surface structure which further increases the effective luminal absorptive surface area. The initial increase in surface area is attributed to folds of mucosa and submucosa called the folds of Kerckring which project into the lumen. These folds are approximately 0.75 cm in height and cover the complete circumference of the small intestine. Further surface area increases are due to the fact that the entire epithelial surface of the small intestine is covered with finger-like projections known as villi. These villi have lengths ranging from 0.5 to 1.5 mm and it has been estimated that 1 mm² of the duodenal mucosal surface contains between 10 - 40 villi. Projecting from the surface of each of the villus are fine microstructures called microvilli. These microvilli are approximately 0.1 µm in length and it has been estimated that each vilus is covered by about 600 microvilli. These microvilli give the intestinal mucosal surface a hairy texture leading to the microvilli region of the small intestine to be referred to as the "brush border" region. When compared to the surface of a smooth cylinder of the same length, the folds of Keckring, villi and microvilli increase the effective surface area of the small intestine by factors of 3, 30 and 600 respectively giving the small intestine a total surface area of approximately 200 m². The corresponding surface area estimate for the stomach varies from 0.1 to 3.5 m² (Rowland and Tozer, 1995; Rouge et al, 1996; Daugherty and Mrsny, 1999). This large surface area enables the small intestine to be the main absorptive site of orally administered drugs, except those designed for buccal, sublingual and colonic preparations (Kim and Singh, 2002).

The ileocaecal junction is the point at which the small intestine ends and the large intestine starts. The large intestine then continues on until the internal anal sphincter where it terminates. The primary functions of the large intestine are the absorption of water and electrolytes and the formation, storage and

elimination of faecal matter. It can be subdivided into four sections - caecum. colon, rectum and anal canal. The colon forms the major portion of the large intestine and is composed of the ascending, transverse, descending and sigmoid colon. From a functional perspective, the proximal half of the large intestine (caecum, ascending colon and part of the transverse colon), is involved in the absorption processes while the distal half (transverse, descending and sigmoid colon, rectum and anal region) is involved with the storage and mass movement of the faecal matter. Unlike the small intestine, the luminal wall of the large intestine is relatively flat with no villous structure present. Subsequently, it has a relatively small surface area and therefore, only around 10% of the absorption of drugs passing through the GI tract occurs in the large intestine (Koch-Wesser and Schechter, 1981). One unique characteristic of the large intestine is the presence of active bacterial microflora which has been exploited in the development of oral drug delivery systems targeting the colon (Ashford and Fell, 1994; Hovgaard and Brondsted, 1996; Chourasia and Jain, 2004).

1.3 Transit through the Gastrointestinal (GI) Tract

The transit rate at which drugs pass through the GI tract has a bearing on the extent and rate of absorption of a drug compound. Studies have shown that the passage of food through the GI tract show changes in the transit times of the different digestive organs and these transit times can be affected by factors such as food intake, timing of administration and effects exerted by certain drugs such as antimotility agents.

1.3.1 Gastric Transit

After ingestion and passage through the oesophagus, food and other ingested substances arrive at the proximal stomach (fundus and body) where it is initially stored. The stomach is capable of expanding from a fasted volume of approximately 150 ml to up to 1.5 l when fed without being accompanied by a significant increase in the gastric intraluminal pressure or emptying rate (Kelly,

1980; van Wynsberghe et al, 1992; Ropert et al, 1993; Kuiken et al, 1999; Liau et al, 2001). During the fed state, the gastric intraluminal pressure is steadily exerted through slow, segmental contractions which cause the gastric contents to be moved towards the distal region of the stomach (antrum). In the antrum, the gastric contents are mixed and triturated. The partially digested semi-solid material (chyme) is then discharged into the duodenum via the pyloric sphincter (Wilding et al, 2001). The stomach is able to retain large undigested particles whilst expelling the small particles via an "antral-sieving" process. In this process, liquids and small particles are able to pass through the partially contracted sphincter while the large particles end up being retropelled back into the main body of the stomach.

At the end of the digestive period, the stomach enters the interdigestive migrating myoelectric cycle (MMC). During this fasted state, the stomach contains swallowed saliva, mucus, cellular debris as well as the large indigestible particles of food left from the previous meal. The MMC empties the stomach of these contents through four consecutive cycles of activity:

- Phase I: contractions of the stomach almost non-existent
- Phase II: contractions occur intermittently
- Phase III: acts as the housekeeping phase whereby contractions occur at a high intensity and the stomach is emptied of all non-digested contents (occurs for 5-15 min)
- Phase IV: interdigestive state

The different phases of this cycle migrate distally from the stomach to the terminal ileum over a 2 hr period; i.e. when Phase III reached the terminal ileum, the cycle re-starts in the stomach. The MMC is immediately suspended on feeding and one meal may disrupt the cycle for approximately 3 - 4 hr.

Studies have shown that the two main parameters which influence the gastric emptying of solid oral dosage forms are the size of the dosage form and the time at which the dosage form is administered; i.e. during the fed or fasted state (Davis, 1987). Generally, large non-disintergrating single unit solid dosage forms such as tablets are treated by the stomach as indigestible material and emptied during the Phase III phase of the MMC (Kaus et al, 1984). If the stomach is maintained in the fed state, the solid dosage form will

remain in the stomach throughout this time period. This extended retention time may be advantageous in cases where the drug is mainly absorbed from the proximal region of the small intestine. Interestingly, Davis et al (1988) showed that when two large tablets (12 mm diameter) were administered together to a fed stomach, the gastric emptying of these tablets did not necessarily occur concurrently, with tablet pairs being emptied up to 480 min apart in some cases. During the fasted state, the gastric emptying of large solid dosage forms is fairly erratic with the emptying rate depending on the time of arrival in the stomach in relation to the contractile activity of the MMC (Park et al, 1984a). Solid oral dosage forms consisting of small pellets or powders of around 1 mm, on the other hand, have been shown to be able to pass through a contracted pyloric sphincter which results in a gradual, steady rate of gastric emptying during the fed state (Hunter et al, 1982). This, in turn, means that the drug particles are well dispersed within the small intestine with minimal potential for mucosal irritation (Bechgaard, 1982). However, the amount of food ingested can influence the emptying rate of these particles. Marvola et al (1989) found that when taken with food, a portion of the ingested pellets remained in the stomach for up to 6 – 8 hr before being emptied by the action of the 'housekeeper wave'. During the fasted state, only a limited dispersion of the pellets occurs. Instead, the material was seen to undergo a bolus emptying (Hunter et al, 1980; O'Reilly et al, 1987).

Although a cut-off size for the gastric emptying of indigestible solids has been believed to determine the rate of emptying, studies have shown that the presence of food is a more important factor with the emptying rate being inversely proportional to the calorific value of the meal; i.e. the greater the calorific value, the longer the gastric retention time (Davis et al, 1984a; Davis et al, 1984b; Wilding et al, 2001).

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1.3.2 Small Intestinal Transit

The partially digested material is released from the stomach through the pyloric sphincter as chyme into the proximal region of the small intestine (duodenum). In the small intestine, the chyme is exposed to segmental and peristaltic contractions, similar to those in the stomach with the intensity of the contractions dependent on the digestive state. The segmental contractions are initiated by the presence of chyme and are responsible for increasing the contact between the intestinal contents and the absorptive surface of the small intestine. The peristaltic contractions, on the other hand, serve to move the unabsorbed chyme distally along the intestine. As the chyme moves along the small intestine, the viscosity of chyme increases and consequently the rate at which it moves down the intestine decreases. This phenomenon has also been observed in the fasted state, whereby the flow rate of the chyme in the jejunum is twice that of the ileum (2.52 ml/min versus 1.23 ml/min) (Soergel, 1971; Kararli, 1995).

Unlike the highly variable gastric transit time, it has been observed that transit times of solid dosage forms along the small intestine are remarkably consistent with a mean transit time of approximately 3 – 4 hr being reported (Davis et al, 1986). The transit time was found not to be affected by the type of dosage form administered (e.g. solution, pellets, small or large tablets), or by the presence or absence of food (Mundy et al, 1989). Pathological conditions (e.g. ulcerative colitis, diarrhoea or constipation), exercise and time of administration also did not cause any significant changes in the intestinal transit time (Ollerenshaw et al, 1987).

1.3.3 Colonic Transit

The ingested material in the distal ileum moves into the colon via the ileocaecal junction. This junction acts as a 'gate' that controls the passage of the ingested material to the colon while preventing bacteria and faecal matter from moving backwards into the small intestines (Phillips, 1983). However, the

transit across the ileocaecal junction is still a poorly understood event (Phillips et al, 1988). Some studies suggest that the junction opens in accordance to peristaltic waves reaching the terminal ileum and allows around 2 ml of the intestinal contents to enter the colon at a time (Aiache and Aiache, 1985). However, other studies propose that the movement through the ileocaecal junction is independent of the MMC but instead determined by the rate at which material accumulates within the ileum (Quigley et al, 1984). The nature of the material at the terminal ileum also appears to play a role in the rate at which it crosses the ileocaecal junction with unabsorbed fats and proteins shown to delay the emptying of the ileum into the colon by initiating a phenomenon known as the ileo-brake mechanism (Spiller et al, 1988). This causes the material to be stagnant in the terminal ileum and it is believed that the physiological function of this stagnation or reservoir effect is to enable efficient absorption of the nutrients from the GI tract. It has been shown that this stasis at the terminal ileum can last from between 2 - 20 hr.

This stagnation also affects solid dosage forms such as small pellets which have been previously dispersed within the small intestines allowing these pellets to regroup at the ileocaecal junction. As stasis can last for a considerable period it can affect drug delivery to the colon. However, on passing the ileocaecal junction, the small pellets get redispersed within the colon (Khosla and Davis, 1989; Price et al, 1991; Wilding et al, 2001). The degree of dispersion is size dependent with the larger particles passing through the colon more rapidly than smaller particles (Hardy et al, 1985; Parker et al, 1988).

The colon also undergoes two distinct motility patterns – segmental and peristaltic contractions. The segmental contractions predominate in the colon and enable the luminal contents to be mixed and spread slowly along the colon. Peristaltic contractions which occur about 3 – 4 times a day are responsible for moving the chyme through the colon (Wilson and Washington, 1989). The chyme moves relatively slowly along the colon thus giving time for maximal water absorption to take place. The movement in the colon occurs at intermittent intervals and therefore, the colon shows highly variable transit times both within and between individuals, with times varying between 1 to

more than 60 hr (Hardy et al, 1985; Hardy et al, 1987). Studies have shown that the peristaltic contractions in the colon are increased after eating due to the gastrocolic response. This response is initiated by a receptor present in the gastroduodenal mucosa and seems to be sensitive to ingested fats and proteins (Wright et al, 1980; Sun et al, 1982). The dietary fibre intake also has an effect on colonic transit times whereby individuals who have higher fibre intake show slower transit times (Price et al, 1991).

1.4 Mechanisms and Pathways for Drug Absorption from the Gastrointestinal (GI) Tract

Once a drug molecule is in solution, it has the potential to be absorbed. In order for absorption to occur, the drug must penetrate through the intestinal "membrane" and enter the systemic circulation, either via the portal blood or the intestinal lymphatic system. The pathway through which the drug enters the systemic circulation partially depends on the hydrophilicity or conversely the hydrophobicity of the drug. Generally, a more hydrophobic compound has a greater chance of being absorbed into the lymphatic circulation than a hydrophilic compound. An advantage of using the lymphatic system as the carrier pathway is that the drug is delivered directly to the vena cava bypassing the liver and therefore avoiding hepatic first pass metabolism (Charman and Porter, 1996). However, drug absorption is not always a straightforward passive process and various absorption routes and mechanisms exist. Although the characteristics of the drug compound, such as its inherent permeability, as well as the characteristics of the surrounding environment, such as the pH, presence of interacting materials and characteristics of the absorbing membrane play a role in determining if drug absorption occurs, the epithelial lining is considered to be the main barrier to the absorption of drugs from the GI tract (Blanchard, 1975). As shown in Figure 1-3, there are two main routes to which drugs can cross the epithelial barrier - paracellular and transcellular (Lee and Yang, 2001; Hillery, 2001).



Figure 1-3: Mechanisms and pathways for drug molecules across the epithelial lining of the GI tract (adapted from Hillery, 2001)

In the paracellular route, the drug crosses the epithelium by passive diffusion through the junction of adjacent epithelial cells and the movement of these drug molecules are driven by a concentration gradient. The main problem of this absorption route is that it is restricted by the presence of junctional complexes encircling the epithelial cells. These complexes seal adjacent cells together and preventing the luminal contents from entering the intercellular spaces. They are located immediately below the luminal surface and comprise of a tight junction, an adherent junction and a desmosome. Under fasting conditions, the diameter of the tight junction pores are around 4 – 8 Å and 10 – 15 Å in human and animals respectively (Fordtran et al, 1968; Schultz et al, 1974). However, these junctions are not completely impermeable and are used for the uptake of water, electrolytes and certain small molecules. The fact that it is used physiologically for water uptake suggests that these junctions are hydrophilic in nature and subsequently, absorption by this route is limited to low molecular weight hydrophilic compounds with the diffusion rate being inversely proportional to the molecular weight of the drug. The paracellular route is not believed to be a major absorption pathway since the tight junctions only make up around 0.1% of the total absorptive surface area (Nellans, 1991; Houin and Woodley, 2002). Examples of drugs which are able to be absorbed paracellular route include atenolol, ranitidine, via the clodronate, hydrochlorothiazide and small peptide drugs (Collett et al, 1996; Gan et al, 1998; Raiman et al, 2003; van der Merwe et al, 2004).

Studies have shown that the absorption rates of small hydrophilic drug molecules are increased significantly when accompanied by the flux of water from the intestinal lumen (Ochsenfahrt and Winne, 1974a; Ochsenfahrt and Winne, 1974b; Mullen et al, 1985). Conversely, flux of water into the lumen reduces the absorption of these drugs. This phenomenon is known as solvent-drag. However, opinion is divided on whether water absorption and solvent drag occur via the paracellular and/or transcellular route.

It is believed that the transcellular route is the predominant route of drug absorption. In this route, drug molecules move through the epithelial cell membrane using either active of passive processes before transversing the cytosol to exit the cell through the basolateral membrane. The cell membrane is a thin (~75 – 100 Å) elastic layer which is composed of globular proteins embedded in a phospholipid bilayer. These charged polar groups of protein project from the surface of the bilayer. The membrane bilayer is a dynamic structure with three important functional characteristics. Firstly, it is lipid in nature; i.e. it is permeable to lipid-soluble molecules but not to polar compounds. Secondly, the membrane contains submicroscopic water-filled pores which allow the passage of water and small water-soluble molecules such as urea and alcohol. Finally, these membranes also have channels that allow substances to cross when combined with a specific carrier (Blanchard, 1975; Kararli, 1989a).

The main form of transcellular absorption is passive diffusion through the lipid bilayer. Like paracellular diffusion, transport by this route does not require energy and is driven by a concentration gradient. However, unlike paracellular passive diffusion which is limited to hydrophilic compounds, this transcellular diffusional route is strictly for lipophilic substances with the absorption rate being affected by the oil-to-water partition coefficient and ionization state of the drug. These factors have been used to form the basis of the pH partition hypothesis which describes the interrelationship of dissociation constant, lipid solubility, pH of the absorption site and absorption characteristics of the drug along the GI tract. Most drugs are weak acids or weak bases which exist in a partially ionised state in aqueous solution. Since unionised molecules are more lipid-soluble, the rate of absorption of these weak acids and bases is related to the fraction of drug that is present as the unionised form. The variation in pH that exists along the GI tract shown in Table 1-1, mean that the degree of ionisation and subsequently absorption of these compounds varies along the GI tract. For example, weak bases are considered to be better absorbed from the small intestine while weak acids absorb better from the stomach (Prescott, 1974; Yu et al, 1996). However, it is important to note that an unionised compound is not necessarily a lipid-soluble compound and that the oil-to-water partition coefficient of a drug also plays a role in determining if diffusion will occur.

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The flux, J, of drug across the membrane can be described using Fick's 1st Law even if the law is based on an inert membrane whereas the lipid bilayer is a fluid and dynamic layer (Blanchard, 1975). Fick's 1st Law states that:

$$J = -DA \times \frac{dc}{dx}$$

Equation 1: Fick's 1st Law, where D is the diffusion coefficient, A is the surface area and $\frac{dc}{dx}$ is the concentration gradient

 $\frac{dc}{dx}$ may be re-written such that Equation 1 becomes:

$$J = -\frac{(DAK)}{h} \left(C_{gut} - C_{plasma} \right)$$

Equation 2: Fick's 1st Law re-written, where K is the membrane/water partition coefficient and C_{gut} is the drug concentration in the gut lumen, C_{plasma} is the drug concentration in the plasma and h is the membrane thickness

By defining the permeability coefficient, P as $-\frac{DK}{h}$, Equation 2 becomes:

 $J = PA(C_{gut} - C_{plasma})$

Equation 3: Fick's 1st Law re-written, using P as the permeability coefficient

Once the drug enters the portal blood, it is quickly redistributed into a very large volume, meaning the drug concentration in the blood is maintained at very low levels compared to the concentration at the absorptive site. Therefore, the drug in the blood 'compartment' is in sink conditions and a large concentration gradient exists across the membrane during the absorption process. Subsequently, $(C_{gut} - C_{plasma})$ can be approximated to C_{gut} and Equation 3 simplified to:

 $J = PA C_{gut}$

Equation 4: Simplified Fick's 1st Law

Besides the globular proteins, the phospholipid bilayer also contains waterfilled pores. These pores are responsible for the continuity which exists between the interior and exterior of the cells. Studies have shown that like the tight junctions (paracellular route), water and low molecular weight compounds with radii of less than 4 Å can cross these pores (Kingham and Loehry, 1976; Lindemann and Solomon, 1962). This means that the majority of drugs are too big to cross the membrane using this pathway.

Active transport is an absorption process where the membrane plays an active role and this system requires carriers with structures or sites that can bind specifically to solute molecules and mediate transfer. Since proteins have the required properties to impart the specificity characteristics required of carrier transport systems, the membrane proteins are widely accepted to be carriers in active transport. These carriers form a complex with the drug molecule at the luminal surface of the intestinal epithelium. The carrier-drug complex formed moves to the other side of the membrane where the drug molecule is liberated. The free carrier then returns to the starting point where the whole process is repeated. Active transport can transfer molecules against the concentration gradient and therefore, enables the membrane to control substances entering and leaving the cell. The energy required for active transport can be obtained from either:

- the negative free energy obtained when solute transport is coupled with a simultaneous movement of a second substrate moving down the concentration gradient either in the same (symport) or opposite (antiport) direction as the first, or
- the hydrolysis of adenosine triphosphate (ATP) or other high energy compounds on the protein surface
The protein carriers in active transport are highly selective with respect to the structure of the substrate although substrates with similar structures are capable of competing for the same sites on the carrier and saturation can occur. Drug uptake using active transport systems can be inhibited with metabolic poisons such fluoride and dinitrophenol as well as lack of oxygen. Examples of substances transported across the membrane by active transport include bile salts, sugars, paraaminobenzoic acid (PABA), biotin, 5-fluororouracil, L-3, 4-dihydrophenylalanine, L-5-hydroxytryptophan cyclacillin and cephalexin (Schanker and Jeffrey, 1961; Shindo et al, 1973; Shindo et al, 1977; Nakashima et al, 1984).

Facilitated diffusion is another route that substances can use to cross the membrane bilayer. This absorption pathway bears similarities to both passive diffusion and active transport. Like passive diffusion, facilitated diffusion does not require external energy input and is driven by a concentration gradient. However, like active transport, it involves specific carrier proteins. These carrier proteins are composed of two or more polypeptide chains that span the membrane. It has been proposed that a water-filled channel exists down the core axis of the aggregate. When a drug compound binds to the carrier protein at an active site, free energy from the binding causes a structural change in the protein. Facilitated diffusion is believed to be the reason why the absorption rate of many substances which are poorly lipid-soluble or charged, such as glucose and Na⁺ is higher than expected (Blanchard, 1975).

Endocytic processes such as pinocytosis can also be used to transport material across the biological membrane. This is a particulate absorption process whereby invagination of the apical cell membrane occurs to form vacuoles which contain material from the GI tract. This process takes place in several steps, starting with the folding of a microscopic portion of the cell membrane followed by local invagination. The invaginated portion of the membrane then buds off within the cell interior with this small sac or vesicle containing the solute. Endocytosis is an active process requiring cellular energy and it exhibits a degree of specificity. Colloids, proteins and fat globules are among the substances transported via endocytosis (Blanchard, 1975).

The majority of substances which are absorbed via the mechanisms and pathways described above enter the systemic circulation through the bloodstream. However, a small proportion of substances are absorbed into the lymphatic circulation. Generally, lymphatic absorption is only important for the absorption of dietary lipids, cholesterol, lipid-soluble vitamins and extremely lipophilic compounds. Unlike absorption into the portal blood vessel, compounds which enter the systemic circulation via the lymphatic vessels do not pass through the liver and thus avoid first pass metabolism. Therefore, the blood concentration levels of a drug substance which is known to undergo hepatic first pass metabolism can be significantly increased by directing the drug into the lymphatic system. Unfortunately, very little is known about the conditions required for the lymphatic absorption of foreign compounds. Studies have suggested that lymphatic absorption seems to depend on the partition coefficient of the compounds, the nature of the carrier solvent and the presence of bile salts (Chainkoff et al, 1952; Gallagher et al, 1965; Fidge et al, 1968; Mezick et al, 1968; Sieber et al, 1974; Blanchard, 1975; Noguchi et al, 1985a; Noguchi et al, 1985b). It should also be noted that although a drug molecule may be predominantly absorbed via one particular route or mechanism, it is extremely likely that suboptimal transportation of the drug also occurs through other routes and mechanisms.

1.5 Factors Affecting Gastrointestinal (GI) Drug Absorption

The degree and rate of absorption of drugs from the GI tract, particularly the small intestine, depend on various factors including the physicochemical properties of the drug, the non-absorptive physiological functions and state of the intestine, the metabolic activity and functions of the absorbing cell as well as the structure of the absorbing surface. Changes in one or more of these factors will result in variation in the drug absorption process (Levine, 1970). The previous section has shown how some of the physicochemical properties of the drug such as ionization state, oil-in-water partition coefficient and molecule size and weight, as well as the physiological conditions along the GI tract such as pH, influence the absorption process. This section will cover additional factors which may affect GI drug absorption.

The pH-partition hypothesis discussed in the previous section states that drug absorption is proportional to the fraction of drug existing in its unionised, more lipid-soluble state. Consequently, the acidic conditions in the stomach mean weakly acidic drugs are better able to cross the epithelial barrier there. However, this is only part of the story since, in order to be able to be absorbed, the drug must be in solution and weak acids are more soluble in basic conditions while weak bases are more soluble in acidic conditions. Also, most lipophilic drugs which permeate the epithelial barrier easily are insoluble in the aqueous medium of the GI tract. Therefore, a balance exists between solubility and permeability. Generally, the absorption of poorly water-soluble drugs is hindered by solubility and so the dissolution rates of these compounds become the rate-limiting step. The dissolution rate of a drug can be modified by forming more soluble salts of the parent drug, e.g. sodium salts of barbiturates or the presence of bile salts and other surface active agents.

The dissolution of a solid into a liquid can be seen as occurring in two consecutive steps (Mosharraf and Nystrom, 1995; Richards, 1996) :

- liberation of the solute molecules from the solid phase (interfacial reaction) followed by,
- transport of molecules into the bulk of the liquid phase by diffusion or convection

The dissolution rate of a solid depends on the speed at which the slowest step occurs. Generally, if no chemical reaction between the solute and solvent takes place, the rate-limiting step is the diffusion of the dissolved solute across the static solvent boundary layer at the solid-liquid interface (Nogami et al, 1966; Carstensen, 1980). In these cases, the Noyes-Whitney equation

(Equation 5) can be used to describe the dissolution process (Noyes and Whitney, 1897):

$$\frac{\mathrm{d}m}{\mathrm{d}t} = kA(C_{\mathrm{s}}-C)$$

Equation 5: Noyes-Whitney equation, where dm/dt is the dissolution rate of the solid, *A* is the effective surface area of the solid in contact with the solvent, C_s is the saturation solubility of the solid in the diffusion layer, *C* is the concentration of the solid in the bulk solution and *k* is the dissolution rate constant

The dissolution constant can be described using the following equation (Equation 6):

$$k = \frac{D}{Vh}$$

Equation 6: Dissolution rate constant, k, where D is the diffusion coefficient of the dissolution medium, V is the volume of the dissolution medium and h is the thickness of the boundary layer

If the solute is removed faster from the dissolution medium than the rate it passes into solution, then *C* can be approximated to zero and the term $(C_s - C)$ to C_s and the Noyes-Whitney equation can be simplified to:

$$\frac{\mathrm{d}m}{\mathrm{d}t} = kACs$$

Equation 7: Simplified Noyes-Whitney equation

From this equation, it can be seen that the dissolution rate is proportional to the saturated concentration or solubility of the solid (Mosharraf and Nystrom, 1995). Conversely, in conditions where *C* is equal or greater than C_s no net dissolution would occur.

Another factor affecting the absorption of drug compounds is gastric emptying and GI tract motility. As the stomach is not a major absorptive site, the rate of drug absorption will depend on how fast the drug is delivered to the small intestines where the majority of absorption takes place; i.e. gastric emptying rate. The rate of gastric emptying is controlled by various factors such as (Levine, 1970; Gibaldi and Feldman, 1970):

- autonomic and hormonal activity
- volume, composition, viscosity, tonicity, pH and temperature of the stomach contents
- the presence of bile salts and surfactants (e.g. dioctyl sodium succinate)

The rate of gastric emptying becomes of clinical significance in cases where rapid onset of action is required or where drugs undergo metabolism or degradation in the stomach such as L-DOPA, methyldigoxin and penicillin (Levine, 1970). In these cases, long gastric retention time would result in delayed onset of action or reduce the amount of active drug available for absorption. Increased peristalsis which shortens GI transit time can cause poorly soluble drugs or compounds such as riboflavin and some digoxin preparations that are absorbed by active transport processes to have insufficient time to undergo complete dissolution or absorption (Levy et al, 1972; Manninen et al, 1973). On the other hand, increased peristalsis can also enhance absorption by aiding the disintegration of tablets and capsules and dissolution.

The rate of drug absorption can also be altered by food. Generally, the absorption of drugs occurs more slowly when taken with food and the amount of drug absorbed is also reduced (Gower and Dash, 1969; McGilveray and Matlok, 1972). Exceptions to this exist however, and an example would be in the case of griseofulvin where absorption is enhanced when taken concurrently with a fatty meal (Crounse, 1961).

Besides food, drug interactions occurring when two or more drugs are taken together can also result in either reduced or enhanced absorption. For example, antacids are known to reduce the absorption of antibiotics such as tetracycline and rifampicin by chelation or complexation (Joint Formulary Committee, 2004). Other drug-drug interactions may alter the degree and/or rate of drug absorption by pH effects on dissolution and ionization, changing the GI motility and gastric emptying, interfering with active transport mechanisms, altering membrane permeability, exerting toxic effects of the GI mucosa or modifying the mucosal and bacterial drug metabolism (Prescott, 1974).

GI diseases such as Crohn's disease, ulcerative colitis and coeliac disease can also affect the absorption of both food and orally administered drugs by altering the GI transit times and/or the permeability of the GI tract membrane.

1.6 Bioavailability and Absorption

The bioavailability of a drug has been defined by the Committee of Proprietary Medicinal Products of the European Medicines Evaluation Agency (EMEA) in its "Note for guidance on investigation of bioavailability and bioequivalence" as "the extent and the rate to which a substance or its therapeutic moiety is delivered from a pharmaceutical form into the general circulation" (EMEA, 1998). Based on this definition, it can be seen that the bioavailability of drugs administered orally is dependent on both the absorption and systemic availability of the drug. The systemic availability of a drug refers to the amount of drug that arrives in the systemic circulation and is affected by factors such as hepatic first pass metabolism (FDA, 1999; Löbenberg and Amidon, 2000). The systemic availability of a drug can be quantified relatively easily using pharmacokinetic parameters such as the maximum plasma concentration of the drug (C_{max}) and area under the curve (AUC). Drug absorption, on the other hand, involves the permeation of a drug through the intestinal mucosa of the GI tract and the rate and extent at which absorption takes place is harder to quantify (Amidon et al, 1995; Lennenäs, 1997). Figure 1-4 gives a schematic illustration of the factors which can affect bioavailability.



Figure 1-4: Schematic diagram showing events in the GI tract following administration which affect bioavailability (Dressman and Reppas, 2000)

In 1995, the Biopharmaceutics Classification System (BCS) was proposed as a method of correlating *in vitro* drug product dissolution and *in vivo* bioavailability based on the absorption characteristics of a drug compound (Amidon et al, 1995). Under this system the dissolution rate and GI permeability of a drug are considered to be the two most important parameters controlling the rate and extent of absorption and subsequently, the BCS categorises drug compounds into four different classes based on their aqueous solubility and permeability (Table 1-2).

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

 Table 1-2: Drug classes under the Biopharmaceutics Classification System

 (Amidon et al, 1995)

The BCS uses three dimensionless numbers – dose number (D_o), dissolution number (D_n) and absorption number (A_n) – to characterise drug substances and determine the appropriate borderlines between the different drug classes. These numbers combine the physicochemical and physiological parameters of the drug in a simplified form thus allowing the assessment of the *in vivo* behaviour of the drug (Amidon et al, 1995; Löbenberg and Amidon, 2000).

The absorption number is the ratio of the effective permeability (P_{eff}) and intestinal radius (R) multiplied by the mean residence time (T_{si}) in the small intestine and can be written as the ratio of mean residence time and absorptive time (T_{abs}):

$$A_n = \frac{P_{\rm eff}}{R} \times T_{\rm si} = \frac{T_{\rm si}}{T_{\rm abs}}$$

Equation 8: Absorption number, A_n

The dissolution number is defined as the ratio of mean residence time to the dissolution time (T_{diss}), which includes solubility (C_s), diffusivity (D), density (ρ) and the initial particle radius (r) of the drug:

$$Dn = \frac{3D}{r^2} \times \frac{Cs}{\rho} \times Tsi = \frac{Tsi}{Tdiss}$$

Equation 9: Dissolution number, D_n

The dose number is described as the ratio of dose concentration (*M*) to drug solubility (C_s):

$$D_{o} = \frac{M / V_{o}}{C_{s}}$$

Equation 10: Dose number, D_o , where V_o is the volume of water taken with the dose

Class I drugs are both highly water soluble and permeable. Drugs in this class have high A_o and D_n but low D_o . The low D_o is a reflection of the good aqueous solubility of Class I drugs. Drugs in this class are generally well absorbed from the GI tract although they may show low bioavailability if susceptible to enzymatic degradation or undergo first pass metabolism. However, for drugs with an extremely fast dissolution rate, the absorption rate will depend on the speed at which gastric emptying occurs and will not show any correlation with the *in* vitro dissolution rate (Amidon et al, 1995). It has been suggested that in order to show bioequivalence, dissolution specifications for immediate release

dosage forms should be set so that 85% of the drug is dissolved in less that 15 min. Examples of Class I drugs include amiloride, primaquine and prednisolone (Lindenberg et al, 2005).

Class II drugs exhibit high permeability but poor aqueous solubility due to their hydrophobic molecular structure. They have a high A_o and D_o but low D_n which is in agreement with the fact that the dissolution rate acts as the rate limiting this step for drugs in this class. For class II drugs, the *in vivo* absorption profile shows a good correlation with the *in vitro* dissolution profile. Classic examples of Class II drugs are griseofulvin and ibuprofen (Yu, 1999).

Class III drugs are the mirror-image of Class II drugs whereby they have high aqueous solubility but poor permeability. As a result, drugs in this class have a low A_o . The poor permeability across the lipid barrier of the intestinal epithelium acts as the rate limiting step to absorption. Therefore, the oral bioavailability of drugs in this class can be improved by maximising the contact times between the dissolved drug compound and the absorbing surface as well as enhancing the effective permeability of the drug using penetration enhancing excipients such as fatty acids and surfactants (Junginger and Verhoef, 1998). Examples of Class III drugs include atenolol and colchicine (Lindenberg et al, 2005).

The final class of drugs, Class IV have both poor aqueous solubility and permeability. As a rule, drugs in this class rarely make it past the development stage although there are some Class IV drugs, such as the anitvirals, nelfinavir and indinavir available on the market (Lindenberg et al, 2005).

1.7 Formulation Approaches for Improving the Bioavaiiability of Poorly Water-Soluble Drugs

For the majority of orally administered drugs which are poorly water-soluble, especially those which are given at a low dose, the dissolution rate is the ratelimiting step to absorption and consequently, is the main factor affecting the bioavailability of these drugs (Rasenack and Müller, 2004). The Noyes-Whitney equation (Equation 7) shows that the two main parameters affecting the dissolution rate is the saturation solubility and effective surface area available for dissolution. Subsequently, the formulation approaches used to improve the dissolution rate and bioavailability of these drugs work on the basis of modifying these factors. The following section will discuss established techniques used to improve dissolution such as milling as well as some of the newer approaches such as evaporative precipitation into liquid solution (EPAS).

1.7.1 Particle Size Modifications

The effective surface area available for dissolution can be increased by decreasing the particle size of a drug. It has also been shown that particles less than 5 μ m are surrounded by a thinner diffusion layer which, in turn, contributes to a faster dissolution rate (Bisrat and Nyström, 1988). The absorption from small particles is also less affected by physiological factors such as gastric emptying times.

1.7.1.1 Milling

Milling is a mechanical micronisation process used to reduce particle size through either friction, attrition, impaction, shearing, pressure or a combination of two or more of these methods. Various types of equipment such as ball mills, air-jet mills and high pressure homogenizers are used for milling. Milling is a well established approach used to improve the dissolution rate of poorly water-soluble substances and has the advantage of being a relatively simple and rapid process which can be carried out either wet or dry.

Unfortunately, milling has many disadavantages, the main one being the limited opportunity to control the important characteristics of the final product such as size, shape, morphology, surface properties and electrostatic charge. Milling cleaves the drug crystal at the face with the smallest attachment energy which is generally, the face containing the lowest energy (Roberts et al, 1994). Subsequently, the properties of the milled product are dominated by the surface properties of this crystal face. However, the newly created surface of a

poorly water-soluble drug, like the unmicronised drug, will remain hydrophobic and exhibit poor wettability.

Another disadvantage is that the surface of the milled product is electrostatically charged and generally milled powders are aggregated due to their cohesive behaviour. The product also shows a broad particle size distribution with heterogenous particle shapes.

Milling is a high energy process and this high energy input disrupts the crystal lattice resulting in the milled product containing disordered or amorphous regions (Saleki-Gerhardt et al, 1994). These regions are thermodynamically unstable and are susceptible to recrystallisation on storage, especially in humid and hot conditions. As a result, the surface of the mechanically micronised powder has a "dynamic nature" (Ward and Schultz, 1995). The change in the surface properties will also change the physicochemical properties of a drug. For instance, milled powders with an activated amorphous region will differ in saturation solubility from a completely crystalline powder, irrespective of particle size. Other physicochemical characteristics such as blending and flow are also affected (Feeley et al, 1998; Mackin et al, 2002) which in turn have a bearing on the formulation process.

Despite these disadvantages, milling is still the most common way of producing drug powders with small particle sizes. However, newer approaches which use controlled particle production processes have been introduced to generate micro- and nano-sized particles.

1.7.1.2 Controlled Particle Production and Particle Engineering

Unlike milling which involves the comminution of large particles, controlled particle production processes produce the particles directly as micro- and nano-sized particles. The production of these micro- and nanoparticles remain a big challenge due to the high surface area which has to be created and stabilised against the tendency of particle growth (Rasenack and Müller, 2004). Most of these techniques therefore involve the use of a large quantity of

stabilising agents. Examples of these processes are precipitation from supercritical fluid (SCF), spray freezing into liquid (SFL) and evaporative precipitation into aqueous solution (EPAS).

Although the basic concept of using SCF for micronisation was first described in 1879 by Hannay and Horthgarth, it is only recently that there has been increased interest in this technology due to the pioneering works of Krukonis and the Battell Institute in understanding and developing the concept (Jung and Perrut, 2001). Carbon dioxide is the most widely used supercritical gas phase due to the fact that it is chemically inert, non-toxic and its supercritical temperature is low (31.1°C) which makes it suitable for heat-sensitive materials.

For drugs which are soluble in the SCF, the drug particles are produced via the rapid expansion of supercritical solutions (RESS) process. In this process, the SCF which has been saturated with the drug compound is rapidly depressurised through a nozzle into a low pressure chamber. This causes an extremely rapid nucleation of the drug substance resulting in small particles. Turk et al (2002) prepared griseofulvin nanoparticles of approximately 150 nm in diameter by this process using trifluromethane as the SCF. Dissolution studies carried out using artificial gut fluid (pH 7.4) showed that the RESS-produced nanoparticles had a faster dissolution rate than both milled and crystalline griseofulvin. However, this technique is restricted to drug compounds that have a reasonable solubility in the SCF.

If the drugs are insoluble in the SCF, another technique is employed whereby the SCF is used as the non-solvent (anti-solvent). In this technique, the drug is dissolved in a solvent which is miscible with the SCF. This drug solution is then precipitated in the SCF. Several variations of this technique have been developed such as gas anti-solvent (GAS), aerosol solvent extraction system (ASES) and solution enhanced dispersion by supercritical fluids (SEDS). In GAS, the drug solution is mixed with a SCF causing the solvent to have a lower solvent strength than the pure solvent. Subsequently, the solution becomes supersaturated and the drug precipitates out as microparticles. ASES, on the other hand, involves the spraying of the drug solution through an

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atomisation nozzle as fine droplets into compressed supercritical gas. The dissolution of the SCF in the solvent together with a large volume expansion result in a sharp rise in supersaturation of the drug-solvent mixture which leads to the formation of small and uniform particles. In the SEDS variation, both the drug solution and the SCF are sprayed through a nozzle with two co-axial passages into a pressure and temperature-controlled vessel. The high velocity of the SCF enables the drug solution to break up into small droplets. Work by Moneghini et al (2003) showed that the GAS technique could be used to produce carbamazepine microparticles. In this set of experiments, dissolved in a variety of solvents carbamazepine was (acetone, dichloromethane and ethylacetate) and carbon dioxide was used as the nonsolvent. They found that all three samples showed a faster dissolution rate than the unprocessed material. However, no significant differences were seen when the dissolution rate of particles produced using different solvents were compared.

However, all these SCF techniques involve specialised equipment and as yet, scale-up has not been successful. Therefore, this together with cost has limited their usage in the pharmaceutics field.

Another approach which can be used to create micro- and nanoparticles is SFL (Hu et al, 2003). In this process, the drug and hydrophilic excipients are dissolved in a solvent to form the feed solution. This feed solution is then sprayed through an atomiser directly into a cryogenic liquid, e.g. liquid nitrogen, to produce frozen nanostructured particles. These frozen nanoparticles are then freeze dried to produce micronised amorphous microparticles. When danazol was prepared using this process with polyvinylpyrrolidone (PVP) K-15 acting as the hydrophilic excipient, particles showing a size distribution ranging from 750 nm to 1.5 μ m were produced. Dissolution studies showed at a danazol/PVP K-15 ratio of 10:1, complete dissolution was achieved within approximately 20 min while the unprocessed material took longer than an hour (Hu et al, 2004).

More recently, EPAS has been introduced (Sarkari et al, 2002). In this approach, the drug compound is dissolved in a solvent with a low boiling point, e.g. dichloromethane before being heated to a temperature above the solvent boiling point. The solution is then sprayed through a fine nozzle immersed in a heated aqueous solution containing hydrophilic stabilising agents such as surfactants. As the nozzle is immersed in the aqueous stabilising solution, the drug particles are rapidly stabilised thus enabling crystallisation and particle growth to be rapidly inhibited. The drug suspension formed is then dried by techniques such snap-freezing followed by freeze drying or spray drying to produce the final particles. In their study, Sarkari et al (2002) showed that 74% of EPAS prepared cabamazepine particles (mean diameter 13 μ m) was released within 10 min compared to around 3.7% of the unprocessed material.

1.7.2 Saturation Solubility Modifications

The water molecule has an unusual structure whereby the oxygen and hydrogen atoms are arranged at the apices of a triangle. This unique structure results in water having many features that distinguishes it from other liquid solvents. Many theories of the water structure have been proposed, with one of the most highly developed theory being the 'flickering cluster' concept. This model describes water as comprising of 'clusters' which are relatively rigid association of water molecules held together by strong hydrogen bonds. These clusters are dynamic with the hydrogen bonds between molecules being broken and formed simultaneously resulting in 'ice-like' water clusters being suspended in a fluid of unbonded water. It is these clusters which are responsible for the solvent characteristic of water such as the high dipole moment and high tension which in turn mean that less polar compounds show poor wettability as well as have low dissolution potential in water (Florence and Attwood, 1998). Techniques such as solubilization and cosolvency involve the addition of excipients which interfere with the structure of water resulting in an improved saturation solubility of poorly soluble drugs while prodrugs and complexation alter the chemical structure of the drug to increase its polarity and therefore increased saturation solubility.

1.7.2.1 Solubilization

Solubilization is generally conceived as the preparation of a thermodynamically stable isotropic solution of a normally poorly soluble or insoluble substance in a given solvent by the introduction of surfactant(s) (Florence, 1981; Florence and Attwood, 1998). Surfactants are amphiphilic molecules consisting of a hydrophilic head group and a hydrophobic tail group. They are classified based on the ionic nature of their hydrophilic head group – anionic, cationic, non-ionic and zwitterionic (amphoteric). Generally, for toxicity and irritancy reasons, non-ionic surfactants are the preferred surfactants for orally administered drug formulations.

When a surfactant dissolves in water, the water molecules structuring around the hydrophobic tail causes an increase in free energy (Δ G) due to the entropy decrease of the system. Subsequently, the system acts to eliminate contact between the hydrophobic surfactant group and water molecules. Hydrophobic interactions as a consequence of van de Waals forces between the hydrocarbon molecules as well as the loss of water structuring acts as the driving force for such mechanisms (hydrophobic effect). This involves the various processes, depending on surfactant concentration as shown in Figure 1-5 (Florence and Attwood, 1998).

Increasing Surfactant Concentration Accumulation of surfactant at water/air or water/oil interfaces Micelle formation (micellization) Formation of rod-like micelles Formation of hexagonal arrays of rod-like micelles Formation of lamellar or neat phase

Figure 1-5: Surfactant behaviour in water with increasing surfactant concentration

Micelles are aggregates of surfactant molecules as illustrated in Figure 1-6. They form once the surfactant concentration in the solvent reaches the critical micelle concentration (CMC). In water, the micelles formed contain a lipophilic core which is generally believed to be the location where hydrophobic solutes are dissolved (solubilised). Therefore, the degree of solubilization is dependent on the volume of the core or the length of the hydrophobic hydrocarbon chain (Mulley, 1964). However, it has been shown that when non-ionic surfactants with a polyoxyethylene (POE) head group are used, the interface between the hydrophobic core and hydrophilic layer of the micelle can act as an alternative site for solubilization (Elworthy et al, 1968).



Increasing Surfactant Concentration

Figure 1-6: Schematic diagram of surfactant aggregation behaviour in water (Bauer et al, 1997)

When surfactants are used at concentrations below CMC, they may still improve the oral bioavailability of a hydrophobic drug by:

- Adsorbing onto the surface of the drug particle and therefore improving wetting and subsequently dissolution (Martis et al, 1972; Short et al, 1972; Rees and Collett, 1974)
- Adsorbing onto and subsequently penetrating into the GI membrane, altering both the fluidity and permeability of the membrane (Elworthy et al, 1968; Gibaldi and Feldman, 1970)

1.7.2.2 Cosolvency

Cosolvency is another effective and readily available method of improving the saturation solubility of poorly water-soluble drugs. This method involves the addition of a cosolvent to an aqueous system (Jouyban-Gharamaleki et al, 1999). Cosolvents are water-miscible substances composed of a hydrogen bond donor and/or acceptor group and a hydrocarbon region. When added to water, the hydrophilic hydrogen-bonding groups ensure water miscibility while the hydrocarbon regions interfere with the clusters of hydrogen-bonded water molecules resulting in a reduction of the overall intermolecular attraction of water. This results in a decrease in the ability of water to 'squeeze out' nonpolar hydrophobic drugs and therefore, increases the saturation solubility of the drug. Cosolvency can also be described as a technique where the water is made more non-polar to aid the solubility of the hydrophobic compound. Due to the fact cosolvency requires an aqueous system, it is used mostly for parenteral dosage forms with the more common cosolvents being ethanol, propylene glycol (PG), glycerin and glycerol (Millard et al, 2002).

1.7.2.3 Prodrugs

Besides altering the saturation solubility of a poorly water-soluble drug by using excipients, there has been work whereby the actual chemical structure of the drug has been altered to form more soluble molecules; i.e. prodrugs. Prodrugs have been defined as a bioreversible chemical derivative of an active parent drug (Anderson, 1985). This approach requires the prodrug to be able to convert back to the active parent drug prior to reaching the site of action (Amidon, 1981). Examples of chemical modifications which have been shown to improve the solubility of drug compounds include the esterification of hydroxyl, amine or carboxyl groups with a moiety (progroup), the formation of the water-soluble salts of the parent drug. Once ingested, the prodrug is converted back to the active drug at certain sites in the body, e.g. small intestine, through

enzymatic, nonenzymatic or a combination of both processes (Testa and Joachim, 2002).

The esterification of drug compounds with progroups to produce a drug derivative or prodrug increases the aqueous solubility of the drug by either introducing an ionisable function or reducing the intermolecular interactions responsible for the low solubility (Fleisher et al, 1996). For instance, fosphenytoin, a sodium phosphate ester derivative of phenytoin shows a 7000-fold increase in aqueous solubility compared to phenytoin (Varia et al, 1984). In some cases where the parent drug shows better permeation characteristics than the prodrug, the latter are designed such that enzymatic reconversion to the parent drug occurs at the mucosal membrane by membrane-bound enzymes to take advantage of this.

1.7.2.4 Complexation

Complexants are hydrophilic molecules which react chemically with the drug compound resulting in an intermolecular complex with increased solubility compared to the parent drug. The complex formed is specific and reversible to enable the drug to be released and to ensure maximum therapeutic efficiency. Examples of complexants include water-soluble macromolecules such as polyvinylpyrrolidone (PVP), polyethylene glycols (PEGs) and cyclodextrins (Stella and Rajewski, 1997; El Arini and Leuenberger, 1998; Pinnamaneni et al, 2002; Emara et al, 2002). Other excipients such as sodium salicylate and sodium benzoate have also been shown to be effective as complexing agents for certain drugs (Saleh et al, 1969; Jain et al, 1988).

1.7.3 Solid Dispersions

The concept of solid dispersions was first introduced in the 1960s when Sekiguchi and Oki produced a eutectic mixture of the drug, sulphathiazide and the water-soluble carrier, urea by melting the physical drug-carrier mixture together before allowing this mixture to cool (Sekiguchi and Obi, 1961). On cooling, the eutectic mixture was milled to reduce the particle size. When compared to conventional preparations, this solid dispersion showed an enhanced dissolution rate as well as better bioavailability. This concept was further developed through the production of solid solutions (Levy, 1963; Kanig, 1964). Eutectic mixtures are composed mostly of a physical mix of fine drug and carrier crystals although depending on the conditions, may contain a fraction of molecularly dispersed drug. Solid solutions, on the other hand, are composed solely of a molecular dispersion of drug within the carrier matrix (Goldberg et al, 1965; Goldberg et al, 1966a). Molecular dispersion is considered to be the ultimate particle size reduction (Goldberg et al, 1966b).

When the eutectic mixtures or solid solutions are exposed to the aqueous media, the water-soluble carrier dissolves leaving a fine colloidal drug dispersion with a high effective surface area for dissolution (Serajuddin, 1999). In solid solutions, dissolution rate is also enhanced by the fact that the drug has no crystal structure, and therefore, no energy is required to break up the crystal structure (Taylor and Zografi, 1997). Furthermore, when the carrier is dissolved, the drug is presented as a supersaturated solution with the dissolved carrier believed to be able to inhibit precipitation of the drug (Simonelli et al, 1976; Hilton and Summers, 1986; Usui et al, 1997). It has also been speculated that if precipitation does occur, the drug precipitates as a metastable polymorph with a higher solubility than the stable polymorph (Ford and Rubinstein, 1978; Hilton and Summers, 1986). Other studies have shown that the water-soluble carriers can act as wetting agents, even those that do not have surface activity such as urea, while surfactant carriers can contribute to solubilising or cosolvent effects (Chiou and Riegelman, 1971; Leuner and Dressman, 2000).

Solid dispersions are basically produced by either melting the drug together with the water-soluble carriers (hot melt method) or dissolving the drug and carrier in a common organic solvent followed by solvent removal by using techniques such as evaporation, spray drying or freeze drying (solvent method).

As mentioned previously, the hot melt method produces eutectic mixtures where part of the drug may be molecularly dispersed. The amount of drug molecularly dispersed depends largely on a combination of three factors - the degree of supersaturation, the rate of cooling and the miscibility of the drug and carrier when heated. When the melted drug-carrier mixture is cooled, supersaturation occurs and the drug precipitates out as crystals. Due to the solidification of the mixture, these crystals remain dispersed within the carrier matrix. In cases where supersaturation is high, the eutectic mixture formed will contain very little molecularly dispersed drug as most of the drug will precipitate out of the mixture. The rate of cooling, on the other hand, affects the time available for recrystallisation to occur. A rapid cooling rate results in a quicker transition from the liquid (molten) to solid state, thus giving the drug less time to recrystallise and therefore, favours molecular drug dispersion (Save and Venkitachalam, 1992; Urbanetz and Lippold, 2005). Finally, in order for drugs to be molecularly dispersed, both drug and carrier must be completely miscible when in the molten state. Miscibility gaps usually leads to a product which is not molecularly dispersed. The main limitation of the hot melt method is that it requires a high temperature which makes this technique unsuitable for thermolabile drugs. As a result in the 1970s and 1980s, the solvent method was more popular for producing solid dispersions. However, the introduction of the hot melt extrusion technique, where the drug-carrier mixture is simultaneous melted, homogenised and extruded in the form of tablets, granules, pellets, sheets, sticks or powders, has resulted in a renewed interest in the hot melt method. One huge advantage of hot melt extrusion is that during this process, the drug-carrier mixture is only subjected to high temperatures for about 1 min thus allowing somewhat thermolabile drugs to be used (Leuner and Dressman, 2000).

The second method of producing solid dispersions, the solvent method was introduced by Tachibani and Nakumara (1965). The main requirement of this method is that both drug and carrier are reasonably soluble in the organic solvent. When first introduced, this method was preferred over the hot melt method as it does not require the use of high temperatures and therefore can be used to produce solid dispersions of thermolabile drugs as well as allowing the use of heat-sensitive carriers and carriers with high melting points such as polyvinylpyrrolidone (PVP) (~ 300°C). However, the main problems with the solvent method are that small variations in the conditions when organic

solvents are removed result in large changes in product performance and the toxicity issues surrounding the use of organic solvents (Chiou and Riegelman, 1971; Ford, 1986; Foster et al, 2002). This, together with ecological and subsequent economical problems related to the use of large quantities of organic solvent, has seen a decrease in popularity of this method (Chiou and Riegelman, 1969; Serajuddin, 1999; Leuner and Dressman, 2000).

Although many studies involving solid dispersions have been carried out, very few solid dispersion systems have been marketed. The latter include a griseofulvin-polyethylene glycol dispersion (Gris-PEG from Norvatis) and a nabilone-povidone dispersion (Cesamet from Norvatis). Another formulation consisting of a troglitazone-PVP dispersion (Romozin from Glaxo-Wellcome) was taken off the market due to toxicity issues related to the drug (Chokshi and Zia, 2004).

1.8 Research Scope

The poor oral bioavailability of Class II drugs is usually a direct consequence of their poor aqueous solubility and therefore, slow dissolution rate. This slow dissolution rate acts as the rate-limiting step to absorption and bioavailability. Subsequently, to increase the bioavailability of drugs in this class, improvements to the dissolution rate must be made.

The work in this thesis aims to improve the dissolution rate and consequently bioavailability of model hydrophobic drugs by decreasing particle size, improving their wetting behaviour and possibly increasing their saturation solubility. It was proposed that the first of these aims be achieved by producing micro- and nanoparticles of the model drug using techniques such as spray drying and solvent-diffusion. Hydrophilic non-ionic surfactants were used to achieve the latter two aims.

The first part of the work concentrated on the particle design process. Different surfactants and surfactant blends were used at various concentrations to assess their suitability for particle formation and wetting ability. Once

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satisfactory particles were produced, they were subjected to *in vitro* studies to determine their dissolution rates and characterised using a variety of techniques including scanning electron microscopy (SEM), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and low angle laser light scattering (LALLS).

The second part of the work focused on the *in vivo* studies using rats as the animal model. The particles which showed enhanced dissolution rates were encapsulated in a gelatin capsule and administered orally to the rats. The drug concentration in the plasma sample was determined and used to calculate the area under the curve (AUC) and maximum plasma concentration (C_{max}). This pharmacokinetic data was then used to assess the bioavailability of the novel particles in comparison to the control.

Chapter 2: Organogels and Amphiphilogels

2.1 Introduction

In recent years, the discovery of a number of biocompatible substances capable of gelling various organic solvents has resulted in the emergence of organogels as novel drug carrier systems (Anand et al, 2001). The following work was aimed at producing organogel microparticles containing the model hydrophobic drug, indomethacin.

2.1.1 Gels

Gels are an intermediate state of matter containing both solid and liquid components. Gels are difficult to define and over the years various statements have been made to define gels in different ways. Herman (1949) defined gels as a colloid disperse system that exhibits solid-like mechanical properties and which consists of at least two different components that extend themselves continuously throughout the whole system. This definition was further extended by Flory (1974) who added that gels must have a continuous structure such as lamellar structures, disordered physically aggregated polymer networks, covalent polymeric networks or particulate structures. However, it has to be said that the statement made by Lloyd in 1926, that gels are a colloidal condition which is easier to recognise than define still holds true today.

Besides classifying gels by their continuous structure, gels can be classified based on criterias such as the nature of its dispersing phase, the type of bonds holding its three-dimensional network together or the number of phases it contains (Table 2-1).

Criteria	Examples of Gel Types
Bond nature	Covalent bonds
	Hydrogen bonds
	van der Waals forces
Number of phases	Single phase
	Two phase
Liquid phase nature	Aqueous
	Organic
Continuous structure type	Lamellar structure
	Disordered physically aggregated polymer
	networks
	Covalent polymeric network
	Particulate structure type

Table 2-1: Methods of classifying gels

Chemical gels are formed when strong covalent bonds hold the gel together while physical gels are gels which rely on hydrogen bonds and electrostatic van der Waals forces to hold them together. A single phase gel is a gel which consists of macromolecules dispersed uniformly within a liquid with no clear boundary between the two components and a two phase gel is a suspension containing discrete solid particles. When a gel contains an aqueous continuous phase, the gel is considered to be a hydrogel. An organogel, on the other hand, is a gel containing an organic solvent as the continuous phase.

2.1.2 Organogels and Amphiphilogels

Organogels are generally formed by decreasing the interaction of the gelator molecules (organogelators) with the organic solvent (Anand et al, 2001). There are a number of organogel systems which have been developed and the organogels can be classified based on the type of gelator molecules used. Some of the more established organogels systems are briefly discussed below, including the non-ionic surfactant-based organogel system which was used for the work in this chapter. Certain compounds with a low molecular mass have been shown to be capable of causing the gelation of organic solvents such as n-alkane and 1-octanol when present in low concentrations ($\leq 2\%$ w/w). These compounds have been termed "low molecular mass organogelators" or LMOGs and organogels formed using LMOGs named LMOG organogels. When LMOGs are dissolved in an inorganic solvent, then cooled, aggregation of the LMOGs occurs. This aggregation eventually leads to the formation of fibres, strands or tapes which interlink to form a three dimensional network that 'encapsulates' the liquid phase to inhibit its flow and therefore cause gelation. The gelation is thermoreversible; i.e. the gels can be cycled repeatedly through their pregelation (sol) phase by heating and cooling. Examples of compounds which can act as LMOGs include cholesteryl 4-(2-anthryloxy)butanoate (CAB), 2,3-di(dodecyloxy)anthracene (DDOA) as well as some long chain n-alkanes; e.g. toluene and n-octacosane (Abdallah and Weiss, 2000a; Abdallah and Weiss, 2000b).

Another class of organogels are the lecithin-based organogels. These gels are produced when trace amounts of a polar substance, e.g. water is added to a reverse micellar solution of lecithin in an organic solvent, e.g. isooctane. The addition of water induces a uniaxial growth of the spherical micelles resulting in cylindrical aggregates. Above a threshold length, these aggregates begin overlapping to form a temporal three dimensional network causing gelation (Scartazzini and Luisi, 1988; Schipunov, 2001).

Organogels can also be produced by adding acidic phenolic compounds such as p-chlorophenol and p-cresol to an anhydrous solution of the twin-tailed anionic surfactant, bis(2-ethylhexyl) sodium sulfosuccinate (AOT) in an organic non-polar solvent such as isooctane. As with lecithin, AOT forms spherical reverse micelles in non-polar solvents. When the phenolic compound is added such that the phenol : AOT ratio is approximately equal, an optically clear organogel forms. Fourier transform infrared (FTIR) experiments have suggested that gelation is the result of hydrogen bonds forming between the AOT molecules and the phenolic species and AOT-phenol organogels have been shown to break down when exposed to trace amounts of moisture (Xu et al, 1993; Singh et al, 2004).

As mentioned earlier, the organogel system used in this work is the non-ionic surfactant-based organogel system. In this system, a hydrophobic non-ionic surfactant such as Span 60 (sorbitan monostearate) (Figure 2-1) is dissolved in a hot organic solvent, e.g. isopropyl myristate, hexane or hexadecane (Murdan et al, 1999b; Murdan et al, 1999d). When this hot solution is cooled, the formation of a gel occurs. These gels are thermoreversible and have been proposed to be used as carriers for vaccines (Murdan et al, 1999c).



Figure 2-1: Chemical structure of Span 60

More recently, a sub-class of these non-ionic surfactant-based organogels have been introduced (Jibry et al, 2004). This novel organogel uses a liquid surfactant as the continuous phase and has been termed amphiphilogels based on the amphiphilic nature of the continuous phase. Amphiphilogels have been shown to be capable of dissolving poorly water-soluble drug compounds such as cyclosporin and ibuprofen for both oral and transdermal purposes. Studies have shown that when cyclosporin-containing amphiphilogels were given orally to mice and dogs, high oral absorption and therefore, good bioavailability was seen (Murdan et al, 1999a; Murdan and Andrýsek, 2003). It has been suggested that the surfactants in the gel are capable of exerting both a solubilising and penetration enhancing effect (Jibry et al, 2004). Another advantage of these amphiphilogels is that since they are composed of nonionic surfactants, they are less likely to cause irritation. Indeed, in a study carried out by Jibry and Murdan (2004), it was shown that amphiphilogels composed of 80% Tween 80 and 20% Span 60 did not cause any more irritation to the skin than the negative control (aqueous cream BP) following repeated applications.

sThe work in this chapter aims to use both non-ionic surfactant-based organogels and amphiphilogels to produce indomethacin-containing microparticles. It was hoped that the surfactants in the gel would aid in drug wetting and solubilization whilst the relatively small size of the microparticles would increase the effective surface area available for dissolution.

2.1.3 Indomethacin – Model Drug

The model drug chosen, indomethacin (Figure 2-2), is a member of the nonsteroidal anti-inflammatory drug (NSAID) class. It has anti-inflammatory, analgesic and antipyretic activity (Joint Formulary Committee, 2004). Indomethacin is indicated for the treatment of pain and moderate to severe inflammation in rheumatic disease and other acute musculoskeletal disorders, acute gout and dysmenorrhea. The recommended oral dose of indomethacin for the treatment of rheumatic disease is 50-200 mg daily in divided doses and for acute gout, 150-200 mg daily in divided doses.



Figure 2-2 : Chemical structure of indomethacin

Like other members of the NSAID class, indomethacin acts by inhibiting prostaglandin biosynthesis. Some of the most common adverse effects of taking indomethacin are gastrointestinal bleeding and ulceration.

Indomethacin is an aryl acetic acid derivative. The chemical name for indomethacin is 1-(p-chlorobenzyl)-5-methoxy-2-methylindole-3-acetic acid. Other chemical and proprietary names for indomethacin are listed in the monograph for indomethacin in the Merck Index (1996).

Indomethacin is a pale yellow to tan crystalline powder with a slightly bitter taste. It is odourless or almost odourless with a molecular weight of 358. It is practically insoluble in water as shown by its high log P hydrophilic/hydrophobic ratio (HHR) value of 4.27 (Fini and Feroci, 1995).

Indomethacin is known to exist in at least two polymorphic forms. Yamamoto (1968) refers to these forms as the γ -type (melting point about 162°C) and α -type (melting point about 155°C). These two forms have also been referred to as Form I and Form II respectively. Form I, the polymorph with the higher melting point and lower solubility is the more thermodynamically stable crystalline form of indomethacin and is therefore more commonly used in pharmaceutical preparations. However, Form I and II are equivalent in terms of bioavailability and activity. In addition to the two polymorphic forms, indomethacin can also form solvates with benzene, t-butyl alcohol and other solvents (O'Brien et al, 1984).

2.2 Materials

Indomethacin, Span 60 (sorbitan monostearate), Tween 80 (polysorbate 80) were obtained from Sigma Aldrich Company (Poole, UK). Dichloromethane was purchased from BDH (VWR International Ltd., Poole UK) and isopropyl myristate was purchased from Fluka Chemika (Germany).

All chemicals were used as purchased unless otherwise stated. The water used in all the experiments was deionised using Elga Option 4 water purifier unless otherwise stated.

2.3 Methods

2.3.1 Preparation of Amphiphilogel (Span 60 and Tween 80)

In the initial experiments, Span 60 was used as the solid organogelator while Tween 80 was chosen as the continuous phase. First, the solid organogelator was weighed out into a glass vial and the required amount of continuous phase was added. The vial was then placed in a water bath heated to 50°C until the organogelator had completely dissolved thus forming the sol phase. The glass vial was removed from the water bath and allowed to stand at room temperature to cool and form the amphiphilogel.

The weight ratio of Span 60 : Tween 80 was varied from 1:1 to 1:9 to find the ratios at which amphiphilogels could be produced. These ratios were then used in subsequent experiments.

The next step was to determine the solubility of indomethacin in these amphiphilogels. The pre-made amphiphilogels were placed in a heated water bath (50°C) until they melted to the sol phase. Indomethacin was weighed into glass vials and a set amount of the sol phase was added to each vial. The vials

were vortexed briefly to ensure the drug was well dispersed within the sol and then returned to the heated water bath. The drug-sol mixture was left in the water bath for 24 hr, with occasional vortexing.

After 24 hr, the drug-sol mixture was removed from the bath and examined visually for the presence of indomethacin particles. It is recognised that this was a crude way of determining indomethacin solubility but it was sufficient for the required purpose. A more accurate determination was limited by the opacity and the solid-like nature of the gel. The saturation solubility of the drug was determined as the concentration between the highest concentration at which the drug dissolved and the lowest concentration whereby it failed to dissolve (James, 1986).

2.3.2 Preparation of Organogel-in-Water Emulsions

The appropriate indomethacin-organogel was selected from the previous experiments, and used to produce an organogel-in-water emulsion. The criteria used for the selection of the gel were that the gel should have a relatively high concentration of indomethacin as well as a suitable hydrophilic-lipophilic balance (HLB) for making the emulsion. The suitable HLB range for an oil-in-water emulsion is 5 to 15.

The indomethacin-organogel and deionised water were heated separately in the water bath (50°C). Once the sol formed, it was added slowly to the warm water while homogenising (Ultra-Turax T25 Basic, IKA Werke) at 24 000 rpm for 3 min to form a coarse emulsion. This coarse emulsion was then microfluidized (Microfluidizer M110S, Microfluidics Corporation, USA) five times at 800 bar to form a fine emulsion. The emulsion was freeze dried (Drywinner 110, Heto-Holten A/S, Gydevang, Denmark) for approximately 12 hr.

2.3.3 Preparation of Organogels and Organogel-in-Water Emulsions using (Span 60 and Cinnamaldehyde)

The experiments described in Section 2.3.1 and 2.3.2 were repeated using Span 60 as the organogelator and cinnamaldehyde as the continuous phase, in place of Tween 80.

2.3.4 Other Organogels

Other organic solvents, such as ethyl acetate, poly(dimethylsiloxane) and 1octanol were added to the organogelator, Span 60, to see if novel non-ionic surfactant-based organogels could be formed. Where appropriate, these gels were then used for further experiments.

2.3.5 Organogel Characterisation

The microstructure of the organogels and amphiphilogels were examined using a light microscope (Nilkon Microphot FXA) fitted with a temperature controlled hot-stage (Linkam BSC 196, England).

2.4 Results and Discussion

2.4.1 Physical Characteristics

2.4.1.1 Amphiphilogels (Span 60 and Tween 80)



Figure 2-3: Span 60/Tween 80 amphiphilogel at room temperature

When Span 60 and Tween 80 were heated in the water bath, Span 60 dissolved to produce a clear, colourless sol. On cooling, this sol formed an opaque, white gel (Figure 2-3). It was found that the hardness of the gel increased as the ratio of Span 60 : Tween 80 increased. When the ratio of Span 60 : Tween 80 was 1:9, no gelation occurred. Instead, a very viscous liquid was produced. Increasing the Span 60 : Tween 80 ratio to 1:1, on the other hand, led to the formation of a very hard gel formed.

When indomethacin was added to the sol phase, the sol turned yellow in colour. On cooling, the amphiphilogels formed were also yellow in colour instead of white (Figure 2-4). The intensity of the colour increased with increasing indomethacin concentration suggesting that indomethacin was responsible for the colour change. It was also found that the saturation solubility of the drug increased as the ratio of Span 60 : Tween 80 decreased and that indomethacin solubility showed a linear correlation with the concentration of Tween 80 present in the amphiphilogel (Figure 2-5).



Figure 2-4: Span 60/Tween 80 amphiphilogel containing indomethacin at room temperature



* Data points show the highest dissolved concentration of indomethacin



The amphiphilogel composed of a Span 60 : Tween 80 ratio of 1:4 was chosen for further experiments because it was the ratio at which the maximum amount of indomethacin could be incorporated while still being able to form a gel.

2.4.1.2 Organogels (Span 60 and Cinnamaldehyde)

As cinnamaldehyde is yellow in colour, a clear yellow sol was formed when it was heated together with Span 60. On cooling, the sol formed an opaque yellow gel with a cinnamon-like smell. As with Tween 80, the hardness of the gel was dependent on the Span 60 concentration. When the Span 60 : cinnamaldehyde ratio was 1:1, the hardest gel formed and when the ratio was 1:9, no gel formed.

When indomethacin was added to the sol phase, the sol became a darker yellow. The saturation concentration of indomethacin varied with the percentage of cinnamaldehyde present in the organogels; saturation solubility increased with increasing cinnamaldehyde concentration.

2.4.1.3 Other Organogels

Further experiments with other solvents as the continuous phase showed that although Span 60 could be dissolved in the more polar solvents such as ethyl acetate and 1-octanol when heated, gelation did not occur on cooling.

Gelation did occur when the solvent poly(dimethylsiloxane) was used as the continuous phase. However, indomethacin was insoluble in this organogel and therefore was not suitable to be used in this work.

2.4.2 Gel Microstructure

2.4.2.1 Amphiphilogels (Span 60 and Tween 80)

The gel microstructure was examined using hot-stage light microscopy. At 25°C, the amphiphilogels consisted of star-like tubules (Figure 2-6). These clusters of approximately 50 μ m in diameter were dispersed relatively uniformly thoughout the gel. As the temperature of the gel was increased to approximately 35 – 40°C, to form the sol phase, these clusters dissolved into the solvent. The temperature at which the gel converted into its sol phase increased with increasing gelator (Span 60) concentration. On cooling, these star-like clusters re-formed as the gel re-solidified. The number of clusters present increased with increasing Span 60 concentration thus suggesting that Span 60 was responsible for the formation of these clusters.

It is proposed that the clusters are linked to one another to form a threedimensional network which is responsible for the gelation of the amphiphilogels. At lower temperatures, the affinity of Span 60 for Tween 80
decreases and therefore, self-assembly of the gelator molecules into the starlike cluster structures occurs. These clusters link together to form a coherent three-dimensional network throughout the continuous phase which immobilises it.





Figure 2-6: Microstructure of Span 60/Tween 80 amphiphilogel at 25°C (top) and 40°C (bottom)

The addition of indomethacin did not appear to significantly change the microstructure of the gel when examined using light microscopy.

Organogels (Span 60 and Cinnamaldehyde)

When examined using hot-stage microscopy, star-like clusters which appeared more tubular that those of the Span 60/Tween 80 amphiphilogels were observed (Figure 2-7). As with the amphiphilogels, these star-like clusters





Figure 2-7: Microstructure of Span 60/cinnamaldehyde organogels at 25°C (top) and 40°C (bottom)

started to dissolve when the microscope stage was heated to approximately 35 - 45°C, around the temperature at which the sol phase started forming. As

before, the conversion from gel to sol phase occurred at higher temperatures when the gelator concentration was increased. On cooling, the star-like clusters re-assembled and the gel phase was re-formed.

The presence of indomethacin at low concentrations (< 10% w/w) did not affect the microstructure of the gel. At higher concentrations, needle-shaped crystals of indomethacin were seen in the gel. The needle-like crystals of indomethacin were capable of 'gelling' pure cinnamaldyde at a concentration of 20%, without the addition of Span 60.

2.4.3 Gel-in-Water Emulsions

2.4.3.1 Amphiphilogels (Span 60 and Tween 80)

When indomethacin-containing amphiphilogels formed from Span 60 and Tween 80 were used added to water (amphiphilogel : water = 3 : 7), homogenised and microfluidised, a milky yellow emulsion was formed. This emulsion was stable for more than 24 hr with no indomethacin crystal formation. However, no distinct interface between the organogel phase and water phase could be seen (Figure 2-8). This is probably due to the high solubility of Tween 80 in water.



Figure 2-8: Span 60/Tween 80 amphiphilogel after microfluidization

After freeze drying the gel-in-water emulsion, a sticky yellow pellet formed. No obvious particles were seen. When the freeze dried product was examined using SEM, no discrete particles were seen. Instead, small particles which were covered by a layer of viscous substance were seen (Figure 2-9). This layer of probably Tween 80 which was not removed by freeze-drying that was covering the Span 60/indomethacin particles.



Figure 2-9: Span 60/Tween 80 amphiphilogel emulsion after freeze drying

When rotary evaporation was used as an alternative method of removing the aqueous phase of the emulsion, long needle-like crystals of indomethacin formed. The combination of heat and slow rate of evaporation allowed crystallisation to take place.

2.4.3.2 Organogel (Span 60 and Cinnamaldehyde)

When the Span 60/cinnamaldehyde organogel was homogenised and microfluidised in the same ratio as the amphiphilogel (Section 2.4.3.1), a pale yellow emulsion which was stable for more than 24 hours formed. Under the light microscope, two distinct phases – organogel and water – were seen with the organogel droplets being less than a micron in diameter (Figure 2-10).

Unfortunately, this emulsion could not be completely dried using the freeze drying process. Despite being a volatile oil, cinnamaldehyde remained on the final product and as a result, a sticky semi-solid paste was produced.



Figure 2-10: Span 60/cinnamaldehyde organogel-in-water emulsion after microfluidization

2.5 Chapter Conclusions

The work in this chapter showed that in addition to using the previously established solvents such as hexane, isopropyl myristate and vegetable oils, other organic solvents such as cinnamaldehyde and poly(dimethylsiloxane) could be gelled by the organogelator, Span 60. It was found that gelation occurred when the less polar solvents were used as the continuous phase since gelation is a consequence of the reduction in affinity between the gelator and the solvent on cooling. When more polar solvents such as ethyl acetate were used, Span 60 was too soluble in them and therefore, remained in solution rather than gelling when cooled.

The model drug, indomethacin was successfully incorporated in the amphiphilogel consisting of Span 60 and Tween 80 as well as the organogel composed of Span 60 and cinnamaldehyde. Both this amphiphilogel and organogel could be microfluidised with water to form a stable emulsion without any precipitation of the drug occurring. However, difficulty arose when trying to remove the aqueous phase from the emulsion leading to the formation of an adhesive pellet/paste rather than discrete gel microparticles.

If these emulsions were administered *in vivo*, these indomethacin-containing emulsions may well enhance the bioavailability of the drug. This is because indomethacin had already dissolved in the gel phase of the emulsion and therefore, dissolution, which is the rate-limiting step to indomethacin absorption when given orally, would not be a factor. However, this was not the aim of this work. Therefore, other techniques, spray drying and solvent-diffusion (Chapter 3) were used to produce microparticles and nanoparticles of indomethacin coated with a hydrophilic surfactant layer.

Chapter 3: Indomethacin – Particle Design and Dissolution Studies

3.1 Introduction

Since particles could not be obtained from organogels and amphiphilogels, alternative approaches were needed to produce the required particles. The two techniques selected, spray drying and solvent-diffusion have been shown to be capable of producing micro- and nanoparticles.

The introduction to this chapter reviews spray drying and solvent-diffusion in relation to particle production. The characteristics of the particles produced from these techniques such as the crystallinity and its possible effects on the dissolution rate will also be discussed.

3.1.1 Spray Drying

Spray drying is a technique which has been used successfully to enhance the dissolution rate of poorly water-soluble drugs and therefore, possibly improve their bioavailability. The improved dissolution rate comes about either through the alteration of physicochemical properties caused by the spray drying process, for example, the transformation of a crystalline material into its amorphous state (Di Martino et al, 2001), or by spray drying the poorly water-soluble drug with hydrophilic polymers such as poly(vinyl pyrrolidone) (PVP) (Paradkar et al, 2004) and polyethylene glycol 6000 (PEG 6000) (Weuts et al, 2005).

The spray drying process converts solutions (Berggren et al, 2004; Asada et al, 2004), emulsions (Hansen et al, 2004; Steckel and Brandes, 2004), slurries (Pham et al, 2002) or pastes (Bhandari et al, 1999) into dry particulate systems. This process takes involves four sequential steps – atomisation, spray-gas contact, drying of the spray and separation of dried sample from the gas (Masters, 1991; Broadhead et al, 1992).

3.1.1.1 Spray Drying Process

The spray drying process starts with the atomisation of the liquid feed where the feed is broken up into individual droplets forming a spray. There are various types of atomisation systems such as rotary atomisation, nozzle atomisation, pneumatic atomisation and two-fluid atomisation. The work carried out in this study uses the pneumatic atomisation system whereby the compressed gas (air from the atmosphere) is used to atomise the feed solution (Masters, 1991).

Atomisation is followed by spray-gas contact where the spray formed is then brought into contact with heated air to start the drying process.

Next, the drying of the spray takes place and this step can be divided into four sequential stages (Figure 3-1).



Moisture Contect (weight of moisture per unit dry product)



In the first stage, the warm up period (a), the droplet comes into contact with the hot air resulting in the evaporation of the solvent from the droplet surface. The temperature of the droplet surface increases until an equilibrium between the surface and the hot air occurs.

This is followed by the constant evaporation stage (b) which continues as long as there is sufficient moisture within the droplet to replenish the moisture lost from the surface. When the so-called critical point occurs; i.e. the moisture content of the droplet is too low to maintain the saturated surface conditions, the third stage (c) begins.

During this stage, the drying rate falls dramatically while the temperature of the droplet increases. If the boiling point of the solvent is higher than the air temperature, the solvent will vaporise and depending on the type of crust formed, the particle may remain intact, fracture, inflate or collapse (Figure 3-2). For instance, a high internal pressure brought about by vaporisation of the solvent would lead to the fracture of the particle with a hard, non-porous crust while a particle with a pliable shell would become inflated (Oakley, 1997).



Figure 3-2: Schematic diagram shows the fate of the droplet through the stages of the spray drying process depending on the nature of the crust formed upon drying (adapted from Oakley, 1997) In the final stage (d), the evaporation rate continues to decrease since the droplet surface provides resistance to drying. This will occur until equilibrium is reached. However, the product is usually collected before equilibrium.

Once the spray is dried, the final step of the spray drying process, product recovery, takes place. As its name suggest, this step involves the separation of the spray dried product from the air stream and it works by cyclonic air flow within the cyclone separator.

3.1.1.2 Factors affecting spray dried product

The type and size of particle formed can be controlled to a certain extent by changing certain variables on the spray dryer. The controllable variables in the spray drying process are the inlet temperature, aspirator speed, air flow rate and liquid feed rate and concentration. However, all these variables are interdependent; i.e. changing one variable will result in changes in the other variables.

The inlet temperature may affect the particle size. However, the effect is dependent on the material being spray dried (Crossby and Marshall, 1958). For instance, the particle size of coffee extract was significantly decreased when the inlet temperature was increased. However, inlet temperature changes did not have any effect on the size of crystalline sodium sulphate (Broadhead et al, 1992).

Aspirator speed affects the degree of separation in the cyclone; the greater the aspirator speed, the higher the separation. However, at very high aspirator speeds, fine particles may be lost in the air stream exhaust.

The air flow rate is the amount of compressed air used to convert the liquid feed into fine droplets. The higher the air flow rate, the smaller the size of the particles produced.

The liquid feed concentration and flow rate affects both particle size and density. Generally, a liquid feed containing a high concentration of dissolved solids will produce larger and more porous particles. The bulk density of the final particles is usually higher when spray drying hydrophobic materials at a fast feed rate (Masters, 1991). Hydrophilic materials usually result in powders with a lower bulk density.

3.1.2 Solvent-Diffusion

Solvent-diffusion, also referred to as solvent displacement or nanoprecipitation, is a technique which was first described and patented by Fessi et al in 1987 (Quintanar-Guerrero et al, 1998). In this process, a synthetic polymer such as poly(lactic acid) (PLA), poly(lactide-co-glycolide) (PLGA) or poly(ε -caprolactone) (PCL) and drug are dissolved in a water-miscible solvent (e.g. ethanol or acetone). This drug/polymer mixture is added to an aqueous solution containing a stabiliser (e.g. poly(vinyl alcohol) (PVA) or poloxamer) under magnetic stirring. Ideally, the drug and polymer are only soluble in the solvent phase and not in the aqueous phase. On mixing, the water-miscible solvent will rapidly diffuse into the aqueous phase, thus resulting in the precipitation of both polymer and drug as nanoparticles, where the polymer surrounds the drug (Quintanar-Guerrero et al, 1998). This is shown schematically in Figure 3-3.





The formation of nanoparticles by this technique involves a complex interfacial hydrodynamic phenomenon (Fessi et al, 1989). The process is governed by the Marangoni effect caused by the interfacial turbulence occurring at the interface of the solvent and non-solvent as a result of complex and cumulative variables such as flow, diffusion and surface tension (Bilati et al, 2004). This turbulence causes a violent spreading due to the mutual miscibility of the solvent and the aqueous phase. As a result, solvent droplets of a nanometrical size are torn from the interface. These droplets are then rapidly stabilised by the stabilising agent until diffusion of solvent is complete and the polymers have aggregated.

The main disadvantage of this technique is that it is only suitable for drugs of a hydrophobic nature with limited solubility in water. This is because drug leakage into the aqueous medium leads to decreased entrapment efficiently. Another problem is finding a combination of drug, polymer, solvent and non-solvent where the drug and polymer are sufficiently soluble in the solvent and non-solvent respectively and solvent and non-solvent are miscible with each other (Quintanar-Guerrero et al, 1998; Bilati et al, 2004).

3.1.3 Crystalline and Amorphous Solids

Drug solids can be crystalline or amorphous depending on the processing methods and storage conditions used. The following section discusses these different solid states and their effects on the dissolution rate and stability of the drug.

3.1.3.1 Crystalline Solids and Polymorphism

A crystal is a solid mass of atoms or molecules which are packed in a definite repeating pattern, showing both long and short-range order. This high degree order results in the development of definite crystal faces leading to the formation of a specific external shape called the crystal habit, to be formed. The crystals may vary in the development of various faces and sizes but the angle between any two adjacent faces is a constant value as described by the law of constant interfacial angles, first proposed by Hauy in 1784 (Mullin, 1997).

The basic unit in a crystal is known as a unit cell and the repetition of this unit cell in three dimensions gives rise to the crystal. Various types of crystal habits for example, needles, lamellar and columnar can be formed depending on the structure of the molecules involved and the crystallisation conditions involved (Florence and Attwood, 1998).

Polymorphism is used to describe the phenomena where the same compound exists in different crystal habits due to different crystal packing arrangements. Subsequently, different polymorphs of the same compound have different chemical and physical properties such as melting point, solubility, dissolution rate and bioavailability (Rustichelli et al, 2000). The most stable polymorph usually has the highest melting point and the lowest solubility which results in it also having the poorest bioavailability. These properties are due to the polymorph having a closer and more stable packing of the molecules. Given a sufficient amount of time, less stable (metastable) polymorphs will eventually convert to the most stable polymorphic form; the rate of conversion depending on the free energy difference between the metastable and stable form (Florence and Attwood, 1998). Indomethacin, as mentioned in the previous chapter (Section 2.1.3), exists in two polymorphic forms, γ and α , with the γ -form being the more stable polymorph.

3.1.3.2 Amorphous Solids

The term amorphous is derived from the Greek word *amorph* meaning 'without shape'. As this suggests, the term amorphous solids is used to describe solids that contain molecules which are randomly arranged. Amorphous solids can be defined in relation to the crystalline solids. Like the crystalline solid, an amorphous solid may show short-range molecular order but unlike the crystalline solid, an amorphous solid does not have any long-range order of molecular packing or arrangement (Yu, 2001). The presence of the short-range

molecular order in amorphous solids makes it different from the gas phase (Figure 3-4).



Figure 3-4: Schematic representation of the structure of an amorphous solid (adapted from Yu, 2001)

Amorphous solids are of a higher energy state that their corresponding crystals. Subsequently, this results in poorly soluble drugs having an enhanced solubility and dissolution rate (Yu, 2001; Ambike et al, 2004). However, the disadvantage of the amorphous solid is that its higher energy state makes it metastable and therefore, it tends to revert back to its more stable crystalline state upon storage. The stability of the amorphous material can be increased by storing below the glass transition temperature (T_g) and protecting it from plastisizers (e.g. water vapour) which can reduce its T_g temperature on storage (Hancock and Zografi, 1997). T_g is the temperature at which an amorphous material changes from its glassy state to the rubbery state or vice versa. Many amorphous substances have a T_g above room temperature, for instance indomethacin has a T_g of 40°C (Matsumoto and Zografi, 1999).

There are various pharmaceutical techniques which can be used to convert a crystalline substance to its amorphous form. They include quench cooling of a melt (Chawla et al, 2003), grinding (Oguchi et al, 2003) and as mentioned previously, spray drying (Di Martino et al, 2001; Ambike et al, 2004).

3.2 Materials

Indomethacin and sodium hydroxide were obtained from Sigma Aldrich Company (Poole, UK). Brij 76 (polyoxyethylene (10) stearyl ether) and Brij 35 (polyoxyethylene lauryl ether) were purchased form Fluka Chemika (Germany). Pluronic F108 and Pluronic F127 were obtained from BASF Chemical Company (Mount Olive, NJ, USA) while Gelucire 50/13 was obtained from Gattefosse Ltd. (Berkshire, UK). Potassium dihydrogen orthophosphate, dichloromethane (analytical grade) and acetone (analytical grade) were purchased from BDH (VWR International Ltd., Poole, UK).

All chemicals were used as purchased unless otherwise stated. The water used for all experimental work was deionised using Elga Option 4 water purifier unless otherwise stated.

3.3 Methods

3.3.1 Microparticle Production

3.3.1.1 Spray Dried Indomethacin

Dichloromethane was chosen as the solvent as it is non-flammable and therefore suitable for use in the Büchi 191 Mini Spray Dryer (Büchi Laboratories-Technik AG, Flawil, Switzerland) (Figure 3-5). Indomethacin has a solubility of 3% w/w in dichloromethane and preliminary experiments carried out showed that varying the indomethacin concentration in dichloromethane showed no significant difference in the size of the particles produced. Subsequently, a 1.5% w/w choosen to ensure that indomethacin did not precipitate out of solution particularly when surfactant was added. As the outlet temperature was dependent on inlet temperature and feed rate, these latter two parameters were adjusted to ensure the Towest outlet temperature could

be achieved in order to prevent the surfactant(s) used from melting. The optimised conditions used are as follows:

- Inlet temperature : 40°C
- Outlet temperature : 35°C
- Aspirator : 80% (-32 mbar)
- Feed rate : 7.5 ml/min
- Air flow rate : 700 NI/hr

The spray dried product was collected and stored in a desiccator at room temperature prior to further use.



Figure 3-5: Schematic diagram of the spray dryer used (adapted from Hill, 1999)

3.3.1.2 Spray Dried Indomethacin with Pluronic F108

Surfactant-coated indomethacin microparticles were produced by adding the non-ionic surfactant, Pluronic F108 to the 1.5% w/v indomethacin in dichloromethane solution. The spray drying conditions used were the same as in Section 3.3.1.1.

The spray dried product was stored in a desiccator at room temperature until ready to be used in further experiments.

3.3.1.3 Indomethacin Nanoparticles produced by Modified Solvent-Diffusion Technique

The theory behind the solvent diffusion technique has been described in the introduction (Section 3.1.2). In this work, the technique was modified such that the use of polymer was eliminated. Instead, a hydrophilic surfactant is used both to stabilise the drug and 'encapsulate' it. Acetone was chosen as the water-miscible solvent as the solubility of indomethacin in ethanol, which could be used as an alternative, is low (0.02% w/v).

Indomethacin was dissolved in acetone to form a 3% w/w solution. Aqueous surfactant solutions containing a range of non-ionic hydrophilic surfactant (e.g. Brij 76, Gelucire 50/13 and Pluronic F108 and Pluronic F127) at different concentrations were made.

The drug solution was slowly added to the surfactant solution while homogenising (T25 Ultra-Turax, IKA, Werke GmbH & Co. KG, Germany) at 24000 rpm for 5 min. The particles within the formed suspension were then examined using light microscopy, scanning electron microscopy (SEM) or when appropriate, transmission electron microscopy (TEM).

The drug suspensions which contained nano-sized particles were freeze dried (Drywinner 110, Heto-Holten A/S, Gydevang, Denmark) for approximately 24 hr or until all water was removed.

3.3.2 Particle Characterisation

3.3.2.1 Light Microscopy

The particles were dispersed in a small volume of water, dropped onto a glass slide, covered with a glass cover slip and examined under the light microscope (Nikon Microphot FXA) for the presence of indomethacin crystals.

3.3.2.2 Scanning Electron Microscopy (SEM)

Indomethacin particles were fixed on an aluminium stub with conductive double sided carbon adhesive tape. The samples were then sputter coated with gold for 3 min at 30mA (Emitech K550, Ashford, Kent, England). Scanning electron micrographs were then taken using a Philips/FEI XL 30 (Philips, Eindhoven, The Netherlands).

3.3.2.3 Transmission Electron Microscopy (TEM)

Indomethacin nanoparticles were stained with phosphotungstic acid (1%) and pipetted onto carbon grids. The grids were then examined under the transmission electron microscope.

3.3.2.4 Particle Size Measurements

The particles produced were sized using the laser light scattering technique. This technique is based on the interaction between the laser light and the particles. Several different systems using laser light scattering exist and the type of system used is dependent on the size range of the particles to be measured. Low angle laser light scattering (LALLS) is based on the principle that light is scattered by particles and the pattern of light intensity waves varies with the angle depending on the particle size; i.e. small particles scatter light at large angles while large particles scatter light at smaller angles. LALLS can be used to measure particles with a size range of 0.1 to 2000 μ m depending on the focal length of the lenses used.

In this system, the light scattered by the particles via an optical arrangement to a series of detectors which record the current proportional to the intensity of the scattered light falling upon them. A correlator then converts the intensity of scattered light into size distributions. Figure 3-6 shows a schematic diagram of the LALLS setup.



Figure 3-6: Schematic diagram of LALLS setup

Unlike LALLS which measures the light scattering of particles at all angles, photon correlation spectropscopy (PCS) measures the light scattering at only one angle. This technique is based around the principle of Brownian motion – the random movement of the particles is due to the bombardment of the solvent molecules that surround them. The larger the particle, the slower the Brownian motion of the particle. As a rule, this technique is used to size submicron particles although this may vary slightly depending on the onset of sedimentation, sample concentration, refractive index, laser power and detector sensitivity.

PCS gives the hydrodynamic diameter which is the diameter of a sphere which has the same translational diffusion coefficient as the particle being measured.

The particle size is calculated from the diffusion coefficient using the Stokes-Einstein equation:

$$d(H) = \frac{kT}{3\pi\eta D}$$

Equation 11: Stokes-Einstein equation where d(H) is the hydrodynamic diameter, D is the diffusion coefficient, k is the Boltzmann constant, T is the absolute temperature and η is the viscosity



Figure 3-7: Schematic representation of speckle pattern

When a sample cell containing stationary samples is illuminated by a laser and viewed with a frosted screen glass, a speckle pattern is seen (Figure 3-7). The bright patches are caused by the light scattered by the particle arriving on screen at the same phase thus interfering constructively. The dark patches are caused by mutually destructive phase additions of the scattered light; i.e. the two phases cancel each other out. If the particles are in Brownian motion, the speckle pattern would change constantly. The rate of this change would be dependent on the rate at which the particles move. As previously mentioned, this in turn, depends on the size of the particles. The rate at which the speckle pattern changes is converted into electrical impulses and fed into a correlator where it is measured and the data obtained is then converted into size

distributions using computational software. Figure 3-8 shows a schematic diagram of a typical PCS setup.



Figure 3-8: Schematic diagram of PCS setup (adapted from Malvern Instruments Ltd. User Guide)

Particles which were determined to be within the micro-sized range with light microscopy and SEM were measured using LALLS. To prepare these samples, the particles were first suspended in water (containing 0.05% w/w Tween) by sonicating briefly (Grant Ultrasonic Bath XB6, Grant Instruments, Cambridge, UK). This suspension was immediately added dropwise into the magnetically stirred small volume diffraction chamber of the Mastersizer S (Malvern Instruments Ltd., UK) until a obscuration level of between 10 - 15% was achieved. The computer software was then used to generate a particle size distribution from the diffraction data obtained.

The nanoparticles produced were measured using the PCS technique (Zetasizer, Malvern Instruments Ltd, UK). First, the nanoparticles were suspended in water and sonicated briefly. The suspension was transferred into a plastic cuvette with four clear sides and placed in the sample holder within the PCS machine and the lid closed. Once the machine had achieved temperature stability, the machine started automatically.

3.3.2.5 Powder X-Ray Diffraction (PXRD)

X-ray diffraction is a technique used to determine the nature of a solid material; i.e. crystalline, amorphous or a combination of both and can also aid in the identification of the material. Crystalline material consists of a three dimensional structure which is defined by regular, repeating planes of atoms that make up the crystal lattice. When a focused X-ray beam interacts with these planes of atoms, the beam can either be absorbed, transmitted, refracted, scattered or diffracted. The diffraction pattern of the X-ray beam is dependent on the type and arrangement of the atoms making up the crystal lattice (http://pubs.usgs.gov/info/diffraction/html).

In PXRD, a current is applied on a filament within a sealed tube under vacuum. This current produces electrons which are then accelerated by applying a high voltage within the tube. X-rays are generated when the electrons hit a target (usually copper). The X-rays are collimated and directed onto the sample. Using the diffraction pattern produced, the distances between the planes of atoms that constitute the sample can be measure using Bragg's Law:

$n\lambda = 2dsin\theta$

Equation 12: Bragg's Law, where *n* is the order of the diffracted beam, λ is the wavelength of the incident X-ray beam, *d* is the distance between the adjacent planes of atoms (the d-spacings) and θ is the angle of incidence of the X-ray beam

The set of d-spacings generated by a typical X-ray scan provides a 'fingerprint' unique to the material being analysed. Crystalline material with its crystal lattice is characterized by a number of sharp, well defined peaks on the PXRD pattern while amorphous material which consists of atoms with no long range ordered pattern show diffuse diffraction pattern with no sharp peaks. Partially amorphous material will produce a pattern containing a mixture of diffuse diffraction patterns and sharp peaks (Viboonkiat, 2003).

Powder X-ray diffraction patterns were collected in transmission using an X-ray diffractometer (Philips PW3710 Scanning X-Ray Diffractometer, Philips, Cambridge, UK) with Cu K_a filter generated at 30 mA and 45 kV. The samples were gently compressed into a round disc sample holder and smoothed with a flat Perspex[®] block. The samples were scanned over a range of 20 values from 5° to 50° at a scan rate of 1.0° 20/sec.

3.3.3 Dissolution Studies

The phosphate buffer which was used as the dissolution medium was made by adding 68.0 g of potassium dihydrogen orthophosphate to 9.4 g of sodium hydroxide to water. The pH of the medium was adjusted to 6.8 (\pm 0.05) (pH211 Microprocessor pH Meter, Hanna Instruments, UK) before being made up to the final volume of 10 l.

Samples containing 30 mg of active drug substance (approximately 5% w/v of saturation solubility) was dropped into dissolution vessels at 0 min. The dissolution rate was measured using a dissolution apparatus (Pharmatest PTWS3C Dissolution Bath, Hainburg, Germany) fitted with a paddle (USP II). The dissolution conditions were as follows:

 Stirring speed 	: 100 rpm
------------------------------------	-----------

- Temperature : 37°C (± 0.5)
- Medium volume : 900 ml

Samples were withdrawn at 10 or 15 min intervals for a total duration of 1.5 hr. The indomethacin concentration in the samples was quantified using UV spectrophotometry (Cecil CE2020 UV Spectrophotometer) at a wavelength of 320 nm.

3.3.4 Calibration and Vaiidation of UV Spectrometry

0.05 g indomethacin was dissolved in absolute alcohol and made up to 100 ml in a volumetric flask to form the stock solution. The stock solution was then

diluted with phosphate buffer pH 6.8 to form standard solutions containing 10, 15, 20 and 50 mg/100ml of indomethacin.

The UV absorbance of these stock solutions was measured spectrophotometrically (Cary 3E UV-Visible Spectrophotometer, Varian Inc. Scientific Instruments, USA) at 320 nm using phosphate buffer pH 6.8 as a blank.

3.4 Results and Discussion

3.4.1 Control

The control referred to throughout this chapter was the indomethacin obtained from Sigma Aldrich, used without any further modification. At room temperature, indomethacin was a white powder which upon closer examination using SEM, appeared as a mixture of large and small crystals with no particular fixed shape (Figure 3-9).



Figure 3-9: Indomethacin control

The PXRD pattern obtained for the sample had narrow peaks indicative of the presence of crystalline material (Figure 3-10).

Solubility experiments carried out showed that indomethacin had a saturation solubility of 1.69 (\pm 0.07) mg/100 ml at 37°C in distilled water. However, it was found that since indomethacin was a weak acid with a pKa value of 4.5 (Somasundaram et al, 1997), the solubility of indomethacin was highly dependent on pH. In a pH 6.8 phosphate buffer at 37°C, indomethacin had a solubility of 41.5 (\pm 4.9) mg/100 ml. Therefore, it was important to ensure that the pH of the dissolution medium was the same in all the dissolution experiments.



Figure 3-10: Powder X-ray diffraction pattern for indomethacin control

3.4.2 Spray Dried Indomethacin Microparticles

Spray drying the indomethacin in a dichloromethane solution produced a pale yellow powder rather than the white powder that was the control. This yellow colour suggested that indomethacin had undergone a physical and perhaps chemical transformation, possibly as a result of a conversion from being crystalline to amorphous. SEM examination of this powder showed that spray drying had successfully changed the indomethacin crystals to spherical, discrete microparticles with a smooth surface (Figure 3-11). These microparticles appeared to be within a fairly narrow size range of between 1 to 5 μ m.



Figure 3-11: Indomethacin spray dried from a dichloromethane solution

The spray dried microparticles produced a diffuse diffraction pattern with no defined peaks when subjected to PXRD examination (Figure 3-12). Spray drying produced indomethacin in an amorphous form and that this was the reason for the observed colour change.



Figure 3-12: Powder X-ray diffraction pattern for spray dried indomethacin

Therefore, to ensure that indomethacin remained amorphous after spray drying, the indomethacin sample was stored in a desiccator at room temperature (below the T_g of indomethacin) and whenever possible used on the same day.

Saturation solubility tests carried out on the spray dried indomethacin in phosphate buffer at pH 6.8 as described in Section 3.4.1 showed that amorphous indomethacin had a higher saturation solubility, 120.0 (\pm 1.4) mg/100 ml when compared to the crystalline indomethacin control. This agrees with the studies that have shown that amorphous indomethacin showed higher saturation solubility than its crystalline equivalent (Hancock and Parks, 2000).

3.4.3 Surfactant-coated Spray Dried Indomethacin Microparticles

The aim of spray drying the hydrophobic indomethacin with a hydrophilic surfactant was to produce a microparticle with a surface which was coated with a layer of hydrophilic surfactant. In theory, the surfactant should move to the gas/liquid interface during the solvent droplet formation and on drying, coating the surface of the drug particle, thus making the surface more hydrophilic. This, in turn, would aid dispersion and wetting during dissolution which would hopefully result in a better dissolution rate.

Non-ionic surfactants were chosen over anionic surfactants such as sodium dodecyl sulphate (SDS) as anionic surfactants have been known to cause damage to the mucosal membrane of the rodent and to the human small intestine (Nadai et al, 1972; Saunders et al, 1975; Gullikson et al, 1977). On the other hand, non-ionic surfactants have been shown to have almost no effect on the intestinal surface (Yonezawa, 1977). The criteria required for the surfactants were that they had to be hydrophilic and exist as a solid at room temperature with a melting point higher than 40°C (i.e., the boiling point of dichloromethane). The surfactants which were found to meet the criteria were Gelucire 50/13, Pluronic F127 and Pluronic F108.



a)

b)

c)

Figure 3-13: Indomethacin spray dried with Gelucire 50/13 at different drug to surfactant ratios (a) 1 : 1, (b) 1 : 0.5 and (c) 1 : 0.05

Gelucire 50/13 is a steroyl macrogol-32 glyceride. At room temperature, it exists as a waxy solid with a faint odour. Gelucires are classified using two numbers, the first referring to the approximate melting point of the surfactant and the second to the HLB value (Sutanata et al, 1995). In this case, Gelucire 50/13 has a melting point of approximately 50°C (46 -51°C) and an HLB value of 13. Several formulations were made with Gelucire 50/13 containing varying drug to surfactant weight ratios, from 1 : 1 to 1 : 0.05. The spray dried products were sticky and when examined under the SEM, no particles could be seen (Figure 3-13). Varying the surfactant concentration did not seem to make any difference to the spray dried product.

Next, the spray dried experiments were carried out using Pluronic F108 in place of Gelucire 50/13. Pluronic F108 is a triblock polymer which is composed of 50 units of poly(propylene oxide) (PPO) and capped on both ends with 132 units of poly(ethylene oxide) (PEO) (Figure 3-14). At room temperature, Pluronic F108 is a waxy white solid with a slight odour. Pluronic F108 has a melting point of approximately 57°C and an HLB of >24.

$$\begin{array}{c} \mathsf{CH}_3 & \mathsf{CH}_3 \\ \mathsf{I} \\ \mathsf{HO}\text{-}(\mathsf{CH}\mathsf{CH}_2\mathsf{O})_{\mathsf{X}}\text{-}(\mathsf{CH}_2\mathsf{CH}_2\mathsf{O})_{\mathsf{Y}}\text{-}(\mathsf{CH}_2\mathsf{CH}\mathsf{O})_{\mathsf{X}}\text{-}\mathsf{H} \end{array}$$

Figure 3-14: Structural formula of Pluronic F108, where x = 132 and y = 50

When the dichloromethane solution of indomethacin and Pluronic F108 was spray dried at a ratio of 1 : 0.5, a sticky yellow paste containing needle-like indomethacin crystals was produced. However, when the drug to surfactant ratio was reduced to 1 : 0.05, a fine yellow powder was formed. When examined under SEM, discrete spherical particles similar to those obtained in the previous section (Section 3.4.2) were seen (Figure 3-15).



Figure 3-15: Indomethacin spray dried with Pluronic F108 at a drug surfactant ratio of 1 : 0.05

PXRD experiments showed that like the indomethacin only microparticles, these surfactant/drug particles were amorphous (Figure 3-16).



Figure 3-16: Powder X-ray diffraction pattern for spray dried indomethacin with Pluronic F108

Saturation solubility studies showed that addition of Pluronic F108 further increased the solubility of the amorphous drug. The surfactant/drug formulation had a saturation solubility of 136.5 (\pm 2.1) mg/100ml. This increase is probably due to solubilization of indomethacin by Pluronic F108. It was decided that this formulation would be used in dissolution studies.

3.4.4 Indomethacin Nanoparticles produced by a Modifled Solvent-Diffusion Technique

The modified solvent-diffusion technique was used with a range of surfactants, such as Gelucire 50/13, Brij 76, Brij 35, Pluronic F58 and Pluronic F108. The results of the experiments are discussed below.

3.4.4.1 Brij 76





When 0.5 ml of a 3% w/w solution of indomethacin in acetone was added while stirring to 25 g 0.1% w/w Brij 76, a milky emulsion formed. This emulsion was then freeze-dried before being examined under the SEM. The freeze dried product consisted of a web-like structure (Figure 3-17a). However, when the freeze dried product was dispersed in water spherical particles were seen (Figure 3-17b). These particles appeared aggregated which could be due to

the surfactant. PCS experiments gave results of 593.5 (\pm 15.3) nm with a polydispersity index of 0.464 (\pm 0.023).

In further experiments, it was found that increasing the surfactant concentration resulted in solubilization of the drug and no particle formation occurred. However, if either the surfactant concentration was decreased, or the drug concentration increased, long needle-like crystals of indomethacin formed. This is probably due to the fact that decreasing the surfactant to drug ratio results in insufficient stabilisation of the droplets before diffusion of the solvent is complete. Therefore, uncontrolled precipitation of indomethacin took place resulting in the formation of large crystals.

Saturation solubility studies in water showed that the nanoparticles had a higher saturation solubility than the control, 6.71 (\pm 0.50) mg/100 ml versus 1.69 (\pm 0.07) mg/100 ml.

3.4.4.2 Brij 76 and Brij 35

Next, a combination of Brij 76 and Brij 35 was studied in a ratio of 1 : 1 in place of Brij 76, as the stabilising agent. No microparticles were seen. Instead, the suspension contained needle-like crystals of indomethacin.

3.4.4.3 Pluronic F127 and Pluronic F108

Experiments involving the two Pluronic surfactants did not yield any nanoparticles. Long needle-like crystals were seen in all the experiments suggesting that the Pluronic surfactants were not suitable as stabilising agents.

3.4.4.4 Gelucire 50/13

When 0.16 ml of a 3% w/w solution of indomethacin in acetone was added to 10 ml 0.1 % w/w Gelucire 50/13 aqueous solution, it was found that nanoparticles with a z-average diameter of 340.0 (\pm 6.8) nm were produced.

These particles had a polydispersity index of $0.204 (\pm 0.05)$ which suggested a relatively narrow size distribution. Figure 3-18 shows an example of a typical size distribution obtained from PCS experiments.



Figure 3-18: An example of a graph obtained from PCS experiments

When the particles were examined using both SEM, the images shown in Figure 3-19 were obtained. The images showed the particles produced were spherical with no sign of needle-like crystals.


Figure 3-19: Nanoparticles of indomethacin produced using a modified solventdiffusion technique with Gelucire 50/13 acting as the stabilising agent

As with Brij 76, when the surfactant concentration was increased or the drug concentration was decreased, solubilization occurred. When the surfactant concentration was decreased or the drug concentration increased, long needle-like crystals formed.

When saturation solubility studies were carried out, it was found that the saturation solubility of the Gelucire 50/13 containing nanoparticles had a lower saturation solubility (4.86 \pm 0.5 mg/100ml) than the nanoparticles made with Brij 76.

It was decided that the Brij 76 nanoparticles would be used in the dissolution studies.

3.4.5 Dissolution Studies

The results of the dissolution studies are summarised in Figure 3-20. The nanoparticles and surfactant-coated spray dried microparticles improved the dissolution rate when compared to the control.

Dissolution rates are affected by both effective surface area and saturation solubility. The improved dissolution rate of the microparticles produced by spray drying with Pluronic F108 can be attributed in part to the improved saturation solubility. Spray drying has also been shown to reduce the tendency of particles to form aggregates compared to micronisation (Hirschorn and Kornblum, 1971). Another possible factor in the dissolution rate enhancement is the presence of Pluronic F108. As discussed earlier, the hydrophilic surfactant should have moved preferentially to the surface of the droplet during the spray drying process forming a hydrophilic layer around the amorphous indomethacin core. This hydrophilic layer improved the wetting ability of the particle as well as aiding the dispersion of the microparticles in the dissolution medium. These latter two factors would lead to an increase in the effective surface area of the particles.

The nanoparticles produced using a modified solvent-diffusion technique showed similar dissolution rates to the spray dried indomethacin/Pluronic F108 microparticles. The improved dissolution rate is probably due to a combination of decreased particle size and large surfactant concentration. Like with the surfactant-coated spray dried microparticles, the high concentration of the hydrophilic Brij 76 aided the wetting and subsequently dispersion of the nanoparticles in the dissolution medium. This combined with the small particle size resulted in a very much increased effective surface area available for dissolution leading to the rapid dissolution rate.

However, due to the fact that indomethacin dissolved so readily in phosphate buffer, it was not possible to differentiate which formulation was better. The ease at which indomethacin dissolved was also a concern because in the duodenum to ileum where the majority of absorption occurs, the pH of the gastrointestinal fluid varies from 5.0 - 7.0. As a result, the dissolution rate of indomethacin is likely to be rapid with the bioavailability probably being or approaching 100%. Therefore, any improvement in bioavailability as a direct result in dissolution rate enhancement would not obvious. At this point, it was decided that a new drug which did not have a pH-dependent solubility profile should be used.



Figure 3-20: Dissolution rate of indomethacin samples (± SD, n=3)

3.5 Chapter Conclusions

Spray drying was capable of successfully converting crystalline indomethacin to its amorphous state. These amorphous microparticles had a higher saturation solubility compared to the control.

At low concentrations, the hydrophilic surfactant, Pluronic F108, could be spray dried in combination with indomethacin to produce discrete, uniform microparticles. Like the non-surfactant containing microparticles, they were amorphous with higher saturation solubility than the control. Higher concentrations of surfactant resulted in a adhesive sticky mass containing long needle-like crystals of indomethacin.

A modified solvent-diffusion technique was used successfully to produce nanoparticles of indomethacin with another hydrophilic surfactant, Brij 76. These particles were spherical and uniform in shape.

Dissolution studies using the particles produced showed that the nanoparticles and surfactant-containing spray dried microparticles improved the dissolution rate of indomethacin when compared to the control. The improved dissolution rate of the nanoparticles was probably due to the combination of a large effective surface area available for dissolution as well as the presence of a fairly high percentage of surfactant which aided wetting and subsequently, dispersion of the nanoparticles in the dissolution medium. The improvement of the spray dried microparticles was attributed in part to the fact these particles were amorphous and had higher saturation solubilities. In addition, the hydrophilic Pluronic F108 improved the wetting ability of the particles and therefore, aided dispersion and subsequently increased the effective surface area available for dissolution.

However, since indomethacin dissolved rapidly in the pH 6.8 phosphate buffer, any subtle differences between the dissolution rate of the microparticles and nanoparticles were not seen. This rapid dissolution rate might also be a problem *in vivo* as it may prevent any dissolution rate related improvements

not to be translated into bioavailability improvements. Therefore, it was decided that the particle design work in this chapter would be transferred to another hydrophobic model drug whose solubility is not pH-dependent.

Chapter 4: Griseofulvin – Particle Design and Dissolution Studies

4.1 Introduction

Griseofulvin is a poorly water-soluble Class II drug. Unlike indomethacin, griseofulvin does not contain any readily ionisable groups and therefore, its solubility is not affected by pH. The bioavailability of griseouflvin has also been shown to reflect changes in its dissolution rate which makes it an ideal model drug for the following work (Ahmed et al, 1998). The aim of the experiments carried out in this chapter is to transfer the methods of particle production used to successfully produce indomethacin micro- and nanoparticles to griseofulvin.

4.1.1 Griseofulvin – Modei Drug

Griseofulvin is an antifungal agent which has been used orally in the treatment of dermatophyte infections of the skin, hair and nails whenever topical treatment has failed or is inappropriate. In most cases, the recommended daily doses is 500 mg in adults (Joint Formulary Committee, 2004).

At room temperature, griseofulvin is a white, odourless, crystalline powder with a molecular weight of 353 (Townley, 1979). It has a melting point of 220°C (Townley, 1979). The chemical structure of griseofulvin is shown in Figure 4-1. As the structure shows, griseofulvin does not contain any readily ionisable functional groups and therefore, the solubility is not affected by change in pH. Griseofulvin is poorly soluble in water with literature reporting aqueous solubility values of approximately 12.9 mg/ml at 37°C (Vojnovic et al, 1993).



Figure 4-1: Chemical structure of griseofulvin

Over the years, several of the techniques described in Section 1.7 have been used to improve the dissolution rate of griseofulvin such as solid dispersions, complexation and particle size modification. For instance, Lo and Law (1996) showed that the dissolution rate could be enhanced by producing griseofulvin solid dispersions using the solvent method. In this study, PEG 6000, talc and the combination of both were used as dispersion carriers. In another study, Saito et al (2002) produced griseofulvin/saccharide mixtures by roll mixing the two components together. The resulting increase in the dissolution rate of griseofulvin was attributed to the fact that roll mixing rendered both griseofulvin and the saccharides amorphous.

Veiga et al (1998) and Dhanaraju (1998) used the complexation approach to improving the griseofulvin dissolution rate. Both studies used cyclodextrin as the complexant. Cyclodextrin is a water-soluble starch-derived cyclic carbohydrate which contains a hydrophobic cavity in which hydrophobic drugs can be included. The main disadvanaage of using cyclodextrin is that it has a limited drug loading capacity.

The micronization technique has also been used successfully to reduce the particle size of griseofulvin, which in turn, led to increased dissolution rates. Reverchon et al (2004) used supercritical assisted atomisation (SAA) to micronise griseofulvin. In this work, supercritical carbon dioxide was solubilised in a liquid solution of griseofulvin. This ternery mixture was then sprayed through a nozzle to produce griseofulvin microparticles which were shown to have an enhanced dissolution rate. Trotta et al (2003), on the other hand, formulated griseofulvin nanoparticles from triacetin-in-water emulsions. These nanoparticles successfully increased the dissolution rate of griseofulvin in water. They attributed the increased dissolution rate to the decreased particle size; i.e. increased available surface area of the drug particles.

4.2 Materials

Griseofulvin and sodium hydroxide were purchased from Sigma Aldrich Company (Poole, UK). Pluronic F127 was obtained from BASF Chemical Company (Mount Olive, NJ, USA) and Brij 76 was obtained from Fluka Chemika (Germany). Potassium dihydrogen orthophosphate, dichloromethane (analytical grade) and acetone (analytical grade) were obtained from BDH (VWR International Ltd., Poole, UK).

All chemicals were used as purchased unless otherwise stated. The water used for all experimental work was deionised using Elga Option 4 water purifier unless otherwise stated.

4.3 Methods

4.3.1 Microparticle Production

4.3.1.1 Spray Dried Griseofulvin

The spray drying technique has been discussed in Section 3.1.1. Griseofulvin was dissolved in dichloromethane to produce a 1.5% w/v solution. The solution was then spray dried using a Büchi 191 Mini Spray Dryer (Büchi Laboratories-Technik, AG, Flawil, Switzerland). The spray drying conditions used were as follows:

- Inlet temperature : 55°C
- Outlet temperature : 39°C
- Aspirator : 80% (-32 mbar)
- Feed rate : 7.5 ml/min
- Air flow rate : 700 NI/hr

The spray dried product was collected and stored in a desiccator at room temperature until it was ready to be used.

4.3.1.2 Spray Dried Griseofulvin with Pluronic F127

A dichloromethane solution containing 1.5% w/v griseofulvin and 0.05 % w/v Pluronic F127 was made. This drug-surfactant solution was then spray dried using the same conditions described in the previous section (Section 4.3.1.1).

The spray dried product was also stored in a desiccator at room temperature prior to analysis.

4.3.1.3 Griseofulvin Microcrystals produced by Solvent-Diffusion

The theory and principles of solvent diffusion have been discussed in Section 3.1.2. Griseofulvin was dissolved in acetone to form a 3.0% w/w solution. An aqueous surfactant solution was made.

The griseofulvin solution was added dropwise to the surfactant solution while homogenising (T25 Ultra-Turax, IKA Werke GmbH & Co. KG, Germany) at 24 000 rpm for 5 min. The suspension formed was then freeze dried (Drywinner 110, Heto-Holten A/S, Gydevang, Denmark) for approximately 24 hr.

The freeze dried product was resuspended in water and centrifuged (SciQuip 3K30, Sigma Laboratory Centrifuges, Poole, UK) at 15 000 rpm for 15 min to remove excess surfactant. This process of resuspending in water and centrifuging was repeated twice. After the final time, the pellet was freeze dried to remove any remaining water.

The product formed was then used in further experiments.

4.3.2 Particle Characterisation

4.3.2.1 Scanning Electron Microscopy (SEM)

The particles were mounted onto an aluminium stub using double-sided adhesive carbon tape and sputter-coated for 3 min at 30 mA (Emitech K550, Ashford, Kent, England) with gold. They were then examined using a scanning electron microscope (Philips/FEI XL30, Eindhoven, The Netherlands).

4.3.2.2 Particle Size Measurements

The theory behind using low angle lase light scattering (LALLS) as a technique to measure particle size has been discussed in the previous chapter (Section 3.3.2.4). LALLS experiments were carried on the MastersizerS (Malvern Instruments Ltd, UK). The particles were suspended in water containing 0.05% w/w Tween 80 (as the wetting agent) by sonicating briefly in an ultrasonic bath (Grant Ultrasonic Bath XB6, Grant Instruments, Cambridge, England) to ensure there was no aggregation.

4.3.2.3 Powder X-Ray Diffractometry (PXRD)

The theory and uses of powder X-ray diffraction (PXRD) has been discussed in Section 3.3.2.5. PXRD patterns were collected using a Philips PW3710 Scanning X-Ray Diffractometer (Philips, Cambridge, UK) with Cu K_a filter generated at 30 mA and 45 kV. The samples were gently compressed into a round disc sample holder and the surface smoothed with a flat Perspex[®] block. The samples were loaded onto the diffractometer and scanned over a range of 20 values from 5° to 50°. The scan rate used was 1.0° 20/sec.

4.3.2.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) can provide useful information about the physical and energetic properties, such as purity and polymorphism, of a pharmaceutical material using a very small amount of sample. It acts by measuring various thermal events, both exothermic (e.g. crystallisation and precipitation) and endothermic (e.g. melting and desolvation) (Clas et al, 1999).

As its name suggests, it is a calorimetric technique whereby energy differences are measured as a function of both time and temperature. The DSC instrument consists of two separate furnaces containing the reference and sample pan (Figure 4-2). The temperature difference between the two pans due to the thermal transitions within the sample is 'compensated' for by varying the heat required to keep both pans at the same temperature. The amount of energy required to maintain this is directly proportional to the changes occurring to the sample. Computer software is then used to plot energy difference versus time or temperature.



Individual heaters



DSC experiments were carried out using a DSC7 (PerkinElmer Instruments, Beaconsfield, Bucks, UK). Before each experimental session, the calorimeter was calibrated using a high purity indium standard with a known melting point onset (156.6°C) and Δ H (28.45 kJ/g). Samples were weighed out accurately in aluminium sample pans (PerkinElmer Aluminium Sample Pans) using a

microbalance (PerkinElmer AD4 Autobalance). An empty aluminium pan was used as the reference pan. The pans were sealed using a crimper press. The sample and reference pan was then placed in the appropriate specimen holder and left to reach thermal equilibrium prior to each run. Nitrogen (flow rate of 20 ml/min) was used as the purge gas throughout the experimental procedure. The samples were scanned over a temperature range of 30°C to 250°C at a rate of 10°C/min.

4.3.2.5 Contact Angle Measurements

The contact angle, θ , is a quantitative measurement of the wetting interaction between a liquid and solid. Geometrically, the contact angle is the angle formed by the liquid at the three phase boundary where the liquid, gas and solid intersect as shown in Figure 4-3.

Low contact angle values indicate that the liquid spreads easily over the solid surface, or wets well while high contact angle values show poor wetting ability. When water is the liquid medium, contact angles can be used to determine the hydrophilicity or conversely the hydrophobicity of a solid; i.e. a low contact angle value would suggest a hydrophilic solid material and a large contact angle value, a hydrophobic solid.



Figure 4-3: Schematic diagram of contact angles between a solid and liquid. The top diagram shows good wetting interaction between the solid and liquid while the bottom diagram shows poor wetting ability



Figure 4-4: Dynamic contact angle analyser

Various techniques based on either goniometry or tensiometry can be used to to measure the contact angles of a substance (Neumann and Good, 1979). The Wilhelmy technique used in this work is an example of the tensiometric approach and was first described in 1863 by Ludwig Wilhelmy (Wilhelmy, 1863; Fabretto et al, 2004). The experimental set up is shown in Figure 4-4. The forces acting on the balance, F, can be summarised by the following equation:

$F = F_W + F_B + F_C$

Equation 13: Forces acting on balance, F, where F_w is the weight of the dry cover slip, F_B is the buoyancy force and F_c is the capillary force

When the cover slip coated with the sample comes into contact with water (liquid medium), a change in the force acting on the plate occurs. By taring the balance before the run, F_W can be removed. F_B can be defined as (Wu, 1982):

$F_B = \rho g A d$

Equation 14: Buoyancy force, $F_{B'}$, where ρ is the density of the liquid, g is the gravitational force, A is the cross sectional area of the plate and d is the immersion depth

At the point where the end of the plate coincides with the air/water interface, $F_B=0$ because d=0. A plot of force versus depth (Figure 4-5) can be extrapolated back to zero so that the F_B term can be eliminated.

The remaining force, F_W , can be defined as:

 $F_W = \gamma P \cos \theta$

Equation 15: Weight of dry cover slip, F_{W} , γ is the liquid surface tension, P is the perimeter of the cover slip and θ is the contact angle

Since the value of γ and p is known, θ can then be calculated.





The contact angle obtained from the data as the cover slip enters the water is called the advancing contact angle and the angle obtained as the cover slip retreats is the receding contact angle. These contact angles are called dynamic contact angles because they are measured during immersion and withdrawal of the cover slip. Since the sample is moving at a slow speed, the viscous drag can be ignored.

The contact angle experiments were carried out using the CAHN Dynamic Contact Angle Analyser DCA 312 (Thermo Cahn, USA). Prior to each session, the surface tension of the water (HPLC grade, Fischer Scientific, Loughborough, UK) used was measured with a clean glass cover slip (VWR International Ltd., Poole, UK).

Next, a glass cover slip was coated with a thin layer of Photo Mounting Adhesive (3M, UK) and dipped into a container containing the sample. Excess sample was removed using a soft brush leaving a thin layer of sample on the cover slip. The cover slip was clipped onto the balance and tared. The cover slip was slowly lowered (151.1 μ m/sec) into a beaker of fresh HPLC grade water. The computer software was used to calculate the advancing contact angle of the sample.

4.3.2.6 Surface Area Analysis

The surface area of the particles was measured using the gas adsorption technique. Adsorption is a phenomenon which occurs whereby a gas (adsorbate) is adsorbed by a solid surface (adsorbent). To some extent, this phenomenon occurs whenever a clean solid surface is exposed to gas. The amount adsorbed is dependent on absolute temperature (T), pressure (P) and the interaction potential (E) between the adsorbate and adsorbent. However, since experimentally, the quantity of gas adsorbed is measured at constant temperature, the variables are reduced to P and E. A plot of weight (W) versus P at constant temperature is called the adsorption isotherm of a particular vapour/solid interface. It was found that all adsorption isotherms can be divided into 5 types based on the conditions involved (Brunauer et al, 1940).

During physical adsorption at low relative pressures, the first sites covered are the high energy ones. It should be noted that although the more energetic sites are covered first, it does not mean that no adsorption occurs at those with lower potential. As the adsorbate pressure is increased, the surface gets progressively more covered and the probability that a gas molecule will be adsorbed onto a previously bound molecule is increased. Therefore, in reality, there is no pressure at which the surface is covered with exactly one monolayer. The Brunauer, Emmett and Teller (BET) theory (Brunauer et al, 1938), is an extension of the Langmuir kinetic theory (Langmuir, 1918) and allows an experimental determination of the number of gas molecules needed to form a single monolayer (Lowell and Shields, 1984).

The determination of specific surface area is made by applying the BET equation (Equation 16):

$$\frac{1}{W[(P_0/P)-1]} = \frac{1}{W_mC} + \frac{C-1}{W_mC} \left(\frac{P}{P_0}\right)$$

Equation 16: BET equation where W is weight, W_m is monolayer weight, C is BET constant, P is pressure and P_0 is saturated vapour pressure

The plot of $\frac{1}{W[(Po/P)-1]}$ versus $\left(\frac{P}{P_0}\right)$ usually produces a straight line in the range of $0.05 \le \left(\frac{P}{P_0}\right) \le 0.35$ (Figure 4-6).



Figure 4-6: A typical BET plot (Lowell and Shields, 1984)

From which, the slope, *s* and intercept, *c* of the BET plot is:

$$s = \frac{C-1}{W_m C}$$

Equation 17: Slope, s, of BET plot

$$c=\frac{1}{W_mC}$$

Equation 18: Intercept, c, of BET plot

From these equations, W_m can be obtained:

$$W_m = \frac{1}{s+i}$$

Equation 19: Monolayer weight, W_m

While the solution for C gives:

$$C = \frac{s}{i} + 1$$

Equation 20: BET constant (C)

The total surface area, SA_t can then be calculated:

$$SAt = \frac{W_m \overline{N}Ac}{M_w}$$

Equation 21: Total surface area, SA_t where \overline{N} is the Avagrado's constant and A_c is the adsorbate cross sectional area

and the specific surface area can be determined by dividing SA_t by the sample weight.

Surface area analysis was carried out using the SA3100 Surface Area and Pore Size Analyser (Beckman Coulter Ltd., Buckinghamshire, England). First, the sample was weighed accurately into the glass sample holder. Then, the sample surface was freed of contaminants by heating under vacuum (degassed) at 30°C for an hour. The sample was re-weighed and degassed again. This process of degassing and weighing was repeated until the sample weight remained constant.

The sample was then frozen in liquid nitrogen and nitrogen was slowly added to the evacuated sample chamber. The surface area was calculated using the software.

4.3.3 Dissolution Studies

The phosphate buffer used as the dissolution medium was made by adding 68.0 g potassium dihydrogen orthophosphate and 9.4 g sodium hydroxide to water and the pH adjusted to 6.8 (\pm 0.05) (pH211 Microprocessor pH Meter, Hanna Instruments, UK). Water was then added to make a final volume of 10 l.

The different griseofulvin formulations were encapsulated in size 9 gelatin capsules (Torpac, USA); each capsule contained 10 mg of the active drug substance.

The dissolution test was carried out using a dissolution apparatus (Pharmatest PTWS3C Dissolution Bath, Hainburg, Germany) fitted with a paddle (USP II). The dissolution conditions used were as follows:

- Stirring speed : 100 rpm
- Temperature : 37°C (± 0.5)
- Medium volume : 1000 ml

Samples were withdrawn at 15 min time intervals for 3 hr and quantification of the amount of drug dissolved in the dissolution medium was carried out photometrically at 295 nm (Cecil CE2020 UV Spectrophotometer).

4.3.4 Calibration and Validation of UV Spectrophotometry

0.05 g of griseofulvin was dissolved in absolute alcohol and made up to 50 ml in a volumetric flask to form the stock solution. This stock solution was then diluted with phosphate buffer pH 6.8 to form standard solutions containing 0.50, 0.75, 1.00 and 1.50 mg/100ml of griseofulvin.

The UV absorbance of these standard solutions was then measured spectophotometrically (Cary 3E UV-Visible Spectrophotometer, Varian, Inc. Scientific Instruments, USA) at 295 nm using phosphate buffer pH 6.8 as a blank.

The measurement of these standards was repeated over a course of 5 days to ensure repeatability of the method.

4.3.5 Statistical Analysis

One-way between-groups analysis of variance (ANOVA) was performed on the results obtained where appropriate using the four different formulations as the independent variable. In cases where statistically significant differences (p<0.05) were found, post-hoc comparisons using Tukey HSD test were conducted to find where the differences lay.

4.4 Results and Discussion

4.4.1 Microparticle Production and Characterisation

4.4.1.1 Control

Figure 4-7 shows an SEM picture of the control griseofulvin crystals. The crystals show a large variation in shape and this is reflected in the size distribution of the particles. The long rod shapes have lengths varying from around $5 - 10 \mu m$ while the smaller spherical crystals appear to be around a micron or less. DLS results showed that the control had a 50% undersize value of 12.61 μm (±1.11).



Figure 4-7: Griseofulvin control

The powder diffraction pattern obtained from XRD showed many narrow peaks indicative of long-range order found in crystals (Figure 4-8). The major peaks obtained occurred at the °20 values expected for a griseofulvin sample (Townley, 1979).

DSC experiments indicated that a single sharp endothermic peak. The onset meting point temperature of the control was 219.1 (± 0.2) °C which falls within the melting ranges reported in literature (Schwarz et al, 1976; 1996; EP, 1997). A corresponding Δ H value of 118.3 (± 1.8) J/g was recorded.

The contact angle experiments gave a value of 92.5 $(\pm 0.9)^{\circ}$ confirming the hydrophobicity of the drug. The advancing contact angle rather than the receeding contact angle was used because following immersion in water, some dissolution might occur. This in turn might change the wetting characteristics of the particle surface. This might result in a significant variation in contact angle values when hydrophilic surfactants, such as Pluronic F127 which dissolved easily in water, are involved.

Preliminary gas adsorption experiments showed that degassing the sample for 3 hr at 30°C was sufficient for removing the impurities on the sample. After degassing, the control was subjected to surface area analysis. The crystals had an average specific surface area of 1.852 (\pm 0.036) m²/g.



Figure 4-8: Powder X-ray diffraction pattern for griseofulvin control

4.4.1.2 Spray Dried Griseofulvin

Spray drying produced a fine white powder with a yield of approximately 30%. An SEM of this product showed that spray drying successfully converted the griseofulvin crystals into microparticles with a fairly uniform spherical shape and size (Figure 4-9). These spherical particles were unlike the indomethacin particles which had a smooth surface. Instead the surface was rough with the larger particles having crater-like holes. The LALLS data obtained gave a D₅₀ value of 8.5 (\pm 0.16) µm.



Figure 4-9: Griseofulvin spray dried from a dichloromethane solution



Figure 4-10: Powder X-ray diffraction pattern for spray dried griseofulvin

PXRD analysis produced a pattern similar to that of the control with peaks indicative of crystalline material (Figure 4-10). This was unexpected given that spray drying is used as a method for producing amorphous material (Yu, 2001; Corrigan et al, 2003) and the indomethacin particles which were spray dried and stored using the same conditions were amorphous (Section 3.4.2). However, work by Ahmed et al (1998) showed that griseofulvin was easily recrystallised and capable of spontaneously recrystallising at room temperature in the absence of moisture (Fletcher, 2005). Therefore, although the spray dried material may have been amorphous immediately after spray drying, it was capable of quickly recrystallising. Also, it should be noted that XRD does not detect amorphous content if it makes up less than 5 - 10% of the total sample (Saleki-Gerhardt et al, 1994; Ahmed et al, 1996; Ahmed et al, 1998). Studies have shown that a drug in its amorphous form has higher saturation solubility than when in its crystalline state (Mosharraf and Nystrom, 2003; Ambike et al, 2004) and this in turn leads to a faster dissolution rate. However, disadvantage of amorphous material the is that it is thermodynamically unstable and tends to revert to the more stable crystalline form during processing (Fromming et al, 1980) or on storage (Hancock and

Zografi, 1997). This instability also increases the tendency for the amorphous material to react with environmental moisture (Ahlneck and Zografi, 1990), degrade (Shalaev and Zografi, 1996) or take part in solid-state reactions (Byrn, 1998) upon storage. Therefore, from stability considerations, it is not altogether disadvantageous that the spray dried product was crystalline; i.e. in a more stable state.

DSC data obtained showed a sharp endothermic peak with an onset of 217.6 $(\pm 0.1)^{\circ}$ C which although significantly different from the control (p<0.05) remained within the melting point range reported in literature. The DSC thermogram did not show any exothermic peaks which could be indicative of the recrystallisation of an amorphous material. However, the Δ H value of 112.5 (± 1.3) J/g obtained was slightly lower than the control (118.3 ± 1.8 J/g). This could be due to residual dichloromethane present in the final spray dried product.

Dynamic contact angle measurements showed that the spray dried griseofulvin had an advancing contact angle of 92.1 (\pm 0.7)°. This value was not found to be significantly different from that of the control (p>0.05) and once again showed the hydrophobic nature of the spray dried product.

Surface area analysis was carried out using the same conditions as the control. The results obtained showed that the spray dried drug with a specific surface area, 1.880 (\pm 0.059) m²/g, was fairly similar to the control, 1.852 (\pm 0.036) m²/g despite the fact that the particle size had been reduced. However, this is probably due to the fact that spray drying produced spherical particles, i.e. they have the smallest surface area per unit volume of all shapes. Therefore, the decrease in particle size was not sufficient to counteract the decrease in specific surface area of spheres.

4.4.1.3 Spray Dried Griseofulvin with Pluronic F127

Griseofulvin was spray dried with the same surfactant and surfactant to drug ratio as with indomethacin in the previous chapter in order to be able to make a

direct comparison between the two drugs. Spray drying produced a white powder similar to that obtained in the previous section (Section 4.4.1.2). The yield obtained was also similar at approximately 30%. However, SEM pictures showed that the two products looked very different (Figure 4-11). Although the microparticles produced were largely discrete, the surface of the microparticles was highly dimpled. Since the addition of surfactant was the only difference between this formulation and the previous one (Section 4.4.1.2), it can be concluded that the surfactant was responsible for the changed surface morphology of the microparticles.

During and following the formation of solvent droplets in the spray drying process, the surfactant moves preferentially to the gas/liquid interface. Although the evaporation process occurs rapidly (Masters, 1991; Millqvist-Fureby et al, 1999), the concentration of surfactant is likely to be much higher on the surface than inside the droplet, forming a 'shell' around the solvent droplet. Since the spray drying process was carried out at a temperature close to the surfactant melting point of 56°C (BASF Pluronic Grid), the shell formed was soft and pliable. On cooling, this shell collapsed resulting in the highly dimpled surface.

The D_{50} value obtained from the LALLS experiments was 11.24 (± 0.11) µm. These particles were therefore larger than those obtained by spray drying without surfactant but were similar in value to the control.



Figure 4-11: Griseofulvin and Pluronic F127 spray dried from a dichloromethane solution

The PXRD pattern obtained from this sample was similar to that of the control and griseofulvin spray dried in the absence of surfactant (Figure 4-12); i.e. the spray dried material was crystalline. The surfactant did not to affect the XRD pattern nor did the presence of the surfactant stabilise the amorphous griseofulvin that could have been produced by spray drying. However, the sample contains only about 3% w/w of surfactant as confirmed by high performance chromatography (HPLC) using the method described in Section 5.3.5.

The DSC trace showed a peak correlating to the melting point with an onset temperature of 217.7 (± 0.2)°C which is similar to the control. The Δ H value obtained, 102.9 J/g, was significantly different (p<0.05) from the control as well as from the previous sample (i.e. spray dried without surfactant). This lower value could be attributed in part to the presence of residual dichloromethane as well as to the presence of the surfactant in the sample.

These microparticles had a contact angle of 81.6 (\pm 2.8)° which is significantly lower than the control and microparticles spray dried without surfactant (p<0.05). The surface of these microparticles was therefore more hydrophilic. This confirms that surfactant is present on the surface of the particles. Pluronic F127, which has an HLB value of 22 (BASF Pluronic Grid), is a hydrophilic non-ionic surfactant, the presence of which on the particle surface would aid the wetting of the particle in water thus reducing the contact angle.



Figure 4-12: Powder X-ray diffraction pattern for griseofulvin spray dried with Pluronic F127

Gas adsorption experiments showed that these particles had a specific surface area of 1.253 (\pm 0.040) m²/g, giving it the smallest specific surface area of all the formulations.

4.4.1.4 Solvent-Diffusion Microcrystals

Solvent-diffusion experiments were initially carried out using the same surfactants and concentrations as used for indomethacin (Section 3.3.1.3). However, it was found that with this combination, griseofulvin nanoparticles could not be obtained. Therefore, other surfactants were screened to test their ability to produce nanoparticles.

Although this screening process was not able to find a surfactant suitable for producing nanoparticles, these experiments led to the discovery that solvent-

diffusion using a surfactant blend of Bij 76 and Tween 80 could be used to produce relatively small, uniform griseofulvin crystals with a particle size of around $2.0 - 2.5 \mu m$ (Figure 4-13). These crystals were bipyramidal in shape with a yield of close to 100%.



Figure 4-13: Griseofulvin crystals produced by solvent-diffusion with Brij 76 and Tween 80 as the stabilising agent

It has been shown that the addition of additives such as surfactant and polymer during the crystallisation process are capable of affecting the various stages of the process such as crystal growth (Davey, 1982) and nucleation (Davey, 1982; Stefen, 1988). Such additives tend to adsorb onto the crystal/liquid interface as the crystal precipitates out of solution (Mackellar et al, 1994). As the acetone diffuses into the water phase, griseofulvin precipitation occurs forming a hydrophobic surface. The related surface energy causes an increase in the energy of the system. Since the surfactant combination present in the aqueous phase had an affinity to the newly formed surface, it was capable of spontaneously covering the newly formed surface. This process reduced the surface energy and the enthalpy of the system. As a result, the small crystals which normally reduce the surface energy are sterically stabilised against crystal growth (Schott, 1985; Raseneck et al, 2003).

It was found that using a 1% w/w surfactant blend produced the smallest crystals. Increasing the surfactant concentration resulted in the dissolution of the drug whilst decreasing the surfactant concentration meant that there was insufficient surfactant to stabilise or control the crystal precipitation; i.e. non-uniform crystals were produced. At this point, it was decided that instead of nanoparticles that were aimed for, these microcrystals produced by solvent-diffusion would be used.

When the crystals were sized using LALLS, a D_{50} value of 2.18 (± 0.12) µm was obtained. This result is in agreement with the size estimates obtained using SEM. Therefore, controlled crystallisation using the solvent-diffusion technique was capable of producing smaller particles than spray drying.



Figure 4-14: Powder X-ray powder diffraction pattern for griseofulvin microparticles produced by solvent-diffusion

The XRD pattern obtained showed some peaks but not as many as in the control suggested a mixture of amorphous and crystalline material (Figure 4-14). This was unexpected as the pictures obtained from SEM suggested a completely crystalline material. However, the amorphous diffraction pattern could be attributed to the relatively large quantity of surfactant remaining on

the surface of the sample. UV spectrophotometry showed that the final product consisted of around 11 – 13% w/w of surfactant. Another possibility is that the surfactant could have been incorporated into the crystal lattice (Fairbrother and Grant, 1978; Fairbrother and Grant, 1979; Chow et al, 1984). Given how quickly griseofulvin is capable of recrystallising, it is unlikely that the drug is the cause of the amorphous pattern seen on the diffractogram.

However, this second possibility was excluded as the only thermal event seen on the DSC thermogram was the endothermic melting point peak which gave an onset temperature of 218.0 (\pm 0.5)°C and Δ H value of 102.9 (\pm 1.7) J/g. The melting point onset was close to the control and both the spray dried formulations suggesting that there was no significant change in the crystal habit. Although the Δ H value obtained was lower than the control, it is more likely due to the presence of the surfactant on the surface of the crystal rather than within the crystal. Work by Mackellar et al (1994) showed that the presence of surfactant on the surface of crystals was capable of reducing the Δ H value of a sample.

Due to the adhesive nature of the freeze dried product, surface area analysis and contact angle studies could not be carried out on the microcrystals.

4.4.1.5 Summary of Particle Characterisation

Table 4-1 summarises the particle characteristics which has been discussed above.

	Control	Spray dried	Spray dried with Pluronic F127	Solvent- diffusion crystals
Mean 50%	12.61	8.51	11.24	2.18
undersize	(± 1.11)	(± 0.16)	(± 0.11)	(± 0.12)
(µm)	()	(,	(,	(,
Drug loading	_	-	97	87 - 89
(% w/w)			01	0. 00
Melting				
point onset	219.1	217.6	217.7	218.0
temperature	(± 0.2)	(± 0.1)	(± 0.2)	(± 0.5)
(°C)				
ΔH (J/g)	118.3	112.5	102.9	102.9
	(± 1.8)	(± 1.3)	(± 1.5)	(± 1.7)
Contact	92.5	92.1	81.6	
angle (°)	(± 0.9)	(± 0.7)	(± 2.8)	
Surface area	1.852	1.880	1.253	
(m²/g)	(± 0.036)	(± 0.059)	(± 0.040)	

Table 4-1: Summary of particle characteristics

4.4.2 Dissolution Studies

The dissolution studies were not carried out under sink conditions, rather they were carried out at 80% saturation solubility. This was due to the fact that the poor solubility of griseofulvin meant that in order to achieve sink conditions such a small amount (~ 1 mg) of griseofulvin was required that would it would be impractical. Furthermore, it would be difficult to detect such small concentrations of drug in the dissolution medium.

The results of the dissolution studies are given in Table 4-3 and graphically in Figure 4-15. The griseofulvin microcrystals produced using the solventdiffusion technique gave the fastest dissolution rate, followed by the microparticles produced by spray drying griseofulvin with Pluronic F127. Next came the microparticles spray dried without surfactant and finally the control which had the slowest dissolution rate.

The Noyes-Whitney equation (Equation 5) shows that the dissolution rate can be affected by the saturation solubility and effective surface area of the solid in contact with the dissolution medium. Therefore, saturation solubility experiments were carried out in phosphate buffer (pH 6.8) at 37°C using a shaking water bath (Clifton Shaking Bath NE5-28D, Nickel Electro Ltd., England) which showed that all four formulations had similar solubilities (Table 4-2). As a result, saturation solubility could be eliminated as a cause of the improved dissolution rate of the samples compared to the control.

	Control	Spray dried	Spray dried with Pluronic F127	Solvent- diffusion crystals
Saturation solubility (µg/ml)	12.2	12.8	13.1	13.9

Table 4-2: Saturation solubility of griseofulvin samples in phosphate buffer (pH6.8) at 37°C

If predictions were to be made using the experimental surface area results obtained (Table 4-1), the microparticles produced by spray drying in the absence of surfactant would be expected to have a faster dissolution rate than those spray dried with Pluronic F127. Clearly, this was not the case with the wetting ability of the particles playing an important role in determining the dissolution rate of the samples.

The two formulations containing surfactant – microparticles spray dried with Pluronic F127 and microcrystals with Brij 76 and Tween 80 – showed the fastest dissolution rates; the solvent-diffusion microcrystals having the faster rate of the two. The hydrophilic surfactants present in both formulations improved the wetting ability of both formulations as well as preventing the aggregation of hydrophobic drug particles in the aqueous medium of the phosphate buffer leading to an increase in effective surface area.

Although the control and particles spray dried without surfactant had a larger surface area than the microparticles spray dried with surfactant, the hydrophobicity of griseofulvin means that it had a tendency to aggregate when in an aqueous medium. This, in turn, would reduce the effective surface area available for dissolution and therefore, result in a slower dissolution rate.

The solvent-diffusion microcrystals showed a faster dissolution rate than the microparticles containing Pluronic F127 probably because it has a higher surfactant concentration (~ 12% versus ~3%) and smaller particle size. Although the difference in specific surface area was not shown experimentally, the fact that the microcrystals are approximately 8 μ m smaller than the spray dried particles, means that the microcrystals are likely to have a greater surface area then the spray dried particles. As previously mentioned, the larger the effective surface area the faster the dissolution rate.

	Concentration (mg/100ml)					
Time			Spray dried	Solvent-		
(min)	Control	Spray dried	with Pluronic	diffusion		
(11111)			F127	crystals		
15	6.84	17.60	47.62	58.18		
	(± 1.0)	(± 4.3)	(± 4.6)	(± 3.0)		
30	11.98	29.55	59.51	69.90		
	(± 1.6)	(± 6.2)	(± 5.3)	(± 3.0)		
AE	15.58	35.82	63.74	74.62		
40	(± 2.0)	(± 6.4)	(± 5.4)	(± 3.2)		
	18.48	39.80	66.33	77.44		
00	(± 2.3)	(± 6.2)	(± 5.5)	(± 3.4)		
75	21.01	42.84	68.18	79.92		
(5	(± 2.4)	(± 6.0)	(± 5.2)	(± 3.2)		
90	23.17	45.47	69.81	81.39		
	(± 2.6)	(± 5.8)	(± 5.1)	(± 3.2)		
105	25.12	47.80	71.06	82.70		
	(± 2.6)	(± 5.4)	(± 4.9)	(± 3.1)		
120	27.02	49.69	72.11	83.87		
	(± 2.7)	(± 6.0)	(± 4.9)	(± 3.2)		
135	28.69	51.23	73.11	84.88		
	(± 2.8)	(± 5.8)	(± 4.7)	(± 3.0)		
150	30.28	52.60	73.89	85.69		
	(± 2.9)	(± 5.6)	(± 4.6)	(± 2.9)		
165	31.71	53.88	74.90	86.32		
	(± 2.9)	(± 5.4)	(± 4.5)	(± 2.8)		
180	33.16	54.84	75.49	86.78		
	(± 2.9)	(± 4.9)	(± 4.3)	(± 2.6)		
		-				


Figure 4-15: Dissolution rate of the griseofulvin samples (± SD, n=5)

4.5 Chapter Conclusions

The work in this chapter showed that the spray drying technique optimised for indomethacin in Chapter 3 could be transferred to griseofulvin without any major modifications. However, the microparticles produced from griseofulvin were crystalline unlike the amorphous particles produced from indomethacin. This was due to the rapidly crystallising characteristic of griseofulvin. The disadvantage of reduced saturation solubility of the crystal drug form was compensated by the increased stability conferred from its crystallinity.

Solvent diffusion could not be used to produce nanoparticles. However, the technique was capable of controlling the crystallisation of griseofulvin leading to extremely uniform microparticles with an average particle size of around 2 μ m.

The dissolution results obtained was promising, and showed that all three formulations had an improved dissolution rate when compared to the control (unprocessed griseofulvin). The microparticles obtained by solvent-diffusion had the fastest dissolution rate followed by the microparticles spray dried with Pluronic F127, then by the particles spray dried without any additional surfactant and finally, the control.

The addition of a hydrophilic surfactant aided the wetting ability of the drug in the aqueous medium of the buffer. This wetting ability was a more important factor in improving the dissolution rate than was increasing surface area. This was seen clearly in the case where the spray dried particles containing Pluronic F127 had a faster dissolution rate than the spray dried particles without surfactant despite having a smaller surface area.

The next section of work will determine whether the improvement in the dissolution rates observed with the novel microparticles will result in comparable improvements in the bioavailability of the animal model.

Chapter 5: Absorption Studies

5.1 Introduction

The absorption studies carried out in this chapter uses the three griseofulvin microparticle and microcrystal formulations which were produced and characterised in the previous chapter. The aim of these experiments was to find if the improved dissolution rate obtained from the three particle formulations would lead to an increase in *in vivo* bioavailability when administered orally to rats.

Before a new drug or drug formulation can be used in clinical trials, animal studies are generally used as a predictive indicator of how the drug or formulation will react in human as well as to gain toxicological information on the drug. Due to the metabolic, anatomical, physiological and biochemical variations which exists between human and animals, no single animal model can be used to replace human studies. However, for predictive purposes, animal models can be used fairly successfully. As a rule, small animals such as rats, mice, guinea pigs and rabbits are more suitable for determining the mechanism of drug absorption and bioavailability values from powder or solution formulations while larger animals like dogs, pigs and monkey are better for assessing absorption from formulations (Kararli, 1995).

5.1.1 Pharmacokinetics of Griseofulvin

The physicochemical characteristics of griseofulvin have been covered in Section 4.1.1. This section will cover the pharmacokinetics (absorption, distribution, metabolism and excretion) of griseofulvin following oral administration.

Griseofulvin is primarily absorbed from the duodenum, with smaller amounts being absorbed from the jejunum and ileum. Some drug may also be absorbed from the stomach and rectum but none is absorbed from the colon (Bedford et al, 1960; Lin et al, 1973; Becker, 1984). The absorption of griseofulvin occurs fairly rapidly with peak plasma concentration occurring between 2 - 9 hr after administration (Becker, 1984). The plasma levels remain elevated for approximately 10 - 20 hr, indicating that prolonged absorption occurs

(Rowland et al, 1968; Hagermark et al, 1987). Griseofulvin has a mean plasma half-life ($t_{1/2}$) of 22 hr in humans (Lin et al, 1973). The poor aqueous solubility of griseofulvin results in the drug showing a highly variable bioavailability with Priestley et al (1982) reporting bioavailability values ranging from 27 – 72% in humans. This variable bioavailability can potentially lead to therapeutic failure with the drug. Studies have shown that griseofulvin absorption can be increased by decreasing particle size or increasing surface area (Becker, 1984), by administering the drug concurrently with fats (Crounse, 1961; Khalafalla et al, 1981) or as an oil-in-water emulsion (Bates and Sequeira, 1975; Bates et al, 1977).

Bedford et al (1960) showed that following a single oral dose of griseofulvin, the drug was found in the heart, lungs, kidney, spleen and testes at concentrations levels similar to that found in the blood. Significantly higher amounts were found in the skeletal muscle, fat, liver and skin. Interestingly, other studies have shown that a higher concentration of griseofulvin was found in the outer layers of the human stratum corneum than in the deeper layers (Becker, 1984; Epstein et al, 2004). It is believed that griseofulvin has also been found to have a higher affinity for diseased skin than normal skin (Scott, 1960). This is useful, given that griseofulvin is used to treat dermatophyte infections in the skin.

Griseofulvin is metabolised by the microsomal system in the liver (Develoux, 2001). The principal inactive metabolites formed are 6-demethylgriseofulvin and its glucoronide conjugate (Becker, 1984).

The excretion of griseofulvin is highly variable even in the same individual. Lin et al (1973) reported that 50% of the oral dose is excreted in the urine and 36% in the faeces within 5 days of administration. However, it is unclear if the fraction excreted in the faeces is unabsorbed drug or whether drug that has been excreted through biliary excretion after being absorbed by the body (Lin and Symchowicz, 1975).

5.1.2 Gastrointestinal (GI) Tract

The human GI tract has been reviewed in Chapter 1. As mentioned earlier, although animal models, including the rat model, share basic structural similarities with human, variations arising from adaptation, nature of food, frequency of food intake, need for food storage, body size and shape mean that animal models cannot be used as a replacement for human studies (Kararli, 1995). However, understanding the differences between the animal and human model will lead to a more accurate prediction of drug behaviour in human. Therefore, the following section focuses on comparing the human and rat GI tract as the rat was chosen as the model in this work to predict griseofulvin absorption in man.



Figure 5-1: Schematic diagram of the rat GI tract (Stevens, 1977)

5.1.2.1 Structure and Anatomy of the Human and Rat Gastrointestinal (GI) Tract

The rat GI tract has the same basic structure as the human GI tract whereby it consists of the oesophagus, stomach, small intestine and large intestine (Figure 5-1). The most obvious difference seen between the rat and human GI tracts is the caecum, which is large and well defined in rats whereas the human caecum is small and continuous with the colon (Figure 1-1).

As with human, the rat stomach functions mainly as a reservoir for ingested material. However, unlike the human stomach which is completely glandular, the rat stomach has both glandular and non-glandular sections (Figure 5-2). The non-glandular sections are usually thin-walled and transparent. These sections are lined with keratinised stratified squamous epithelial cells and while the glandular part is thick-walled and covered with columnar epithelial cells. The glandular section is used for storage and digestion of food. In the glandular section, the lamina propria is occupied by simple tubular gastric glands which contain mucus secreting neck cells, pepsinogen secreting chief cells and hydrochloric acid secreting parietal cells (Stevens, 1977).



Human Stomach



Rat Stomach



Figure 5-2: Diagram of human and rat stomach (not shown to scale) (Stevens, 1977)

The rat small intestine is similar to human in that it consists of three regions – duodenum, jejunum and ileum and acts as the major absorptive site for ingested materials, including drugs among which, the model drug used in this

work, griseofulvin. The surface of both sets of small intestines is covered with villi and microvilli that increase the surface area on which nutrient and drug absorption can occur. In human, these villi are finger-shaped while those found in rats are tongue-shaped (Dressman and Yamada, 1991). A comparison of the relative lengths of the small intestines of human and rats obtained from post-mortem examinations are shown in Table 5-1. It appears that post-mortem dimensions are bigger than those obtained *in vivo*. For instance, post-mortem study suggests that the human small intestine is approximately 7 m in length while an *in vivo* estimation suggests that the length is closer to 3 m (Fordtran and Locklear, 1966).

	Human		F	Rat
	Length (mm)	Diameter (mm)	Length (mm)	Diameter (mm)
Duodenum	250	50	95-100	2.5-3
Jejunum	3000	50	900-1350	4-5
lleum	3000	50	25-35	3.5
Caecum	100-300	70	50-70	10
Colon	1500	50	90-110	3-10
Rectum	150-190	25	80	3-10

Table 5-1: Post-mortem lengths and diameters of the human (Ritschel, 1991) and rat (Hebel and Stromberg, 1976) GI tract

The small intestine is linked to the colon by the caecum. As mentioned previously and as shown in Table 5-1, the rat caecum is disproportionally large compared to the human caecum. This is because rats are herbivores and the caecum, which contains microorganisms, functions as a site for cellulose digestion.

In the colon, the absorption of water, Na⁺ and other minerals take place. The colon contains the largest proportion of microorganisms and is the main site of

production and absorption of volatile fatty acids (Stevens, 1978). The main difference between human and rat colon is the human colon is sacculated while the rat colon is unsacculated.

	Human	Rat
Stomach	1.5-3.5	
- forestomach		4.5
- glandular stomach		2.3
Duodenum	5.0-7.0	6.9
Jejunum-ileum	6.0-7.0	7.8
Caecum		6.7
Colon	5.5-7	7.1
Rectum	7.0	

5.1.2.2 Physiology of the Human and Rat Gastrointestinal (GI) Tract

Table 5-2: pH values of human (Gruber et al, 1987) and rat GI fluids (Ward andCoates, 1987) along the GI tract

The pH of the GI fluids can affect the solubility and dissolution rate of weakly acidic or basic drugs such as indomethacin. Subsequently, the degree of absorption of these drugs varies as the pH changes along the GI tract. Table 5-2 compares the pH along the human and rat GI tract. Overall, it can be seen that the fluid in the rat GI tract has a higher pH than the fluids in the human GI tract. However, as mentioned in the previous chapter, one of the reasons griseofulvin was chosen as the model drug was its non-ionizable nature. Therefore, the results obtained from the following study should not be affected by pH variations between the human and rat GI tract.

The presence of bile salts can also affect nutrient and drug absorption from the GI tract. Bile salts (cholates, chenodeoxycholates and deoxycholates) are

formed when the liver breaks down chlolesterol and are part of the bile fluid which is excreted by the liver. Bile salts can improve the intestinal absorption of lipophilic compounds by enhancing dissolution rate and solubility. They also play an important part in the lymphatic uptake of lipids and lipophilic drugs (Charman, 1992). The enhanced dissolution rate of hydrophobic drugs can be attributed to micellar solubilization and/or an improved wetting effect; i.e. the reduction in surface tension between the drug and dissolution medium (Miyazaki et al, 1979). In the *in vitro-in vivo* correlation of highly lipophilic drugs, it is important to know if the drug is micellarly solubilised (Kararli and Stolzenbach, 1992). Bile is secreted through the hepatic duct into the gallbladder. After food intake, the chyme entering the stomach stimulates the secretion of secretin and cholecystokinin which in turn, stimulates the secretion of bile fluids into the duodenum. The circulation of bile salts is confined to the enterohepatic circulation – liver, gall bladder, biliary tract and portal venous system.

In human, the common bile duct enters the duodenum at the duodenal papilla. A sphincter, known as the sphincter of Oddi, at the junction of the common bile duct and pancreatic duct with the duodenum, controls the flow of bile into the duodenum and prevents the reflux of duodenal contents (Toouli and Baker, 1991). In rats, there is a strict interdependence between the biliary and pancreatic systems, shown by the fact that pancreatic ducts flow straight into the common bile duct (Mann et al, 1920). Besides food intake, the rate of bile fluid secretion in a given animal depends on the circadian rhythm of the animal (Vonk et al, 1978). In animals without a gall bladder, such as rats, bile fluid is secreted continuously in dilute form and large volumes. Table 5-3 compares the flow rate and concentration of bile salts in human and rat.

	Human	Rat
Bile flow (ml/d.kg)	2.2-22.2	48-92
Total bile salts (mmol/L)	3-45	17-18

Table 5-3: Bile flow rate and quantity of bile salts in human (Dressman andYamada, 1991) and rats (Kararli, 1989b)

The surface of the GI tract is covered by water-insoluble, free-flowing viscous gel known as mucus. Mucin which is the main component of mucus is made up of a large protein core with an average of one oligosaccharide side chain (1-30 units) attached to every fourth amino acid in the backbone. Nacetylgalactosamine, N-acetylglucosamine, D-galactose, L-frucose and sialic acid constitute the sugar molecules in the oligosaccharides. The last two sugars occupy the free end of the oligosaccharide moiety (Park et al, 1984b; Montagne et al, 2004). The anionic L-fructose and sialiac acid at the end groups give mucin an overall anionic character. Mucus acts as a lubricant to protect the underlying mucosal cells from mechanical abrasion. It also protects the mucosa from luminal acids and pepsins by providing an unstirred layer where the neutralisation of the acids by the mucosal bicarbonate can occur in both the stomach and duodenum (Allen and Gardner, 1980). The thickness of this mucus layer varies between species. In drug delivery, mucus plays an important role in the mucosal adhesion of dosage formulations which in turn may result in longer GI transit times.

5.1.2.3 Gastrointestinal (GI) Motility and Transit in Human and Rats

Although, usually no significant absorption of food and drugs occur in the stomach, gastric emptying is considered important for absorption as it significantly affects the absorption in the small intestine (Kararli, 1995). As discussed in depth in Section 1.3, gastric motility can be altered by the presence of food. During the fasted state, the indigestible contents of the stomach are emptied via the migrating myoelectric cycle (MMC). This cycle

has been found to be common to both humans and typical laboratory animals (Dressman and Yamada, 1991).

The MMC is suspended on eating with mixing and grinding of the ingested material taking its place. The time required for the ingested material to exit the stomach and enter the duodenum is related to the particle size and the size of the animal stomach. In both humans and rats, the intestinal transit time of the proximal small intestine was faster than that of the distal intestine (Soergel, 1971; Lennernas and Regardh, 1993).

5.2 Materials

Griseofulvin and diazepam were purchased from Sigma Aldrich Company (Poole, UK). Pluronic F127 was obtained from BASF Chemical Company (Mount Olive, NJ, USA) and Brij 76 was obtained from Fluka Chemika (Germany). Dichloromethane (analytical grade) and acetone (analytical grade) were obtained from BDH (VWR International Ltd., Poole, UK). Acetonitrile (HPLC grade) was obtained from Fisher Chemicals Ltd. (UK).

All chemicals were used as purchased unless otherwise stated. The water used for all experimental work was deionised using Elga Option 4 water purifier unless otherwise stated.

5.3 Methods

5.3.1 Preparation of Dosage Forms

The spray dried samples (with and without surfactant) and the microcrystals produced by modified solvent-diffusion were prepared as described in Section 4.3.1.3.

These samples were encapsulated in size 9 gelatin capsules (Torpac, USA) (Figure 5-3) using the filling funnel and tamper provided. Each capsule contained 12.5 (\pm 0.5) mg of the active drug (Table 5-4). The control for all the experiments was the griseofulvin which was used as received.

Sample	Sample Weight (mg)/capsule
Control	12.5
Spray Dried	12.5
Spray Dried with Pluronic F127	12.8
Solvent-diffusion Microcrytals	14.2

Table 5-4: Weight of sample containing 12.5 mg griseofulvin



Figure 5-3: Size 9 gelatin capsule containing griseofulvin

5.3.2 Absorption Studies

Male Wistar rats weighing between 160-180 g (Harlan, UK) were used for the *in vivo* experiments and were allowed free access to both food and water prior to and during the study.



Figure 5-4: Oral gavage syringe – to eject capsule, push the plunger in the direction of the arrow.

Each rat was given one griseofulvin-containing capsule by oral gavage using a special gavage needle which is shown in Figure 5-4. After dosing, the rats were placed in a cage where they were allowed to move freely.

Approximately 0.3 ml of blood was collected from the tail vein of the rats into heparinised tubes (Microvette CB300, Sarstedt, UK) at times 0.75, 1.5, 2.5, 3.5, 5.0 and 8.0 hr post-administration. At 24 hr, 2 ml of blood was obtained via cardiac puncture and the animals were sacrificed by a Schedule 1 method. The blood samples were centrifuged at 3 000 rpm for 10 min (Eppendorf Centrifuge 5415D, Eppendorf AG, Hamburg, Germany fitted with a fixed angle rotor 45°; 24 X 1.5-2.0 ml with polypropylene lid) within 24 hr of sampling. 0.1 ml of the plasma (supernatant) was measured accurately and placed into a 1.5 ml Eppendorf tube and immediately frozen (-20°C) prior to analysis.

5.3.3 Bioavailability Studies

Preparations of an intravenous (iv) solution of propylene glycol, absolute alcohol and water were mixed together in the following ratio -4:1:5.

Griseofulvin was then added to form a drug concentration of 0.5 mg/ml and the mixture was sonicated in an ultrasonic bath to ensure complete dissolution. The drug solution was then filtered through a 0.25 μ m syringe filter (Millex-HA, Millipore, Carrigtwohill, Co., Cork, Ireland).

0.5 ml of this solution was administered intravenously through the tail vein of male Wistar rats weighing between 160-180 g. The rats were then bled and the blood processed as described in the Section 5.3.2.

5.3.4 Analysis of Plasma Samples

The frozen plasma samples were thawed by standing them at room temperature for 30 min. Griseofulvin was extracted from the plasma by protein precipitation. 1.0 ml acetonitrile was added to 0.1 ml plasma and the mixture was sonicated in an ultrasonic bath (Grant Ultrasonic Bath XB6, Grant Instruments, Cambridge, England) for 30 s and left to stand for 30 min. The samples were then centrifuged at room temperature for 10 min at 10 000 rpm. 0.9 ml of the supernatant was transferred into a glass test tube and allowed to evaporate to dryness under a stream of nitrogen at 90°C using a DriBlock DB3 heater fitted with a SC-3 sample concentrator (Techne, Cambridge, England). The residue was reconstituted using 0.18 ml mobile phase (65% acetonitrile and 35% water) containing 15.0 μ g/ml diazepam (internal standard).

The plasma samples were analysed using a high performance liquid chromatographic (HPLC) method modified from Hackett and Dusci (1978). The chromatographic system used consisted of Hewlett-Packard 1050 Series HPLC system (Agilent Technologies UK Ltd.) and the peaks obtained were integrated using PC/Chrom+ software (H&A Scientific Inc., USA). 50 μ I of the reconstituted sample was injected onto a 250 mm x 4 mm column (5 μ m C18, Macherey-Nagel GmbH & Co., KG Düren, Germany) fitted with a guard column at room temperature. An isocratic system was used and the flow rate of the mobile phase was set at 1.3 ml/min. The mobile phase used consisted of an organic solvent, i.e. 65% acetonitrile in water (35%). The mobile phase was filtered through a 0.2 μ m nylon membrane filter (Whatman International Ltd.,

Maidstone, England) and was degassed by bubbling helium through prior to use.

Two standard solutions were injected prior to each run to validate the run. The retention times of griseofulvin and diazepam were 3.7 and 5.1 min. respectively.

5.3.5 Calibration and Validation of HPLC Method

A series of standard solutions were prepared by dissolving known drug amounts in mobile phase containing 15 μ g/ml diazepam. The standards were injected onto the column using the conditions above and a calibration curve produced.

The assay was also validated by spiking drug-free plasma with a standard ethanolic griseofulvin solution in a concentration range of 0.1-3.0 μ g/ml. The drug concentration in each sample was measured using HPLC under the same conditions. Recovery and precision studies were performed with blank plasma samples spiked with the drug in a concentration of ranging from 0.1 to 3.0 μ g/ml and comparing the height ratios obtained to those obtained from the standards.

5.3.6 Statistical Analysis

One-way between-groups analysis of variance (ANOVA) was performed using the four different formulations as the independent variable and the area under the curve (AUC), maximum drug concentration in the plasma (C_{max}) and time to C_{max} (T_{max}) as the dependent continuous variables. In cases where statistically significant differences (p<0.05) were found, post-hoc comparisons using Tukey HSD test were conducted to find where the differences lay.

5.4 Results and Discussion

5.4.1 HPLC Calibration and Assay Validation

The HPLC assay was investigated for its sensitivity to detect the presence of griseofulvin in the samples. It was found that the HPLC assay gave baseline resolved peaks with retention times of 3.7 and 5.1 min for griseofulvin and diazepam respectively (Figure 5-5). The assay was found to be specific and no interference was seen at the retention times of both griseofulvin and the internal standard. Standard curves were produced based on peak height ratios of griseofulvin to diazepam and was found to be linear between a concentration range of $0.1 - 3.0 \mu g/ml$. The correlation coefficient (r²) was 0.9997.



Figure 5-5: Chromatogram of griseofulvin and internal standard in mobile phase



Figure 5-6: Chromatogram of griseofulvin and internal standard extracted from plasma

When griseofulvin spiked plasma samples were analysed, the results indicated that the extraction procedure was capable of recovering 99.3% at 0.16 μ g/ml 104.5% at 0.6 μ g/ml and 97.4% at 1.75 μ g/ml. As before, the chromatogram was devoid of any interference at the retention times of griseofulvin and the internal standard (Figure 5-6).

The detection limit of griseofulvin (4.5 times the noise level) was 0.06 μ g/ml. The lowest detectable level was 0.1 μ g/ml which was approximately 7 times the noise level.

5.4.2 Absorption Studies

As mentioned before, griseofulvin is a model Class II drug displaying poor solubility but high permeability and the dissolution rate acts as the rate limiting step in absorption. In the previous chapter, it was shown that all three formulations displayed an increased *in vitro* dissolution rate when compared to the control. Therefore, it might be anticipated that this increased dissolution rate would give rise to an improved absorption and subsequently improved bioavailability *in vivo*.

Generally, oral *in vivo* studies in rats are carried out by administering the drug formulation dissolved or suspended either in an aqueous medium or in oil. However, in this set of experiments, administration as a solid dosage form for was chosen two reasons. First, solid dosage forms are the preferred way of administering a drug because it is more convenient and practical in real life and a more uniform dose can be maintained compared to when giving drugs as a suspension. Secondly, it was believed that the increase in dissolution rate of the surfactant-coated spray dried microparticles and microcrystals produced by modified solvent-diffusion was due in part to their improved wetting characteristics and a solid dosage form was more suitable for showing this effect. Therefore, the solid dosage form was more suitable for this purpose than a suspension.

The individual and mean plasma concentration versus time profiles obtained for the different formulations and the control are shown in Figure 5-7 to Figure 5-10 (oral) and Figure 5-11 (iv). The mean plasma concentration versus time profile of all the *in* vivo studies together with their respective standard deviations is summarised in Figure 5-12 and Figure 5-13. The pharmacokinetic data for both the oral and intravenous administration is shown in Table 5-5 and Table 5-6.

The absolute oral bioavailability is determined as the dose ratio of the iv and oral treatment multiplied by the ratio of the infinite areas under the plasma concentration time curve (AUC_{∞}) obtained for the orally administered treatment and the AUC_{∞} obtained after intravenous administration (Equation 22).

Absolute oral bioavailability (F) = $\frac{\text{Dose}_{iv}}{\text{Dose}_{oral}} \times \frac{\text{AUC}_{oral}}{\text{AUC}_{iv}} \times 100\%$

Equation 22: Absolute oral bloavailability (F), where AUC is the area under the curve



Figure 5-7: Plasma concentration-time profile for the control



Figure 5-8: Plasma concentration-time profile of the spray dried griseofulvin



Figure 5-9: Plasma concentration-time profile of surfactant-coated spray dried griseofulvin



Figure 5-10: Plasma concentration-time profile of griseofulvin microcrystals produced by solvent-diffusion



Figure 5-11: Plasma concentration-time profile of griseofulvin administered intravenously



Figure 5-12: Mean (±SD) plasma concentration-time profiles after oral administration



Figure 5-13: Mean (±SD) plasma concentration-time profile after intravenous administration

Figure 5-12 shows that the microparticles which were produced by spray drying with the hydrophilic surfactant, Pluronic F127, gave the highest bioavailability. This was followed by the spray dried microparticles without surfactant and the control. The microcrystals produced by solvent-diffusion gave rise to a highly variable and unpredictable bioavailability.

Statistical analysis using one-way between-groups ANOVA showed that significant differences existed between the samples when the AUC and C_{max} were compared. Post-hoc analysis using Tukey HSD showed that both the AUC and C_{max} of particles spray dried with Pluronic F127 were significantly higher (p<0.05) than those of the control, the spray dried griseofulvin microparticles (without the surfactant) and the microcrystals obtained through solvent-diffusion. The time require to reach C_{max} was fairly similar for all four formulations and no significant differences (p>0.05) were seen.

Time (hr)	Control	Spray Dried	Spray Dried with Pluronic F127	Solvent-diffusion Microcrystals
0	0	0	0	0
0.75	0.171 (± 0.053)	0.264 (± 0.127)	0.597 (± 0.174)	0.422 (± 0.659)
1.5	0.260 (± 0.121)	0.635 (± 0.285)	1.094 (± 0.191)	0.234 (± 0.259)
2.5	0.483 (± 0.190)	1.121 (± 0.428)	1.636 (± 0.049)	0.510 (± 0.348)
3.5	0.749 (± 0.228)	0.964 (± 0.505)	2.125 (± 0.115)	0.229 (± 0.267)
5.0	0.620 (± 0.110)	0.582 (± 0.232)	1.425 (± 0.888)	0.318 (± 0.094)
8.0	0.460 (± 0.147)	0.200 (± 0.058)	0.456 (± 0.238)	0.194 (± 0.193)
24	0	0	0	0.294 (± 0.188)

 Table 5-5: Summary of plasma concentrations at different times (± SD) following oral administration

Time (hr)	Plasma concentration (µg/ml)
0	3.423 (± 0.414)
0.75	1.648 (± 0.070)
1.5	0.871 (± 0.050)
2.5	0.377 (± 0.161)
3.5	0.131 (± 0.034)
5.0	0
AUC (µg.hr/ml)	3.823 (±0.175)

Table 5-6: Summary of intravenous pharmacokinetic data (\pm SD, n=3) (Dose: 0.250 mg/rat)

	AUC	Bioavailability	C _{max}	T _{max}
	(µg.hr/ml)	(%)	(µg/ml)	(hr)
Control	7.54	3.94	0.82	3.9
Control	(± 2.00)	(± 1.04)	(± 0.11)	(± 0.75)
Spray Dried	6.74	3.53	1.22	2.8
Spray Drieu	(± 2.17)	(± 1.13)	(± 0.44)	(± 0.5)
Spray Dried	13 23	6.92	2 18	39
with Pluronic	(+ 2 78)	(+ 1.08)	(+ 0.18)	(+ 0.75)
F127	(± 3.70)	(± 1.90)	(± 0.18)	(± 0.75)
Solvent-	6.23	3 35	0.81	8.06
diffusion	(1,2,00)		(1.0.40)	(1.40.77)
crystals	(± 3.09)	(1.90)	(± 0.48)	(± 10.77)

5.4.2.1 Control and Spray Dried Microparticles without Surfactant

Although the spray dried formulation (without surfactant) showed a higher mean C_{max} value as well as a faster mean time to T_{max} , the results obtained for both were found to be statistically similar. This was contrary to the results obtained for the *in vitro* dissolution tests.

Although spray drying changed the shape, and to a lesser extend the size of the drug particles, griseofulvin was still crystalline (as revealed by PXRD) and its innate hydrophobic character remained unchanged. Also, as the characterisation work (Section 4.4.1.2) carried out showed, although the particle size of the spray dried formulation decreased, the total surface area remained similar to the control (Section 4.4.1.1). Therefore, both formulations were in effect presenting the same effective surface area available for dissolution. Also, it is possible that the hydrophobicity of the drug surface caused aggregation within the GI tract and therefore resulted in a reduced effective surface area available for drug dissolution.

5.4.2.2 Spray Dried Microparticles with Pluronic F127

The results obtained showed that this formulation gave rise to the highest C_{max} as well as the largest AUC value.

As discussed in Section 4.4.1.3, the contact angle results, which showed that these particles had a higher wettability, suggested that the hydrophilic surfactant was coating the surface of the microparticles. This would prevent the aggregation of the microparticles in the aqueous medium of the GI fluid. This, in turn, would increase the effective surface area available for the dissolution of griseofulvin and together with improved wetting would lead to an improved dissolution rate. Therefore, although this formulation had a smaller effective surface area than either the control or the microparticles spray dried without surfactant, it could dissolve faster *in vivo* and this could be the reason why it showed a higher C_{max} and better AUC values.

5.4.2.3 Microcrystals produced by Solvent-Diffusion

These microparticles gave rise to the lowest bioavailability of all the formulations. This was quite unexpected because it showed the fastest *in vitro* dissolution results. The poor *in vivo* results could be due to a combination of reasons.

In the *in vitro* experiments, a large volume (1 I) of buffer was used to dissolve a small quantity (10 mg) of drug. Therefore, this allowed for greater volume in which the drug can be dispersed. Also, the paddle speed used (100 rpm) might have provided better agitation than in the rat's stomach which may have further added drug dispersion.

The lack of sink conditions and poorer agitation in the GI tract of the rat, combined with the fact that the microcrystal formulation appeared as a cohesive pellet, might have resulted in poor and non-uniform dispersion within the GI tract. Any of these factors could have been responsible for the observed highly variable plasma drug concentrations.

If the particle 'pellet' did not break apart but stayed as a large pellet, it could have been retained in the stomach for a long period of time. As Bedford et al (1960) showed, minimal amounts of griseofulvin are absorbed from the stomach. As the pellet slowly breaks apart, the drug is released into the small intestine. In rats where griseofulvin was seen in the plasma at the 24 hr time point, it could be that it took the pellet a long time to disperse and for the griseofulvin crystals to pass into the small intestines to subsequently dissolve and be absorbed.

In the rats where pulsed concentrations of griseofulvin were seen in the plasma, it could be that parts of the pellet had broken apart and drug was released into the small intestines a little at a time. In other words, whenever bits of the pellet were released from the stomach into the small intestine, a rise in griseofulvin plasma levels were seen.

The poor bioavailability could also be related to the relatively high surfactant amount present in the microcrystal formulation. When dissolved in the rat GI tract fluids, the final surfactant concentration was much higher than in the *in vitro* dissolution studies. This high surfactant concentration can cause an increase in the viscosity of the GI fluids which in turn causes a decrease in drug solubility (Florence, 1991).

These hypotheses could be further investigated by carrying out *in vitro* dissolution tests using small volumes of buffer as well as slower paddle speeds.

5.4.2.4 Absolute Oral Bioavailability

The absolute oral bioavailability of all three formulations and the control is shown in Table 5-7. The bioavailability values obtained are rather low when compared to the 27 – 78% values reported in the literature (Lin et al, 1973; Gupta et al, 1994). However, it should be noted that these values were obtained from human studies.

These bioavailability values however, show relatively close agreement when compared to other *in vitro* studies involving rats. For instance, Bloedow and Hayton (1976) reported bioavailabilities ranging from 4 - 19% in experiments where 225 - 350 g male Sprague-Dawley rats were given griseofulvin doses of 50 mg/kg as oral suspensions containing 5 ml/kg lipids (hexadecane, oleyl alcohol, polysorbate 80, trioctanoin or triolein) or as an aqueous suspension with 0.5% methylcellulose. It should be noted that in these experiments, differing percentages (from < 0.01% in hexadecane to 94% in polysorbate 80) of griseofulvin had dissolved in the lipid phase and the starting particle size of the griseofulvin was smaller (0.43 μ m ± 1.5) than in the current set of experiments. Also, the rats used were fasted prior to and during the study and in general, it has been shown that food has an inhibitory effect on the GI absorption of most drugs (Bates and Gibaldi, 1970).

In another set of experiments carried out by Bates and Carrigan (1975), 50 mg/kg micronised griseofulvin was administered as a corn oil-in-water emulsion (25 mg griseofulvin and 25 mg polysorbate 80/ml) to non-fasted rats (250 - 325 g). They reported a mean AUC value of 13.4 µg.hr/mL, which is similar to the AUC value obtained for the rats dosed with the microparticles spray dried with Pluronic F127. Although this paper did not offer any bioavailability values, an approximation of the bioavailability can be made using the iv data shown in Table 5-6. For these calculations, it was assumed that the rats used were 290 g and given a griseofulvin dose of 14.5 mg. By putting these values into Equation 22, a bioavailability value of 6.04% was obtained.

Bioavailability comparisons with other *in vivo* studies are summarised in Table 5-8. It should be noted that these comparisons are not exact as assumptions are being made about the exact weight of the rats and therefore, the oral dose used for bioavailability calculations. Also, the iv data was obtained from the experiments carried out in this work so different methods of determining griseofulvin plasma had been used.

The low bioavailability obtained from *in vivo* studies using rats compared to human could be in part due to the shorter small intestine length and smaller diameter and therefore, the smaller surface area available for drug absorption. Griseofulvin may also undergo a greater degree of metabolism in rats than human and this too may account for the lower bioavailability values seen.

Reference	Rat weight (g)	Formulation	Mean AUC (µg.hr/ml)	Bioavailability (%)	Notes ^e
		Aqueous suspension ^c : • 10 mg griseofulvin • 10 mg polysorbate 60	7.95	3.78ª	
Carrigan and Bates (1973)	250 – 300 (275⁵)	Corn oil suspension ^c : • 25 mg griseofulvin • 25mg polysorbate 60	12.1	5.76ª	
		 Oil-in-water emulsion^c: 10 mg griseofulvin 10 mg polysorbate 60 10 mg edible fats 	19.8	9.42ª	
Bates and	250 – 325	 Aqueous suspension^c : 10 mg griseofulvin 10 mg polysorbate 60 	6.42	2.90ª	
Carrigan (1975)	(290 ^b)	Oil-in-water emulsion ^c :	17.70	7.98ª	
		 10 mg polysorbate 60 10 mg edible fats 	13.40	6.04ª	Rats were unfasted prior to and during the experiment

Table 5-8: Bioavailability data calculated from literature AUC values

Reference	Rat weight	Formulation	Mean AUC	C Bioavailability (%)	Notes ^e
	(g)		(µg.hr/ml)		
		Aqueous suspension ^c :			
		10 mg griseofulvin	9.43	12 ^d (4.65 ^a)	
		0.5% methycellulose			Rats were anaesthetised
		Hexadecane suspension ^c :	4 50	$6^{d}(2,22^{a})$	prior to dosing
		10 mg griseofulvin	1.00	0 (2.22)	Rats were anaesthetised prior to dosing
	225 – 350 (265 ^b)	Oleyl alcohol suspension ^c :	3.16	4 ^d (1.67 ^a)	
Bloedow and Hayon (1976)		 10 mg griseofulvin 			
		Polysorbate 80suspension ^c :	15.77	19 ^d (7.78 ^a)	
		 10 mg griseofulvin 			
		Trioctanoin suspension ^c :	5.11	6 ^d (2.52 ^a)	
		10 mg griseofulvin			
		Triolein suspension ^c :	3.36	4 ^d (1.66 ^a)	
		10 mg griseofulvin			
Kadir et al (1986)		Aqueous suspension ^c :			
	200 – 230 (215 ^ь)	25 mg griseofulvin	2.28	1.39ª	
		1% sodium			
		carboxymethycellulose			

Table 5-8: Continued

Reference	Rat weight	Formulation	Mean AUC	Bioavailability (%)	Notes*
	(g)		(µg.hr/ml)		
Kadir et al (1986)	200 – 230 (215 ^ь)	 Oil-in-water emulsion^c: 20 mg griseofulvin 20 mg polysorbate 80 20 mg sesame oil 0.4 ml corn oil 	6.78	4.13ª	
		Methyl propionate ^c : 25 mg griseofulvin	11.42	6.95ª	
		Oleic acid ^c : • 25 mg griseofulvin	0.96	0.58ª	

Table 5-8: Continued

^a bioavailabillty calculations made using iv data shown in Table 5-6

^b weight used for bioavailability calculations in brackets; the dose for all the rats was 50 mg/kg

^c per ml of the solution/suspension/emulsion

^d bioavailability values obtained from paper

^e unless otherwise stated, the rats were fasted prior and during the experiments

5.5 Chapter Conclusions

From the *in vivo* results, it can be concluded that spray drying the model Class II drug, griseofulvin, with a small percentage (3.33% w/w) of a hydrophilic surfactant successfully improved the absorption and subsequently the bioavailability of the drug. The bioavailability obtained was very similar to that of the emulsion produced by Carrigan and Bates (1973) and Bates and Carrigan (1975) which has been considered the 'gold standard' for comparing griseofulvin absorption. The increased absorption occurred despite the fact the surface area of the drug was smaller than that of the control and of the spray dried griseofulvin without surfactant. This suggests that the wetting ability of the particle plays a larger role in increasing the dissolution rate of griseofulvin than the particle size/surface area.

The absorption results obtained from the microcrystals produced by solventdiffusion was disappointing given the excellent *in vitro* dissolution results obtained in Chapter 4. This is thought to be due to the cohesiveness of the pellet produced after freeze drying. Further work would need to be carried out to conclusively identify the reason for the failure to reproduce the *in vitro* results *in vivo*.

Chapter 6: General Discussion

6.1 Introduction

Drugs are rarely administered solely as pure chemical substances, but are almost always given in formulated preparations (York, 1988). These preparations or dosage forms are either solids (e.g. tablets and capsules) or liquids (e.g. solutions and suspensions) and can be administered by various routes such as oral, parenteral, respiratory and rectal routes. However, all these dosage forms are designed with the aim of getting the active drug into the body in a safe, efficient, reproducible and convenient manner (Aulton, 1988).

The oral route is the most frequently used route for drug administration as it is the simplest, most convenient and safest of all the routes. With the exception of dosage forms designed to target the buccal cavity or the so-called "GI drugs" such as anti-motility agents and laxatives, orally administered drugs are usually required to enter the systemic system in order to exert the intended effect. Orally administered drugs enter the systemic system by absorption from the GI tract and the rate and extent of absorption depends on the physicochemical characteristics of the drug and the conditions within the GI tract. In order for absorption to occur, several events must take place. First, the drug must be dissolved in the GI fluids. Next, the dissolved drug must then permeate through the GI "membrane" to enter the systemic circulation (either the portal blood or intestinal lymphatic system). Therefore, drug solubility and permeability are considered to be the two most important parameters in determining if absorption will occur and this has been recognised by the BCS which uses these two parameters to classify drugs based on their likelihood of being absorbed (Charman, 2000).

The work in this thesis was focused on the particle design of poorly aqueoussoluble drugs in order to enhance their absorption and the bioavailability. Poor aqueous solubility is a growing problem in the pharmaceutical industry as an increasing number of new drug entities show low aqueous solubility. A recent study stated that 41% of failures in new drug development of seven UK-owned companies have been attributed to poor biopharmaceutical properties,
including low aqueous-solubility. The rate-limiting step to absorption of poorly water-soluble drugs is their slow dissolution rate. From the Noyes-Whitney equation, it can be seen that the dissolution rate can be increased by decreasing particle size to increase the effective surface area available for dissolution and by increasing the saturation solubility of the drug.

The aim of the work carried out in this thesis was to increase the dissolution rate and subsequently the oral biovailability of a model poorly water-soluble drug by:

- forming micro- and nano-particles with a increased effective surface area
- increasing saturation solubility of the drug by use of hydrophilic surfactants

The presence of the surfactants may also result in increased wetting of the particles which, in turn, may lead to improved dispersion of the drug particles when exposed to the aqueous medium.

In the first part of the study, indomethacin was used as the poorly watersoluble model drug. Indomethacin was later replaced by griseofulvin as indomethacin is a weak acid which shows pH-dependent solubility, and its saturation solubility increased with increasing pH. Due to the variations of pH in the GI tract, it was believed that griseofulvin would better illustrate any solubility-related bioavailability improvements than indomethacin.

6.2 Organogels and Amphiphilogels

The initial plan was to improve the dissolution rate of the model drug using non-ionic surfactant-based organogels and amphiphilogels. As their name suggest, these gels are composed of non-ionic surfactants; these can aid wetting and have the potential to increase saturation solubility and therefore, dissolution. Amphiphilogels have also been shown to be able to improve the oral bioavailability of the poorly water-soluble drug, cyclosporin (Murdan et al, 1999a; Murdan and Andrýsek, 2003). It was planned that organogels and

amphiphilogels would be used to produce microparticles which could then be administered in capsules.

Preliminary experiments showed that the model drug, indomethacin could be incorporated into amphiphilogels composed of Tween 80 and Span 60 as well as organogels composed of cinnamaldehyde and Span 60 without drug crystallisation occurring. These experiments also found that the concentration of indomethacin incorporated was proportional to the concentration of the continuous phase (Tween 80 or cinnamaldehyde) of the gel, i.e. it is quite likely that indomethacin was dissolved in the continuous phase. This was advantageous as having the drug in solution would eliminate the need for dissolution in the GI tract following oral administration and subsequently dissolution rate would not act as the rate-limiting factor to absorption.

The drug-loaded gels were then emulsified in water to form microsized droplets which were then dried by freeze drying or rotary evaporation. Unfortunately, the cohesiveness of the gel meant that that the gel droplets could not be extracted as discrete particles after emulsification and therefore, were not suitable for the purposes of this study. Subsequently, alternative methods of particle production were explored; i.e. spray drying and solvent-diffusion.

6.3 Spray Dried Microparticles

Spray drying is a technique which converts a fluid feed to a dry particulate state. Spray drying is a fairly well established technique which has been used by the pharmaceutical industry to lyophilise blood plasma and serum, hormones, vaccine and vitamins. Spray drying has also been used as a means of producing drug/excipient products with altered dissolution rates. In this study, spray drying was used as a means of:

- Reducing particle size (increasing effective surface area)
- Producing the particles coated with hydrophilic non-ionic surfactant to improve wetting behaviour and possibly saturation solubility

Unlike solid dispersions, where the poorly water-soluble drug is spray dried together with high percentages of an inert hydrophilic carrier such as PEG 6000, hydroxypropylcellulose (HPC) and PVP (Ambike et al, 2004; Weuts et al, 2005), the drug/surfactant particles produced in this thesis contained minimal amounts of surfactant (<5% w/w of the final product).

Spray drying was used successfully to produce discrete microparticles (both with and without surfactant) which showed a relatively narrow size distribution. Although spray drying is often used as a means of producing amorphous material, the results obtained showed that spray dried griseofulvin (with and without surfactant) was crystalline which suggests that this is not always the case. Both the amorphous and crystalline states have their advantages and disadvantages. Amorphous materials generally shows better saturation solubility as can been seen in this work where amorphous indomethacin had a saturation solubility of 120.0 ± 1.4 mg/100 ml in phosphate buffer (pH 6.8) while the crystalline indomethacin had a saturation solubility of only 41.5 ± 4.9 mg/100 ml. Unfortunately, the amorphous material is in a higher, less stable energy state and amorphous materials tend to revert to the more stable crystalline state upon storage and saturation solubility is decreased. The crystalline griseofulvin microparticles have an advantage over the amorphous indomethacin microparticles, as their saturation solubility is not likely to change upon storage.

The characteristics of the spray dried indomethacin and griseofulvin particles produced are discussed in the relevant chapters and therefore, will not be discussed here. However, the dissolution results obtained suggest that the improved wetting ability achieved by the addition of hydrophilic surfactant was ultimately more important in improving the dissolution rate than increasing effective surface area. The griseofulvin microparticles spray dried with Pluronic F127 had better wetting as shown by the lower contact angle (81.6 ± 2.8°) than the non-surfactant containing spray dried microparticles (92.1 ± 0.7°) and a faster dissolution rate than the particles spray dried without surfactant despite having a lower effective surface area (1.253 ± 0.040 m²/g versus 1.880 ± 0.059 m²/g). It is likely that the poorer wetting ability of the particles without surfactant

led to the aggregation of the particles in the aqueous buffer which would cancel out the advantage offered by the greater surface area.

6.4 Solvent-Diffusion Particles

Although spray drying proved to be effective in reducing the particle size around 1 μ m, further reductions in size could not be achieved using this technique. Therefore, in order to form particles in the nanometer range, an alternative technique, solvent-diffusion was used. The solvent-diffusion technique used in this work was a modified version of the one introduced by Fessi et al in 1987 (Quintanar-Guerrero et al, 1998). Unlike the original technique which required the use of both a polymer for encapsulation and stabilising agents (e.g. surfactants) to prevent particle growth, the technique used in this work used non-ionic hydrophilic surfactants for both stabilisation and encapsulation. Previous studies have shown that as the hydrophobic drug precipitates out of solution, the newly formed hydrophobic surface causes an increase in the energy of the system. Subsequently, stabilising agents such as surfactants are preferentially adsorbed onto the surface to reduce the surface energy. These stabilising agents may also prevent crystal growth and therefore result in smaller particles.

Particle formation is however a complex process and the production of small particles required both the right surfactant choice and drug/surfactant ratio. With indomethacin, it was found that spherical nanoparticles could be formed using the non-ionic hydrophilic surfactants Brij 76 (z-average diameter 593.5 nm) and Gelucire 50/13 (z-average diameter 340 nm). When the surfactants Brij 35 and Pluronic F108 and F127 were used or if the surfactant : drug ratio was decreased, needle-like crystals were seen. On the other hand, increases in the surfactant : drug ratio led to drug solubilization.

When the indomethacin-optimised technique was transferred to griseofulvin, it was found that nanoparticles could not be formed. A surfactant blend of Tween 80 and Brij 76 (ratio 0.5:9.5) was needed and small (D_{50} 2.18 µm) bipyramidal

crystals with a narrow size distribution could be formed. These crystals were used for the subsequent dissolution and absorption studies.

Dissolution studies with the indomethacin nanoparticles and griseofulvin microcrystals showed that both particle formulations improved the dissolution rate of the drugs when compared to the control. The indomethacin nanoparticles showed dissolution rates similar to those of the surfactant-containing spray dried indomethacin particles while the griseofulvin microcrystals showed the fastest dissolution rate of all the griseofulvin samples tested.

6.5 In Vivo Absorption Studies

As mentioned earlier, drug dissolution and permeation are essential for drug absorption and the clinical response for all orally administered drugs, bar the so-called GI drugs such as antimotility agents, adsorbants and some laxatives. This was recognised by the BCS which was introduced as a means of predicting the *in vivo* drug absorption based on *in vitro* drug dissolution results. However, this paradigm is not perfect due to the complexity of the processes occurring in the GI tract and the pharmacokinetics of the drug administered which cannot always be taken into account. Generally though, a good correlation between the *in vitro* dissolution and *in vivo* absorption has been seen with Class II drugs including griseofulvin. Therefore, based on the *in vitro* dissolute from the three griseofulvin formulations produced, it was expected that all three formulations would improve the absolute bioavailability of griseofulvin compared to the control following oral administration in capsules to male Wistar rats.

Surprisingly, the *in vivo* results showed that only the griseofulvin formulation produced by spray drying with Pluronic F127 significantly improved the bioavailability of griseofulvin (6.92%) when compared to the control (3.94%). Although the particles formed by spray drying without surfactant showed a higher C_{max} (1.22 µg/ml), this was not found to be significantly different from the control (0.82 µg/ml) and the absolute bioavailability (3.53%) was also

similar. The lack of improvement is thought to be due to the innate physicochemical properties of griseofulvin (poor solubility and wettability) which remained unchanged by the spray drying process in the absence of surfactant. The poor wettability may also have lead to aggregation within the GI tract and subsequently to a low effective surface area for dissolution.

Particles produced using the modified solvent-diffusion method also did not show a improved bioavailability. This was highly unexpected given the excellent *in vitro* dissolution results. Although due to time constraints, further investigation to find the cause of this could not be carried out, several hypotheses were made in Section 5.4.2.3.

The mean AUC value obtained from the microparticles spray dried with Pluronic F127 (13.23 μ g.hr/ml) was found to be similar to the values obtained by Bates and Carrigan (1975) following the administration of a single oral dose of an oil-in-water emulsion containing 10mg of griseofulvin (13.40 μ g.hr/ml). This is extremely promising as this oil-in-water emulsion is considered to be 'gold standard' in obtaining maximal griseofulvin bioavailability. Furthermore, this spray dried formulation is fairly straightforward to produce and the low non-ionic surfactant concentration (3.33% w/w) is safe and non-toxic for oral use, the LD₅₀ of Pluronic F127 in rats being 15 g/kg.

6.6 General Conclusions

From the results obtained from this study, it can be concluded that spray drying a poorly water-soluble drug such as griseofulvin with low concentrations of a non-ionic hydrophilic surfactant such as Pluronic F127 can be used successfully to improve the dissolution rate of the drug. This improved dissolution rate, in turn, may lead to improved oral bioavailability.

6.7 Further Work

Although the results obtained from this study look promising, further experiments are needed in order to obtain more definitive reasons for some of the results as well as further optimisation of the particle production methods.

The low contact angle of the surfactant-containing spray dried griseofulvin particles suggests that the surfactant forms a coating on the particle surface. This can be further investigated using techniques such as X-ray photoelectron spectroscopy (XPS) which utilises X-rays to determine the material present on the surface of particles. Work by Scholes et al (1999) has shown that XPS can be used to identify the presence of Pluronic F127 (the surfactant used for spray drying in this study) on the surface of a particle.

The *in vivo* work was carried out on a relatively small sample group. Ideally, the sample size would be increased to get a more accurate picture of the absorption and absolute bioavailability of the formulations produced in this work.

In addition, the unexpected bioavailability results obtained for the griseofulvin microcrystals produced using the modified solvent-diffusion technique should be further investigated to find the cause. Using the hypotheses made about the cause of these results (Section 5.4.2.3), *in vitro* dissolution experiments can be carried out using a small volume of buffer and a lower paddle speed. If the results obtained show the same unpredictability as the *in vivo* data, further work can be carried out to address this issue.

Stability studies also need to be conducted on all the formulations produced. As the indomethacin spray dried particles produced were amorphous, they are quite likely to be converted to the more stable crystalline state on storage, especially if subjected to high humidity and/or high temperatures. In contrast, the griseofulvin particles produced might show relatively good stability as the particles were in the more stable crystalline state. Finally, the techniques used in this study can be repeated using other hydrophobic Class II drugs such as phenytoin and carbamazepine (Lindenberg et al, 2005) followed by appropriate *in vitro* and *in vivo* studies to determine if the method can be transferred to other drugs to test the general applicability of the processing methods.

References

Abdallah, D. J., Weiss, R. G., n-alkanes gel n-alkanes (and many other organic liquids). *Langmuir*, 16, (2000a) 352-355.

Abdallah, D. J., Weiss, R. G., The quest for the simplest possible organogelators and some properties of their organogels. *Journal of the Brazilian Chemical Society*, 11, (2000b) 209-218.

Ahlneck, C., Zografi, G., The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid-state. *International Journal of Pharmaceutics*, 62, (1990) 87-95.

Ahmed, H., Buckton, G., Rawlins, D. A., The use of isothermal microcalorimetry in the study of small degrees of amorphous content of a hydrophobic powder. *International Journal of Pharmaceutics*, 130, (1996) 195-201.

Ahmed, H., Buckton, G., Rawlins, D. A., Crystallisation of partially amorphous griseofulvin in water vapour: determination of kinetic parameters using isothermal heat conduction microcalorimetry. *International Journal of Pharmaceutics*, 167, (1998) 139-145.

Aiache, J. M., Aiache, S., The fate of dosage forms in the gastrointestinal-tract. *Pharmacy International*, 6, (1985) 18-21.

Allen, A., Gardner, A., Mucus and bicarbonate secretion in stomach and their possible role in mucosal protection. *Gut*, 21, (1980) 249-262.

Ambike, A. A., Mahadik, K. R., Paradkar, A., Stability study of amorphous valdecoxib. *International Journal of Pharmaceutics*, 282, (2004) 151-162.

Amidon, G. L., Drug derivatation as a means of solubilization: Physicochemical and biochemical strategies. In: Yalkowsky, S. H. (Ed.), Techniques of solubilization of drugs. Marcel Dekker Inc., New York, (1981), 183-211.

Amidon, G. L., Lennenäs, H., Shah, V. P., Crison, J. R., A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharmaceutical Research*, 12, (1995) 413-420.

Anand, B., Pisal, S. S., Paradkar, A. R., Mahadik, K. R., Applications of organogels in pharmaceuticals. *Journal of Scientific and Industrial Research*, 60, (2001) 311-318.

Anderson, B. D., Prodrugs for improved formulation properties. In: Bundgaard, H. (Ed.), Design of prodrugs. Elsevier, Amsterdam, (1985), 243-270.

Asada, M., Takahashi, H., Okamoto, H., Tanino, H., Danjo, K., Theophylline particle design using chitosan by the spray drying. *International Journal of Pharmaceutics*, 270, (2004) 167-174.

Ashford, M., Fell, J. T., Targeting drugs to the colon: delivery systems for oral administration. *Journal Of Drug Targeting*, 2, (1994) 241-257.

Aulton, M. E., 1988. Pharmaceutics: The science of dosage form design. Churchill Livingstone, London.

Bates, T. R., Carrigan, P. J., Apparent absorption kinetics of micronized griseofulvin after its oral administration on single- and multiple-dose regimens to rats as a corn oil-in-water emulsion and aqueous suspension. *Journal of Pharmaceutical Sciences*, 64, (1975) 1475-1481.

Bates, T. R., Gibaldi, M., In: Swarbrick, J. (Ed.), Current Concepts in the Pharmaceutical Science: Biopharmaceutics. Lea & Febiger, Philadelphia, PA, (1970), 78.

Bates, T. R., Pineiaszek, H. J., Sequeira, J. A., Rasmusen, J. E., Gastrointestinal absorption of griseofulvin from corn oil-in-water emulsion. *Archives of Dermatology*, 113, (1977) 302-306.

Bates, T. R., Sequeira, J. A., Bioavailability of micronised griseofulvin from corn oil-in-water emulsion, aqueous suspension, commercial tablet dosage forms in humans. *Journal of Pharmaceutical Sciences*, 64, (1975) 793-797.

Bauer, K. H., Frömming, K. -H., Führer, C., 1997. Pharmazeutische Technologie. Gustav Fischer Verlag, Stuttgart.

Bechgaard, H., Design of controlled release products as adapted to gastrointestinal pH and transit time. *Acta Pharmacologica Technologica*, 28, (1982) 149-157.

Becker, L. E., Griseofulvin. Dermatologic Clinics, 2, (1984) 115-120.

Bedford, C., Busfield, D., Child, K. J., MacGregor, I., Sutherland, P., Tomich, E. G., Studies on the biological disposition of griseofulvin, an oral antifungal agent. *Archives of Dermatology*, 81, (1960) 735-745.

Berggren, J., Frenning, G., Alderborn, G., Compression behaviour and tabletforming ability of spray-dried amorphous composite particles. *European Journal Of Pharmaceutical Sciences*, 22, (2004) 191-200.

Bhandari, B. R., D'Arcy, B. R., Padukka, I., Encapsulation of lemon oil by paste method using beta-cyclodextrin: encapsulation efficiency and profile of oil volatiles. *Journal Of Agricultural And Food Chemistry*, 47, (1999) 5194-5197.

Bilati, U., Allemann, E., Doelker, E., Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. *European Journal of Pharmaceutical Sciences*, In Press, Corrected Proof, (2004).

Bisrat, M., Nyström, C., Physicochemical aspects of drug release. VIII. The relationship between particle size and surface specific dissolution rate in agitated suspensions. *International Journal of Pharmaceutics*, 47, (1988) 223-231.

Blanchard, J., Gastrointestinal absorption: I. Mechanims. *American Journal of Pharmaceutics*, 147, (1975) 135-146.

Bloedow, D. C., Hayton, W. L., Effects of lipids on bioavailability of sulfisoxazole acetyl, dicumarol, and griseofulvin in rats. *Journal of Pharmaceutical Sciences*, 65, (1976) 328-334.

Broadhead, J., Rouan, S. K. E., Rhodes, C. T., The spray drying of pharmaceuticals. *Drug Development and Industrial Pharmacy*, 18, (1992) 1169-1206.

Brunauer, S., Deming, L. S., Deming, W. S., Teller, E., On a theory of the van de Waals adsorption of gases. *Journal of the American Chemical Society*, 62, (1940) 1723-1732.

Brunauer, S., Emmett, P. H., Teller, E., Adsorption of gasses in multimolecular layers. *Journal of the American Chemical Society*, 60, (1938) 309-319.

Byrn, S. A. S., Solid state chemistry - regulations and reactions. (1998). SanDiego, Molecular Simulations Inc and Silicon Graphics Computer Systems.Proceedings of the Internation Symposium, New Approaches inPharmaceutical Development. 19-2-0098.

Carrigan, P. J., Bates, T. R., Biopharmaceutics of drugs administered in lipidcontaining dosage forms I: GI absorption of griseofulvin from an oil-in-water emulsion in the rat. *Journal of Pharmaceutical Sciences*, 62, (1973) 1476-1479.

Carstensen, J. T., 1980. Solid pharmaceutics: Mechanical properties and rate phenomena. Academic Press, London.

Chainkoff, I. I., Bloom, B., Sperstein, M. D., Kiyasu, J. Y., Reinhardt, W. O., Dauben, W. G., Eastham, J. F., Lymphatic transport of absorbed cholesterol-C¹⁴. *Journal of Biological Chemistry*, 194, (1952) 407-412.

Charman, W., Classifying drugs by their ease of absorption. *Pharmaceutical Journal*, 265, (2000) 576-577.

Charman, W. N., Lipid vehicle and formulation effects on intestinal lymphatic drug transport. In: Charman, W. N., Stella, V. J. (Eds.), Lymphatic Transport of Drugs. Chemical Rubber Company, Boca Raton, FL, (1992), 113-180.

Charman, W. N., Porter, C. J. H., Lipophilic produrugs designed for intestinal lymphatic transport. *Advanced Drug Delivery Reviews*, 19, (1996) 149-169.

Chaumeil, J. C., Micronization: A method of improving bioavailability of poorly soluble drugs. *Methods and Findings in Experimental and Clinical Pharmacology*, 20, (1998) 211-215.

Chawla, G., Gupta, P., Thilagavathi, R., Chakraborti, A. K., Bansal, A. K., Characterization of solid-state forms of celecoxib. *European Journal of Pharmaceutical Sciences*, 20, (2003) 305-317.

Chiou, W. L., Riegelman, S., Preparation and dissolution characteristics of several fast-release solid dispersions of griseofulvin. *Journal of Pharmaceutical Sciences*, 58, (1969) 1505-1509.

Chiou, W. L., Riegelman, S., Pharmaceutical applications of solid dispersion systems. *Journal of Pharmaceutical Sciences*, 60, (1971) 1281-1302.

Choi, J., Jo, B. W., Enhanced paclitaxel bioavailability after oral administration of pegylated paclitaxel prodrug for oral delivery in rats. *International Journal of Pharmaceutics*, 280, (2004) 221-227.

Chokshi, R., Zia, H., Hot-melt extrusion technique: A review. *Iranian Journal of Pharmaceutical Research*, 3, (2004) 3-16.

Chourasia, M. K., Jain, S. K., Polysaccharides for colon targeted drug delivery. *Drug Delivery*, 11, (2004) 129-148.

Chow, E. Y. J., Go, J., Mehdizadeh, M., Grant, D. J. W., Modification of adipic acid crystals: influence of growth in the precense of fatty acid additives on crystal properties. *International Journal of Pharmaceutics*, 20, (1984) 3-24.

Clas, S.-D., Dalton, C. R., Hancock, B. C., Differential scanning calorimetry: applications in drug development. *Pharmaceutical Science and Technology Today*, 2, (1999) 311-320.

Collett, A., Sims, E., Walker, D., He, Y. L., Ayrton, J., Rowland, M., Warhurst, G., Comparison of HT29-18-C1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption. *Pharmaceutical Research*, 13, (1996) 216-221.

Corrigan, D. O., Healy, A. M., Corrigan, O. I., The effect of spray drying solutions of bendroflumethiazide/polyethylene glycol on the physicochemical properties of the resultant materials. *International Journal of Pharmaceutics*, 262, (2003) 125-137.

Crossby, E. J., Marshall, W. R. Jr., Effects of drying conditions on the properties of spray dried particles. *Chemical Engineering Progress*, 54, (1958) 56-63.

Crounse, R. G., Human pharmacology of griseofulvin: The effect of fat intake on gastrointestinal absorption. *Journal of Investigative Dermatology*, 37, (1961) 529-533.

Cull, P., 1989. The sourcebook of medical illustrations. Parthenon, Boca Raton.

Cundy, K. C., Annamalai, T., Bu, L., De Vera, J., Estrela, J., Luo, W., Shirsat, P., Torneros, A., Yao, F., Zou et, a., XP13512 [(+/-)-1-([(alphaisobutanoyloxyethoxy)carbonyl] aminomethyl)-1-cyclohexane acetic acid], a novel gabapentin prodrug: II. Improved oral bioavailability, dose proportionality, and colonic absorption compared with gabapentin in rats and monkeys. *The Journal Of Pharmacology And Experimental Therapeutics*, 311, (2004) 324-333.

Daugherty, A. L., Mrsny, R. J., Transcellular uptake mechanisms of the intestinal epithelial barrier Part one. *Pharmaceutical Science & Technology Today*, 2, (1999) 144-151.

Davey, J. R., The role of addiives in precipitation processes. In: Jancic, S. J., De Jong, E. S. (Eds.), Industrial crystallisation. North-Holland, Amsterdam, (1982), 123-135

Davis, S. S., The design and evaluation of controlled release dosage forms for oral drug delivery. *STP Pharma*, 3, (1987) 412-417.

Davis, S. S., Fara, J., Hardy, J. G., Transit of pharmaceutical dosage forms through the small intestine. *Gut*, 27, (1986) 886-892.

Davis, S. S., Hardy, J. G., Taylor, M. J., Whalley, D. R., Wilson, C. G., A comparative study of the gastrointestinal transit of a pellet and tablet formulation. *International Journal of Pharmaceutics*, 21, (1984a) 167-177.

Davis, S. S., Hardy, J. G., Taylor, M. J., Whalley, D. R., Wilson, C. G., The effect of food on the gastrointestinal transit of pellets and an osmotic device (Osmet). *International Journal of Pharmaceutics*, 21, (1984b) 331-340.

Develoux, M., Griseofulvin. *Annales de Dermatologie et de Venerologie*, 128, (2001) 1317-1325.

Dhanaraju, M. D., Kumaran, K. S., Baskaran, T., Moorthy, M. S., Enhancement of bioavailability of griseofulvin by its complexation with beta-cyclodextrins. *Drug Development and Industrial Pharmacy*, 24, (1998) 583-587.

Di Martino, P., Scoppa, M., Joiris, E., Palmieri, G. F., Andres, C., Pourcelot, Y., Martelli, S., The spray drying of acetazolamide as a method to modify crystal properties and to improve compression behaviour. *International Journal of Pharmaceutics*, 231, (2001) 209-221.

Dressman, J. B., Reppas, C., In vitro-in vivo correlations for lipophilic, poorly water-soluble drugs. *European Journal of Pharmaceutical Sciences*, 11, (2000) S73-S80.

Dressman, J. B., Yamada, K., Animal models for oral drug absorption. In: Wellington, P., Tse, F. L., Shrikant, V. D. (Eds.), Pharmaceutical bioequivalence. Marcel Dekker, Inc., New York, (1991), 235-266.

El Arini, S. K., Leuenberger, H., Dissolution properties of praziquantel-PVP systems. *Pharmaceutica Acta Helvetiae*, 73, (1998) 89-94.

Elworthy, P. H., Florence, A. T., MacFarlane, C. B., 1968. Solubilization by surfactant active agents. Chapman and Hall, London.

Emara, L. H., Badr, R. M., Elbary, A. A., Improving the dissolution and bioavailability of nifedipine using solid dispersions and solubilizers. *Drug Development and Industrial Pharmacy*, 28, (2002) 795-807.

EMEA. Note for guidance on the investigation of bioavailability and bioequivalence (CPMP/EWP/QWP/1401/09). (1998).

EP. European Pharmacopoeia. 3rd Ed., (1997). Strausbourg.

Epstein, W. L., Shah, V. P., Riegelman, S., Griseofulvin levels in the strateum corneum. Study after oral administration in man. *Archives of Dermatology*, 106, (2004) 344-348.

Evans, D. F., Pye, G., Bramley, R., Clark, A. G., Dyson, T. J., Hardcastle, J. D., Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut*, 29, (1988) 1035-1041.

Fabretto, M., Ralston, J., Sedev, R., Contact angle measurements using the Wilhelmy balance for assymetrically treated samples. *Journal of Adhesion Science and Technology*, 18, (2004) 29-37.

Fairbrother, J. E., Grant, D. J. W., The crystal habit modification of a tablet lubricant, adipic acid. *Journal of Pharmacy and Phamacology*, 30, (1978) 19P.

Fairbrother, J. E., Grant, D. J. W., Crystal engineering studies with an excipient material (adipic acid). *Journal of Pharmacy and Phamacology*, 31, (1979) 27P.

FDA. Draft - guidance for industry: BA and BE studies for orally administered drug products - general considerations. (1999).

Feeley, J. C., York, P., Sumby, B. S., Dicks, H., Determination of surface properties and flow characteristics of salbutamol sulphate, before and after micronisation. *International Journal of Pharmaceutics*, 172, (1998) 89-96.

Fessi, H., Puisieux, F., Devissaguet, J. Ph., Ammoury, N., Benita, S., Nanocapsule formulation by interfacial polymer deposition following solvent displacement. *International Journal of Pharmaceutics*, 55, (1989) R1-R4.

Fidge, N. H., Shiratori, T., Ganguly, J., Goodman, D. S., Pathways of absorption of retinal and retinoid acid in the rat. *Journal of Lipid Research*, 9, (1968) 103-109.

Fini, A., Feroci, G., Solubility and solubilization properties of non-steroidal antiinflammatory drugs. *International Journal of Pharmaceutics*, 126, (1995) 95-102.

Fleisher, D., Bong, R., Stewart, B. H., Improving oral drug delivery: solubility limitations overcome by the use of prodrugs. *Advanced Drug Delivery Reviews*, 19, (1996) 115-130.

Fletcher, J., Griseofulvin recrystallisation. (2005). Personal communication.

Florence, A. T., Drug solubilization in surfactant systems. In: Yalkowsky, S. H. (Ed.), Techniques of solubilization of drugs. Marcel Dekker Inc., New York, (1981), 15-89.

Florence, A. T., Attwood, D., 1998. Physiochemical principles of pharmacy. 3rd Ed., Macmillan Press Ltd, London.

Flory, P. J., Introductory lecture. *Discussions of the Faraday Society*, 57, (1974) 7-18.

Ford, J. L., The current status of solid dispersions. *Pharmaceutica Acta Helvetiae*, 61, (1986) 69-88.

Ford, J. L., Rubinstein, M. H., Phase equilibria and dissolution rates of indomethacin-polyethylene glycol 6000 solid dispersions. *Pharmaceutica Acta Helvetiae*, 53, (1978) 327-332.

Fordtran, J. S., Locklear, T. W., Ionic constituents and osmolality of the gastric and small intestinal fluids after eating. *American Journal of Digestive Diseases*, 11, (1966) 503-521.

Fordtran, J. S., Rector, F. C., Carter, N. W., The mechanism of sodium absorption in human small intestine. *Journal of Clinical Investigation*, 47, (1968) 884-900.

Foster, A., Rades, T., Hempenstall, J., Selection of suitable drug and excipient candidates to prepare glass solutions by melt extrusion for immediate release oral formulations. *Pharmaceutical Technology Europe*, (2002) Oct 1.

Fromming, K. H., Kreuschner, K., Hosemann, R., Hasse, J., Stability of phenylbutazone and griseofulvin in solidified melts with urea. *Acta Pharmaceutica Technologica*, 26, (1980) 69-73.

Gallagher, N., Webb, J., Dawson, A. M., The absorption of ¹⁴C oleic acid and ¹⁴C triolein in bile fistula rats. *Clinical Sciences*, 29, (1965) 73-82.

Gan, L. S., Yanni, S., Thakker, D. R., Modulation of the tight junctions of the Caco-2 cell monolayers by H2-antagonists. *Pharmaceutical Research*, 15, (1998) 53-57.

Gibaldi, M., Feldman, S., Mechanisms of surfactant effects on drug absorption. *Journal of Pharmaceutical Sciences*, 59, (1970) 579-589.

Goldberg, A. H., Gibaldi, M., Kanig, J. L., Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures I -

theorectical considerations and discussion of the literature. *Journal of Pharmaceutical Sciences*, 55, (1965) 1145-1148.

Goldberg, A. H., Gibaldi, M., Kanig, J. L., Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures II - experimental evaluation of an eutectic mixture: urea-acetaminophen system. *Journal of Pharmaceutical Sciences*, 55, (1966a) 482-487.

Goldberg, A. H., Gibaldi, M., Kanig, J. L., Mayersohn, M., Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures IV - chloramphenicol-urea system. *Journal of Pharmaceutical Sciences*, 55, (1966b) 581-583.

Gower, P. E., Dash, C. H., Cephalexin: Human studies of absorption and excretion of a new cephalosporin antibiotic. *British Journal of Pharmacology*, 37, (1969) 738-747.

Gruber, P., Longer, M. A., Robinson, J. R., Some biological issues in oral, controlled drug delivery. *Advanced Drug Delivery Reviews*, 1, (1987) 1-18.

Gullikson, G. W., Cline, W. S., Lorensonn, V., Benz, L., Olsen, W. A., Bass, P., Effects of anionic surfactants on hamster small intestinal membrane structure and function: relationship to surface activity. *Gastroentology*, 73, (1977) 501-511.

Gupta, A. K., Saunder, D. N., Shear, N. H., Antifungal agents: An overview Part I. *Journal of the American Academy of Dermatology*, 30, (1994) 677-698.

Gursoy, R. N., Benita, S., Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drug. *Biomedicines and Pharmacotherapy*, 58, (2004) 173-182.

Hackett, L. P., Dusci, L. J., Determination of griseofulvin in human serum using high-performance liquid chromatography. *Journal of Chromatography*, 155, (1978) 206-208.

Hagermark, O., Berlin, A., Wallin, I., Plasma concentration of griseofulvin in healthy volunteers and out-patients treated for onchomycosis. *Acta Dermatol-Venereologica*, 56, (1987) 289-296.

Hancock, B. C., Parks, M., What is the true solubility advantage for amorphous pharmaceuticals? *Pharmaceutical Research*, 17, (2000) 397-404.

Hancock, B. C., Zografi, G., Characteristics and significances of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Science*, 62, (1997) 87-95.

Hansen, T., Holm, P., Schultz, K., Process characteristics and compaction of spray-dried emulsions containing a drug dissolved in lipid. *International Journal of Pharmaceutics*, 287, (2004) 55-66.

Hardy, J. G., Healey, J. N. C., Lee, S. W., Reynolds, J. R., Gastrointestinal transit of an enteric-coated delayed-release 5-aminosalicylic acid tablet. *Alimentary Pharmacology and Therapeutics*, 1, (1987) 209-216.

Hardy, J. G., Wilson, C. G., Wood, E., Drug delivery to the proximal colon. *Journal of Pharmacy and Pharmacology*, 37, (1985) 874-877.

Hebel, R., Stromberg, M. W., Digestive system. In: Hebel, R., Stromberg, M.W. (Eds.), Anatomy of the Laboratory Rat. Williams and Wilkins, Baltimore, (1976), 43-52.

Herman, P. H., Gels. In: Kruyt, H. R. (Ed.), Colloid Science. Elsevier Publishing Company, Amsterdam, (1949), 483-651.

Hill, V. L., An investigation into the use of MTDSC as a technique for the characterization of pharmaceutical materials. (1999). University of London.

Hillery, A. M., Drug Delivery: The Basic Concepts. In: Hillery, A. M., Lloyd, A.W., Swarbrick, J. (Eds.), Drug Delivery and Targeting for Pharmacists andPharmaceutical Scientists. Taylor and Francis, London and New York, (2001),1-48.

Hilton, J. E., Summers, M. P., The effect of wetting agents on the dissolution of indomethacin solid dispersion systems. *International Journal of Pharmaceutics*, 31, (1986) 157-164.

Hirschorn, J. O., Kornblum, S. S., Dissolution of poorly water-woluble drugs. II. Excipient dilution and force of compression effects on tablets of a quinazolinone compound. *Journal Of Pharmaceutical Sciences*, 60, (1971) 445-448.

Houin, G., Woodley, J., Séance thématique: Le franchissement des barrières digestives. *Annales Pharmaceutiques Francaises*, 60, (2002) 365-371.

Hovgaard, L., Brondsted, H., Current applications of polysaccharides in colon targeting. *Critical Reviews in Therapeutic Drug Carrier Systems*, 13, (1996) 185-223.

Hu, J., Johnston, K. P., Williams III, R. O., Spray freezing into liquid (SFL) particle engineering technology to enhance dissolution of poorly soluble drugs: organic solvent versus organic/aqeuous co-solvent systems. *European Journal of Pharmaceutical Sciences*, 20, (2003) 295-303.

Hu, J., Johnston, K. P., Williams, I. I. I., Rapid dissolving high potency danazol powders produced by spray freezing into liquid process. *International Journal of Pharmaceutics*, 271, (2004) 145-154.

Hu, L., Prodrugs: effective solutions for solubility, permeability and targeting challenges. *IDrugs: The Investigational Drugs Journal*, 7, (2004) 736-742.

Hunter, E., Fell, J. T., Sharma, H., In vivo disintergration of hard gelatin capsules in fasting and non-fasting subjects. *International Journal of Pharmaceutics*, 4, (1980) 175-183.

Hunter, E., Fell, J. T., Sharma, H., The gastric emptying of pellets contained in hard gelatin capsules. *Drug Development and Industrial Pharmacy*, 8, (1982) 751-757.

Jain, N. K., Patel, V. V., Taneja, L. N., Hydrotropic solubilization of nifedipine. *Die Pharmazie*, 43, (1988) 194-196.

James, K. C., 1986. Solubility and related properties. Marcel Dekker, New York.

Jibry, N., Heenan, R. K., Murdan, S., Amphiphilogels for drug delivery: formulation and characterization. *Pharmaceutical Research*, 21, (2004) 1852-1861.

Jibry, N., Murdan, S., In vivo investigation, in mice and in man, into the irritation potential of novel amphiphilogels being studied as transdermal drug carriers. *European Journal of Pharmaceutics and Biopharmaceutics*, 58, (2004) 107-119.

Joint Formulary Committee, 2004. British National Formulary. 48th Ed., The Pharmaceutical Press, London.

Jouyban-Gharamaleki, A., Valaee, L., Barzegar-Jalali, M., Clark, B. J., AcreeJr., W. E., Comparison of various cosolvency models for calculating solute solubility in water-cosolvent mixtures. *International Journal of Pharmaceutics*, 177, (1999) 93-101. Jung, J., Perrut, M., Particle design using supercritical fluids: Literature and patent survey. *The Journal of Supercritical Fluids*, 20, (2001) 179-219.

Junginger, H. E., Verhoef, J. C., Macromolecules as safe penetration enhancers for hydrophilic drugs--a fiction? *Pharmaceutical Science & Technology Today*, 1, (1998) 370-376.

Kadir, S., Murakami, T., Higashi, Y., Yata, N., Gastrointestinal absorption of griseofulvin from liquid organic acids and esters in rats. *International Journal of Pharmaceutics*, 33, (1986) 235-242.

Kanig, J. L., Properties of fused mannitol in compressed tablets. *Journal Of Pharmaceutical Sciences*, 53, (1964) 188-192.

Kararli, T. T., Gastrointestinal absorption of drugs. *Critical Reviews in Therapeutic Drug Carrier Systems*, 6, (1989) 39-86.

Kararli, T. T., Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharmaceutics and Drug Disposition*, 16, (1995) 351-380.

Kararli, T. T., Stolzenbach, J., Rate-limiting steps in oral absorption of leucotriene D4 antagonist in the beagle dogs. *Journal of Pharmaceutical Sciences*, 81, (1992) 875-878.

Katchen, B., Symchowicz, S., Correlation of dissolution rate and griseofulvin absorption in man. *Journal of Pharmaceutical Sciences*, 56, (1967) 1108-1111.

Kaus, L. C., Fell, J. T., Sharma, H., Taylor, D. C., On the intestinal transit of a single non-disintergrating object. *International Journal of Pharmaceutics*, 20, (1984) 315-323.

Kelly, K. A., Gastric emptying of liquids and solids: role of proximal and distal stomach. *American Journal of Physiology*, 239, (1980) G71-G76.

Khalafalla, N., Elgholmy, Z. A., Khalil, S. A., Influence of high fat diet on GI absorption of griseofulvin tablets in man. *Pharmazie*, 36, (1981) 692-693.

Khosla, R., Davis, S. S., Gastric emptying and small and large bowel transit of non-disintegrating tablets in fasted subjects. *International Journal of Pharmaceutics*, 52, (1989) 1-10.

Kim, K. H., Singh, B. N., Drug delivery - oral route. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker Inc., New York, (2002), 886-909.

Kingham, J. G., Loehry, C. A., Permeability of the small intestine after intraarterial injections of histamine type mediators and irradiation. *Gut*, 17, (1976) 517-526.

Koch-Wesser, J., Schechter, P. J., Slow release preparation in clinical perspective. In: Prescott, L. F., Nimmo, W. S. (Eds.), Drug absorption. MTP Press, Lancaster, (1981), 217-227.

Kuiken, S. D., Samson, M., Camilleri, M., Mullan, B. P., Burton, D. D., Kost, L. J., Hardyman, T. J., Brinkmann, B. H., O'Connor, M. K., Development of a test to measure gastric accomodation in humans. *American Journal of Physiology*, 277, (1999) G1217-G1221.

Langmuir, I., The adsorption of gases on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society*, 40, (1918) 1368-1403.

Lee, V. H. L., Yang, J. J., Oral Drug Delivery. In: Hillery, A. M., Lloyd, A. W., Swarbrick, J. (Eds.), Drug Delivery and Targeting for Pharmacists and Pharmaceutical Scientists. Taylor and Francis, London and New York, (2001), 145-184.

Lennenäs, H., Human jejunal effective permeability and its correlation with preclinical drug absorption models. *Journal of Pharmacy and Pharmacology*, 49, (1997) 627-638.

Lennernas, H., Regardh, C.-G., Regional gastrointestinal absorption of the beta-blocker Pafenolol in the rat and intestinal transit rate determined by movement of ¹⁴C-polyethylene glycol (PEG 4000). *Pharmaceutical Research*, 10, (1993) 130-135.

Leuner, C., Dressman, J., Improving drug solubility for oral delivery using solid dispersions. *European Journal of Pharmaceutics and Biopharmaceutics*, 50, (2000) 47-60.

Levine, R. R., Factors affecting the gastrointestinal absorption of drugs. *Digestive Diseases*, 15, (1970) 171-188.

Levy, G., Effect of particle size on dissolution and gastrointestinal absorption rates of pharmaceuticals. *American Journal Of Pharmacy And The Sciences Supporting Public Health*, 135, (1963) 78-92.

Levy, G., Gibaldi, M., Procknal, J. A., Effect of an anticholinergic agent on riboflavin absorption in man. *Journal of Pharmaceutical Sciences*, 61, (1972) 798-799.

Liau, S.-S., Camilleri, M., Kim, D.-Y., Stephens, D., Burton, D. D., O'Connor, M. K., Pharmacological modulation of human gastric volumes demonstrated noninvasively using SPECT imaging. *Neurogastroenterology and Motility*, 13, (2001) 533-542.

Lin, C. C., Magat, J., Chang, R., MacGlotter, J., Symchowicz, S., Absorption, metabolism and excretion of ¹⁴C griseofulvin in man. *Journal of Pharmacology and Experimental Therapeutics*, 187, (1973) 415-422.

Lin, C. C., Symchowicz, S., Absorption, distribution, metabolism, and excretion of griseofulvin in man and animals. *Drug Metabolism Reviews*, 4, (1975) 75-95.

Lindemann, B., Solomon, A. K., Permeability of luminal surface of intestinal mucosal cells. *Journal of General Physiology*, 85, (1962) 801-810.

Lindenberg, M., Kopp, S., Dressman, J. B., Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58, (2005) 265-278.

Lloyd, D. J., In: Alexander, J. (Ed.), Colloid Chemistry. The Chemical Catalog Company, New York, (1926), 767.

Lo, W. Y., Law, S. L., Dissolution behavior of griseofulvin solid dispersions using polyethylene glycol, talc, and their combination as dispersion carriers. *Drug Development and Industrial Pharmacy*, 22, (1996) 231-236.

Löbenberg, R., Amidon, G. L., Modern bioavailability and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *European Journal of Pharmaceutics and Biopharmaceutics*, 50, (2000) 3-12.

Lowell, S., Shields, J. E., 1984. Powder surface area and porosity. 2nd Ed., Chapman and Hall, London and New York.

Mackellar, A. J., Buckton, G., Newton, J. M., Chowdry, B. Z., Orr, C. A., The controlled crystallisation of a model powder: 1. The effects of altering the

stirring rate and supersaturation profile, and the incorporation of a surfactant (Poloxamer 188). *International Journal of Pharmaceutics*, 112, (1994) 65-78.

Mackin, L., Sartnurak, S., Thomsa, I., Moore, S., The impact of low levels or amorphous material (<5%) on the blending characteristics of a direct compression formulation. *International Journal of Pharmaceutics*, 231, (2002) 213-226.

Mann, F. C., Brimhall, S. D., Foster, J. P., The extrahepatic biliary tract in common domestic and laboratory animals. *Anatomical Record*, 18, (1920) 47-66.

Manninen, V., Melin, J., Apajalahti, A., Karesoja, M., Altered absorption of digoxin in patients given propantheline and metoclopramide. *Lancet*, 1, (1973) 398-400.

Martis, L., Hall, N. A., Thakkar, A. L., Micelle formation and testosterone solubilization by sodium glycocholate. *Journal of Pharmaceutical Sciences*, 61, (1972) 1757-1761.

Marvola, M., Kannikoski, K., Aito, H., Nykanen, S., Kokkonen, P., The effect of food on gastrointestinal transit and drug absorption of a multiparticulate sustained release verapamil formulation. *International Journal of Pharmaceutics*, 53, (1989) 145-155.

Masters, K., 1991. Spray drying handbook. 4th Ed., Longman Scientific and Technical, Harlow.

Matsumoto, S. T., Zografi, G., Pjysical properties of solid molecular dispersions of indomethacin with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinyl-acetate) in relation to indomethacin crystallisation. *Pharmaceutical Research*, 16, (1999) 1722-1728.

Mayersohn, M., Principles of Drug Absorption. In: Banker, G. S. (Ed.), Modern Pharmaceutics. Marcel Dekker, New York, (2002), 23-66.

McGilveray, I. J., Matlok, G. L., Some factors affecting the absorption of paracetamol. *Journal of Pharmacy and Pharmacology*, 24, (1972) 615-619.

Merck Index, 1996. An encyclopedia of chemicals, drugs and biologicals. 12th Ed., Budavari, S. (Ed.). Whitehouse Station, NJ (US).

Mezick, J. A., Tompkins, R. K., Cornwell, D. G., Absorption and intestinal lymphatic transport of ¹⁴C menadione. *Life Sciences*, 7, (1968) 153-158.

Millard, J., Alvarez-Nunez, F., Yalkowsky, S., Solubilization by cosolvents. Establishing useful constants for the log-linear model. *International Journal of Pharmaceutics*, 245, (2002) 153-166.

Millqvist-Fureby, A., Malmsten, M., Bergenstahl, B., Spray-drying of trypsin -surface characterisation and activity preservation. *International Journal of Pharmaceutics*, 188, (1999) 243-253.

Miyazaki, S., Inoue, H., Yahamira, T., Nadai, T., Interaction of drugs with bile components, effect of bile salts on the dissolution behaviour of indomethacin and phenylbutazone. *Chemical Pharmaceutical Bulletin*, 27, (1979) 2468-2472.

Moneghini, M., Kikic, I., Voinovich, D., Perissutti, B., Alessi, P., Cortesi, A., Princivalle, F., Solinas, D., Study of the solid state of carbamazepine after processing with gas anti-solvent technique. *European Journal of Pharmaceutics and Biopharmaceutics*, 56, (2003) 281-289.

Montagne, L., Piel, C., Lalles, J. P., Effect of Diet on Mucin Kinetics and Composition: Nutrition and Health Implications. *Nutrition Reviews*, (2004) March. Mosharraf, M., Nystrom, C., The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. *International Journal of Pharmaceutics*, 122, (1995) 35-47.

Mosharraf, M., Nystrom, C., Apparent solubility of drugs in partially crystalline systems. *Drug Development and Industrial Pharmacy*, 29, (2003) 603-622.

Mullen, T. L., Mullen, M., Van Bruggen, J. T., Role of solvent drag in intestinal transport. *Journal of General Physiology*, 85, (1985) 347-363.

Mulley, B. A., Solubility in systems containing surface-active agents. In: Bean,H. S., Beckett, A. H., Carless, J. E. (Eds.), Advances in pharmaceutical sciences. Academic Press, London, (1964), 86-194.

Mullin, J. W., 1997. Crystallisation. 3rd Ed., Butterworth-Heinmann, Oxford.

Mundy, M. J., Wilson, C. G., Hardy, J. G., The effect of eating on transit through the small intestine. *Nuclear Medicine Communications*, 10, (1989) 45-50.

Murdan, S. and Andrýsek, T. Novel amphiphilogels for the oral delivery of cyclosporine A. Proceedings of the 30th Annual Meeting of the Controlled Release Society. (2003) Poster 708.

Murdan, S., Arunothayanun, P., Ford, J., and Florence, A. T. Amphiphilogel systems as oral delivery vehicles for cyclosporine A: preliminary *in vivo* results. *Proceedings of the Symposium on Lipid and Surfactant Disperse Systems*, (1999a) 237-238.

Murdan, S., Gregoriadis, G., Florence, A. T., Novel sorbitan monostearate organogels. *Journal of Pharmaceutical Sciences*, 88, (1999b) 608-614.

Murdan, S., Gregoriadis, G., Florence, A. T., Sorbitan monostearate/polysorbate 20 organogels containing niosomes: a delivery vehicle for antigens? *European Journal Of Pharmaceutical Sciences*, 8, (1999c) 177-186.

Murdan, S., Gregoriadis, G., Florence, A. T., Interaction of a nonionic surfactant-based organogel with aqueous media. *International Journal of Pharmaceutics*, 180, (1999d) 211-214.

Nadai, T., Kondo, R., Tatematsu, A., Sezaki, H., Drug-induced histological changes and its consequence on the permeability of the small intestinal mucosa. I. EDTA, tetracycline and sodium lauryl sulfate. *Chemical and Pharmaceutical Bulletin*, 20, (1972) 1139-1144.

Nakashima, E., Tsuji, A., Mizuo, H., Yamana, T., Kinetics and mechanism of in vitro uptake of amino- β -lactam antibiotics by rat intestine and relation to the intact-peptide transport system. *Biochemical Pharmacology*, 33, (1984) 3345-3352.

Naseem, A., Olliff, C. J., Martini, L. G., Lloyd, A. W., Effects of plasma irradiation on the wettability and dissolution of compacts of griseofulvin. *International Journal of Pharmaceutics*, 269, (2004) 443-450.

Nellans, H. N., Mechanisms of peptide and protein absorption (1) Paracellular intestinal transport: modulation of absorption. *Advanced Drug Delivery Reviews*, 7, (1991) 339-364.

Neumann, A. W., Good, R. J., Techniques of measuring contact angles. In: Good, R. J., Stromberg, R. R. (Eds.), Surface and Colloid Science. Plenum Press, New York and London, (1979), 31-91. Nogami, H., Nagai, T., Sasuta, A., Powder preparations. XVII. Dissolution rate of sulfonamides by rotating disc method. *Chemical and Pharmaceutical Bulletin*, 14, (1966) 329-338.

Noguchi, T., Charman, W. N. A., Stella, V. J., Lymphatic appearance of DDT in thoracic or mesentric lymph duct cannaluted rats. *International Journal of Pharmaceutics*, 24, (1985a) 185-192.

Noguchi, T., Charman, W. N. A., Stella, V. J., The effect of drug lipophilicity and lipid vehicles on the lymphatic absorption of various testosterone esters. *International Journal of Pharmaceutics*, 24, (1985b) 173-184.

Noyes, A., Whitney, W., The rate of solution of solid substances in their own solutions. *Journal of the American Chemical Society*, 19, (1897) 930-934.

O'Brien, M., McCauley, J., Cohen, E., Indomethacin. *Analytical Profile of Drug Substances*, 13, (1984) 211-238.

O'Reilly, S., Wilson, C. G., Hardy, J. G., The influence of food on multiparticulate dosage forms. *International Journal of Pharmaceutics*, 34, (1987) 213-216.

Oakley, D. E., Produce uniform particles by spray drying. *Chemical Engineering Progress*, 93, (1997) 48-54.

Ochsenfahrt, H., Winne, D., The contribution of solvent drag to the intestinal absorption of the acidic drugs benzoic acid and salicylic acid from the jejunum of the rat. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 281, (1974a) 197-217.

Ochsenfahrt, H., Winne, D., The contribution of solvent drag to the intestinal absorption of the basic drugs amidopyrine and antipyrine from the jejunum of

the rat. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 281, (1974b) 175-196.

Oguchi, T., Sasaki, N., Hara, T., Tozuka, Y., Yamamoto, K., Differentiated thermal crystallization from amorphous chenodeoxycholic acid between the ground specimens derived from the polymorphs. *International Journal of Pharmaceutics*, 253, (2003) 81-88.

Ollerenshaw, K. J., Norman, S., Wilson, C. G., Hardy, J. G., Exercise and small intestinal transit. *Nuclear Medicine Communications*, 8, (1987) 105-110.

Paradkar, A., Ambike, A. A., Jadhav, B. K., Mahadik, K. R., Characterization of curcumin-PVP solid dispersion obtained by spray drying. *International Journal of Pharmaceutics*, 271, (2004) 281-286.

Park, H. M., Chernish, J. M., Rosenbek, B. D., Brunelle, R. L., Hargrove, B., Wellman, H. N., Gastric emptying of enteric coated tablets. *Digestive Diseases and Sciences*, 29, (1984a) 207-212.

Park, K., Ch'ng, H. S., Robinson, J. R., Alternative approaches to oral controlled drug delivery, bioadhesives and in-situ systems. In: Anderson, J. M., Kim, S. W. (Eds.), Recent Advances in Drug Delivery. Plenum, New York, (1984b), 163-183.

Parker, G., Wilson, C. G., Hardy, J. G., The effect of capsule size and density on transit through the proximal colon. *Journal of Pharmacy and Pharmacology*, 40, (1988) 376-377.

Pham, H. H., Luo, P., Genin, F., Dash, A. K., Synthesis and characterization of hydroxyapatite-ciprofloxacin delivery systems by precipitation and spray drying technique. *AAPS PharmSciTech*, 3, (2002) E1.

Phillips, S. F., Diarrhoea: Role of the ileocaecal sphincter. In: Barbara, L., Miglioli, M., Phillips, S. F. (Eds.), New trends in pathophysiology and therapy of the large bowel. Elsevier, Amsterdam/Oxford, (1983).

Phillips, S. F., Quigley, E. M. M., Kumar, D., Kamath, P. S., Motility of the ileocolonic junction. *Gut*, 29, (1988) 390-406.

Pinnamaneni, S., Das, N. G., Das, S. K., Formulation approaches for orally administered poorly soluble drugs. *Pharmazie*, 57, (2002) 291-300.

Prescott, L. F., Gastrointestinal absorption of drugs. *Medical Clinics of North America*, 58, (1974) 907-916.

Price, J. M. C., Davis, S. S., Wilding, I. R., The effect of fibre on gastrointestinal transit times in vegetarians and omnivores. *International Journal of Pharmaceutics*, 76, (1991) 123-131.

Priestley, G. C., Brown, J. C., Inhibited proliferation of human scleroderma skin fibroblasts and rheumatoid synovial cells with griseofulvin in vitro. *Acta Dermatol-Venereologica*, 62, (1982) 167-169.

Quigley, E. M. M., Borody, T. J., Phillips, S. F., Wienbeck, M., Tucker, R. L., Haddad, A., Motility of the terminal ileum and ileocecal sphincter in healthy humans. *Gastroenterology*, 87, (1984) 857-866.

Quintanar-Guerrero, D., Allémann, E., Fessi, H., Doelker, E., Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. *Drug Development and Industrial Pharmacy*, 24, (1998) 1113-1128.

Raiman, J., Tormalehto, S., Yritys, K., Junginger, H. E., Monkkonen, J., Effects of various absorption enhancers on transport of clodronate through Caco-2 cells. *International Journal of Pharmaceutics*, 261, (2003) 129-136.

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Rasenack, N., Müller, B. W., Micron-sized drug particles: common and novel micronization techniques. *Pharmaceutical Development and Technology*, 9, (2004) 1-13.

Rasenack, N., Steckel, H., Müller, B. W., Preparation of microcrystals by in situ micronization. *Powder Technology*, 143-144, (2004) 291-296.

Raseneck, N., Hartenhauer, H., Müller, B. W., Microcrystals for dissolution rate enhancement of poorly water-soluble drugs. *International Journal of Pharmaceutics*, 254, (2003) 137-145.

Rees, J. A., Collett, J. H., The dissolution of salicylic acid in micellar solutions of polysorbate 20. *Journal of Pharmacy and Pharmacology*, 26, (1974) 956-960.

Reverchon, E., Della Porta, G., Spada, A., Antonacci, A., Griseofulvin micronization and dissolution rate improvement by supercritical assisted atomization. *Journal of Pharmacy and Pharmacology*, 56, (2004) 1379-1387.

Richards, J. H., Physicochemical principles of pharmaceutics. In: Aulton, M. E. (Ed.), Pharmaceutics: The science of dosage form design. Churchill Livingstone, London, (1996), 15-128.

Ritschel, W. A., Targeting the gastrointestinal tract: new approaches. *Methods and Findings in Experimental and Clinical Pharmacology*, 13, (1991) 313-336.

Roberts, R. J., Rowe, R. C., York, P., The relationship between indentation hardness of organic solids and their molecular structure. *Journal of Materials Science*, 29, (1994) 2289-2296.

Ropert, A., des Varannes, S. B., Bizais, Y., Roze, C., Galmiche, J. P., Simultaneous assessment of liquid emptying and proximal gastric tone in humans. *Gastroenterology*, 105, (1993) 667-674. Rouge, N., Buri, P., Doelker, E., Drug absorption sites in the gastrointestinal tract and dosage forms for site-specific delivery. *International Journal of Pharmaceutics*, 136, (1996) 117-139.

Rowland, M., Riegelman, S., Epstein, W. L., Absorption kinetics of griseofulvin in man. *Journal of Pharmaceutical Sciences*, 57, (1968) 984-989.

Rowland, M., Tozer, T. N., 1995. Clinical pharmacokinetics: Concepts and applications. 3rd Ed., Williams and Wilkins, Baltimore.

Rustichelli, C., Gamberini, G., Ferioli, V., Gamberini, M. C., Ficarra, R., Tommasini, S., Solid-state study of polymorphic drugs: carbamazepine. *Journal of Pharmaceutical and Biomedical Analysis*, 23, (2000) 41-54.

Saleh, A. M., el Shibini, H. A., Motawi, M. M., The mechanism of solubilization of khellin by sodium benzoate and salicylate. *Die Pharmazie*, 24, (1969) 753-756.

Saleki-Gerhardt, A., Ahlneck, C., Zografi, G., Assessment of disorder in crystalline solids. *International Journal of Pharmaceutics*, 101, (1994) 237-247.

Sarkari, M., Brown, J., Chen, X., Swinnea, S., Williams III, R. O., Johnston, K. P., Enhanced drug dissolution using evaporative precipitation into aqueous solution. *International Journal of Pharmaceutics*, 243, (2002) 17-31.

Saunders, D. R., Sillery, J., Rachmilewitz, D., Effect of dioctyl sodium sulfoccinate on structure and function of rodent and human intestine. *Gatsroentology*, 69, (1975) 380-386.

Save, T., Venkitachalam, P., Studies on solid dispersions of nifedipine. *Drug Development and Industrial Pharmacy*, 18, (1992) 1663-1679.
Scartazzini, R., Luisi, P. L., Organogels from lecithins. *Journal of Physical Chemistry*, 92, (1988) 829-833.

Schanker, L. S., Jeffrey, J. J., Active transport of foreign pyramidines across the intestinal epithelium. *Nature*, 190, (1961) 727.

Schipunov, Yu. A., Lecithin organogel. A micellar system with unique properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 183-185, (2001) 541-554.

Scholes, P. D., Coombes, A. G. A., Illum, L., Davis, S. S., Watts, J. F., Ustariz, C., Vert, M., Davies, M. C., Detection and determination of surface levels of poloxamer and PVA surfractant on biodegradable nanospheres using SSIMS and XPS. *Journal of Controlled Release*, 59, (1999) 261-278.

Schott, H., Colloidal dispersions. Remington's Pharmaceutical Sciences. The Philadelphia College of Pharmacy and Science, Philadelphia, (1985), 286-289.

Schultz, S. G., Frizzell, R. A., Nellans, H. N., Ion transport by mammalian small intestine. *Annual Review of Physiology*, 36, (1974) 51-92.

Schwarz, H. J., Waldman, B. A., Madrid, V., GLC determination of griseofulvin in human plasma. *Journal of Pharmaceutical Science*, 65, (1976) 370-372.

Scott, A., Behaviour of radioactive griseofulvin in skin. *Nature*, 187, (1960) 705-707.

Sekiguchi, K., Obi, N., Studies on absorption of eutectic mixtures. I. A comparison of the behaviour of eutectic mixtures of sulphathiazole and that of ordinary sulphathiazole in man. *Chemical and Pharmaceutical Bulletin*, 9, (1961) 866-872.

Serajuddin, A. T. M., Solid dispersions of poorly water-soluble drugs: Early promises, subsequent problems and, recent breakthroughs. *Journal of Pharmaceutical Sciences*, 88, (1999) 1058-1066.

Shalaev, E. Y., Zografi, G., How does residual water affect solid-state degradation of drugs in the amorphous state. *Journal of Pharmaceutical Science*, 85, (1996) 1137-1141.

Shindo, H., Komai, T., Kawai, K., Studies on the metabolism of D- and Lisomers of 3,4-dihydroxyphenyl-alanine (DOPA). V. Mechanism of intestinal absorption of D- and L-DOPA-¹⁴C in rats. *Chemical and Pharmaceutical Bulletin*, 21, (1973) 2031-2038.

Shindo, H., Komai, T., Kawai, K., Mechanism of intestinal absorption and brain uptake of L-5-hydroxytryptophan in rats, as compared to those of L-3,4dihydroxyphenylalanine. *Chemical and Pharmaceutical Bulletin*, 25, (1977) 1417-1425.

Short, M. P., Sharkey, P., Rhodes, C. T., Dissolution of hydrocortisone. *Journal of Pharmaceutical Sciences*, 61, (1972) 1732-1735.

Sieber, S. M., Cohn, V. H., Wynn, W. T., The entry of foreign compounds into the thoracic duct lymph of rat. *Xenobiotica*, 4, (1974) 265-284.

Simonelli, A. P., Mehta, S. C., Higuchi, W. I., Dissolution rates of high energy sulfathiazole--povidone coprecipitates II: characterization of form of drug controlling its dissolution rate via solubility studies. *Journal of Pharmaceutical Sciences*, 65, (1976) 355-361.

Singh, M., Tan, G., Agarwal, V., Fritz, G., Maskos, K., Bose, A., John, V., McPherson, G., Structural evolution of a two-component organogel. *Langmuir*, 20, (2004) 7392-7398. Soergel, K. H., Flow measurements of test meals and fasting contents in the human small intestine. In: Demling, L. (Ed.), Proceedings of the International Symposium on Motility of the Gastrointestinal Tract. Thieme, Stuttgard, (1971), 81-86.

Somasundaram, S., Rafi, S., Hayllar, J., Macpherson, A., Mahmod, T., Scott, D., Wrigglesworth, J. M., Bjarnason, I., Mitochondrial damage: a possible mechanism of the 'topical' phase of NSAID induced injury to rat intestine. *Gut*, 41, (1997) 344-353.

Spiller, R. C., Trotman, I. F., Adrian, T. E., Bloom, S. R., Misiewicz, J. J., Silk, D. B. A., Further characterisation of the 'ileal brake' reflex in man - effect of ileal infusion of partial digests of fat, protein, and starch on jejunal motility and release of neurotensin, enteroglucagon, and peptide YY. *Gut*, 29, (1988) 1042-1051.

Steckel, H., Brandes, H. G., A novel spray-drying technique to produce low density particles for pulmonary delivery. *International Journal of Pharmaceutics*, 278, (2004) 187-195.

Stefen, M., Controlled precipitation: a method to produce small drug particles and increase bioavailability. *J. Galen*, 181, (1988) 45-53.

Stella, V. J., Rajewski, R. A., Cyclodextrins: Their future in drug formulation and delivery. *Pharmaceutical Research*, 14, (1997) 556-567.

Stevens, C. E., Comparative physiology of the digestive system. In: Swenson,M. J. (Ed.), Dukes' Physiology of Domestic Animals. Comstock PublishingAssociates, Ithaca, NY and London, (1977), 216-232.

Stevens, C. E., Physiological implications of microbial digestion in the large intestine of mammals: relation to dietary factors. *Americal Journal of Clinical Nutrition*, 31, (1978) S161-S168.

Sun, E. A., Snape, W. J., Cohen, S., Renny, A., The role of opiate receptors and cholinergic neurons in the gastrocolic response. *Gastroenterology*, 82, (1982) 689-693.

Sutanata, W., Craig, D. Q., Newton, J. M., An evaluation of the mechanisms of drug release from glyceride bases. *Journal of Pharmacy and Pharmacology*, 47, (1995) 182-187.

Tachibana, T., Nakamura, A., A method for preparing an aqueous colloidal dispersion of organic materials using water-soluble polymers: dispersion of beta-carotene by polyvinylpyrrolidone. *Kolloid-Zeitschrift und Zeitschrift für Polymere*, 203, (1965) 130-133.

Taylor, L. S., Zografi, G., Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharmaceutical Research*, 14, (1997) 1691-1698.

Testa, B., Joachim, M., Prodrug design. In: Swarbrick, J., Boylan, J. C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker Inc., New York, (2002), 2304-2311.

Thompson, D. O., Cyclodextrins - Enabling excipients: their present and future use in pharmaceutics. *Critical Reviews in Therapeutic Drug Carrier Systems*, 14, (1997) 1-104.

Toouli, J., Baker, R. A., Innervation of the sphincter of Oddi: physiology and considerations of pharmacological intervention in biliary dyskinesia. *Pharmacology and Therapeutics*, 49, (1991) 269-281.

Townley, E. R., Griseofulvin. *Analytical Profiles of Drug Substances*, 8, (1979) 219-249.

Trotta, M., Cavalli, R., Chirio, D., Griseofulvin nanosuspensions from triacetinin-water emulsions. *STP Pharma Sciences*, 13, (2003) 423-426.

Trotta, M., Gallarate, M., Carlotti, M. E., Morel, S., Preparation of griseofulvin nanoparticles from water-dilutable microemulsions. *International Journal of Pharmaceutics*, 254, (2003) 235-242.

Urbanetz, N. A., Lippold, B. C., Solid dispersions of nimodipine and polyethylene glycol 2000: dissolution properties and physico-chemical characterisation. *European Journal of Pharmaceutics and Biopharmaceutics*, 59, (2005) 107-118.

Usui, F., Maeda, K., Kusai, A., Nishimura, K., Keiji, Y., Inhibitory effects of water-soluble polymers on precipitation of RS-8359. *International Journal of Pharmaceutics*, 154, (1997) 59-66.

van der Merwe, S. M., Verhoef, J. C., Verheijden, J. H. M., Kotze, A. F., Junginger, H. E., Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, 58, (2004) 225-235.

van Wynsberghe, D., Noback, C. R., Carola, R., 1992. Human anatomy and physiology. 3rd Ed., McGraw-Hill Inc., New York.

Vander, A., Sherman, J., Luciano, D., The digestion and absorption of food. In: Human Physiology: The Mechanisms of Body Function. McGraw-Hill, New York, (2001), 553-592.

Varia, S. A., Schuller, S., Sloan, K. B., Stella, V. J., Phenytoin prodrugs III: Water-soluble prodrugs for oral and/or parenteral use. *Journal of Pharmaceutical Sciences*, 73, (1984) 1068-1073. Veiga, M. D., Diaz, P. J., Ahsan, F., Interactions of griseofulvin with cyclodextrins in solid binary systems. *Journal of Pharmaceutical Sciences*, 87, (1998) 891-900.

Viboonkiat, R., Methods to prepare amorphous material for rapid dissolution solid dosage forms. (2003). University of London.

Vojnovic, D., Fubessa, F., Bogotaj, M., Mrhar, A., Formulation and evaluation of vinyl pyrrolidone/vinyl acetate copolymer microspheres with griseofulvin. *Journal of Microencapsulation*, 10, (1993) 89-99.

Vonk, R. J., Van Doorn, A. B. D., Strubbe, J. H., Bile secretion and composition in the freely moving, unanesthetized rat with a permanent biliary drainage: influence of food intake on bile flow. *Clinical Science and Molecular Medicine*, 55, (1978) 253-259.

Ward, F. W., Coates, M. E., Gastrointestinal pH measurements in rats: influence of the microbial flora, diet and fasting. *Laboratory Animals*, 21, (1987) 216-222.

Ward, G. H., Schultz, R. K., Process-induced crystallinity changes in albuterolsulfate and its effect on powder physical stability. *Pharmaceutical Research*, 12, (1995) 773-779.

Weuts, I., Kempen, D., Verreck, G., Decorte, A., Heymans, K., Peeters, J., Brewster, M., Van den Mooter, G., Study of the physicochemical properties and stability of solid dispersions of loperamide and PEG6000 prepared by spray drying. *European Journal of Pharmaceutics and Biopharmaceutics*, 59, (2005) 119-126.

Wilding, I. R., Coupe, A. J., Davis, S. S., The role of γ -scintigraphy in oral drug delivery. *Advanced Drug Delivery Reviews*, 46, (2001) 103-124.

Wilhelmy, L., Ueber die abhangugkeit der capillaritats-constanten des alkohols von substanz und gerstalt des benetzten festen korpers. *Annalen der Physik und Chemie*, 119, (1863) 177-217.

Wilson, C. G., Washington, N., 1989. Physiological pharmaceutics: Biological barriers to drug absorption. Ellis Horwood Ltd., Chichester.

Wright, R. A., Snape, W. J., Battle, W., Cohen, S., London, R. L., Effect of dietary components on the gastrocolic response. *American Journal of Physiology*, 238, (1980) G228-G232.

Wu, S., 1982. Polymer interface and adhesion. Marcel Dekker, New York.

Xu, X., Ayyagari, M., Tata, M., John, V. T., McPherson, G. L., Formation of novel organogels by the addition of phenols to AOT micelles in isooctane. *Journal of Physical Chemistry*, 97, (1993) 11350-11353.

Yamamoto, H., 1-Acyl-indoles II. A new synthesisof 1-(*p*-chlorobenzyl)-5methoxy-2-methyl-3-indolyacetic acid and its polymorphism. *Chemical and Pharmaceutical Bulletin (Tokyo)*, 16, (1968) 17-19.

Yonezawa, M., Basic structure of the intestinal adsorption. I. Changes to the rabbit mucosa adter exposure to various surfactants. *Nihon University Journal of Medicine*, 19, (1977) 125-141.

York, P., The design of dosage forms. In: Aulton, M. E. (Ed.), Pharmaceutics: The science of dosage form design. Churchill Livingstone, London, (1988), 1-13.

Yu, L., Amorphous pharmaceutical solids: preparation, characterization and stabilization. *Advanced Drug Delivery Reviews*, 48, (2001) 27-42.

Yu, L. X., An integrated model for determining causes of poor oral drug absorption. *Pharmaceutical Research*, 16, (1999) 1883-1887.

Yu, L. X., Lipka, E., Crison, J. R., Amidon, G. L., Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Advanced Drug Delivery Reviews*, 19, (1996) 359-376.