EXPLORING THE POTENTIAL OF OPTIMISED STARCH PARTICULATES AS PLATFORM FOR ORAL DELIVERY OF A MODEL GASTROLABILE DRUG CEFOTAXIME

by

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LIST OF SYMBOLS

Symbol	Description
∞	Infinity
μ	micro
σ	standard deviation of intercept

LIST OF ABBREVIATIONS

Abbreviation	Description
Adeq Pre	adequate precision
Adj R^2	adjusted correlation coefficient
ANN	artificial neural network
ANOVA	analysis of variance
AOT	aerosol OT (1,4-bis(2-ethylhexyl) sodium sulfosuccinate)
ATR-AOT	combination of surfactants AOT and ATR
ATR	aerosol TR (sodium 1,2-bis (tridecoxycarbonyl) ethanesulfonate)
$AUC_{0-\infty}$	area under the curve from zero to infinity
$AUC_{0-\infty, norm}$	dose and weight normalised area under the curve
AUC_{0-t}	area under the curve from zero to time t
AUFS	absorbance units full scale
BBD	box Behnken Design
BCS	biopharmaceutical classification system
BOS	based on starch
°C	Degree Celsius
$C_{AQUEOUS}$	control containing cefotaxime sodium in aqueous solution
CCD	central composite design
CI	confidence interval
C_{max}	peak concentration
$C_{max, norm}$	dose and weight normalised peak concentration
CS	corn starch
CTAB	cetyl trimethyl ammonium bromide
$C_{VEHICLE-PS}$	control containing vehicle for potato starch
$C_{VEHICLE-SS}$	control containing vehicle for soluble starch
CYP 450	cytochrome P 450
DDS	drug delivery system
DMSO	dimethyl sulfoxide
DoE	Design of experiment
DX®	Design Expert®
EDTA	ethyl diamine tetra acetate
FCD	face-centre composite design

Abbreviation	Description
$F_{CS\text{-}SpD}$	microparticles of cross-linked, spray-dried corn starch
FDA	food and drug regulation authority
FEG-ESEM	field-emission gun environmental scanning electron microscopy
FI	factor interaction
$F_{PS\text{-}CL}$	nanoparticles of cross-linked potato starch
$F_{PS\text{-}UCL}$	nanoparticles of uncross-linked potato starch
$F_{SS\text{-}UCL}^{-}$	nanoparticles of uncross-linked anionic soluble starch
$F_{SS\text{-}UCL}^{+}$	nanoparticles of uncross-linked cationic soluble starch
$F_{SS\text{-}UCL}^{0}$	nanoparticles of uncross-linked neutral soluble starch
F value	Fisher's statistics value
g/mol	gram per mole
GIT	gastro intestinal tract
HLB	hydrophilic-lipophilic balance
HPLC	high performance liquid chromatography
Hr	hour
Hz	hertz
ICH	International committee on harmonisation
K_{elim}	elimination rate constant
Kg	Kilogram
kPA	kilopascal
LOD	Limit of detection
LOQ	Limit of quantification
M	molar
M-cells	modified epithelial cells
MDT	mean dissolution time
mg	milligram
min.	minute
ml	millilitre
mm	Millimetre
MRPs	Multidrug resistance proteins
mV	milli electron volt

Abbreviation	Description
MW	molecular weight
n	release exponent
N	normal; number of replicate/samples
nER	number of experimental runs
nF	number of factors
nL	number of levels of the factors
nm	nanometre
O/W	oil in water
OFAT	one factor at a time
p	probability
PAS	publicly available standard
PDI	polydispersity index
PE	processing element
PEG	polyethylene glycol
P-gp	p-glycoprotein
pH	power of hydrogen ion concentration
PI	prediction index
Pred R^2	predicted coefficient of correlation
PS	Potato starch
PSA	polar surface area
R^2	correlation coefficient
RES	reticuloendothelial system
RPM	revolution per minute
RSD	relative standard deviation
RSM	response surface methodology
SEM	scanning electron microscopy
SLNs	solid lipid nanoparticles
SS	soluble starch
T_g	transition temperature
T_{max}	time for peak concentration
VIF	variation inflation factor
v/v	volume per volume
W	Watt
W/OW	water in oil in water
w/w	weight by weight

MENGKAJI POTENSI ZARAH KANJI TEROPTIMUM SEBAGAI PELANTAR UNTUK PENYAMPAIAN ORAL SATU MODEL DRUG GASTROLABILE SEFOTAKSIM NATRIUM

ABSTRAK

Penggunaan zarah adalah pendekatan utama untuk sistem penghantaran kejuruteraan bagi mengatasi rintangan-rintangan di gastrousus. Kanji mempunyai ciri-ciri yang sesuai diperlukan sebagai satu zarah bahan pembuatan. Kanji mudah dibioteruraikan, selamat, murah dan banyak walaubagaimanapun, kurang digunakan sebagai bahan penyelidikan, berbanding biopolymer yang lain. Kerja ini melaporkan kaedah-kaedah yang mudah dan berinovasi termasuk pengeringan sembur, ultrasonikasi dan pengisaran nano untuk produksi saiz micron dan submicron zarah kanji. Zarah kanji teroptimum direka melalui kaedah pemprosesan dan formulasi menggunakan algoritma dengan bantuan komputer.

Ultrasonifikasi gagal menghasilkan zarah kanji secara berulang dengan menggunakan bantuan pemprosesan. Pengeringan sembur menghasilkan partikel dengan saiz 21.64 ± 1.36 μm (keseragaman 0.56 ± 0.135) bagi kanji jagung. Kaedah pengisaran nano kelihatan menjanjikan untuk penghasilan partikel submikron secara berulang dengan kawalan mengenai ciri-ciri zarah melalui modulasiparameter proses dan formulatif . Kanji kentang menghasilkan partikel bersilang dengan saiz dan indeks serakan berganda (PDI) masing-masing 200 nm dan 0.25. Potensi zeta untuk kanji kentang adalah > –21 mV. Kanji terlarutkan yang tidak bersilang menghasilkan partikel yang bersifat kation, anion dan neutral dengan potensi masing-masing +8.59 ± 0.45, –10.5 ± 0.20 dan 2.00 ± 0.30 mV, saiz partikel tersebut < 200 nm dan PDI < 0.25. Kanji jagung pula menghasilkan partikel tidak bersilang dengan saiz sekitar 500 nm dengan PDI > 0.40, iaitu satu nilai yang sedikit tinggi daripada nilai PDI yang boleh diterima, oleh itu partikel kanji jagung tidak dioptimakan. Persilangan kanji kentang dan jagung berciri hidrofobik.

Semburan kering kanji jagung, pengisaran nano silang- dan tidak bersilang kanji kentang dan beranion, kation dan neutral kanji terlarut dipilih untuk kajian *in vitro* dan *in vivo*. Kentang dan kanji terlarut menunjukkan keberkesanan muatan > 9.55%. Kanji jagung menunjukkan keberkesanan muatan yang rendah iaitu 2.30%, mungkin disebabkan hasil rendah kanji jagung (27.77 ± 1.48%) berbanding kanji kentang dan kanji jagung (> 84%). Pertumbuhan saiz zarah telah diperhatikan selepas tempoh waktu tertentu, dalam zarah kanji terlarut. Pembebasan *in vitro* zarah mematuhi model tertib sifar dengan eksponen resapan <1, menggambarkan sifat pembebasan oleh resapan bengkak. Perbandingan bagi sefotaksim natrium bebas, kanji kentang tidak bersilang dan kanji terlarut kation menunjukkan 4.82 dan 2.59 lipatan peningkatan dalam ketersediaan biohayati, masing-masing. Oleh itu, kanji mungkin mempunyai potensi bagi menyampaikan gastrolabile drug, sefotaksim natrium dan dengan itu, untuk meningkatkan keterbiosediaan oral.

Sebagai kesimpulan, partikel kanji bersaiz mikron dan submikron yang teroptimumkan boleh disediakan menggunakan kaedah fizikal melalui gabungan sains farmaseutikal dengan kaedah pengoptimuman dengan bantuan komputer. Zarah adalah mampu untuk meningkatkan keterbiosediaan sefotaksim natrium. Walaubagaimanapun, kestabilan fizikal zarah perlu terus untuk diperbaiki.

EXPLORING THE POTENTIAL OF OPTIMISED STARCH PARTICULATES AS PLATFORM FOR ORAL DELIVERY OF A MODEL GASTROLABILE DRUG CEFOTAXIME SODIUM

ABSTRACT

Use of particulates is a prominent approach for engineering delivery systems to overcome gastrointestinal barriers. Starch has desired properties necessary for a particulate fabrication material. It is biodegradable, safe, cheap and abundant however, has attracted less research attention, comparative to other biopolymers. This work reports simple and innovative top-down physical methods including spray-drying, ultrasonication and nanomilling for production of micron and submicron size starch particles. The optimised starch particulates were fabricated with use of processing and formulative aids employing computer-aided algorithm.

Ultrasonication was unsuccessful in generating reproducible starch particles with processing aids used. Spray-drying produced particle size of 21.64 ± 1.36 µm (dispersion in uniformity of particle size 0.56 ± 0.135) for corn starch. Nanomilling seemed promising for production of reproducible submicron particles with control over particle characteristics by modulation of process and formulative parameters. Potato starch produced cross-linked particles with size and polydispersity (PDI) of 200 nm and 0.25, respectively. The zeta potential of potato starch was > –21 mV. Uncross-linked soluble starch yielded cationic, anionic and neutral particles with charges +8.59 ± 0.45, –10.50 ± 0.20 and 2.00 ± 0.30 mV, respectively with particle size < 200 nm and PDI < 0.25. Corn starch uncross-linked particle size was around 500 nm with PDI > 0.40 which was slightly higher than generally accepted PDI values, thus corn starch particles were not optimised. Cross-linked potato and corn starch were somewhat hydrophobic.

The spray-dried corn starch, nanomilled cross- and uncross-linked potato starch and anionic, cationic and neutral soluble starch were selected for *in vitro* and *in vivo* studies.

Potato and soluble starch demonstrated loading efficiency > 9.55 %. Corn starch showed low loading efficiency of 2.30 %., probably due to its lower yield (27.77 ± 1.48 %) as compared to that of potato and corn starch (> 84 %). Particle size growth was observed over time prominently, in soluble starch particulates. The *in vitro* release of particulates followed zero order model with diffusion exponent < 1, characterising release by swelling-diffusion. Comparative to free cefotaxime sodium, the uncross-linked potato starch and cationic soluble starch showed 5.28 and 3.11 folds increase in bioavailability, respectively. Thus, starch may have potential to deliver gastrolabile drug, cefotaxime sodium and thereby, to enhance its oral bioavailability.

In conclusion, the micron and submicron size optimised starch particles can be prepared using physical methods by blending pharmaceutical science with computer-aided optimisation. The particulates are capable to enhance bioavailability of cefotaxime sodium. However, physical stability of particulates is needed to be improved further.

CHAPTER 1
GENERAL INTRODUCTION AND AIMS OF STUDY

1.1 THE ORAL ROUTE OF ADMINISTRATION

Among the routes of drug administration, per oral administration is the most dominant, convenient and preferred portal by which drugs are presented for systemic effects (Davenport, 1982; Lee and Yang, 2001). It is relatively safe and affords high acceptability, convenience and maximum compliance, in combination with the relative low cost, simple and cost-efficient manufacturing process (Lee and Yang, 2001). Nevertheless, the route is not without limitations, which prohibit the accomplishment of the full benefits of the route (Mahato *et al.,* 2003). Substantial number of existing and new pharmaceuticals moieties exhibits poor oral absorption (Amidon *et al.*, 1995; Hoerter and Dressman, 1997; Devane, 1998). Despite of the above limitation, the design of an oral dosage form is usually the first choice for the new medicinal entities and aims to reformulate and/or optimise dosage forms of the existing drugs. The objective of the current oral formulation design is to develop a dosage form capable of absorption into blood in right time and amount after administration (Macheras *et al.*, 1995). The drug absorption after oral administration is a complex process, requiring series of events (Ungell, 1997).

1.1.1 Drug absorption after oral route of administration

The small intestine usually is the most favourable site for drug absorption due to its large surface area endowed by microvilli and, therefore, delivery of drug to this region is usually a prerequisite for absorption (Davis *et al.*, 1986). The microvilli consist of loose connective tissue and are supplied with blood and lymphatic system at their base (DeMarco and Levine, 1969). If absorbed into blood stream, the drug passes through the liver before its entry into the systemic circulation. However, the drug taken up by lymph accesses the systemic circulation without passing through liver (O'Driscoll, 1992; Ungell, 1997; Martinez and Amidon, 2002). Drug absorption after oral administration depends on physicochemical, pharmaceutical and physiological factors and on the efficiency with which a drug crosses the

several barriers prior to reaching into the systemic circulation (Kinget *et al.,* 1998; Ashford, 2002a; Ashford, 2002b; Mahato *et al.,* 2003; Barrett, 2004). In line with the scope of the present study, only the physiological factors will be discussed under barriers to drug absorption after oral administration (Section 1.1.2).

1.1.2 Barriers to drug absorption after oral administration

GIT provides a variety of morphological (spatial) and physiological (functional) barriers to the arrival of a drug to systemic circulation (Ashford, 2002b). Morphological barriers are mucus and epithelial and endothelial cells while the physiological factors are pH, enzymes, presystemic biotransformation, enterohepatic circulation and counter absorption mechanisms (Shargel *et al.,* 2004). The above barriers are given in Figure 1.1.

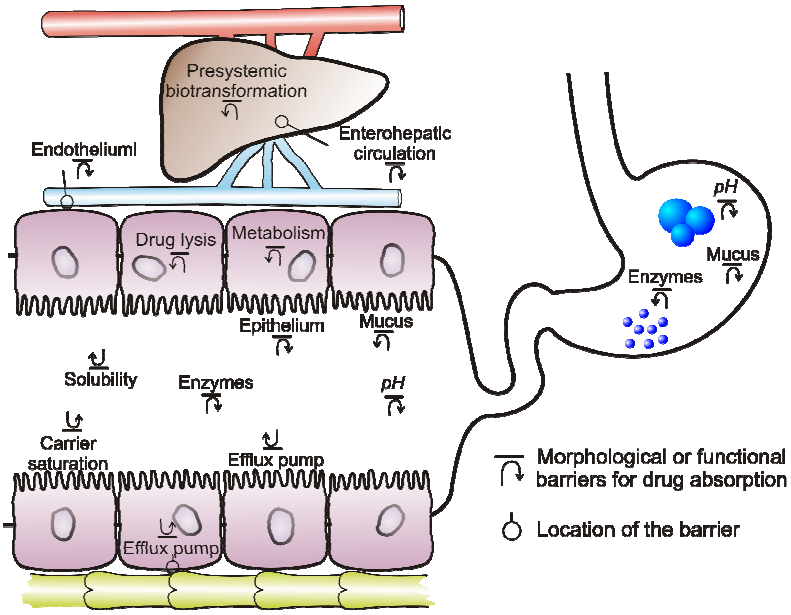

Figure 1.1: Morphological and functional barriers for drug absorption after oral administration

1.1.2(a) Gastrointestinal tract milieu

The pH, enzymes, secretions and foods in gastrointestinal tract (GIT), together make the GIT milieu. The pH varies along the length of GIT, from very slightly acidic mouth region to very acidic region of stomach followed by another change to the neutral pH in the proximal small intestine (Davenport, 1982). Thus, the drug is exposed to fluctuating pH

while it passes along the GIT. The GIT pH influences or completely halts the absorption of drugs by affecting their chemical stability and ionisation or by acid- or base-mediated drug inactivation (Lee and Yang, 2001). Pepsin is the primary enzyme in the stomach while lipase, amylases and proteases are secreted from pancreas into the small intestine (Davenport, 1982). Drugs, particularly peptides, nucleotides and fatty acids are susceptible to enzymatic degradation (Lee, 1991; Wilson and Washington, 2001). The presence of food interferes with the drug absorption by retarding the gastric emptying time, provision of viscous environment and/or provoking the enzyme and acid secretions (Toothaker and Welling, 1980; Lee and Yang, 2001).

1.1.2(b) Mucus

Mucus on the lining of GIT epithelial cells is secreted by the mucosal cells of GIT epithelia. It consists of glycoprotein and glycocalyx (sulphated mucopolysaccharides) and is hydrophilic which is attributed to 95 % of water it contains (Csaky, 1984). Its most important property is the viscoelasticity, enabling it to act as a physical diffusion barrier for drug or to make complexes to some drugs thus, leading to impaired absorption (Gu *et al.,* 1988; Norris *et al.,* 1998; Ashford, 2002b).

1.1.2(c) Epithelial cells – the mechanical barrier on the lumen side

The small intestine is lined with a single layer of tightly joined columnar epithelial cells, called enterocytes (Squier, 1992; Lennernas, 1998). Higher molecular weight, lack of lipophilicity, presence of charge or epithelial binding prohibits translocation of certain drugs across intestinal epithelium (Gu *et al.,* 1988; Tenhoor and Dressman, 1992; Squier, 1992; Amidon *et al.,* 1995; Hoerter and Dressman, 1997).

1.1.2(d) Epithelial permeation, transport pathways and mechanisms

The uptake pathways, permeation mechanisms, transporters and efflux pumps have been regarded as biophysical and biochemical barriers. In the intestinal epithelium, the

possible mechanisms for the permeation of drugs are passive diffusion, carrier-mediated and endocytosis through the paracellular and transcellular pathways (Squier, 1992; Tsuji and Tamai, 1996). Paracellular drug flux is limited to small molecules and is of limited relevance in the most cases (Carino and Mathiowitz, 1999). The transcellular route is available for diffusion of small lipophilic molecules. The hydrophilic compounds are transported by carrier-mediated transport or by endocytosis (Carino and Mathiowitz, 1999; Ashford, 2002b).

In endocytosis, the particles are internalised by phagocytosis or pinocytosis and this occurs at specialised regions of membrane. Thus, drug absorption depends upon the region where they are present (Russell-Jones, 2004; Belting *et al.,* 2005). After internalisation, the molecule may be acted upon by a range of destructive processes or substances including proteases and nucleases or is extruded out by efflux pumps. Distribution, affinity, capacity, specificity and direction of epithelial transporters and pumps play an important role in drug absorption (Ayrton and Morgan, 2001; Steffansen *et al.,* 2004). Absorption of the following drugs is particularly affected; a) drugs having affinity to the intestinal transporters and b) drugs for which the passive diffusion is of no relevance due to higher molecular weight or low partition coefficient (Tsuji and Tamai, 1996; Ayrton and Morgan, 2001; Daniel, 2004; Steffansen *et al.,* 2004).

1.1.2(e) Efflux transporters and pumps

The epithelial or endothelial permeability of a drug depends on the membrane carriers, known as “efflux transporters” which recognize substrate drugs and restrict their transport. The most documented efflux transporters belong to ATP-binding cassette, super family of transporters, including P-glycoprotein (P-gp) and multidrug resistance proteins (MRPs) (Kleinzeller, 1999; Westpahal *et al.,* 2000; Walgren *et al.,* 2000). The P-gp acts as a barrier by preventing drugs from crossing GIT epithelium into blood or extruding them from blood into bile. The transporters include a broad structural diversity of substrates and thus,

affect several therapeutic classes (Leonard *et al.,* 2002; Chan *et al.,* 2004). The MRPs confer resistance to substrate lipophilic anions. Many structurally diverse drugs are the substrates for MRPs (Borst *et al.,* 1999; Bakos *et al.,* 2000; Miller, 2001; Kim, 2003; Kruh and Belinsky, 2003).

1.1.2(f) Endothelial cells – the mechanical barriers on apical side

The endothelium, a cellular lining of the blood vessels underlying the GIT mucosa has fenestrations with pores of 80-100 nm size that allow paracellular transport. However, the presence of P-gp efflux in endothelium is highly restrictive for the transport of drugs using the transcellular route (Firth, 2002; Feng *et al.,* 2002).

1.1.2(g) Presystemic cellular metabolism

The enterocytes contain phase I drug metabolizing enzymes, dominantly Cytochrome P450 (Wacher *et al.,* 1996; Watkins, 1997) and hydrolytic and phase II drug metabolizing enzyme such as, acetyltransferases and sulfotransferases (Krishna and Klotz, 1994; Wacher *et al.,* 1998). The substrate drugs for the Cytochrome P450 (CYP450) enzyme undergo extensive metabolism after uptake in the intestinal wall (Kolars *et al.,* 1991; Wacher *et al.,* 1996; Benet *et al.,* 1996; Wacher *et al.,* 1998). The co-localisation of efflux transporters and metabolizing enzymes in the enterocytes, together with the substantial overlap in their substrate specificity, seems to indicate the existence of interplay between drug transporters and metabolizing enzymes suspected to synergize recycling of the substrate over the apical membrane. This increases the exposure of substrate drugs to the metabolizing enzymes (Cummins *et al.,* 2002; Benet *et al.,* 2004; Chan *et al.,* 2004).

1.1.2(h) Presystemic hepatic metabolism

The heptoportal blood exposes the drugs to liver on its way to systemic circulation and the substrate drugs are extensively metabolised via oxidation by CYP450 enzyme or phase II reactions arising from conjugation with glutathione, sulphate or glucuronic acid.

Only CYP3A4, a predominant member of the CYP450 mixed-function oxidase enzyme, metabolizes 50 % of all the drugs currently prescribed (Evans and Relling, 1999; Clarke and Jones, 2002). Thus, liver extraction and thereby premetabolism substantially reduces the systemic availability of susceptible drugs (Arimori and Nakano, 1998).

1.1.2(i) Enterohepatic shunt

The enterohepatic shunt is a cycle of events after drug absorption from intestine, where a drug secreted into the bile is presented to the intestine for absorption again and then secretion into bile. This cycle entails the increased persistence of drug in the body or inactivation of drug (Shargel *et al.,* 2004).

1.2 STRATEGIES TO OVERCOME BARRIERS FOR ORAL DELIVERY

To be able to permeate through intestinal membrane, a drug must have following biopharmaceutical properties; ability to withstand the insults with appropriate stability in the hostile GIT environment, appropriate hydrophilicity to dissolve in the gastric fluids and sufficiently lipophilic to permeate through the cellular barrier (Thompson, 1997). Furthermore, it should not be a substrate for presystemic cellular or hepatic inactivation of efflux pumps (Ashford, 2002b). Several strategies have been proposed to generate or enhance the above biopharmaceutical features in the dosage form for oral delivery. Among many absorption barriers, the gastric liability has been addressed as one of the most important barriers in drug absorption (Amidon *et al.,* 1995; Hoerter and Dressman, 1997; Macheras and Iliadis, 2005). In line with the scope of the present work, only the strategies to overcome insufficient gastric stability will be discussed.

1.2.1 Approaches addressing gastric liability

The drugs with adverse biopharmaceutical properties are usually delivered through parenteral route or are enteric-coated (Moes, 1993; Hillery *et al.,* 2001). The parenteral route is invasive, usually with brief effects and impossible to terminate drug action, if required

(Robinson and Lee, 1987; Evers, 1997). The enteric coatings contain indigestible solids and make product often of considerable size, prohibitive for patients with gastric hypomotility or pyloric channel narrowing. The location where an enteric coating starts dissolving is uncertain and release may occur within the small intestine or deep in the colon. The large inter- and intra-patient variations leads to a varied drug release (Lee and Yang, 2001). To avoid the above limitations the recent research has been directed towards the use of convenient alternatives to parenteral route, such as sublingual, pulmonary and vaginal route for gastric liable drugs (MacGregor and Graziani, 1997) and preferably, to develop successful oral delivery systems (Lee, 1991; Zhou, 1994; Hillery *et al.,* 2001).

1.2.1(a) Chemical modification – prodrug and drug analogue

A drug can be made enzymatically stable by presenting it as a prodrug with the substitution of chemical group(s) in the drug molecule (Vilhardt, 1990; Lloyd and Smith, 1998). Recently PEGylisation, a chemical modification approach using conjugation or cross-linking of drug with polyethylene glycol (PEG) has gained significant interest (Hinds, 2005). The approach prevents the degradation of PEGylised drug from proteolytic enzymes, reduces drug clearance rate and offers a shield to uptake by the reticuloendothelial system (RES) (Roberts *et al.,* 2002). Nevertheless, prodrug strategy might not be adequate because a prodrug is considered as new chemical entity, thus requires the preclinical and clinical investigations. Therefore, approaches involving the change of formulation or engineering of the drug absorption are feasible (Hillery *et al.*, 2001).

1.2.1(b) Lipidisation strategies

Lipidisation involves the formation of drug in appropriate pre-solubilised lipid phase, which by the metabolism of the lipids in GIT facilitates drug absorption (Humberstone and Charman, 1997). It may be achieved by incorporation of drug into lipid vehicle such as oils, surfactants, dispersions, micelles of mixed bile salt, liposomes, or emulsifying systems (Gershanik and Benita, 2000; Frokjaer *et al.,* 2005). Such systems have

reported to improve bioavailability due to impeded gastric emptying time, increased permeability and facilitated lymphatic uptake mediated by special carriers, chylomicrons and very low density lipoproteins in epithelial cells (Sernka *et al.,* 1979; Schilling and Mitra, 1990; Macheras *et al.,* 1995).

1.2.1(c) Polymeric coating, encapsulation or entrapment

Coating with, encapsulation and entrapment of drug in polymer is used to protect the gastric labile drugs. Depending upon polymeric materials used, they may form coating film, hydrogels and matrices (Grahan and McNeil, 1984; Kopecek *et al.,* 1992; Brondsted and Kopeck, 1992). Several pH-dependent polymeric materials are available which are stable at acidic pH and dissolve in the intestinal or colonic pH. Thus, coating with such polymer protects the gastric unstable drugs (Touitou and Rubinstein, 1986; Saffran, 1991). Hydrogels are the cross-linked hydrophilic polymers able to swell in aqueous environment. This swellability is controllable by pH, degree of cross-linking and temperature. Hydrogels encapsulated drugs can be targeted to specific pH and thus, are attractive as drug delivery systems able to protect drugs at acidic pH (Frokjaer *et al.,* 2005).

1.2.1(d) Drug targeting to the lymphatics

Oral route has proven feasible for the lymphatic uptake of lipophilic drugs and their subsequent access to the systemic circulation without passing through liver. The total flow rate of the lymph in the thoracic duct is approximately 100-200 ml/hr, 500 times less than the blood flow in portal vein (O'Driscoll, 1992; Porter and Charman, 2001). Thus, the lymph portal may be used to achieve sustained drug release and to avoid first pass metabolism. The endothelial layer of lymphatic capillaries is discontinuous or without basement membrane thus, allowing a free permeation, independent of the molecular size (Hastewell *et al.,* 1991).

A drug with molecular weight greater than 16,000 reaches systemic circulation through the lymph (Porter and Charman, 2001; Swartz, 2001; Feng *et al.,* 2002). Delivery

systems can selectively redirect drug absorption into the lymphatics. Drugs in lipid-based particles or oil enhances lymphatic uptake, while macromolecules and colloidal particles, after endocytosis or persorption (Figure 1.2) may enter the lymphatic system through clefts in the terminal vessels (Muranishi, 1997; Nishioka and Yoshino, 2001; Wassan, 2002).

1.2.1(e) Drug targeting to the Peyer's patches

Peyer's patches, concentrated in the ileum are the collection of large lymph tissues in the intestinal mucus-secreting lining and appear as the elongated thickened areas lacking villi (Figure 1.2). The patches are associated with the immune system and contain large number of lymphocytes. Specialised antigen-presenting epithelial cells, called modified epithelia (M) cells cover the patches. Features of M-cells, important in connection with drug absorption can be discriminated from that of the enterocytes. The M-cells have apical microfolds and lack or if have, under-developed microvilli and the mucus covering is assumed to be attenuated to allow antigenic uptake perhaps by adsorptive and/or receptor-mediated process. Thus, M-cells are capable of relatively extensive macromolecular endocytosis (Eldridge *et al.,* 1990; Walgren *et al.,* 2000). The phagocytotic potential of lymphocytes provides a concentration gradient for drug permeation.

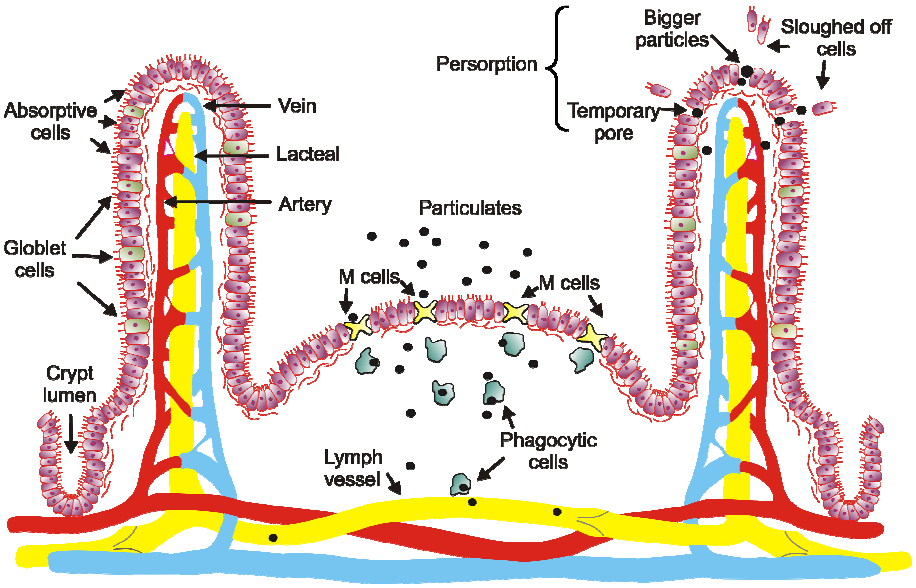

Figure 1.2: Microvilli showing epithelial cells and underneath two circulatory systems, blood and lymphatic stream. A special transport mechanism, persorption is also shown

Targeting the Peyer's patches helps an enhanced drug uptake into lymphatics that depends on particles size, surface hydrophobicity and availability of specific targeting ligands (Eldridge *et al.,* 1990; Muranishi and Yamamoto, 1994). Particle with size range of 50-3,000 nm are taken up by the Peyer's patches and through the lymphatics, subsequently translocated to blood. The particles of size 0.5–1.0 µm are phagocytised by lymphocytes, which migrate to the draining lymph nodes (Jani *et al.,* 1992; Randolph *et al.,* 1999; Jung *et al.,* 2000). The particles of size 3-10 µm are often retained within the Peyer's patches. Particles of size 2-3 µm exhibit the maximal phagocytosis (Champion *et al.,* 2008). A higher uptake is generally observed for more hydrophobic microparticles. Monoclonal antibodies which bind to the specific M-cells-bound carbohydrate residues may increase microparticulates uptake (Eldridge *et al.,* 1990).

1.2.1(f) Targeted drug delivery to the colon

A couple of strategies have been explored for targeting drug to the colon. One of the colonic drug delivery strategies is based on the design of a prodrug which is metabolised by enzymes found exclusively in the colon (Lee and Yang, 2001; Chourasia and Jain, 2003). Azoreduction of polymeric coating is a further important approach used for targeted drug delivery to the colon. The colonic anaerobic bacteria reductively cleave azo bonds leading to the disruption of the polymer network in the coating to release drugs (Van Den-Mooter *et al.,* 1992). Coating of azo polymeric systems onto pellets and solid unit dosage forms is another novel approach and shown to promote the oral administration of insulin and desmopressin (Saffran, 1991; Kopecek *et al.,* 1992).

Hydrogels are reported to have excellent persistence properties in the lower part of the rectum and hence demonstrate a potential of enhancing the colonic absorption of drugs (Grahan and McNeil, 1984; Brondsted and Kopecek 1992; Wilson *et al.,* 1997). Dosage forms using pH-sensitive polymers and hydrogels have also been used for the drug targeting to desirable pH (Saffran *et al.,* 1986; Chiu *et al.,* 1999; Khan *et al.,* 1999). Some other

approaches such as conjugation with glycoside, glucuronide, cyclodextrins, dextrans, amino acid and time-release system and bioadhesive systems have also been reported for the colonic drug delivery by Chourasia and Jain (2003). Impermeability of the colonic epithelia for drugs and long transit time of the colon are the major limitations for colonic drug delivery (Sangalli *et al.,* 1999; Lee and Yang, 2001; Washington *et al.,* 2001).

1.2.1(g) General use of particulate drug carriers

The colloidal particulates are becoming increasingly prominent in the novel drug delivery systems because of their potential for drug delivery, targeting, protecting gastric labile drugs and in parallel, protecting the GIT from drug toxicity and capacity for permeation into membranes (Ferrari, 2005). They are translocated in enterocytes, Peyer's patches and M-cells simultaneously by para- and intra-cellular pathways and subsequently, gain access to lymphatics (Walgren *et al.,* 2000; Feng *et al.,* 2002). Several particulate carrier systems have been described including liposomes, nanoparticles, microparticles, microspheres, dendrimers and Ferro fluids (Artursson *et al.,* 1984; Levy and Andry, 1990; Fournier *et al.,* 1994; Jameela and Jayakrishnan, 1995). In accordance with the scope of the present study, the particulates will be emphasised more in Section 1.3.

1.3 PARTICULATE DELIVERY SYSTEMS

There is no agreed international definition of a nanoparticle, though Kreuter (1983a), Couvreur *et al.* (1986) and Allemann *et al.* (1993) have defined nanoparticles as the particles ranging in size from 10-1000 nm. A review of the internet (http://www.sciencedirect.com accessed on 27 May, 2006), revealed that the majority of the researchers are partial towards the above definition. However, according to the document PAS71 by Publicly Available Standard in collaboration with British Standards Institution, a nanoparticle is a particle having one or more dimensions of the order of 100 nm or less with the properties different from that of the bulk material (http://www.malvern.co.uk; accessed on 27 May, 2006). The nanoparticles are typically made of a single material in which a drug is entrapped,

encapsulated or adsorbed onto the surface (Kreuter, 1993; Kreuter, 1994). Generally, the spherical formulation with a particle size above 1 μm, with a distinguishable coating and core regions are referred to as microcapsules while matrix type structures are the microspheres or microparticles. Micelles are self-assembled block copolymers that form a hydrophilic layer and an inner hydrophobic core or vice versa. Dendrimers are monodispersed symmetric macromolecules with a large number of reactive end groups (Passirani and Benoit, 2005). The terms used to describe particulate carriers vary among the authors but herein the "particulate" will collectively be used for the systems of colloidal particulate intended for drug delivery. However, the references sustain the original terminology used by the authors.

1.3.1 Liposomes

Liposomes are microscopic vesicles composed of one or more lipid bilayers surrounding an aqueous space (Gregoriadis, 1991). Phospholipids are the most commonly used materials of fabrication for liposomes (Knight, 1981; Barratt, 2000). Both water and lipid soluble drugs can be entrapped within the aqueous compartment or lipid bilayers, respectively (Betageri *el al.,* 1993a; Sharma and Sharma, 1997). Surface modification has improved the stability of liposomes in the gut and has facilitated permeation of drugs from gastrointestinal mucosa (Morgan and Williams 1980).

Liposomes protect drug against enzymatic degradation and improve drug hydrophilicity (Al-Meshal *et al.,* 1998; Bayomi *et al.,* 1998; Weiner and Chiang, 1988; Kisel *et al.,* 2001). Liposome-entrapped cefotaxime sodium has been reported to be a potential delivery system for oral route as indicated by its enhanced bioavailability (Betageri *et al.*, 1993b; Rojers and Habib, 1999; Ling *et al.,* 2006). The phospholipids are readily hydrolysed or oxidised, thus liposomes require storage under nitrogen atmosphere. The liposomes are not compatible with several coatings of surfactants and are instable against the bile salts (Betageri *et al*., 1993c; Sharma and Sharma, 1997).

1.3.2 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are the colloidal carrier for lipophilic drugs and composed of a high melting point lipid as a solid core coated with surfactants (Gassco, 1993; Muller *et al.,* 1995; Chen *et al.,* 2001). SLNs, the promising alternative delivery system to emulsions, liposomes and polymeric nanoparticles, can be administered through parenteral, oral or dermal route (Siekman and Westesen, 1992; Gassco, 1993) and share all the advantages of above in one carrier (Schwarz and Mehnert, 1999; Muller *et al.,* 2000).

1.4 GENERAL METHODS FOR PREPARATION OF PARTICULATES

Nano- and microparticulates are formed by bottom-up or top-down procedures, the former procedure being used more frequently. In bottom-up procedure, the particulates are produced by polymerisation of monomers (dispersion polymerisation) or by shaping or condensation of macromolecules (coacervation, spray-drying, or solvent evaporation).

1.4.1 Bottom-up procedures

1.4.1(a) Emulsion/dispersion polymerisation

In emulsion-polymerisation, particles are formed by chemically induced polymerisation of monomers dispersed in a continuous phase (Kreuter, 1983a). The polymerisation media may be aqueous or organic, employed for water-insoluble and soluble monomers, respectively (Gassco and Trotta, 1986; Krause *et al.,* 1986). The polymer, on reaching certain molecular weight, form particles and becomes insoluble, leading to phase separation (Kreuter, 1982). The chemical initiator may be ammonium or potassium peroxodisulphate, bases or basic drugs (Kreuter, 1990; Kreuter 1992).

The surfactants solubilise or disperse the insoluble monomers in continuous phase and stabilize the particulates while the cross-linker links the monomers (Schwarts and Rembaum, 1985). Emulsifiers are not mandatory for a hydrophilic macromolecule since it acts as dispersant (Kreuter, 1983a; Al-Khouri Fallouh *et al.,* 1986). The drugs can either be

incorporated in particles during polymerisation process or adsorbed onto the formed nanoparticles (Allemann *et al.,* 1993). The limitations of the applications are the conditions and solvents with respect to the stability of incorporated drug, drug-polymer reaction and the practically difficult removal of contaminants, un-reacted monomers or reaction initiator residues from the product (Fessi *et al.,* 1989; Quintanar-Guerrero *et al.,* 1996).

1.4.1(b) Preparation from preformed polymers

Use of the preformed and biodegradable macromolecules to prepare particulate carriers may prevent the problems associated with emulsion-polymerisation method. The principal methods for production of particulates from preformed polymers are emulsification-solvent evaporation, precipitation, or denaturation (Tice and Gilley, 1985; Fessi *et al.,* 1989). Solvent evaporation is usually referred to as the phase separation when a water insoluble polymer is in question, while the term coacervation is used for separation of water-soluble macromolecules (Fessi *et al.,* 1989; Jeffery *et al.,* 1991; Jeffery *et al.,* 1993).

I Emulsification-solvent evaporation

In the emulsification-solvent evaporation, a polymer dissolved in a water-immiscible volatile organic solvent is emulsified in the aqueous medium to form oil-in-water emulsion. The subsequent evaporation of the organic solvent, by heating or reduced pressure solidifies the organic phase droplets to particles. Drug is incorporated during the production as dissolved or suspended in the polymer solution in the organic solvent (Tice and Gilley, 1985; Al-Khouri Fallouh, 1986). The final product may require centrifugation, washing and lyophilisation (Krause *et al.,* 1985; Jeffery *et al.,* 1991).

Double emulsion solvent-evaporation, a modification of the emulsification solvent technique improves the loading efficiency of hydrophilic drugs but yields relatively larger particle size. In this approach, an aqueous drug solution is first emulsified in an organic solution of polymer to yield primary emulsion, which then is poured to the larger volume of

aqueous phase (Blanco-Prieto *et al.,* 1994; Rafati *et al.,* 1997; Delie *et al.,* 2001). Limitations of the method are the same as those mentioned for emulsion polymerisation (Verrecchia *et al.,* 1995).

II Coacervation (phase separation) method

Coacervation involves a separation of a polymer from its solution and deposition around a drug to be coated. In this process, a synthetic polymer is dissolved in a water-immiscible organic solvent and the separation is induced by the addition of another organic solvent or oil (Ruiz *et al.,* 1989; Sampath *et al.,* 1992). The polymer may also be dissolved in a water miscible solvent and then poured into a non-solvent (usually water with surfactant). The polymer separation is also induced by a change of surface charge by ionic gelation, temperature, or pH and addition of salt (salting out) or incompatible polymer (Calvo *et al.*, 1997). Addition of desolvating agents causing the so-called salting out phenomenon can also be used to form coacervate. The organic solvent is evaporated under a reduced pressure (Oppenhiem *et al.,* 1984). Hardening is achieved by addition of a cross-linker, thus, purification from hardening agent is a disadvantage of this method (Fessi *et al.,* 1989). Recently, a complex coacervation or polyelectrostatic complexation method has been reported for the preparation of nanoparticles in which the polymer-polymer or polymer-drug complex is formed by electrostatic interactions (Liu *et al.,* 2007; Bayat *et al.,* 2008a; Bayat *et al.,* 2008b).

1.4.1(c) Solvent deposition/displacement (nanoprecipitation)

Nanoprecipitation is a simple, one-step and straightforward technique for preparation of nanoparticles. It is carried out with two miscible solvents, the first one must be the solvent ideally for both polymer and drug while the second is a non-solvent for both. Addition of polymer solution to the non-solvent leads to nanoprecipitation by a rapid diffusion of polymer-containing solvent into the dispersing medium (also called desolvation of polymer). The drug is encapsulated in the polymer (Fessi *et al.*, 1989; Bilati *et al.*, 2005).

1.4.1(d) Emulsification-cross-linking

In this method, the aqueous solutions of polymer and of the drug to be incorporated into the particles are emulsified in oil, which result in the formation of droplets. The droplets can then be hardened by addition of cross-linkers or by denaturation of the molecules at high temperatures (Gupta *el al.,* 1986; Kreuter, 1992). The addition of surfactants stabilizes the emulsion and helps re-suspension of the final dry nanoparticles in water (Widder *et al.,* 1979; Gupta *et al.,* 1986).

1.4.1(e) Desolvation of macromolecules

Particles can be produced after dissolution of relatively hydrophilic copolymers or macromolecules in water-miscible solvents. The solutions of the polymer and of the drug to be entrapped in the above solvents are then poured into water, resulting in the spontaneous formation of particles due to desolvation. The particles produced by this method however, are not redispersible in water after spray- or freeze-drying (Bodmeir *et al.,* 1991). Addition of desolvating agents results in precipitation of macromolecules or coacervation as a new phase. The polymer may be gelatine, human serum albumin, bovine serum albumin, casein and ethyl cellulose. In some cases, surfactants are required in order to solubilise certain drugs or to facilitate the redispersion of the final freeze-dried product (Marty *et al.,* 1978).

1.4.2 Supercritical fluid technology

Use of organic solvents/or some chemical reaction stabilizing the colloid structure and thus, the need of extensive purification can be avoided by use of supercritical fluid technology. The supercritical fluid technology yields organic solvent-free and pure particles thus, is highly environment-friendly. In this technology, the solution of material of interest in a supercritical fluid is expanded through a nozzle. An abrupt decrease in the solvent power on expansion results in precipitation of solute (Tom and Debenedetti, 1991). Nevertheless, insolubility of polymers (MW >10,000 D) in supercritical fluid had made this technique impractical (Tom *et al.,* 1994).

1.4.3 Top-down procedure

Top-down procedures employ the physical energy or stress to reduce the particle size of a coarse polymer. Extrusion and wet-grinding (nanomilling) are the two common physical methods and require the coarse polymer to be grinded in a dispersion medium containing surfactants, stabilizers and other adjunctive materials. Size reduction is achieved by shearing forces (tearing apart), influencing forces (crushing and impinging) and attrition (tearing-impacting combined) (Yilmaz *et al.,* 2001).

1.4.3(a) Extrusion

Extrusion is employed by an instrument, called extruder that has two counter rotating screws through which the material is passed to reduce the size (Patent No WO 00/40617, 2000). Extrusion is cost effective, environment-friendly technology and thus, is an attractive method to encapsulate a great variety of bioactive substances in starch matrices. Yilmaz *et al.* (2001) has comprehensively reviewed the use of extrusion for this purpose. Perhaps, use of a higher volume is a dominating reason for its limited use in the current research.

1.4.3(b) Wet-grinding (nanomilling)

Wet-grinding or nanomilling, using media-mills requires small volume, this coupled with efficiency in reducing the particle size and use of wet-grinding is gaining attraction in size reduction. So far, application of the wet-grinding has been confined only to the material research and there are a few reports on its use for pharmaceutically applicable nanomaterials (Merisko-Liversidge *et al.,* 1996; Merisko-Liversidge *et al.,* 2003).

1.4.4 Materials of fabrication of particulates

Natural polymeric materials or their derivatives such as dextran, maltodextrin, mannan, or other starch derivatives, albumin and gelatine have been studied for developing particulate delivery systems (Schroder *et al.,* 1985; Artursson *et al.,* 1987; Artursson *et al.,*

1988; Fahlvik *et al.,* 1990). However, the synthetic polymeric materials are usually preferred due to their consistent properties, biodegradability, biocompatibility and compatibility with drugs. Copolymers such as poly (lactic-co-glycolic acid) or poly(lactic acid)-polyethylene oxide, have been well-documented (Vert, 1986; Kissel and Koneberg, 1996). Majeti and Kumar (2000) reviewed materials currently used in preparation of particulate materials. Some of the synthetic materials are costly while others have some degree of toxicity (Kreuter, 1992; Kreuter, 1993; Kreuter, 1994). Several features of starch, stated in Section 1.6.4 have motivated the use of starch as the fabrication material.

1.5 GENERAL APPLICATIONS OF PARTICULATES

1.5.1 Oral delivery of peptides, gastrolabile drugs and vaccines

Presenting the gastric instable drugs in particulates offers gastric stability, improves cellular uptake and due to their access to lymphatics, avoids first pass effects (Gautier *et al.,* 1992; Bayat *et al.,* 2008a Bayat *et al.,* 2008b). Particulate-bound gastric labile and water insoluble drugs exhibit enhanced bioavailability (Eldridge *et al.,* 1990; Jani *et al.,* 1992). Recently, Pereira (2008) demonstrated an enhanced absorption of Eudragit-loaded cefotaxime sodium in rats. A drug in nanoparticles may have a long-term effect due, in part to the formation of a depot in the gut wall, Peyer's patches, lymphatic system and/or other body compartments (Grangier *et al.,* 1991).

1.5.2 Targeting infected and inflamed cell

The nanoparticles are readily taken up by phagocytic cells, Kupffer cells and monocytes (Lam and Mathison, 1982), making targeting possible for nanoparticle-bound drug to these cells and the alveoli with an improved drug efficacy (Eltahawy, 1983). The infected reticuloendothelial system (RES) cells with human immunodeficiency virus or leishmaniasis and inflamed cells show preferentially higher phagocytosis of nanoparticles-bound drugs (Mizushima, 1985; Fouarge *et al.,* 1989; Meltzer *et al.,* 1990; Losa *et al.,* 1991).

1.5.3 Targeting cytostatics to tumours

The particulates accumulate in a number of tumours, may be due to their increased bioadhesiveness towards the tumours (Gipps *et al.,* 1986; Alpar *et al.,* 1989; Illum *et al.,* 1989). It may also be attributed to endocytosis of particles by endothelial cells lining the tumour vasculature or escape of particles through leaky or open blood vessels (Couvreur *et al.,* 1982; Widder *et al.,* 1983; Gupta *et al.,* 1989). Nanoparticles have prolonged persistence time in a variety of tumours and metastases with enhanced efficacy and decreased toxicity of a number of cytostatics (Brasseur *et al.,* 1980; Couvreur *et al.,* 1986; Ferrari, 2005).

1.5.4 Ophthalmic delivery

Ocular drug in a particulate delivery system increases the residence time of drug as compared to the conventional ocular delivery systems that have fast washout due to rapid tear turnover, lachrymal drainage and dilution of tears (Lee and Robinson, 1979; Losa *et al.,* 1993; Zimmer *et al.,* 1995). The persistence time of drug can be further enhanced by using a strong bioadhesive substance for nanoparticle fabrication (Gurny *et al.,* 1987).

1.5.5 Vaginal drug delivery

Microparticulate systems have been investigated for drug and vaccine delivery through vaginal mucosa (Okada, 1991; Hagan *et al.*, 1993). Starch microspheres, 40 µm in diameter, were shown to be capable of enhancing the vaginal absorption of insulin (Okada, 1991).

1.5.6 Enhancing drug safety

Due to the selective localisation in the infected cell and tumours, the particulate drug delivery systems reduce drug delivery to the non-targeted tissues (Lam and Mathison, 1982; Eltahawy, 1983). Thus, such systems have potentials to improve efficacy, safety and reduce the chances of drug resistance (Fouarge *et al.,* 1989; Schafer *et al.,* 1992).

1.5.7 Adjuvants for vaccines

Nano- and microparticles claim most of the desired features that the vaccine carriers must have (Wikingsson and Sjoholm, 2002), thus are promising for use as adjuvant for vaccines (Chen *et al.,* 1998; Singh *et al.,* 2000; Briones *et al.,* 2001). Their slow degradation offers long persistence in body, prolonged contact of antigen with immunocompetent cells and thus able to maintain a long immunity (Kreuter *et al.,* 1986; Hedley *et al.,* 1999; Herrman *et al.,* 1999; Cui and Mumper, 2003).

1.6 STARCH AS THE MATERIAL OF PARTICULATE FABRICATION

Starch, the material of fabrication for particulate carrier in this study is the major carbohydrate reserve in plant. It is a semi-crystalline material extracted from a variety of sources, maize being the predominant followed by wheat potato and rice barley (Buléon *et al.*, 1998; De-Baere, 1999; Gordon, 1999; Wesslén and Wesslén, 2002). Chemically, starch is a polysaccharide that consists of repeating glucose units (Li and Yeh, 2001; Singh *et al.*, 2003). Starch is abundantly available, cheap and is widely used polymer. The properties of native starch, being dependent upon the source are inconsistent but the commercially available starch is with consistently uniform quality (Gordon, 1999) as freely flowing white coloured powder.

Starch has found diverse and wide range of applications in pharmaceutical and non-pharmaceutical fields. It is used as staple food, raw material (Aime *et al.*, 2001), source of energy (calories), thickener, gelling agent (Aime *et al.*, 2001; Tester and Karkalas, 2002) and as a dietary fibre (www.Isbu.ac.uk/water/hysta.html; Accessed on 7 December, 2005). In pharmaceutical field, starch is used conventionally as a thickener, tablet binder, diluent, disintegrant, stabilizer, gelling agent and as a drug carrier. In topical preparations, it is used as dusting-powder for its absorbency , as a protective covering in ointment formulations and as an emollient (Bayazeed *et al.*, 1989; Whaley *et al.*, 1999; Schmidt *et al.*, 2001; Jeffcoat *et al.*, 2002; Lind *et al.,* 2002).

1.6.1 Starch composition and molecular structure

Though varied in structure and composition, each starch granule primarily contains blends of two polyglucans, amylose and amylopectin, both being associated through hydrogen bonding and arranged in layers to form granules (Hovenkamp-Hermelink *et al.*, 1987; Tomlinson *et al.*, 1997; Li and Yeh, 2001; Vorwerg *et al.*, 2002).

1.6.1(a) Amylose

Amylose is mostly linear fraction consisting of about 500 – 20000 α-D-glucose units linked through α (1→ 4) linkage and accounting for 20-30 % of the total starch (Li and Yeh, 2001). It has lower molecular weight (10^5–10^6 g/mol) but has a relatively extended shape with radius of 7-22 nm and generally tends to wind up into single or double helical junction zones. Hydrogen bonding is also present between the aligned chains (Morrison *et al.*, 1986; Morrison *et al.,* 1993a; Hoover, 2001).

1.6.1(b) Amylopectin

Amylopectins are branched fractions containing short chains linking linear chains via α (1→ 6) linkages, forming branching point at one residue in every twenty and some cases, every thirty residues, thus making it a huge but compact molecule with molecular weights between 10^6 and 10^7 g/mol (Hoover, 2001; Li and Yeh, 2001). Each amylopectin molecule contains up to two million glucose residues and has a hydrodynamic radius of 21-75 nm (Parker and Ring, 2001). It accounts for 70-80 % of total starch. Hydrogen bonding between sequential residues encourages a helical conformation in amylopectin (French *et al.*, 1984; Li and Yeh, 2001; Parker and Ring, 2001).

1.6.1(c) Molecular arrangement of amylose and amylopectin

Amylose and amylopectin molecules are oriented as alternating semi-crystalline (dark) and amorphous layers, forming concentric regions in granules. In semi-crystalline layer, the ordered regions compose of double helices of short amylopectin branches. The

amorphous regions of the semi-crystalline layers and the amorphous layers are composed of amylose and non-ordered amylopectin branches (Yamaguchi *et al.*, 1979; Jenkins *et al.*, 1993; Donald *et al.*, 1997; Gallant *et al.*, 1997; Baldwin *et al.*, 1998; Baker *et al.*, 2001; Parker and Ring, 2001).

1.6.1(d) Other minor components

Starch may have some minor components such as lipids, fatty acids, phosphorus and proteins (Morrison, 1993a; Lim *et al.*, 1994; Hizukuri *et al.*, 1996; Jane *et al.*, 1996; Ellis *et al.*, 1998; Tester and Karkalas, 2002).

1.6.2 Types of starches

Based on source, the starches are grouped as corn (maize), potato, rice, tapioca and wheat. Depending on contents, starches are waxy when the amylose to amylopectin ratio is low (≈ 15 %), normal when amylose represents ≈ 16-35 % and high-amylose when amylose content exceed ≈36 % (Morrison, 1993b). The common corn starch has 25 % amylose while the waxy corn starch is almost totally made up of amylopectin. The granules of high-amylose starch tend to be smooth and have a narrower size range. Potato starch has about 20 % of amylose (Jane *et al.*, 1994).

1.6.2(a) Modified starches

Starch is modified to enhance and expand its functionality and is achieved chemically, enzymatically, physically, or by biotechnological approach due to rupture of all or part of starch granules (Jobling, 2004). Physical modification can be achieved by milling to produce damaged starch granules. Pregelatinised starch is obtained using a physical method by the simultaneous gelatinisation and drying of aqueous starch dispersions. In this process, heating of starch in excess water induces many physical changes such as loss of crystallinity, swelling and rupturing of starch granules (Blanshard, 1987; Tester, 1997). Gelatinised starch is easily dispersed in cold water. Extrusion of starch is another physical

method (Giezen *et al.*, 2004). Other methods include enzymatic modification by α- β-amylase and amyloglucosidase are used to hydrolyse starch. The cross-linked starches are produced by chemical modification (Tester and Karkallas, 2002).

1.6.3 Functionality of starch

1.6.3(a) Gelatinisation

On heating starch in excess water, starch granules absorb water and while hydrogen bonding between amylose and amylopectin maintains granule integrity, it begins to swell from hilum (centre). Granules lose organised structure, crystallinity and become amorphous. Ultimately, granule structure is completely lost resulting into a thin paste (<4 %) or gel (>4 %). Typically, this process begins at 45°C, peaks at 60°C and completes at 75°C. Gelatinisation renders starch easy deformation and complete digestibility. Degree of swelling dictates the extent of starch water binding capacity (Jane *et al.*, 1996; Li and Yeh, 2001; Rosa, 2004; Tester *et al.*, 2004). After gelatinisation starch, which is otherwise water insoluble, becomes water soluble (Dziechciarek *et al*., 1998; Jane *et al.,* 1999; Tester and Sommervillie, 2003). The common solvents for starch are sodium or potassium hydroxide and dimethyl sulfoxide (Radosta *et al.*, 2001). Higher amylose content, owing to the extensive hydrogen bonding, requires more energy to gelatinise the starch. Thus, high amylose starch have less swelling power and higher gelatinisation temperatures (Li and Yeh, 2001; Singh *et al.*, 2003). The presence of phosphate groups in starch increases its swelling power. Certain salts such as calcium chloride and lithium chloride promote gelatinisation of starch at room temperature (Jane, 1994; Jane *et al.,* 1999; Pan and Jane, 2000).

1.6.3(b) Hydrocolloidal functionality

Starch functions as hydrocolloid due to the presence of amylose and amylopectin. The extended and loose helical amylose chains have a relatively hydrophobic inner surface. Similarly, helical structure in amylopectin may present continuous hydrophobic surface (King, 1995; Whaley, 1999).

1.6.3(c) Thickening agent

Viscosity is purely a function of molecular weight of substances. The branched structure of amylopectin with all attached chains yields much larger molecule than amylose. Consequently, it builds higher viscosity thus the waxy starches have higher viscosity. Amylopectin interferes with the interaction between amylose chains and its solution that can lead to an initial loss in viscosity followed by a more slimy consistency. After gelatinised, the swollen granules increase the viscosity of dispersion (Bayazeed *et al.,* 1989).

1.6.3(d) Gel and film formation

In a solution, amylose molecules easily align themselves with one another and associate through hydrogen bonding to form gels or films. This property is useful for several applications such as coating and for the inclusion of fats and flavours in starch (Shinsato *et al.,* 1999). In general, higher degree of polymerisation (length of amylose in starch) gives greater gel strength due to its increased ability to associate through hydrogen bonding. High amylose starches show better film forming properties. Gelatinisation creates more tendencies for association of granules to form gels and films (Roper and Koch, 1990; Zeller *et al.,* 1999; Haugaard *et al.,* 2001).

1.6.3(e) Retrogradation

Tendency of re-association of the gelatinised starch molecules with one another on cooling and thereby, forcing water out of the molecule, causing starch to recrystallise is called retrogradation. This unique property of starch is due to the presence of amylose. Branched amylopectin impedes retrogradation but the long branched amylopectin are susceptible to retrogradation. Mixing with carrageenan, alginate and xanthan gum and low molecular weight sugars can reduce retrogradation. On release of water, the aligned chains may form double stranded crystallites that are resistant to amylase and due to extensive inter- and intra-strand hydrogen bonding makes hydrophobic structure of low solubility (Shogren *et al.,* 1992; www.lsbu.ac.uk/water/hysta.html: Accessed on 7 December, 2005).