SYNTHESIS AND EVALUATION OF CROSS-LINKED DISULPHIDE CONTAINING POLYMERS FOR COLONIC DRUG DELIVERY

by

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Dedicated to my parents, Brahma of my heart…

Do you remember the night,
You lullabied me to sleep,
Turning my tears to comfort and to laughter,
Do you remember the day
Being the flowers in the sky,
You showed me wisdom and guided me all the way

You have sacrificed for me, leaving heart print of your love,
Brahma of my home, teachers and my saviour,
You're the hero of my life,
Give me everything and more,
Brahma of my joy, Brahma of my heart

I will remember your hopes,
I will make your dreams come true,
I will turn your fears into courage and compassion,
I promise to take care of you,
Share with you all the love I have,
Please do believe me my love for you shines true

You have sacrificed for me,
Leaving heart print of your love,
Brahma of my home, teachers and my saviour,
You're the hero of my life,
Give me everything and more,
Brahma of my joy, Brahma of my heart

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ABBREVIATIONS

DMSO	Dimethylsulphoxide
DCCI	N, N'-dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	dicyclohexylurea
NADPH	nicotinamide adenine dinucleotide phosphate
TES	triethylsilane
TFA	trifluoroacetic acid
SEM	scanning electron microscope
EDX	energy-dispersive X-ray spectroscopy
THF	tetrahydrofuran
CHNS	carbon, hydrogen, nitrogen, sulphur elemental analysis
Trt	Trityl or methyltriphenyl
EtOAc	ethyl acetate
MeOH	methanol
GI	gastrointestinal
IBD	inflammatory bowel disorder
TLC	Thin layer chromatography
UV	Ultra violet
FTIR	Fourier transform infrared
^{1}H-NMR	Proton nuclear magnetic resonance
LC-MS	Liquid chromatography-Mass spectroscopy
s	Singlet
d	Doublet
t	Triplet
et al.	et alii, others
h	hour
M	molar
mM	milimolar
min	minute
R_f	Retention factor
mL	Milliliter (s)
Hz	Hertz

˚C	Degree Celsius
L	Liter
mm	Millimeter
etc	Et cetera

SINTESIS DAN PENILAIAN POLIMER DISULFIDA RANGKAI SILANG UNTUK PENYAMPAIAN DRUG KEPADA KOLON

ABSTRAK

Kolon telah dilaporkan sebagai tapak sasaran yang sesuai bagi penyerapan sistemik protein dan peptida terapeutik drug kerana aktiviti peptidase yang lebih rendah, meghampiri pH neutral dan keadaan 'perbalahan' yang kurang jika dibandingkan dengan kawasan lain daripada saluran pencernaan. Bentuk dosaj yang diformulasikan mesti melalui saluran pencernaan bahagian atas dalam bentuk utuh sebelum penghantaran drug kepada kolon. Penggunaan polimer disulfida sebagai satu penghantaran responsif bakteria merupakan salah satu strategi untuk menyasarkan drug kepada kolon bagi melepaskan drug secara khusus dalam kolon. Oleh itu, objektif kajian ini adalah untuk mensintesis satu polimer disulfida berangkai silang yang baru berasaskan asid amino sisteina untuk sistem penghantaran drug kepada kolon dan menilai polimer-polimer ini di bawah keadaan keupayaan redoks yang rendah. Kajian dimulakan dengan sintesis polimer disulfida rantai bercabang berasaskan asid amino sisteina. Sisteina terlindung bertindak balas dengan boron trifluorida dietil etherat dalam tindak balas perangkaian dengan dua nilai setaraan asid (trifenilmetil) thiopropanoik untuk menghasilkan satu monomer yang mengandungi satu kumpulan thiol terlindung pada setiap tiga rantai bercabang. Proses penyahlindungan dijalankan dengan menggunakan asid trifluoroasetik dan trietilsilana untuk menghasilkan monomer trithiol. Monomer trithiol ini kemudiannya mengalami pempolimeran melalui pengoksidaan udara dengan 1,2-ethanadithiol mengikut gabungan nisbah tertentu untuk menghasilkan empat jenis polimer, iaitu

P10, P11, P151 dan P15. Semua sebatian yang disintesiskan telah dikenalpasti dengan NMR, IR, LC-MS, analisis CHNS, spektroskopi Raman, SEM-EDX dan peta elemental. Polimer-polimer yang telah disintesiskan dinilai dengan ujian penurunan kimia dengan menggunakan larutan zink/asid asetik. Kesesuaian polimer ini sebagai sasaran penghantaran drug kepada kolon telah diuji secara *in vitro* menggunakan kaedah keadaan simulasi dalam kolon. *Bacteroides fragilis* telah digunakan dan kepekatan thiol diukur dengan reagen Ellman. Tempoh masa inkubasi dijalankan pada masa 5, 30 dan 180 jam. Keputusan daripada spektroskopi Raman menunjukkan ketiadaan puncak –SH dan kehadiran puncak S-S membuktikan bahawa polimer disulfida telah disintesiskan. Ujian penurunan kimia menunjukkan semua polimer telah mengalami penurunan selepas 0.5-1.0 jam tetapi kepekatan thiolnya dikesan pada kadar berlainan bagi polimer-polimer berlainan. Keputusan SEM-EDX menunjukkan morfologi permukaan yang berlainan bagi polimer-polimer yang telah disintesiskan. Peta elemental menunjukkan penyerakan elemen-elemen yang sekata dalam polimer-polimer tersebut. Dalam ujian degradasi bakteria, polimer-polimer ini telah dibiodegradasikan di dalam medium bakteria kolon secara anaerobik. Degradasi rangkaian polimer "longgar" adalah lebih berkesan dengan penurunan polimer P15 (1.0 mol monomer trithiol : 5.0 mol monomer dithiol) menunjukkan bacaan tertinggi (118.6×10^{-6} M pada masa 180 jam) jika dibandingkan dengan polimer-polimer lain daripada ujian Ellman bagi thiol. Keputusan ini berhubungkait dengan persetujuan umum bahawa biodegradabiliti bergantung kepada kebolehkembangan polimer-polimer ini dalam keadaan akues. Kesimpulannya, polimer disulfida berangkai silang telah berjaya dikembangkan di mana ia mempunyai aplikasi untuk penghantaran drug kepada kolon secara selektif.

SYNTHESIS AND EVALUATION OF CROSS-LINKED DISULPHIDE CONTAINING POLYMERS FOR COLONIC DRUG DELIVERY

ABSTRACT

Colon has been reported as a favourable target site for the systemic absorption of therapeutic protein and peptide drugs, because of its lower peptidase activity, near neutral pH and less hostile conditions as compared with other regions of gastrointestinal tract. The formulated dosage form must pass through the upper gastrointestinal tract in intact form before delivering the drug to the colon. The use of disulphide polymers, a bacteria responsive delivery, is one of the strategies for targeting drugs to the colon and to release drug specifically in the colon. Therefore, the objective of this study was to synthesise a new cross-linked disulphide containing polymer based on amino acid cysteine for colon drug delivery system and to evaluate the polymers under the condition of a low redox potential. The work was initiated with the synthesis of a branch-chained disulphide polymer based on the amino acid cysteine. The protected cysteine was reacted with boron trifluoride diethyl etherate in a coupling reaction with two equivalents of (triphenylmethyl) thiopropanoic acid, giving rise to a monomer that contained a protected thiol group on each of its 3 branching chains. The deprotection process was conducted using trifluoroacetic acid and triethylsilane which afforded the trithiol monomer. The trithiol monomers were polymerised by air-oxidation with 1,2-ethanedithiol using various ratio combinations to yield four types of polymers mainly, P10, P11, P151 and P15. All compounds synthesised were characterised by NMR, IR, LC-MS, CHNS analysis, Raman spectrometry, SEM-EDX and elemental mapping. The synthesised polymers were

evaluated in chemical reduction studies which were performed in zinc/acetic acid solution. The suitability of the polymer to be used in a colon-targeted drug delivery was investigated *in vitro* using simulated conditions of the colon. *Bacteroides fragilis* was used and the thiol concentrations were detected by Ellman's reagent. Incubation periods were 5, 30 and 180 hours. The Raman spectroscopy results showed the absence of –SH peak and the presence of S-S peak, indicating that the disulphide polymers were synthesised. Chemical reduction studies showed that all polymers were reduced after 0.5-1.0 hour but detected at different thiol concentrations for different polymers. SEM-EDX results showed different surface morphologies of the polymers synthesised. Elemental mapping exhibited homogeneous distribution of the elements in the polymers. In the bacterial degradation studies, the polymers were shown to be biodegraded in the anaerobic colonic bacterial medium. Degradation was more pronounced in polymers with "looser" polymeric networks, with the reduction of polymer P15 (1.0 mol trithiol monomer : 5.0 mol dithiol monomer) indicated the highest reading (118.6×10^{-6} M at 180 hours) when compared with other polymers from the Ellman's test for thiol. This result complements the general consensus that biodegradability relies on the swellability of polymers in an aqueous environment. In summary, a cross-linked disulphide containing polymer for colonic drug delivery has been successfully developed, which has application for selective delivery of drugs to the colon.

CHAPTER ONE

INTRODUCTION

1.1 Background

Oral drug delivery is the most traditional and widely accepted route of drug administration (Ritschel, 1991; Mrsny, 1992; Lai *et al.,* 2008) due to its convenience and patient-friendly route of drug administration. Orally administered drugs are usually intended for rapid dissolution in the upper gastrointestinal (GI) tract, where many drugs are most effectively absorbed. However, for local treatment of conditions of the lower GI tract or delivery of biotechnology products such as proteins and peptides, this approach is inadequate, costly and associated with undesirable adverse effect. Hence, the idea of specifically targeting drugs to the colon itself has stimulated great interest, with much work already carried out in this area of drug delivery (Ritschel, 1991; Mrsny, 1992; Siccardi *et al*., 2005; Lai *et al.,* 2008). The recognition of the importance of this region of the GI tract, not only for local but also for systemic therapy could be the main reason (Tozer *et al.,* 1995).

At present, the specific drug delivery to the colon has secured prominence primarily (Ibekwe *et al.,* 2006) because of the therapeutic benefits to be gained from topical treatment of local disorders such as irritable bowel disease, ulcerative colitis, carcinomas, inflammatory bowel disease, Crohn's disease and infection (Yeh *et al*., 1995; Schact *et al.,* 1996; DiPirio and Bowden, 1997; Gupta *et al.,* 2001). The colon is evaluated as a more favourable target site for the systemic absorption of therapeutic protein and peptide drugs (Ashford and Fell, 1994) because of its lower peptidase activity, a near neutral pH, less hostile conditions as well as an increased

responsiveness to absorption enhancers that would otherwise be inactivated in the upper gastrointestinal regions (Rubinstein, 1995; Van den Mooter and Kinget, 1995; Watts and Illum, 1997).

1.2 Colonic anatomy, physiology and its microbial distribution

1.2.1 Anatomy of the gastrointestinal tract

The gastrointestinal digestive tract, which is also known as the alimentary canal is a system of organs within multicellular animals that take in food, digest to extract energy and nutrients, and expels the resulting waste (Figure 1.1). The normal human adult male GI tract consists of the upper and lower GI tracts and is approximately 6.5 meters long (Anthea *et al.,* 1993).

The colon, or large intestine, forms the lower part of the GI tract and extends from the ileocecal junction (shared with the small intestine) to the rectum and finally anus as shown in Figure 1.2. The colon is composed of the caecum (with its associated vermiform appendix), three relatively straight segments; the ascending segment, transverse segment and descending segment, and sigmoid region. Sigmoid colon is the terminal portion of the colon and is S-shaped, which empties into the rectum, the last part of the intestinal tract. The colon is approximately 1.5 m in length in the adult human and has an average diameter of about 6.5 cm. However, the diameter varies from approximately 9 cm in the caecum to approximately 2 cm in the sigmoid colon (Mrsny, 1992). Unlike the small intestine, the colon does not have villi although it does demonstrate crescentric folds, which modestly increase the internal surface area of the colon to roughly 1300 cm^2 (Cummings and MacFarlane, 1991). Although rodents and guinea pigs are commonly used as models to study colonic drug

delivery, the anatomy in these two animals are different (compared to humans) with shorter colon in guinea pig and slightly longer in rats (Pettersson *et al.*, 1976). Guinea pig has a large caecum, about three times the size of its stomach (Friend, 1991) and rodent has a different mucosa-associated flora from human (Bitton and Marshall, 1980).

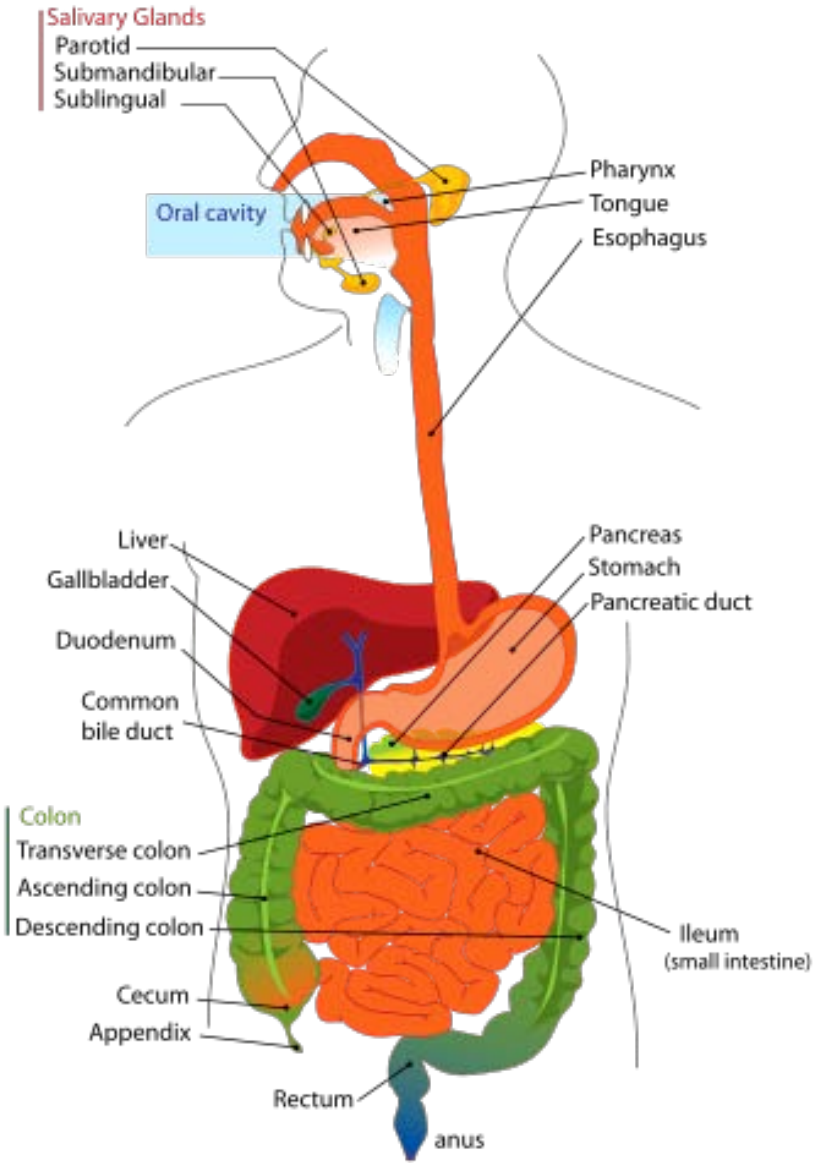

Figure 1.1 : Upper and lower gastrointestinal tract (after Friend, 1991).

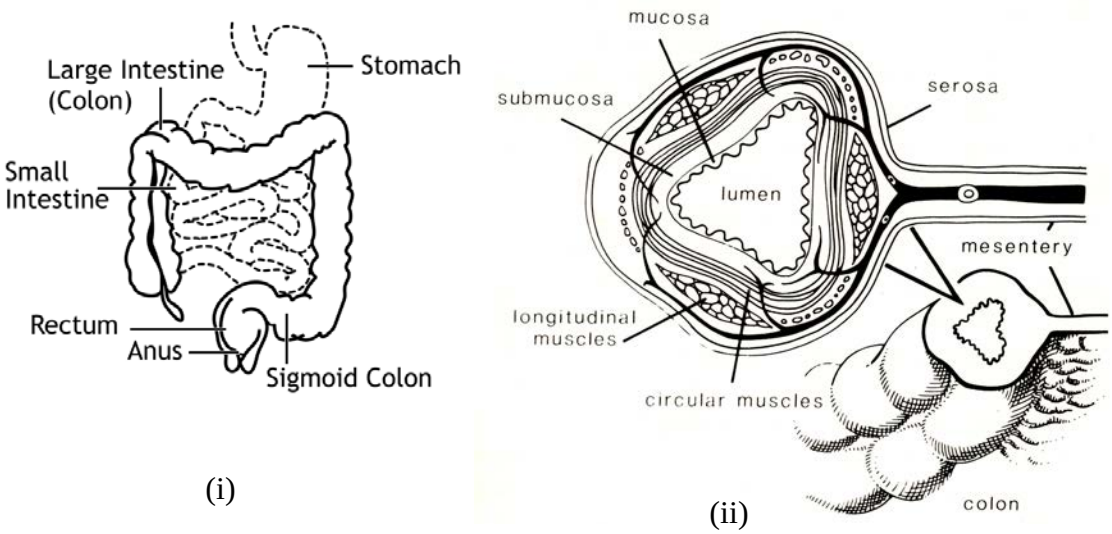

Figure 1.2 : (i) Structure of the large intestine (after Friend, 1991) and
(ii) layers of the colon that make up the bowel wall.

The surface area of the colon is low if compared to the small intestine which plays a role to digest foods and absorb nutrients. The presence of villi and microvilli, as a result of crescentric folds, makes absorption more efficient (Steed *et al.*, 1989). The colon is involved in the fermentation of polysaccharides and proteins, consolidation of the intestinal contents into faeces by the absorption of water and electrolytes and the formation, storage and elimination of faecal material (Edwards, 1997). The distal colonic contents becoming more viscous as a result of rapid water absorption in the ascending colon which estimated that the human colon contains only 220g of wet contents which is equivalent to just 35 g of dry matter (Cummings *et al.*, 1990). The circulation of chyme across the colonic mucosa by segmenting movements helps the absorption of fluid and salt. As potassium and bicarbonate ions are secreted, sodium and chloride ions are absorbed in the healthy human colon (Binder and Sandle, 1994).

1.2.2 Microbial and gastrointestinal system

The number of microbial are restricted in the human stomach due to the pH of the stomach contents (as low as pH 2) and the relatively swift flow (transit time of 4-6 hours) of the digestion through the stomach and small bowel (Gibson and Macfarlene, 1995). Lactobacilli and streptococci are the principal microbial types encountered in the stomach and upper small bowel (Lee, 1985). Unlike the majority of microbes entering the GI tract through indigested food, lactobacilli and streptococci are acid-tolerant bacteria and can survive passage through the stomach. The lactobacilli and streptococci which are carried into the GI tract from the oral cavity and pharynx by saliva are considered to be merely passing through the upper GI tract and they are described as transients (Tannock, 1995). There has been a view considering that the healthy human stomach is never to be colonised by microbes (Lee *et al.,* 1993). However, this view has been altered by the long-term association of a spiral-shaped motile bacterium called *Helicobacter pylori* with the mucosal surface of the stomach antrum. These bacteria are considered as pathogens rather than normal microflora because of their involvement in the causation of inflammation of the stomach (chronic active gastritis) and in the formation of peptic ulcers (gastric or duodenal ulcers). Figure 1.3 shows the distribution of selected bacteria in the GI tract.

The ileum, as the last third of the small bowel, harbours a large number of microbes than are found in the upper regions of the GI tract. The large bowel consists of at least 400 to 500 different species of bacteria, as well as yeast, fungi and protozoa and is considered the most densely colonised region of the digestive tract of humans. The large number of bacteria (Table 1.1) includes a very complex population of aerobes,

facultative anaerobes and strictly anaerobic species with the non sporing anaerobes predominating. There are four microhabitats identified in the colon, namely, the surface of the epithelial cells, the mucus gel overlying the villi, the mucus gel within crypts and luminal contents and the role of each microhabitat varies significantly (Freter, 1983).

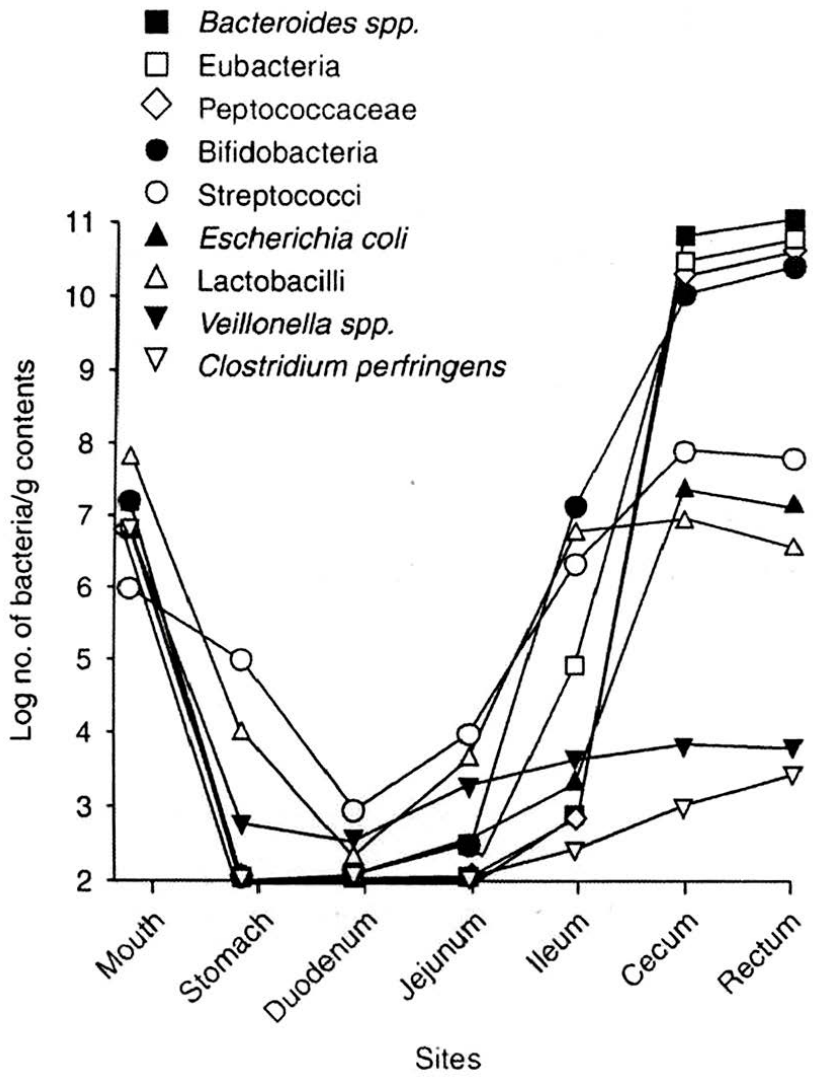

Figure 1.3 : Distribution of selected bacteria in the GI tract (after Basit, 2005).

Table 1.1 : The 25 most prevalent bacterial species in the faeces of human subjects consuming a Western diet (10^{9-10} bacteria per gram wet weight).

1. Bacteroides vulgatus	*14. Peptostreptococcus productus*
2. Bacteroides species, other	*15. Eubacterium lentum*
3. Bacteroides fragilis	*16. Facultative streptococci, other*
4. Bacteroides thetaiotaomicron	*17. Fusobacterium russii*
5. Peptostreptococus micros	*18. Bifidobacterium adolescentis A*
6. Bacillus species (all)	*19. Bifidobacterium adolescentis C*
7. Bifidobacterium adolescentis D	*20. Bacteroides clostridiiformis ss. clostridiiformis*
8. Eubacterium aerofaciens	*21. Peptostreptococcus prevotii*
9. Bifidobacterium infantis, other	*22. Bifidobacterium infantis ss . liberorum*
10. Ruminococcus albus	*23. Clostridium indolis*
11. Bacteroides distasonis	*24. Enterococcus faecium*
12. Peptostreptococcus intermedius	*25. Bifidobactrium longum ss. longum*
13. Peptostreptococcus sp. 2	

1.2.3 Factors affecting gastrointestinal microflora

There is a natural resistance to the alteration of the flora by introduction of bacteria even if they are of species commonly encountered in the intestine. The changing of this resistance is understandable; the bacteria that comprise the flora have adapted to life in the intestine whereas freshly introduced strains require time to adapt. The important role in preventing the flora overrunning the host and determining the distribution of the flora within the intestine must be played by the intestinal physiology and host defence mechanisms. There are some bacteria which are considered beneficial (bifidobacteria and lactobacilli) while others are benign (saccharolytic species of clostridia and bacteroides) and are thought to suppress the overgrowth of those that are harmful to human health (clostridium species and enterobacteriaceae) (Kolida *et al.,* 2000).

Alteration in the composition or the metabolic activity of intestinal microflora can also be linked to diet. The nature of the meal affects gastric emptying and therefore indirectly affects the distribution of bacteria. Nevertheless, some studies of the effect of diet on the composition of the flora in human suggested that diet has little effect on the composition of intestinal flora (Moore and Holderman, 1975; Mitsuoka, 1978). Studies on people living on different diets in various countries under different environmental circumstances have shown some differences in the relative numbers of some of the groups of bacteria present in their faeces. The few attempts to change the composition of the flora by controlled changes in diet have, in general, been unsuccessful (Drasar *et al.,* 1976; Cummings *et al.,* 1978; Drasar, 1981). Suggestions have been made on the observations that colonic bacteria obtain most nutrients from gut secretions and shed mucosal cells rather than from unabsorbed residues in the GI tract. Furthermore, it is difficult to demonstrate the changes in bacteria species while changes in the bacterial metabolic activities occur (Simon and Gorbach, 1987). Table 1.2 shows the factors influencing the gut microflora.

Microbial flora in the gut can be influenced by certain diseases or treatment with antibiotics (Friend and Tozer, 1992). Bacterial growth in the stomach may increase if the hydrochloric acid secretion is decreased caused by certain diseases (Rowland, 1988). The microbial colonisations affected by host factors are related to the flow of contents, oxygen tension and pH. Microbial multiplication cannot usually overcome the rate during peristalsis at the top of the small intestine where flow rate is the greatest (Drasar and Barrow, 1985). Peristalsis slows down to almost motionless upon reaching the distal ileum and when approaching ileo-caecal valve and the rise in bacterial population within this region can be seen. Gross changes of some

microorganisms in the environment within the gut can occur when antibiotic treatments are given where it suppresses the bacterial growth (Goldin and Grobach, 1977).

Table 1.2 : Factors influencing the gut microflora (after Rowland, 1988).

Host factors	i) Individual difference in strain and species: Acid or alkali secretion Intestinal motility Intestinal structure Levels of endogenous nutrients Redox potential Bile salts Antibodies ii) Age iii) Gastrointestinal disorders
Environmental factors	i) Drugs ii) Diet iii) Xenobiotics
Bacterial factor	i) Bacterial metabolites ii) Bacterial interactions iii) pH

1.2.4 Metabolic activities-role of microflora

The massive microflora in the colon fulfills its energy needs by fermenting the various types of substrates that have been left undigested in the small intestine. Glucose is considered one of the substrates that can be easily utilised by the microorganisms and barely reaches the colon since glucose and other easily digestible substrates are well absorbed or utilised in the upper GI tract. The physiological roughage (McBee, 1970) including di, tri-polysaccharides, and

mucopolysaccharides (Rubinstein, 1990) are the indigestible portion of the food that reach the colon. Bacteria produce a wide range of reductive and hydrolytic enzymes to utilise this roughage as a source of carbon such as β-xylosidase, β-glucuronidase, β-galactosidase, α-arabinosidase, azoreductase, nitroreductase, urea hyroxylase and deaminase (Scheline, 1973; Kinget *et al*., 1998).

Anaerobic bacteria of the colon have been found to be able to react to the constantly changing mixture of complex carbohydrates entering the colon by recognising a variety of substrates and producing the appropriate digestive enzyme (Salyers *et al*., 1978). Due to this, various systems have been developed for drug delivery to this part of the GI tract such as prodrugs (Sinha and Kumria, 2001a) and systems based upon biodegradable polymers which are specifically degraded by digestive enzymes (Van den Mooter *et al*., 1995; Sinha and Kumria, 2001b).

The presence of the vast microflora in the colon results in changes in redox potential. The redox potential which are considered as an expression of total metabolic and bacterial activity has been found to be -67$\pm$ 90 in the proximal small bowel; -196$\pm$ 97 in the distal small bowel and -415$\pm$72 in the right colon (Stirrup *et al*., 1990; Wilding *et al.*, 1994). According to Grim and Kopecek (1991), redox mediators such as benzyl viologen and flavin mononucleotide, act as shuttles between the intracellular enzymes and the extracellular substrates. One of the highly specific mechanisms used for targeting drugs to the large bowel is the microflora-induced changes in the redox potential which causes reduction of bonds like the azo bonds and disulphide bonds. Therefore, numerous drugs are being linked to such carrier moieties with these bonds where these linkages are increasingly being exploited for drug delivery

to this part of the GI tract. In addition, various drug-carriers for colon specific drug delivery have been designed which either utilise the presence of enzymes or the redox potential of this particular part of the GI tract for drug targeting (Sinha and Kumria, 2003).

1.3 Colonic drug targeting

The concept of drug targeting to the desired site of drug action is not new. The interest in the treatment of colonic disorders and the delivery of peptide drugs to the colon has become more attractive to researchers. Colon-specific drug delivery has met the challenge of drug delivery to the small intestine as it can be easily achieved by using enteric coating polymers that are soluble in the neutral environment of the small intestine. The formulated dosage form for colon-specific drug delivery must pass through the upper GI tract in intact form before delivering the drug to the colon. After all, numerous investigations have shown that some inflammatory (Wilson and Washington, 1989) and antidiabetic (Gleiter *et al.*, 1985) drugs are better absorbed from the colon rather than the small intestine.

1.3.1 Colonic absorption

The colon plays an important role in the reabsorption of water and the elimination of undigested material includes cellulose, desquamated epithelial cells, unabsorbed remains of intestinal secretions and bacteria (Philips, 1984). The dehydration of faecal material happens when a variety of active and passive transport processes are involved in the events of massive water resorption. In the outlook of this case, human colon receives approximately 1L of chyme from the small intestine, while about 150 ml of faeces are eliminated per day (Rangachari, 1990).

The lubricating actions of mucus secreted by the colonic mucosa assist the elimination of undigested material such as faeces. Colonic mucus consists of rather large (up to 2 x 10^6 Da), carbohydrate-rich (approximately 80% dry weight) and negatively charged glycoproteins (Allen, 1982). The mucus becomes hydrated to form a gel with unique chemical and physical properties resulting from the secretion of colonic goblet cells. The remarkable properties of this mucus are the ability to bind macromolecules, coat bacteria, cushion particulate matter and most importantly to protect the colonic mucosa from the dehydrated luminal contents when moving towards the rectum. The mucus layer is highly charged with a sieve-like nature, due to this, it can affect the transit of large, negatively-charged drug molecules. Such drug-mucus repulsion could prevent the drug from approaching the epithelial surface hence impeding drug absorption. In contrast, drug binding to mucus might facilitate even longer residence time, thereby possibly increasing drug absorption. However, the enhanced effects of enzymes and environmental degradation which might accompany the long residence time must not be ignored (Le, 1998).

The transport pathways of the colon provide for rapid and specific active bi-directional transport of ions across the epithelial layer. The colon has no active transporters for organic nutrients in the mature organ and hence, no chance for drug molecules to be absorbed back in transport compared to the small intestine. In the small intestine, examples of this kind of active absorption of drugs are seen for 5-fluorouracil (Bronk and Hastewell, 1987; Bronk *et al.,* 1987) on the pyrimidine transporter and antibiotics on the peptide transporters (Iseki *et al.,* 1989; Sinko and Amidon, 1989; Dantzig and Bergin, 1990). The drug absorption in the colon is limited due to the lack of such transporters and hence, limits the scope for drug

design with respect to mediated transport across the epithelial barrier. The potential for drug design with respect to carrier mediated transport across the colon may be restricted due to the apparent lack of organic nutrient transporters. However, the active transport pathways of the colon have been reviewed (Hastewell *et al.,* 1991) and reported that the transmucosal and membrane potential differences may be of significance in the absorption of ionised or ionisable drugs (Hogben *et al.,* 1958; Schanker *et al.,* 1958). The absorption of the drug will be improved as the bulk water absorption in this region of the intestine will provide scope for solvent drag.

The passive and active transport processes in the colon may indirectly enhance the uptake of water soluble drug molecules. These processes occur with the net secretion of potassium and bicarbonate and the net absorption of sodium and chloride, resulting in dehydration of colonic contents (Guyton, 1986). Consequently, the drift of water can act as a driving force for the uptake of water-soluble drug molecules. Residual fatty acids are absorbed by the colon after assimilation of lipids in the small intestine which will provide a similar driving force scenario and hence, furnish another route for drug absorption. The paracellular (the most promising means of general drug delivery in the colon) transport to water and small cation is limited due to the occluding tight junctions at the locating neck of colonic cells (Rangachari, 1990; Nellans, 1991). Thus, transcellular ionic gradients is driving the movements of ions and water and giving a ‘leaky’ condition at the proximal colon than the distal colon (Luciano *et al.,* 1984). In order to maximise local delivery as well as enhance absorption, it is desirable that drugs delivered to the colon are maintained in the proximal colon (Wills *et al.,* 1984).

Drugs that are absorbed in the small intestine across the submucosa into venous capillaries are transported to the liver via the hepatic portal vein. In the liver, significant first-pass metabolism occurs which therefore retards the amount of drugs reaching the systemic circulation to exert their effects. In the colon, drugs absorbed can be taken up by venous and/or lymphatic capillary beds. The venous drainage of colon will go into the hepatic-portal circulation after passing through the superior and inferior mesenteric veins and there is no direct systemic venous drainage from any region of the colon (Muranishi, 1989). On the other hand, uptake of drug molecules into the lymphatic system allows direct entry into the systemic circulation bypass immediate transport to the liver which results in less metabolic degradation of the absorbed drugs. This phenomenon indirectly gives one potential advantage of delivering drugs in their intact form to a site of absorption in the colon. The suggestion of increasing drug uptake can occur by increasing lymphatic uptake (Schuette and Rose, 1986) are proven by the increment in the colonic absorption of 1,2-dimethylhydrazine following high levels of dietary fats (Kvietys *et al*., 1981). Thus, peptide absorption may be feasible since lymphatic uptake of large sized molecules may occur.

One of the targets is to use the colon as a site for oral absorption of therapeutic peptides and proteins as they can be absorbed intact from the GI tract (Smith *et al.*, 1992; Garnder, 1994). The bioavailability of therapeutics peptides and proteins administered through colon is extremely low because the colonic epithelium is poorly permeable and does not allow sufficient transport of most drugs, particularly proteins and peptides (Woodley, 1994). There has been limited success in increasing the amount of drug absorbed, which has been achieved by using absorption

enhancers and protease inhibitors to assess their effectiveness in promoting colonic permeability (O'Hagen *et al.*, 1987). Some prominent examples are *β*-lactam antibiotics (Mrestani *et al.,* 2006), calcitonin (Kamei *et al.,* 2009), cyclosporin (Zhou *et al.*, 2009), renin inhibitors (Staessen, 2006), somatostatin octapeptide analogue (Prieto *et al.*, 1999), octreotide (Cervin *et al.*, 2009), salicylates, water-oil-water emulsions, surfactants, bile salts, combinative promotion effects of azone and fusogenic fatty acids and lipids (Mackay *et al.,* 1997). Protection of the peptide from the digestive functions of the stomach and small intestine must be ensured in the mode of delivery or even circumvent the harsh condition entirely by accessing the large intestine via the rectum.

1.3.1.1 Barriers to colonic absorption

The rapid and nonselective uptake of molecules from the colonic lumen is limited by various hypothetical physical and enzymatic barriers (Figure 1.4). Enzymatic or environmental degradation of the drug can occur in the lumen of the colon by resistant bacteria or released bacterial products. Selective or nonselective drug binding by the renewal and continuously flowing barrier produced by goblet cell exocytosis will give a difficult physical barrier to drug uptake. Examples of drugs that can bind to mucus are penicillins, cephalosporins and aminoglycosides (Nubuchi *et al.,* 1986). The release of mucus from goblet cells is stimulated by particular drugs which may hinder their absorption. Furthermore, the diffusion of large delivery structures or molecules might face difficulty if they pose a similar charge as the negatively charged mucin glycoprotein matrix. Although the uptake of drugs could be stimulated by modification of this layer using mucolytic agents, the function of normal colon requires an intact mucus layer (Mack and Sherman, 1991) which will

implicate a diversity of disease processes and pathological conditions (Chadee *et al.*, 1991). The absorption of lipophilic drugs will also be affected by the unstirred water layer between the mucus layer and the epithelial cell surface (Rahman *et al.*, 1986).

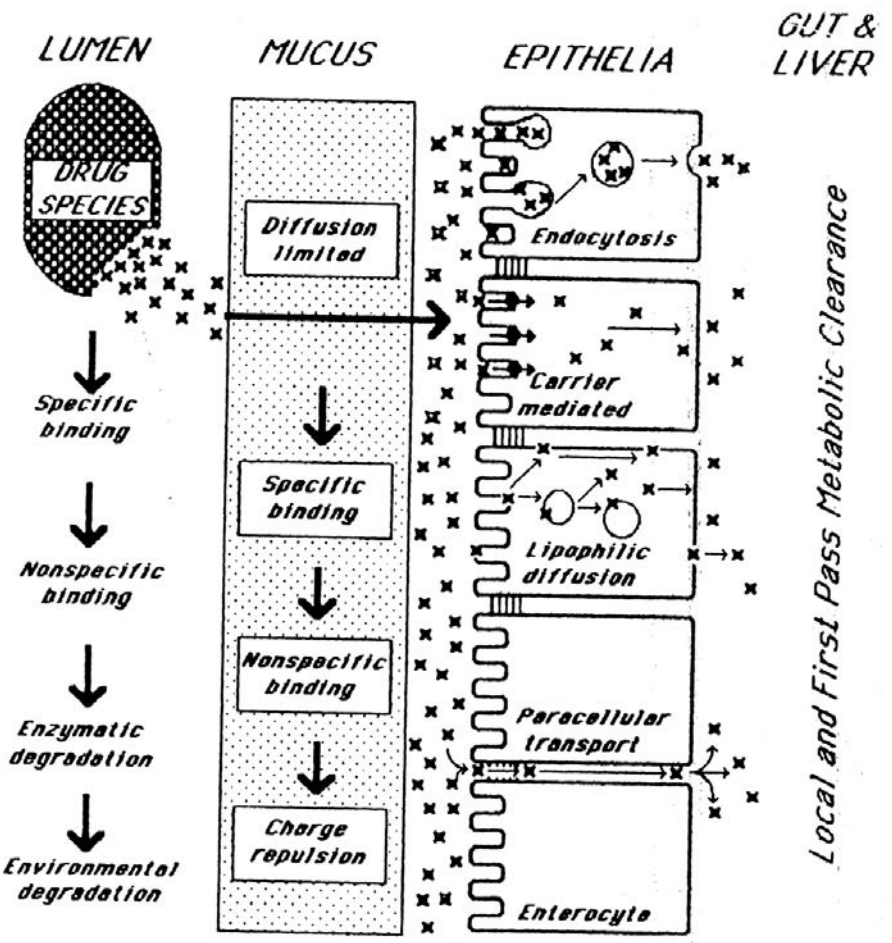

Figure 1.4 : Barriers to the colonic absorption of drugs (after Mrsny, 1992).

The level of the epithelia is observed as the most problematic site of drug delivery in the colon. There are enzymatic activities related with colonocytes and are mostly associated with the proximal vs. distal colon (Brasitus and Dudeja, 1985). The transepithelial movement is limited by the intercellular junction tight complex

(occluding junctions) of essentially biomolecules that avoid enzymatic destruction. There are two possible routes that drugs are successfully transported across the epithelia, namely, transcellular and paracellular. Most drugs are successfully delivered in the colon by the passive transcellular process. Small and amphipathic drugs will readily diffuse across colonic epithelial cells by a series of partitions between membrane lipid and the aqueous environments. Nevertheless, the drug needs to be stable to the environments it encounters on its transit through the colonic epithelial cell upon successful passive transcellular. Passive drug absorption in the proximal colon will be increased by the membrane lipid fluidity of proximal colonocytes which is greater than distal colonocytes (Brasitus and Dudeja, 1985). Nonetheless, thermodynamic barriers of passive diffusion between lipid membranes and aqueous compartments can become too large to overcome (Cooper and Kasting, 1987) and diffusional model breaks down as drug size and hydrophilicity or hydrophobicity increases (Jackson, 1987).

Unlike passive transport, pathways for active transcellular transport of large drug molecules are highly planned out. The uptake of drugs at the luminal surface was initiated at carrier-mediated uptake, pinocytotic endocytosis or receptor-mediated endocytosis. In order to gain a successful transcellular transport of a molecule entering the ephithelial cell via a carrier-mediated transporter event, the compound must be stable in the cyctoplasm. Furthermore, it must also be capable of migrating to the basolateral membrane and then must find some means of traversing this membrane to gain access to the submucosal space (Mrsny, 1992). The endocytotic event will follow a constitutive pathway where it fuses with a lysosome (Mellman *et al.*, 1986; 1987). Although in the work carried out by Kopecek (1990) using prodrugs

that become activated upon reaching the hostile environment of the lysosomal compartment was unclear. Leupold *et al*. (2009) has developed an apolipoprotein E-derived peptide, A2 that efficiently translocates across cell membranes which is mediated by endocytotic processes.

1.3.2 Absorption of drugs from the colon

1.3.2.1 Conventional drugs

With a number of exceptions, the majority of drugs are absorbed from the GI tract by passive diffusion. Small intestine will tend to absorb the di- and tripeptides which are generated from protein digestion. This means that certain drugs contain chemical structures, which allow them to be carried across the small intestine wall by the di- and tripeptide active transport mechanisms, for example, angiotensin converting enzyme (ACE) inhibitors and β-lactam antibiotics (Smith *et al.*, 1992). There will be possibility of some drugs with high lipophilicity epithelial cells being absorbed into the systemic circulation via the lymphatic system (Wilson *et al.*, 1989).

Drugs can be absorbed passively by two routes, i.e. paracellular or transcellular (Figure 1.5). The transport of drug molecules through the tight junctions between cells and most applicable for the hydrophilic drugs is called paracellular absorption, while transcellular absorption involves the passage of drugs through cells and is the route for most lipophilic drugs. According to studies in rat performed by Taylor *et al.* (1989), paracellular absorption was found to be constant throughout the small and large intestine, but transcellular absorption appears only to the small intestine. Although the absorption of paracellular of many drugs in the colon is poor due to the tight epithelial cell junctions (Powell, 1981) and lower surface area, the colon can be

a more selective site for absorption compared to the small intestine. Some examples of drugs shown to be well absorbed are ibuprofen (Moustafine *et al*., 2006), theophylline, glibenclamide (Brockmeier *et al.*, 1985), diclofenac (Ambrogi *et al.*, 2008), oxprenolol (Verhoeven *et al.*, 1989) and metoprolol (Dahan *et al.*, 2009). However, there are a number of drugs which are not absorbed by the colon including lithium, cimetidine, buflomedil (Wilson *et al.*, 1991), piretanide (Brockmeier *et al.*, 1986), furosemide (Bieck, 1989) and chlorothiazide (Riley *et al.*, 1992).

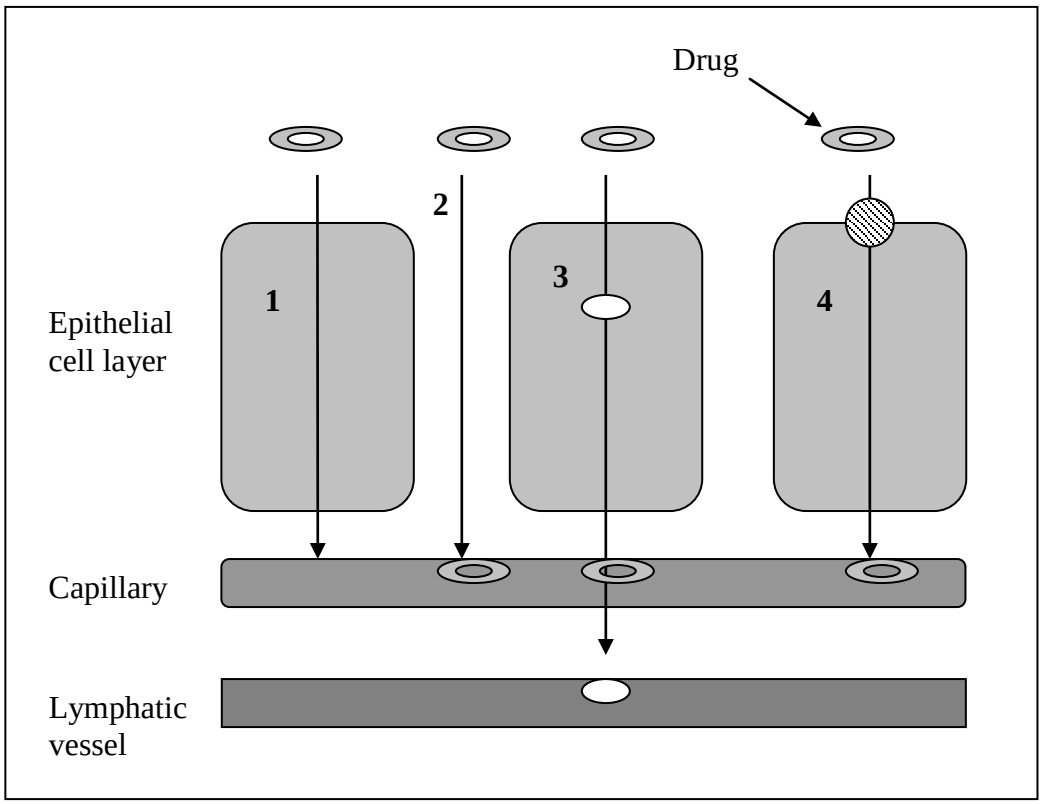

Figure 1.5 : Illustration of the main pathways of intestinal drug absorption: (1) Transcellular absorption; (2) paracellular absorption; (3) transcellular absorption followed by incorporation into chylomicron and transport into lymphatic system; (4) Active transport

Many sustained-release dosage forms rely on a degree of colonic absorption to remain therapeutically effective (Wilson *et al.,* 1989). In a study using an osmotic tablet formulation containing oxprenolol, the drug availability was higher in subjects where tablets resided longer in the colon (Bieck *et al.,* 1989). Thereupon, drug therapy could be compromised by using a once-a-day sustained-release formulation in cases of abnormality rapid GI transit. As insufficient colonic absorption has prevented and will continue to impede the development of sustained-release dosage forms for many drugs, therefore adequate knowledge of colonic absorption is important when developing related formulations.

1.3.2.2 Metabolically labile drugs

Peptide and protein drugs are expected an ever-increasing interest as novel and effective class of therapeutic agents (Cohen and Bernstein, 1996). However, their use is limited by the rapid clearance from body compartments, which can lead to the administration of exceptionally high doses to maintain an acceptable therapeutic level which may cause toxic effects. Therefore, the realisation of smart systems that allow site-specific administration of protein drugs has led to intense investigation. There are some specific sites of action such as organs, tissues, cells and molecular targets that protein and peptides have also been delivered (Hirabayashi *et al.*, 1996). In consideration of targeting a specific site of action, it depends on the aid of a delivery vehicle that relies on specific properties of the protein or peptide to be delivered and the unique properties of tissues being targeted.

The oral absorption of peptide and protein drugs is mostly limited by degradation in the acidic environment of the stomach, low mucosal permeability, extensive first

pass metabolism by the absorbing membrane and liver, enzymatic degradation in the small and large intestine and rapid small intestinal transit (Watts and Illum, 1997). In comparison to the stomach and small intestine, colon relatively lacks degradative enzymes and which is one of the most attractive properties of the colon as a site for peptide and protein delivery. A group of enzymes called peptidase that tend to break down peptides is found in highest concentrations in the intestine and lowest in the colon. As discussed earlier, the role of microflora is important as there is significant protease and peptidase enzymes activity in the colon. Therefore, poor stability of peptide and protein drugs can result within the colon and the opportunities for absorption are still relatively limited, although better than in the small intestine (Rama Prasad *et al.,* 1996).

There has been reported work on human calcitonin where the peptide was directly instilled into the distal colon using a colonoscope following administration of an enema to clear faecal matter (Antonin *et al.,* 1992). The therapeutic dose for calcitonin is delivered once a day. This greatly simplifies the dosing requirements and is becoming a particularly attractive peptide for GI tract delivery. The direct administration of human calcitonin into a colonic loop in anaesthetised rats to examine the bioavailability was performed (Hastewell *et al.,* 1992). This was compared to the pharmacodynamic effect, detectable in normal juvenile animals, of a reduction in plasma calcium levels in response to human calcitonin. This study was related to administering of human calcitonin in the transverse colon of patients (Antonin *et al.,* 1996) where the mean bioavailability was higher than found to be in a previous study (Antonin *et al.,* 1992). It was concluded that the transverse colon

was a better absorption site for human calcitonin than the distal colon although there are differences in the luminal environment between patients and healthy subjects.

Further studies have been conducted in human to test hypotheses concerning the optimisation of colonic delivery of peptides (Hastewell *et al*., 1995). Although the colon has been shown to be lower in peptidase activity than the proximal GI tract, the traces of pancreatic enzymes were retained by the colon. Results proved that low concentrations of human calcitonin are rapidly degraded by human faecal material (Hastewell *et al*., 1995). There was a dose dependent effect on the time taken for 50% degradation at 37°C and varied in the presence of 16 000 units of aprotinin, a protease inhibitor. In the presence of aprotinin, the bioavailability showed a decrease leading to the difficulty in explanation. The author suggested that aprotinin may have caused precipitation of the dose from solution within the colonic lumen. It is concluded that care must be taken in co-administration of compounds which may affect the intraluminal physiochemical environment of the dose although metabolism within the colonic lumen may restrict absorption of intact peptide. In another study, the bioavailability of salmon calcitonin showed a 7.1-fold increase by administration in the presence of taurodeoxycholate (TDC) and was further increased in the form of TDC proliposomes (Song *et al.,* 2005). This indicated that the validity of the hypothesis that the local delivery of a drug to the site of absorption may be favourable for its efficient absorption in the colon.

Colonic peptide delivery gives some advantages such as longer residence time, low metabolic activity, colonic bacterial enzymes present may offer targeting opportunities, responsive to absorption enhancers, the bulk water absorption in this

region of the intestine may provide scope for solvent drag and the transmucosal and membrane potential differences may be of significance in the absorption of ionised or ionisable drugs. However, despite the colon's apparent attractiveness as a route for the oral delivery of peptides, only very limited bioavailability can be demonstrated for this route *in vivo.*

1.3.3 Pathological processes in the colon

The term inflammatory bowel disease (IBD) is defined as a group of illnesses affecting the GI tract and manifested as inflammation of the bowel. Although inflammation is a primary process and usually confined to the digestive organs, the disease may affect almost any area of the body as an indirect consequence of the inflammation. It can be also associated with malnutrition and infection, or as a result of the side effects of drugs prescribed for treatment. The idiopathic IBD consists of ulcerative colitis and Crohn's disease (Haeberlin and Friend, 1992). These diseases are primarily treated with various corticosteroids, mesalazine and immunosuppressants.

Ulcerative colitis refers to an inflammation of the colon (large intestine or bowel) and does not spread to other areas of the intestines (Hanauer and Kirsber, 1985). Chronic ulcerative colitis affects the rectum or the sigmoid colon and progresses proximally to involve the entire left side of the colon. The first site of cell damage and death is the colonic crypts and the disease primarily involves the mucosal layer of the intestine resulting in chronic diarrhoea and abdominal pain. Crohn's disease is granulomatous and can affect any part of the GI tract. In Crohn's disease, the inflammation extends through all layers of the intestinal wall, which the mucosal

surface is reddened, nodular and cobblestone-like, with multiple linear ulcerations. The inflammatory infiltrate will thicken the mucosal layer, then the submucosa and serosa by fibrosis and finally the serosa by hypertrophy (Molema and Meijer, 2001).

1.4 Strategies for targeting drugs to the colon

The main purpose of the designation of colonic drug delivery systems is to take advantage of the conditions that are specific to the colon to gain release of drugs locally. However, targeting this region of the gut can be problematic as the colon represents the most distal segment of the GI tract. The most direct route for delivery of drugs into colon is by rectal administration via suppositories and enemas (Jay *et al.,* 1985), but such formulations rarely succeed in spreading beyond the descending colon, with little or no drug reaching the proximal colon (Hardy *et al.,* 1986). As most patients reject the rectal route, the oral route is preferable as mode of administration where it offers greater convenience, less pain, higher likelihood of compliance and reduced risk of cross-infection (Florence and Jani, 1993; Chen and Langer, 1998; Liu *et al.,* 2003).

The concept of oral route in colon-specific drug delivery is simple where the formulation must delay drug release in the stomach as well as small intestine but allow release in the colon. As a result, the design of oral controlled release products is relying on gross characteristics of the GI tract, which is usually empirically based, rather than using cellular and molecular information. This is because a good formulation is needed to overcome the obstacles of conditions and environments on passage down the gut, including pH, enzymes, electrolytes, transit time and pressure (Basit, 2005). With the concept of drug targeting, which is based on the identification