SYNTHESIS, CHARACTERISATION AND IN VITRO EVALUATION OF DISULPHIDE CROSS-LINKED POLYMERS AS POTENTIAL CARRIERS FOR COLON SPECIFIC DRUG DELIVERY SYSTEM

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by

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To my beloved parents, Lau Leong Bee and Lim Guat Hong,

and my brother, Lau Kian Khee

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GI	Gastrointestinal
IBD	Inflammatory bowel disease
5-ASA	5-aminosalicylic acid
HEMA	2-hydroxyethyl methacrylate
MMA	Methylmethacrylate
BMAAB	bis(methacryloylamino)azobenzene
SHIME	Simulated human intestinal microbial ecosystem
TFA	Trifluoroacetic acid
TES	Triethylsilane
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
TNB^{2-}	5-thio-2-nitrobenzoic acid
SEM	Scanning electron microscopy
EDX	Energy dispersive X-ray
TLC	Thin layer chromatography
FT-IR	Fourier transform infrared
NMR	Nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon nuclear magnetic resonance
LC-MS	Liquid chromatography-Mass spectroscopy
EDC	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
DMSO	Dimethylsulphoxide
DCC	N, N"-dicyclohexylcarbodiimide
DCM	Dichloromethane

DCU	Dicyclohexylurea
HOBt	1-hydroxybenzotriazole hydrate
EDTA	Ethylenediaminetetraacetic acid
WCAB	Wilkens-Chalgren Anaerobic Broth
CMM	Robertson's cooked meat media
ANOVA	One-way analysis of variance
HSD	Honestly Significant Difference
s	Singlet
d	Doublet
t	Triplet
m	Multiplet
et al.	et alii, others
h.	hour
min	minute
R_f	Resolution factor
ml	Milliliter (s)
MHz	Mega Hertz
^oC	Degree Celsius
L	Liter
mm	Millimeter

SINTESIS, PENCIRIAN DAN PENILAIAN SECARA IN VITRO BAGI POLIMER DISULFIDA BERANGKAI-SILANG SEBAGAI PENGANGKUTAN BERPOTENSI UNTUK SISTEM PENYAMPAIAN UBAT KHAS KEPADA KOLON

ABSTRAK

Sistem penyampaian drug ke kolon adalah sangat berguna untuk menyampaikan drug untuk rawatan penyakit kolon setempat seperti penyakit keradangan usus, ulser kolitis dan penyakit Crohn. Polimer disulfida berangkai-silang telah mendapat perhatian kerana keupayaannya untuk bertindak sebagai polimer sensitif redoks dan ikatan disulfida hanya akan dilekangkan oleh persekitaran yang mempunyai potensi redoks yang rendah di dalam kolon. Kebelakangan ini, polimer rantai bercabang disulfida telah menerima perhatian yang lebih disebabkan oleh hakikat bahawa polimer ini kurang terdedah untuk degradasi dalam keadaan pH rendah di perut berbanding dengan polimer rantai linear disulfida. Oleh itu, tujuan kajian ini ialah untuk mensintesiskan monomer trithiol berasaskan asid trikarbalilik untuk pempolimeran ke polimer rantai bercabang disulfida. Polimer sintetik telah dicirikan, dikaji secara kimia dan dinilai dengan menggunakan kajian disintegrasi secara *in vitro*. Monomer ini telah disintesiskan melalui tindak balas gandingan amida antara asid trikarbalilik dan (trifenilmetil) thioetilamina dengan menggunakan dua langkah sintesis. Monomer tersebut dinyahlindungkan dengan menggunakan asid trifluoroasetik dan trietilsilana untuk mendedahkan atom sulfur dalam persediaan untuk pempolimeran selanjutnya. Serbuk pepejal putih diperolehi dengan hasil

lebih kurang 20-25%. Keputusan spektroskopi dan analisis unsur CHNS mengesahkan monomer yang dikehendaki. FT-IR menunjukkan kehadiran puncak amida dan disokong oleh analisis jisim menggunakan LC-MS. Pempolimeran secara pengoksidaan *N*,*N*',*N*''-tris(2-sulfaniletil)propana-1,2,3-trikarboksamida (monomer trithiol) dan 2,2'- (etilenadioksi)dietanathiol (monomer dithiol) dengan pelbagai nisbah molar menghasilkan enam (6) polimer yang berbeza, dinamakan, P10, P11, P12, P15, P21 dan P51. Spektroskopi Raman menunjukkan kemunculan ikatan disulfida. Kajian reduksi kimia menunjukkan bahawa semua polimer disulfida telah diturunkan dengan lengkapnya selepas 1 jam dengan kepekatan thiol yang berbeza. Dalam keadaan simulasi gastrik dan usus kecil, semua polimer menunjukkan kepekatan thiol yang rendah jika dibandingkan dengan kepekatan thiol yang dikesan dalam keadaan simulasi kolon dengan kehadiran *Bacteroides fragilis*. Keputusan ini membuktikan bahawa semua polimer disulfida dapat bertahan dalam persekitaran gastrik dan usus kecil yang kasar dan degradasi hanya akan berlaku dengan kehadiran bakteria anaerobik di kolon. Degradasi didapati lebih ketara dalam polimer P15 menunjukkan kepekatan thiol yang tertinggi, iaitu 57×10^{-6} mol L^{-1} berbanding polimer yang lain. Keputusan ini memberi persetujuan umum bahawa biodegradabiliti bergantung pada kebolehkembangan polimer-polimer ini dalam keadaan akues. Penyelidikan ini telah mengidentifikasikan polimer berangkai silang disulfida yang mungkin mempunyai potensi untuk sasaran ke kolon. Walau bagaimanapun, kajian selanjutnya diperlukan bagi mencapai kestabilan dan penggunaannya sebagai polimer dalam bentuk dosaj farmaseutikal.

SYNTHESIS, CHARACTERISATION AND IN VITRO EVALUATION OF DISULPHIDE CROSS-LINKED POLYMERS AS POTENTIAL CARRIERS FOR COLON SPECIFIC DRUG DELIVERY SYSTEM

ABSTRACT

Colon drug delivery system is very useful to deliver drugs for treatment of localised colonic diseases such as inflammatory bowel disease, ulcerative colitis and Crohn's disease. Disulphide cross-linked polymer has received much attention because of its ability to act as a redox sensitive polymer and will only be cleaved by the low redox potential environment in the colon. Recently, branch-chained disulphide polymers had received more attention due to the fact that it is less susceptible for degradation in low pH condition of the stomach compared to linear-chained disulphide polymers. Therefore, the aim of this work is to synthesise tricarballylic acid based trithiol monomer for polymerisation into branch-chained disulphide polymers. The synthesised polymers were characterised, studied chemically and evaluated using *in vitro* disintegration studies. The monomer was synthesised by amide coupling reaction between tricarballylic acid and (triphenylmethyl) thioethylamine by using two synthetic steps. The monomer was deprotected using trifluoroacetic acid and triethylsilane to expose the sulphur atoms in preparation for further polymerisation. White powdery solid was obtained with yield around 20-25%. Spectroscopic and CHNS elemental analysis results complemented with the desired monomer. FT-IR showed the presence of amide peaks and supported by the mass analysis using LC-MS. The oxidative polymerisation of *N*,*N*',*N*''-tris(2-sulfanylethyl)propane-1,2,3-tricarboxamide (trithiol monomer) and 2,2'-(ethylenedioxy)diethanethiol (dithiol

monomer) of various molar ratios resulted six (6) different polymers, namely, P10, P11, P12, P15, P21 and P51. Raman spectroscopy demonstrated the emergence of disulphide bond. Chemical reduction studies showed that all the disulphide polymers were completely reduced after 1 hour of reduction with different thiol concentrations. In simulated gastric and intestinal condition, all the polymers showed low thiol concentrations when compared to the detected thiol concentrations in simulated colon condition with the presence of *Bacteroides fragilis*. The results proved that all the disulphide polymers were able to withstand the harsh environment of the gastric and intestinal condition and degradation will only occur in the colon condition with the presence of anaerobic colonic bacteria. Disintegration was found to be more pronounced in polymer P15 as the highest thiol concentration of 57×10^{-6} mol L^{-1} among all the other polymers. This result correlated the general consensus that biodegradability relies on the swelling ability of polymers in an aqueous environment. This work has successfully identified a disulphide cross-linked polymer, which might be potential for colon specific targeting. However, further study on these polymers is required to establish the stability and use of the polymer within a pharmaceutical dosage form.

CHAPTER ONE

INTRODUCTION

1.1 Background

To date, oral drug delivery is the most preferred, common, convenience and widely accepted route among many other routes of drug administration (Rubinstein, 1995; Laura *et al.*, 2011). Upper gastrointestinal (GI) tract appeared to be the major region for dissolution and absorption of orally administered drug. However, this approach is not suitable for drugs to be absorbed in lower GI tract or advanced biotechnology products such as peptides and proteins, whereby undesirable side effects and treatment failure will occur. Therefore, studies had been done in developing specific drug targeting especially to lower GI tract. Colon specific drug delivery had received much interest with much work already been carried out in this area of drug delivery (Yan *et al*., 2010; Kenawy *et al*., 2011; Ursekar *et al*., 2012; Lim *et al.*, 2013). The role of colon specific drug delivery is not only limited for localised treatment but also crucial for systematic treatment (Tozer *et al*., 1995). Although colon specific drug delivery can also be achieved via rectal route but this route appeared to be less readily accepted and less appealing to patients. Moreover, study showed that it is difficult to deliver drug to the proximal colon via the rectal route (Ritschel, 1991).

Colon specific drug delivery is particularly important in localised treatment of colon diseases such as inflammatory bowel disease (IBD), ulcerative colitis, Crohn's

disease and colorectal cancer (Gupta *et al*., 2001; Rubinstein, 2005; Yan *et al*., 2010). The nature of colon which is less hostile has a near neutral pH and lower activity of peptidase enzyme favours the systemic absorption of protein and peptide drugs (Maroni *et al*., 2012).

1.2 Anatomy of the colon

The GI digestive tract is a system of organs in human which responsible in taking food, digesting it to extract energy and nutrients and eliminating waste. 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, is located at the distal end of the GI tract (Figure 1.1). It is a muscular tube with a length of approximately 1.5 m long, has an average diameter of approximately 6.5 cm, with a diameter that varies from 2 cm in the sigmoid colon to 9 cm in the caecal region, running from the ileocaecal valve to the anus (Cummings and MacFarlane, 1991). The human colon is composed of the caecum, ascending segment, transverse segment, descending segment and sigmoid region. Colon plays an important role in extracting water and salt from solid wastes before they are eliminated from the body, storing waste, absorbing some vitamins and providing a site for flora-aided fermentation. Unlike the small intestine, the colon does not play a major role in absorption of foods and nutrients. Colon does not have villi although it does demonstrate crescentric folds, which increase the internal surface area of the colon to approximately of 1300 cm^2 (Cummings and MacFarlane, 1991). Guinea pigs and rodents models were commonly used in the study of colonic

drug delivery (Friend, 1991). However, there are differences between the animal models with normal human. The guinea pig has a very large caecum whereas humans have a poorly defined caecal region continuous with the colon (Friend, 1991). The transit time was found to be shorter in guinea pigs and slightly longer in rats if compared to humans (Pettersson *et al.*, 1976). The mucosa of the colon is lined almost entirely by goblet mucus secreting cells, whereby the large amount of bicarbonate ions from the mucus maintains the basic pH of the colonic region (Binder and Sandle, 1994).

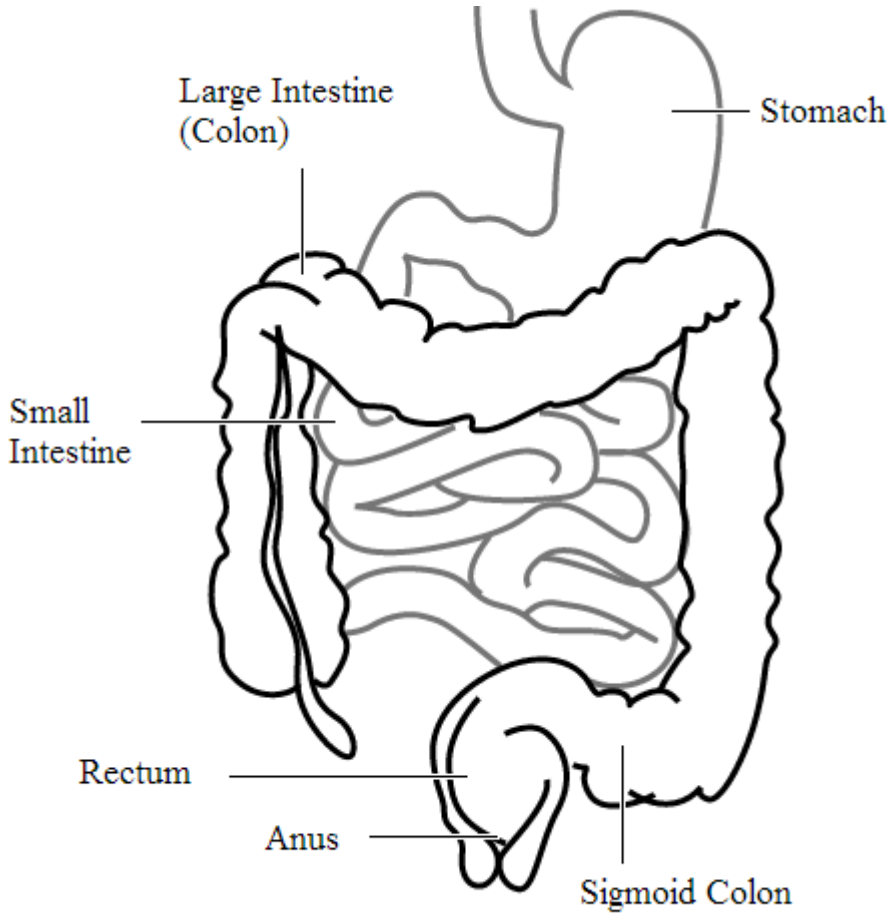


Figure 1.1 : Structure of the colon (large intestine) (after Wikipedia, 2014).

1.3 Microflora of the gastrointestinal tract

The upper GI tract is sparsely populated with micro-organisms. The acidic environment of the stomach exhibits a strong bactericidal activity, whereby the microbial concentration is usually less than 10^3 cfu/ml (Ilett *et al.*, 1990). Acid-tolerant micro-organisms such as gram-positive aerobic streptococci and lactobacilli are the main inhabitants in the stomach (Hao and Lee, 2004). The upper two-thirds of the small intestine which includes duodenum and jejunum contain similar bacterial concentration to the stomach, between 10^3 to 10^4 cfu/ml. However, gram-negative bacteria begin to outnumber gram-positive bacteria in the distal ileum. The reduction in acidity and lower redox potentials play a major role in maintaining a more diverse microflora and higher bacteria population (Tannock, 1983).

Colon is the primary site of microbial colonisation in human body. The colon contains a very large quantity of bacteria from various species. The bacterial concentration in the colon is around 10^{11} to 10^{12} cfu/ml and approximately one third of the fecal dry weight consists of bacteria (Simon and Gorbach, 1984). The increase in bacterial concentration in this region is due to a near neutral pH caused by neutralisation of the bowel contents by intestinal juice and lower motility rate of the colon. However, 99.9 % of colonic microflora is obligate anaerobes (Hao and Lee, 2004). There are over 400 different bacterial species found in the colonic region. The predominant species isolated include *Bacteroides*, *Eubacterium*, *Bifidobacterium* (Hill and Drasar, 1975). Anaerobic gram-positive *Cocci*, *Enterococci*, *Clostridia* and other species of *Enterobacteriaceae*, *Coliforms*, *Staphylococci*, *Lactobacillus*, *Spirochetes*, *Pseudomonas*, *Proteus*, *Clostridium*, *Actinomyces*, *Borrelia*,

Fusobacterium and *Diphtheroides* species are also found. However, *Bacteroides* species account for nearly 32 % of all bacterial flora isolated from the GI tract (Finegold *et al*., 1977). The comparison of microbial concentration in different regions of GI tract is shown in Figure 1.2.

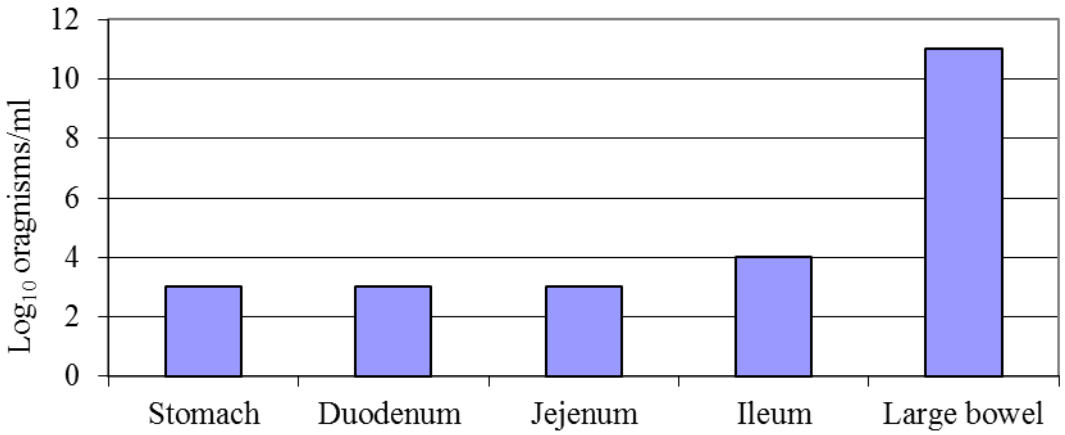


Figure 1.2 : Concentration of microflora in different regions of gastrointestinal tract (taken with modification from Basit, 2005).

Certain diseases or antibiotic treatment will influence the microbial flora in the gut (Friend and Tozer, 1992). The nature of certain disease, where reduction of gastric acid secretion occurred, will lead to increase in bacterial growth in the stomach (Rowland, 1988). The speed of peristalsis movement decreases from the jejunum to upper ileum and the distal ileum. As it is approaching the ileo-caecal valve, peristalsis slows down to an almost stagnant state and this has been the major factor which cause the drastic rise in the bacterial population in this area. The bacteria

populations found in this area are mainly *Bacteroides*, *Enterobacteria* and *Bifidobacteria.* Huge changes of some organisms in the region within the gut can occur when antibiotic treatments are introduced where it has bactericidal effect and suppresses the bacterial growth (Goldin and Grobach, 1977).

Colon is well known for its reductive environment, where the redox potential becomes more negative from the small intestine to the large bowel. The redox potential of proximal small bowel is -67 ± 90 mV, -196 ± 97 mV in the distal small bowel and -415 ± 72 mV in the colon (Stirrup *et al*., 1990). The presence of the bacterial microflora causes the changes in the redox potential along the GI tract. Due to its low redox potential, the colonic region is very active in reductive and degradative reactions (Rowland, 1986).

Bacterial microflora in the GI tract produce a wide range of reductive and hydrolytic enzymes which include β-glucoronidase, β-galactosidase, β-xylosidase, azoreductase, nitroreductase, urea hyroxlase and α-arabinosidase (Kinget *et al*., 1998). The ability of colonic anaerobic bacteria to produce appropriate digestive enzyme in order to digest the constantly changing carbohydrates complex was exploited for the development of enzyme-sensitive prodrugs to achieve colonic drug targeting (Sinha and Kumria, 2001a; Sinha and Kumria, 2001b).

1.4 Colon-specific drug targeting

The concept of delivering drug to the desired site is not new. All drugs exert their beneficial effects along with undesirable side effects to varying degrees of severity. Therefore, site-specific drug delivery is important by enhancing the beneficial effects and limiting the side effects. The formulated dosage form for colon-specific drug delivery must pass through the upper GI tract intact before delivering the drug to the colonic region.

Physiological factors in the upper GI tract such as extreme acidity in stomach, degradative enzyme activities and presence of bile salts will destroy the drug molecules that are intended to colon, especially peptides and proteins. This will result in poor bioavailability of drugs and treatment outcome. Due to the barriers of the upper GI tract, colon has been focused as the targeting site for orally administered drug. Numerous investigations have shown that some anti-inflammatory (Wilson and Washington, 1989) and antidiabetic (Gleiter *et al*., 1985) drugs are better absorbed from the colon rather than the upper GI tract.

The colon is found to have prolonged residence time for the colonic contents (Coupe *et al*., 1991). The average transit time through the colon is 22-36 hours and it is much higher if compared to the transit time in the stomach (0.5-3 hours) and the small intestine (1-6 hours) (Coupe *et al*., 1991). On top of that, colonic transit time has been shown to be independent of size and density of the dosage form (Parker *et al*., 1988). The ideal property for a colon-specific drug delivery system is to spend as little time as possible in the upper GI tract (Friend, 1991). Therefore, the

prolonged colonic transit time provides a longer time interval for drug absorption in the colonic region. This will compensate the smaller surface area for absorption in the colon compared to the small intestine.

1.5 Strategies for targeting drugs to the colon

The main responsibility of a colonic drug delivery system is to utilise the special characterisitics of the colon in order to achieve localised drug release. The main concept of orally administered colon-specific drug delivery is where the dosage form must be able to withstand the harsh environment of the stomach and small intestine but to achieve drug release in the colon. The major characteristics of the GI tract include pH, transit time, enzymes, bacteria and pressure (Basit, 2005; Bhalerao and Patel, 2012) were taken into considerations in order to develop a good formulation for colonic drug delivery. These strategies are currently being studied and will be further discussed below.

1.5.1 pH responsive delivery

pH in human GI tract changes when it shifts from one region to another. The environment of the stomach is acidic (pH 1-2) and the pH increases in the small intestine region (pH 6.5-7). The pH in the terminal ileum and colon (pH 7-8) is the highest in the human GI tract. The concept of pH responsive delivery was designed by using materials that are insoluble in acidic pH but dissolve in neutral to slightly basic pH (Ibekwe *et al*., 2006). However, pH in the terminal ileum region is higher than in the caecum. Thus, the dosage forms are often delayed at the ileocecal

junction. In short, the concept of using polymers that are sensitive to higher pH was employed and adapted for colonic drug delivery instead of conventional enteric coating (Edsbacker and Anderson, 2004).

Acrylic polymers from the Eudragit® range are commonly used for this purpose because of its solubility when expose to pH above 6 (Basit, 2005). Eudragit-based polymers can be made to dissolve at various basic pH by modifying the methylester content (Peeters and Kinget, 1993). Eudragit® S and Eudragit® L were designed to be specifically dissolved in pH 7 and pH 6 respectively. Dew et al. (1982) were the first to employ Eudragit® S as a capsule coating by utilising gastrointestinal pH as a trigger for drug release in the distal gut. The results were positive in most patients as the capsule disintegrated in the distal gut. However, Ashford *et al*. (1993a, 1993b) found that the drug release of Eudragit® S-coated tablets in the colon of healthy volunteers was not adequately reproducible. The reason behind was due to the decrease in pH after passage through the ileocaecal valve. Therefore, Eudragit® S coating dissolves and releases its drug content in the ileum rather than colon.

Despite such evidence of premature release of drug contents in the ileum, preparations include Asacol® (Eudragit® S) and Claveral® (Eudragit® L) have been formulated using which have approval for human use. The preparations were found to be successful in treatment of IBD (Hardy *et al*., 1987). In order to compensate the low release pH of Eudragit® L, a thicker layer was formulated. The release of drug contents (5-aminosalycilic acid) from both preparations is reported to take place at the distal ileum and right colon.

1.5.2 Time responsive delivery

The size, shape and density of the dosage form and the feed status of the individual are the major factors which will affect the GI transit time (Davis *et al*., 1984; Devereux *et al*., 1990). Residence time in the stomach varies from a few seconds to few hours. However, small intestine transit time was found to be more consistent at 3-4 hours, without being affected by feed status of the individual (*Davis et al*., 1986). The consistent transit time of small intestine has been exploited in developing colon drug delivery system. Time responsive delivery is formulated to release their drug contents after a predetermined lag time. Assumption was made by researchers, whereby 5-6 hours are needed for the ingested dosage form to reach the colonic region. Therefore, a nominal lag time of 5 hours is usually considered sufficient (Davis *et al*., 1986).

The pioneer time responsive delivery system was developed by Wilding *et al*. (1992), namely, Pulsincap™ device. The device consists of an impermeable hard gelatin capsule sealed at one end with hydrogel plug. Upon contact with gastrointestinal fluid, the plug hydrates and begins to swell and after a pre-set lag time, ejects from the capsule body, enabling drug release to occur. The size and composition of the plug are crucial in controlling the lag time of the entire device. Two different Pulsincap™ devices with different pre-set lag time of 5 and 6 hours respectively were studied in fasted human subjects using gamma scintigraphy (Hebden *et al*., 1999). The authors found that the location of the device varied in human body at the time of release. Further study reported that the device exhibits poor emptying feature

especially in distal bowel. Lack of fluid in the distal bowel appeared to be the major factor which caused the drawback (Wilding, 2000).

Newer colon drug delivery system was described by merging of both pH and time responsive mechanisms in order to increase the effectiveness (Ishibashi *et al*., 1998). The system comprises of a conventional hard gelatin capsule which contains mixture of drug content and organic acid. The outer surface of the capsule is coated with three layers, including outer enteric layer, hydrophilic layer and acid-soluble layer. The entire system was designed to survive in the harsh condition of the stomach. Subsequently, the capsule will reach a higher pH condition in the small intestine, whereby the outer enteric layer will dissolve followed by the intermediate hydrophilic layer. The inner acid-soluble layer which dissolves at pH below 5 will survive the condition of the intestine. However, fluid will enter the capsule and lead to the dissolution of the organic acid. pH reduction occurs followed by the dissolution of the acid-soluble layer, leading to drug release from the entire system. Study was done on fasted and fed volunteers but the results showed variability for the site of disintegration (Ishibashi *et al*., 1998).

From the report of several studies, individual variability is a major influence towards the gastrointestinal transit time (Coupe *et al*., 1991). Moreover, transit was found to be faster in the morning if compared to the evening (Hebden *et al*., 1999). Time responsive delivery system was unable to detect individual's gastrointestinal transit, which limits the practicality of the formulation.

1.5.3 Pressure responsive delivery

Higher pressures are encountered in the colon as a result of peristalsis movement. The grinding, propulsion and motility of the luminal contents were found to be generating pressure due to the muscle contraction of the intestinal wall. However, the intensity and duration of the generated pressure varies throughout the GI tract (Takaya *et al*., 1995). The colon is believed to exert a higher effective luminal pressure in a viscous environment. A capsule utilising pressure responsive system was developed by Takaya *et al*. (1995), using a hydrophobic polymer ethycellulose. Drug release can be achieved after the successful disintegration of the capsule due to the luminal pressure of the colon. The thickness of the ethylcellulose layer plays a crucial role for the disintegration of the dosage form.

Several works had been done on methods of manufacturing pressure responsive delivery system. Takaya *et al*. (1995) had successfully coated the inner surface of the gelatin capsule with hydrophobic ethylcellulose, Hu *et al*. (2000) reported a low temperature coating of the capsule-shaped pieces of suppository base. The dipping method into both ethanolic ethylcellulose and alkalised enteric polymer solution was conducted by Jeong *et al*. (2001). Investigations were made for these formulations which involved pharmacokinetic evaluation. Correlation between times for appearance of drug in plasma with literature values for colon arrival times had been done. However, the results did not conclusively show successful disintegration in the colon due to gastric and intestine transit times are known to be variable.

1.5.4 Bacteria responsive delivery

Majority of the bacteria in the human colon are anaerobic in nature. These anaerobic bacteria are responsible in producing various types of enzyme to metabolise substrates, such as proteins and carbohydrates, which escape from the upper GI tract (Cummings *et al*., 1989). Materials which are susceptible to the enzymatic degradation in the colon but able to survive the condition of gastric and small intestine, could be designed as carriers for colonic drug targeting. These enzyme sensitive materials depend heavily on the enzyme-generating microflora in the colon. The enzymes generated include azoreductase, peptidase, esterase and glycosidase. To date, bacteria responsive colon drug delivery has received much attention (Vandamme *et al*., 2002; Sinha and Kumria, 2003; Chourasia and Jain, 2004; Ibekwe *et al*., 2008).

1.5.4.1Azo-containing prodrugs

Sulphasalazine appeared to be the first bacteria responsive colon drug delivery system being invented. It is a prodrug consisting of the active drug content (mesalazine) linked by an azo bond to a carrier molecule (sulphapyridine) (Peppercorn and Goldman, 1972; Svartz, 1988). From the study done by Myers *et al*. (1987), mesalazine was found to be rapidly absorbed from the small intestine. However, absorption is inhibited in the gastric and small intestine until it reaches colon when the prodrug is ingested. Bacteria in the colon will release enzyme such as azo-reductase to cleave the azo bond of the prodrug, leading to release of the active drug content of 5-aminosalicylic acid (5-ASA) at the inflammation region.

Klotz (1985) reported that approximately 85% of an orally administered sulphasalazine survived unabsorbed into the colonic region.

Enzymatic reduction will break down sulphasalazine into 5-aminosalicylic acid (5-ASA) and sulphapyridine as shown in Figure 1.3. 5-ASA is generally unabsorbed in the colon where it is responsible in providing topical anti-inflammatory activity (Peppercon, 1984). However, sulphapyridine is well absorbed in the colon (Klotz, 1985) and causes undesirable side effects such as allergic reactions, which occurs in up to 50% of recipients.

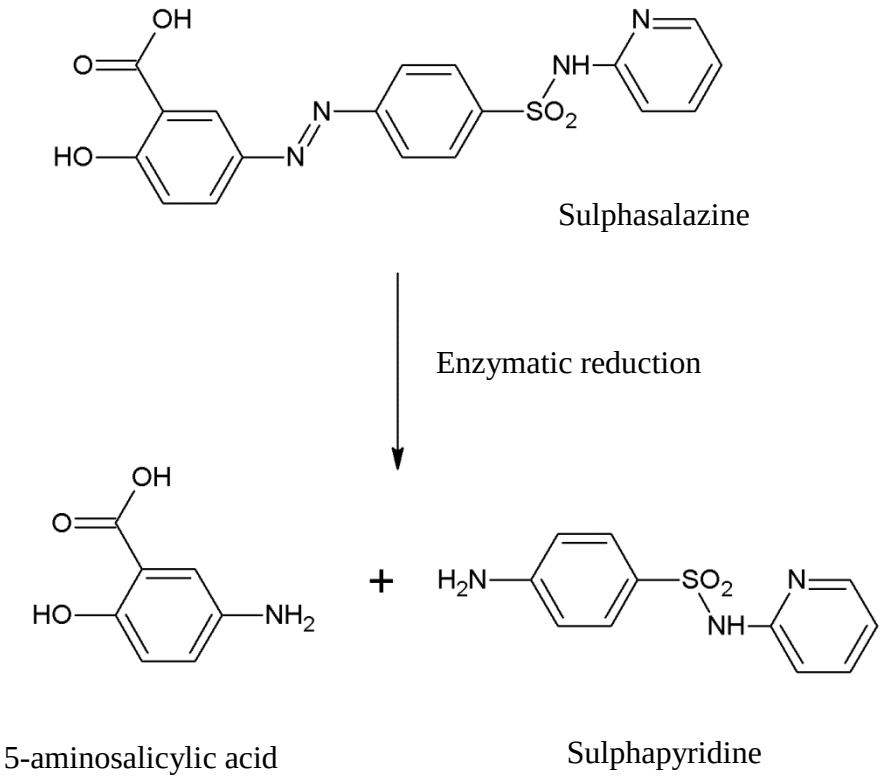


Figure 1.3 : Mechanism of enzymatic reduction of sulphasalazine in the colon.

In order to solve the toxicity issue of sulphapyridine, mesalazine has been azo bonded to another mesalazine molecule, namely olsalazine (Campbell and Berlingdh, 1998) or inert carrier, namely balsalazide and ipsalazide (Chan *et al*., 1983). Molecular structures of olsalazine, balsalazide and ipsalazide are shown in Figure 1.4. These methods provide alternative prodrugs for use in IBD which can prevent the toxicity problem of sulphapyridine.

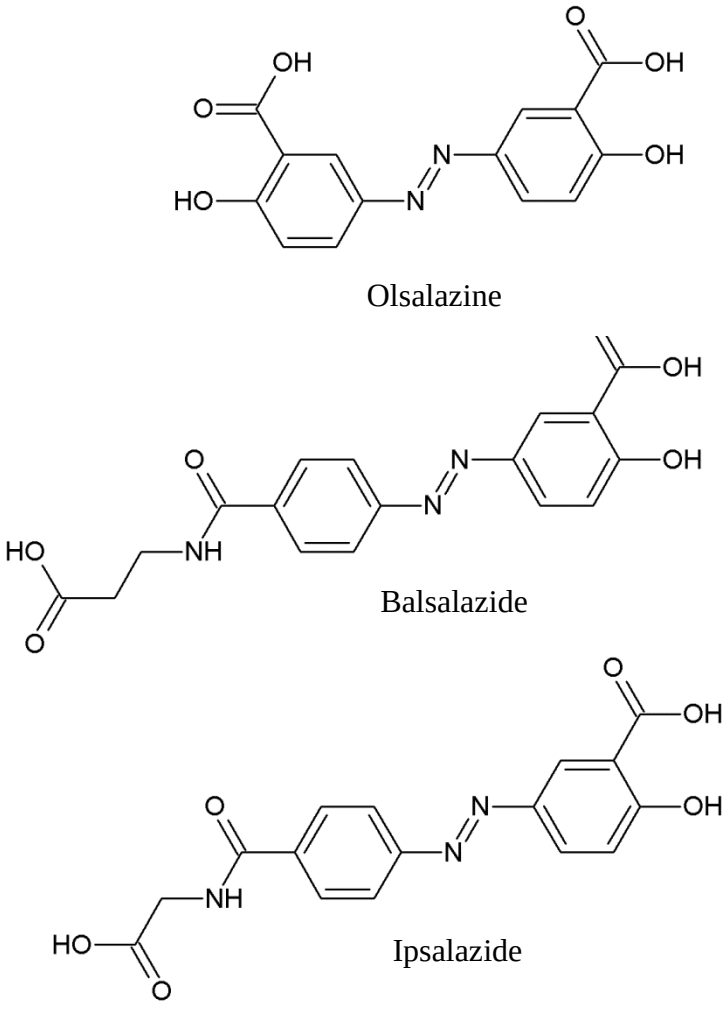


Figure 1.4 : Molecular structure of new-generation prodrugs of 5-ASA.

1.5.4.2 Azo-polymers

Azo-reductase is a type of enzyme which is commonly found in human colon. The use of bacterial azo-reductase to split prodrug sulphasalazine into sulphapyridine and 5-ASA was the first colon specific targeting system. Subsequently, several studies were carried out by exploiting the azo-reductase enzyme as a release mechanism for drugs within the colonic region. Coating of peptide insulin with polymers cross-linked with azoaromatic group has been discovered to protect insulin from the harsh environment of stomach and small intestine. Thereafter, azo bonds were reduced in the colon, leading to the release of insulin (Saffran *et al*., 1986). Further study was conducted to deliver similarly coated insulin dosage forms to the colon of diabetic dogs (Saffran *et al*., 1990) and the release mechanism was concluded to be caused by the enzymatic degradation of the azo-polymer coatings in the colon. The increase in insulin level and reduction in glucose production and glucose level were found to be statistically significant.

Copolymers were employed for potential colon drug targeting by Van den Mooter *et al*. (1992, 1993 and 1995). Copolymers composing of 2-hydroxyethyl methacrylate (HEMA) and methylmethacrylate (MMA) were prepared in the presence of a bifunctional azo compound, which is bis(methacryloylamino)azobenzene (BMAAB). Different molar ratios of HEMA and MMA were used in order to obtain different copolymers. Copolymer comprised of 1:6 molar ratio of MMA/HEMA and polymer without the addition of MMA showed significant bacterial reduction both *in vitro* and *in vivo*. Both of the polymers demonstrated good resistant in simulated gastric

acid and intestinal fluid. Conclusions were made that the monomer composition has a greater influence than the structure of the cross-linking agent.

Hydrophilic azo-polyamides were studied as a potential colonic drug targeting by Schact *et al*. (1991). The films were found to be completely dissolved under reductive medium (-430 mV) and simulated human intestinal microbial ecosystem (SHIME). The degradation was detected by observing the colour changes from pink-orange to white/colourless. Lloyd *et al*. (1994) suggested that complete reduction of azo bonds to amine would be favorable for effective drug release. Hydrophilic polymers appeared to meet the requirement because of reduction of such polymers are reported to go toward amine formation. Meanwhile, hydrophobic polymers reduction stops at the hydrazo stage which appeared to be undesirable (Schact *et al*., 1996). Recent studies showed similar results in degradation of azo dyes by bacteria (Pandey *et al*., 2007; Hong *et al*., 2008).

1.5.4.3 Glycosidic prodrugs

Colonic bacteria provide glycosidase activity by generating glycosidase enzyme which forms the fundamental of a colon specific drug delivery system. Friend and Chang (1984 and 1985) utilised corticosteroid prodrugs as glycosidic carrier by the attachment of the active agent. Drug glycosides are poorly absorbed from small intestine due to its hydrophilic nature. The drug glycosides can be cleaved by glycosidase enzyme upon reaching colonic region, releasing the active drug contents which will be absorb by the colonic mucosa. Several studies have reported that

absorption occur for corticosteroids obtained from hydrolysis of glycosidic prodrug in the colonic region.

Strategy of glycosidic prodrug was evaluated in rats with two steroid prodrugs, prednisolone 21-β-D-glucoside and dexamethasone 21-β-D-glucoside (Tozer *et al*., 1991). The potential of glycosidic prodrug is assessed by determining the rates of its hydrolysis down the alimentary canal of the guinea pig. It was found that the caecum and colon contents showed greater hydrolytic activity than the stomach and small intestine contents. On top of that, the movement and hydrolysis of the prodrug down the GI tract of the guinea pig were also examined. The authors reported approximately 20 to 30 % of an oral dose appeared to reach the caecum, whereby the prodrug was rapidly hydrolysed to the active form. Greater site-specific delivery is expected for human due to less glucosidase activity in the small intestine.

1.5.4.4 Polysaccharides

Natural or synthetic polysaccharides have received much attention for colon specific drug delivery. The feature of natural polysaccharides which can be degraded by the local bacterial enzyme upon entering colonic region was exploited for colon drug delivery (MacFarlane and Cummings, 1991). These natural polysaccharides are present in normal dietary food and usually are resistant to digestive enzyme in the upper GI tract. Many of these polymers are already used as pharmaceutical excipients in formulations. Therefore, problems associated with toxicity and biocompatibility are much simplified. However, the hydrophilic nature of the polysaccharides would cause swelling in the upper GI tract and eventually lead to

premature drug release. Thus, methods were developed to prevent premature drug release by either chemically modified the polymers and mixed with hydrophobic polymers. This will prevent the swelling in the upper GI tract, but still retaining the solubilisation feature of the coating in the colon due to bacterial degradation.

Several studies have been done on the use of dextrans in colonic drug delivery (Harboe *et al*., 1988; McLeod *et al*., 1993). Dextran has potential to act as a carrier for drugs such as naproxen and dexamethasone. The authors reported that degradation of dextran was significantly higher in colon if compared to upper GI tract. Modification has been made for dextran by esterification with fatty acids in order to reduce its solubility (Hirsch *et al*., 1995). *In vitro* degradation studies showed that the modified dextran resisted the harsh condition of the upper GI tract, but sensitive to degradation by colonic microflora. However, increment in degree of crosslinking will render the reduction of colonic degradability of the polymer.

Pectinolytic enzymes found in the colonic region are responsible in the degradation of pectin (Rubinstein and Sintov, 1992). This unique feature was exploited by the researchers which pectin was used as a carrier for colonic drug delivery (Ashford *et al*., 1994). Pectin Type 170 (30-40 % methoxylated) and Pectin USP (70 % methoxlated) were used as a compression coating. A minimum thickness of 0.9 mm (700 mg) of Pectin USP coat was found to be viable in resisting the intestinal condition. However, a thicker coat of 1000 mg showed poor degradation in pectinolytic enzyme medium due to poor penetration of fluid to hydrate the coating for enzyme entry. From the scintigraphic result, pectin coating is able to protect the

drug until colonic region and majority of the release occurred in caecum and ascending colonic region. Modification was made by cross-linking calcium with pectin, yielding calcium pectinate (Adkin *et al*., 1997). The authors reported that calcium pectinate tablets were degraded in rat caecum, whereby such modification has potential for colonic drug delivery.

Guar gum is a galactomannan which has high viscosity. This polysaccharide has potential to be used as a carrier, delivering drugs to the colon without undesirable release in the upper GI tract. The potential of guar gum has been studied in the form of a matrix and compression coat (Rubinstein and Gliko-Kabir, 1997; Krishnaiah *et al*., 1998a and 1998b; Tugcu-Demiroz *et al*., 2004). A study utilising guar gum compression coat of 400 mg that applied to tablet cores of 300 mg, containing 250 mg of ornidazole was found to be significantly delay the onset of drug release and absorption (Krishnaiah *et al*., 2003). However, the drug from the coated formulation was found to be absorbed in a slower rate from the colon.

Amylose is one of the major components of starch, apart from amylopectin (Biliaderis, 1991). Amylose is susceptible to fermentation by a broad range of colonic bacteria (Salyers *et al*., 1977), where more than half of the bacterial population is able to digest amylose (MacFarlane and Englyst, 1986). The polysaccharide amylose has been exploited as a carrier for colonic drug delivery in the form of film coating (Siew *et al*., 2000; Leong *et al*., 2002). Addition of hydrophobic ethylcellulose as a structuring agent is essential due to the fact that amylose films tend to swell in aqueous media. Various ratios of amylose with

ethylcellulose were employed to investigate the applicability in colonic drug delivery. Drug release from the coated formulations was reported in fermentation environment of the colon. Therefore, amylose coated formulations have potential to become a carrier for drug delivery to the colon.

1.5.4.5 Disulphide polymers

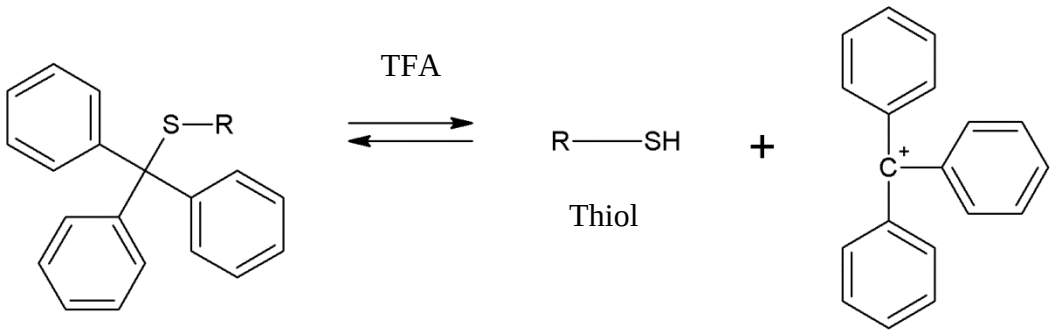
To date, disulphide polymers have received much interest due to its potential as carrier for colonic drug delivery. Disulphide polymer was found to be resistant in the upper GI tract but susceptible to degradation in the colonic region. Low redox potential environment of the human colon is the key feature for the entire mechanism, where disulphide bond will be reduced. It has been proposed that linear polymers containing disulphide backbones could be degraded in the low redox potential environment of the human colon (Schact and Wilding, 1991). Watts and Illum (1997) reported that the tablets coated with polymer rapidly disintegrated, achieving complete dissolution in the testing medium.

Recently, studies were focused on employing branch-chained disulphide polymers instead of linear-chained. The major reason was due to the insolubility features of the branch-chained polymers. Linear-chained polymers are easily degraded in low pH condition and more soluble if compared to branch-chained polymers (Sahudin, 2001; Lim *et al.*, 2013). The ability of disulphide polymers to degrade in low redox potential of the colonic environment while sustaining the harsh environment of the upper GI tract, have been exploited as a redox-sensitive polymers in colon-specific drug delivery.

1.6 Literature review

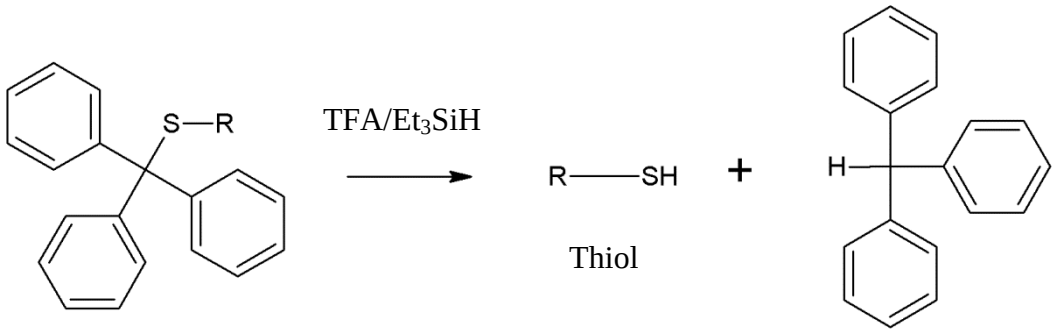
Protection of the thiol group is crucial in many areas of organic research especially in peptide synthesis. The thiol groups were found to be very reactive and therefore the protection is necessary for the sulphur atom. A free thiol group can be protected as a thioether or thioester. The protecting group can be easily cleaved via acidic hydrolysis or by using sodium/ammonia (Wuts and Greene, 2006). Triphenylmethyl thioether protecting group which is used in this synthesis can be remove by using iodine, thallium trifluoroacetate and trifluoroacetic acid (TFA). Iodine and thallium are not favorable as a deprotection agent because they will effect simultaneous disulphide bond formation (Fields and Noble, 1990). Relatively, TFA is a more favorable agent because it generates free thiol groups without forming disulphide bond (Atherton and Sheppard, 1987). However, the using of TFA alone for the deprotection procedure is not sufficient. The cleavage reaction is reversible due to the high stability of the trityl cation and strong nucleophilic nature of the thiol group. Hence, the use of triethylsilane (TES) scavengers appeared to be crucial in the deprotection process. TES will quench the trityl carbocation to form triphenylmethane and make the entire process irreversible (Figure 1.5) (Pearson *et.al*. 1989).

a)



Trityl protected thiol Trityl cation

b)



Trityl protected thiol Triphenylmethane

+ $Et_3SiOCOCF_3$

Figure 1.5 : TFA deprotection of triphenylmethyl (a) and TFA/TES scavenger deprotection of triphenylmethyl (b).

Linear disulphide polymers have been studied shown to be biodegradable in colonic bacterial culture (Le, 1998). Such polymers have been suggested as the coating materials for colon drug delivery system (Schacht and Wilding, 1991). However, linear-chained disulphide polymers were found to be easily degraded in low pH condition of the stomach (Sahudin, 2001). Therefore, branch-chained disulphide polymers had received more attention due to less susceptible for degradation in low pH condition of the stomach and more insoluble compared to linear-chained disulphide polymers. Nevertheless, the insolubility feature of branch-chained disulphide polymers would appear to be desirable in the stomach and small intestine if the polymerisation of the polymers can be controlled. The important goal of the polymer is to maintain the dosage form in an intact state and should be readily degraded in low redox potential environment of the human colonic region.

Oxidative polymerisation method can be used for synthesis of disulphide cross-linked polymer where the monomer is placed in a mild basic condition, exposed to open air and with the presence of oxidising agent such as dimethyl sulphoxide (DMSO) (Tan *et al*. 1991). DMSO is miscible with water at all concentrations, thus it helps to improve the solubility of thiol monomers in aqueous solution. The reaction mechanism of oxidation by DMSO was shown in Figure 1.6.