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# Design and development of a stimuli-responsive oral tablet system for the treatment of ulcerative colitis

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# DESIGN AND DEVELOPMENT OF A STIMULI-RESPONSIVE ORAL TABLET SYSTEM FOR THE TREATMENT OF ULCERATIVE COLITIS

**PRIYA BAWA**

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Pharmacy

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**Johannesburg, 2011**

## DECLARATION

I, Priya Bawa, declare that this dissertation is my own work. It has being submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…….

This …….. day of May 2011

## RESEARCH OUTPUTS

### 1. Research Publications

a. Priya Bawa, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Stimuli-responsive polymers and their applications in advanced drug delivery. *Biomedical Materials.* 2009. 4 (2): 1-15 (Abstract in Appendix A1).

b. Priya Bawa, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M. K. Ndesendo and Pradeep Kumar. A Composite Polyelectrolytic Matrix for Controlled Oral Drug Delivery. *AAPS Pharmaceutical Sciences and Technology*. 2011. 12(1): 227-238 (Abstract in Appendix A2).

c. Priya Bawa, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Leith C.R. Meyer. Formulation design and *in vitro* and *in vivo* evaluation of a novel crosslinked stimuli-responsive colon-targeted drug delivery system, submitted (Abstract in Appendix A3).

d. Rubina Shaikh, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo, Priya Bawa and Shivaan Cooppan. A Review of Multi-Responsive Membranous Systems for Rate-Modulated Drug Delivery. *AAPS Pharmaceutical Sciences and Technology.* 2010. 11 (1): 441-459 (Abstract in Appendix C1).

e. Ndidi Ngwuluka, Viness Pillay, Lisa C. du Toit, Valence M.K. Ndesendo, Yahya E. Choonara, Girish Modi, Dinesh Naidoo, Priya Bawa and Shivaan Cooppan. Levodopa delivery systems: advancements in delivery of the gold standard. *Expert Opinion on Drug Delivery*. 2010. 7 (2): 203-224 (Abstract in Appendix C2).

f. Kovanya Moodley, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo, Priya Bawa and Shivaan Cooppan. Oral Drug Delivery Systems Comprising Altered Geometric Configurations for Controlled Drug Delivery, submitted. (Abstract in Appendix C3).

### 2. Poster Presentations

a. Priya Bawa, Viness Pillay and Yahya E. Choonara. Effect of the degree of esterification and amidation on drug release from crosslinked zinc pectinate matrices. Poster presented at 28$^{th}$ Annual Conference of the Academy of Pharmaceutical Sciences, 4-7 September 2007, Cape Town (Abstract in Appendix B1).

b. Priya Bawa, Viness Pillay and Yahya E. Choonara. The influence of the choice of solvent in wet granulation as a means to retard drug release in the stomach. Abstract submitted to 2nd Annual Symposium of the Biomaterials Association of South Africa (BioMatASA), University of Witwatersrand, 26th November 2007 (Abstract in Appendix B2).

c. Priya Bawa, Viness Pillay and Yahya E. Choonara. A grafted co-polymerized poriferous matrix for oral drug delivery. Poster presented at 29th Annual Conference of the Academy of Pharmaceutical Sciences, 22-26 September 2008, Rustenburg (Abstract in Appendix B3).

d. Priya Bawa, Derusha Frank, Viness Pillay and Yahya E. Choonara. Comparison of crosslinked and non-crosslinked quaternised chitosan matrices for effectual gastric accentuation of drug release. Poster presented at 29th Annual Conference of the Academy of Pharmaceutical Sciences, 22-26 September 2008, Rustenburg (Abstract in Appendix B4).

e. Priya Bawa, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Valence M. K. Ndesendo. Formulation and evaluation of a novel buoyant prolonged release drug delivery system. Poster presented at 2008 American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition, 16-20 November 2008, Atlanta, United States of America (Abstract in Appendix B5).

f. Priya Bawa, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. An Active Pre-Programmed Gastro-Stimulus Drug Delivery System. Poster presented at Faculty Research Day and Postgrad Expo, University of Witwatersrand, 20 August 2008 (Abstract in Appendix B6).

g. Priya Bawa, Angeliki Stephanou, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Physicomechanical and physicochemical analysis of novel chemically modified lyophilized polymeric matrices. Poster presented at the 2009 5th ICPPS conference, 23-26 September 2009, Potchefstroom (Abstract in Appendix B7).

h. Priya Bawa, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Synthesis of chemically modified polymeric matrices for potential drug delivery applications. Poster presented at 2009 American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition, 8-12 November 2009, Los Angeles, United States of America (Abstract in Appendix B8).

i. Priya Bawa, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M. K. Ndesendo and Pradeep Kumar, A Novel Enzyme-Responsive Colon-Targeted Drug Delivery System for the Treatment of Local Colonic Pathologies, Poster presented at the 6th International Symposium on Stimuli-Responsive Materials, 26-27th October 2010, University of Southern Mississippi, United States of America (Abstract in Appendix B9).

j. Priya Bawa, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M. K. Ndesendo and Pradeep Kumar, A Pepsin-Responsive Polymeric Drug Delivery System for the Targeted Delivery of Loperamide HCl to the Small Intestine. Poster presented at the 6th International Symposium on Stimuli-Responsive Materials, 26-27th October 2010, University of Southern Mississippi, United States of America (Abstract in Appendix B10).

**3. Podium Presentations**

a. Priya Bawa, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Formulation And Evaluation Of A Novel Stimuli-Responsive Colon-Targeted Drug Delivery System For The Treatment Of Local Colonic Pathologies. Podium presentation at the School of Therapeutic Sciences Research Day, University of Witwatersrand, 12 August 2009.

b. Priya Bawa, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Formulation And Evaluation Of A Novel Stimuli-Responsive Colon-Targeted Drug Delivery System For The Treatment Of Local Colonic Pathologies. Podium presentation at the 2009 5th ICPPS conference, Young Scientist Competition, 23-26 September 2009, Potchefstroom.

**4. Additional Research Outputs**

a. Priya Bawa, Zaheeda Khan, Caragh Murphy, Deshika Reddy and Shivaan Cooppan. Biomaterials Association of South Africa (BioMatASA) Members newsletter editorial committee, Newsletter 1, Issue 2, 2007.

b. Priya Bawa, Zaheeda Khan, Caragh Murphy, Deshika Reddy and Shivaan Cooppan. Biomaterials Association of South Africa (BioMatASA) Members Newsletter editorial committee, Newsletter 2, Issue 1, 2008.

**5. Patent application**

a. Priya Bawa, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Pharmaceutical dosage form for the site-specific delivery of more than one active pharmaceutical ingredient. Publication no.: WO/2009/153633, International Application no.: PCT/IB2009/005830.

## SUMMARY

*Ulcerative colitis (UC), notorious for its unpredictable attacks of inflammation of the large intestine, is estimated to affect as many as 1.4 million people in the USA and 2.2 million people in Europe with 15000-30000 new cases being diagnosed annually worldwide. The chronic inflammatory process is limited to various regions of the colonic mucosa and is postulated to occur due to a dysregulated mucosal response in the intestinal wall, facilitated by defects in the protective barrier function of the intestinal epithelium and mucosal immune system. Due to the range and extent of disease manifestations the goals of UC therapy are broad and non-specific. The focus of therapy is thus primarily placed on the treatment of active disease by ameliorating the signs and symptoms characteristic of the disease state with concurrent adjunctive and anti-inflammatory therapy. Thus, ideally a delivery system should facilitate a reduction in the pill burden, daily dosing requirements and allow for concurrent adjunctive and anti-inflammatory therapy with a single delivery system administration. Therefore, essentially the purpose of this work was to develop a novel stimuli-responsive oral tablet system (SROT) that provided targeted drug delivery of 5-ASA to the colon and loperamide HCl to the small intestine with a single delivery system. For this purpose, the employment of polymers that are termed 'stimuli-responsive' or 'smart' were established to be the most attractive approach for 'activating' drug release at the desired site in response to the pre-determined reliable stimulus. Thus, advantage is taken of the over 400 distinct species of anaerobic bacteria and their corresponding enzymatic activities in the colon.*

*Investigations performed according to a Box-Behnken experimental design exposed an optimum enzyme-responsive colon-targeted tablet that effectively inhibited premature 5-ASA release in conditions simulating the upper gastrointestinal tract, whilst enabling an immediate initiation of drug release on exposure to colonic enzymes. The enzyme-responsiveness of the tablet was a direct result of the employment of only naturally-derived polysaccharides that were susceptible to colonic degradation. Furthermore, and more importantly, the prevention of premature drug release was achieved by the enzyme-responsive hydrophobic coating consisting of pectin and an aqueous ethycellulose dispersion that was applied to tablets until a ±10% total weight gain was achieved. In addition the in situ crosslinking between pectin and $BaCl_2$ in the tablet matrix as well as the crosslinked 5-ASA-loaded granules resulted in a zero-order drug release throughout the 18 hour period in the simulated colonic environment containing enzymes.*

*The development of the outer pepsin-responsive small intestinally-targeted coating was also conducted according to the Box-Behnken experimental design. Extensive investigations revealed an optimized pepsin-responsive coating after conducting the relevant studies on the 15 statistically-derived formulations. Essentially, the tablets coated with the 40%$^{w}/_{v}$ gelatine solutions resulted in the greatest increase in weight and shell thickness of the formulations however these were the least responsive to pepsin. The optimum pepsin-responsiveness was achieved from a gelatine coating of 14.379%$^{w}/_{v}$ which was crosslinked for 6 hours in a glutaraldehyde-lactose dry mixture. Furthermore, the novel crosslinking method ensured that no entrapped loperamide HCl was prematurely lost during the crosslinking process. In addition, the optimum formulation also achieved 100% drug release in the small intestine-at its site of therapeutic action.*

*In vivo investigations of the SROT in the large white pig model explicated the colon-targeting ability of the 5-ASA-loaded tablet as well as the benefits of the SROT compared to the conventional commercially available system, Asacol® (Aventis Pharma (Pty) Ltd.,Midrand, Johannesburg, South Africa). The success of the loperamide-loaded coating was evident from the minimal presence of loperamide HCl in plasma in the first 2 hours post-dosing compared to its commercially available counterpart Imodium® (Janssen Pharmaceutica (Pty) Ltd., Woodmead, Johannesburg, South Africa).*

*Investigations into an alternate colon-targeted drug delivery system revealed 3 novel composite polyacrylamide-polysaccharide hydrolyzed electrolytic matrices consisting of either pectin, chitosan or a combination of both, complexed with hydrolyzed polyacrylamide. Each matrix presented with varying surface area and porositometric properties which influenced their drug release behaviour. These formulations hold potential for numerous controlled drug delivery applications and are not exclusively limited to colon-targeting.*

## ACKNOWLEDGEMENTS



***On the absolute reality and its planes,***
***On that finest spiritual light,***
***We meditate, as remover of obstacles***
***That it may inspire and enlighten us.***

-Gayatri Mantra (Hindu prayer)-

In completion of this work, the author would like to convey her sincerest gratitude and appreciation to various individuals that have assisted and guided her towards this goal.

To my parents, this work is as a direct result of your never faltering love and faith in me. Thank you for supporting me without question or hesitation.

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## DEDICATION

*This work is dedicated to my parents, Bhagoobhai and Chandravati Bawa, the source of my being and the grounds behind my character.*

## ANIMAL ETHICS DECLARATION

I hereby confirm that the following study entitled “An *in vivo* assessment of novel biocompatible polymeric drug delivery systems in pigs” had received the approval from the Animal Ethics Committee of the University of the Witwatersrand with ethics clearance number 2007/56/04 (see Appendix D1).

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## CHAPTER 1

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# CHAPTER 1

# THE RATIONALE FOR THE DEVELOPMENT OF A NOVEL STIMULI-RESPONSIVE ORAL TABLET SYSTEM (SROT) FOR THE SIMULTANEOUS ANTI-INFLAMMATORY AND SYMPTOMATIC TREATMENT OF ULCERATIVE COLITIS

## 1.1 Introduction

According to the Centre for Disease Control (CDC) (2007) it is estimated that the overall healthcare cost associated with Inflammatory Bowel Disease (IBD) approximates to $1.7 billion USD and it is one of the five most prevalent gastrointestinal disease burdens in the United States of America. IBD is a generic classification for the group of non-specific, idiopathic inflammatory disorders of the gastrointestinal tract (GIT) (Young and Koda-Kimble, 1995; Abdel-Hady and Bunn, 2004). By convention IBD comprises of three main entities namely, Ulcerative Colitis (UC), Crohn's Disease (CD), and indeterminate colitis. The latter of which represents a patient group that cannot be classified as either UC or CD, but which constitutes about 10% of IBD sufferers (van Bodegraven and Wijmenga, 2009). Despite the similarities in the natural history and clinical features of CD and UC, there are significant differences in the pathophysiology, anatomic distribution and clinical course of these diseases.

Early medical literature on IBD provided scant scientific data and writers often applied free reign to their imaginations when explaining this condition. The first documented case of UC was in 1859 and was described by Sir Samuel Wilks. Similarly, various cases of CD had appeared in literature even before Crohn and co-workers published data regarding this condition. In the following two decades the distinction between UC and CD was further elaborated in macroscopic terms and in 1960, the nature of the inflammatory processes underlying these conditions was explicated (Moum and Ekbom, 2002). According to most recent literature, CD is identifiable as chronic inflammation typically affecting the terminal ileum and right colon, but almost always involves the entire GIT, whereas UC is exclusively localized to the colon (Van Assche *et al.*, 2009). Table 1.1 systematically differentiates UC and CD based on their pathophysiological characteristics.

Research has shown that in the past 50 years the incidence of CD has increased 8 to 10 fold, whereas the incidence of UC has remained stable (Bach, 2002). CD is found to be prevalent in urbanized, developed countries and its change in incidence is thought to be attributable to the improved hygiene and social standards of these typically western societies. However UC is more of a global disease (Karlinger *et al.*, 2000). van Bodegraven

and Wijmenga (2009) later suggested that the incidence of IBD thus varies according to geographic location, industrialization, and the availability of diagnostic means.

***Table 1.1:*** *Pathophysiologic differences between Ulcerative Colitis and Crohn's Disease (Young and Koda-Kimble, 1995; Mpofu and Ireland, 2006; Merck Manual, 2007; van Bodegraven and Wijmenga, 2009)*

| Characteristics | Ulcerative Colitis (UC) | Crohn's Disease (CD) |
|---|---|---|
| **World-wide incidence (per year)** | 6.4/100,000 | 5.5/100,000 |
| **Anatomical location** | Colon and rectum<br><br>Rectosigmoid is invariably involved; colonic involvement is usually left-sided. | Mouth to anus, small bowel involvement in 80% of cases.<br>Rectosigmoid is often spared; colonic involvement is usually right-sided. |
| **Distribution** | Continuous, diffuse, mucosal | Segmental, focal, transmural, rigid, thick, edematous, and fibrotic |
| **Bowel wall** | Shortened, loss of haustral markings, generally not thickened. Bowel wall is affected symmetrically and uninterruptedly from the rectum proximally. | Bowel wall is affected asymmetrically and segmentally, with "skip areas" between diseased segments. |
| **Gross rectal bleeding** | Common | Infrequent, except in 75 to 85% of cases of Crohn's colitis. |
| **Crypt abscesses** | Common | Infrequent |
| **Fissuring with sinus formation** | Absent | Common |
| **Noncaseating granulomas** | Absent | Common |
| **Strictures** | Absent | Common |
| **Abdominal mass** | Absent | Common |
| **Abdominal pain** | Infrequent | Common |
| **Toxic megacolon** | Occasional | Rare |
| **Bowel carcinoma** | Greatly increased | Slightly increased |
| **Perianal lesions** | Significant perianal lesions never occur. | Perianal lesions are significant in 25 to 35% of cases. |

## 1.2 Ulcerative Colitis – Delineating the Disease

UC, a relapsing and remitting disease characterized by chronic non-infectious inflammation of the colorectal mucosa boasts an annual incidence rate of approximately 10-20 cases to 100,000 of the population in the United Kingdom with over 200 people per 100,000 living with UC at any given time (Probert *et al.*, 1992; Ghosh *et al.*, 2000, Loftus *et al.*, 2000; Carter *et al.*, 2004). The clinical pattern of UC is based on the extent of colonic involvement i.e. if only the rectal mucosa is affected it is termed 'proctitis'; if it extends into the sigmoid colon it is termed 'proctosigmoiditis', and if the entire colon is involved it is termed 'colitis' or 'pancolitis' (Mpofu and Ireland, 2006).

The characteristic manifestations of UC exist as spells of bloody diarrhoea, increased urgency, abdominal pain, fever, fatigue and weight loss, however extra-intestinal

manifestations of UC may present as arthritis, ocular inflammation, liver and biliary disease, skin rashes and numerous other pathologies as indicated in Figure 1.1, all of which significantly reduce quality of life during active phase of the disease (Cohen, 2006).

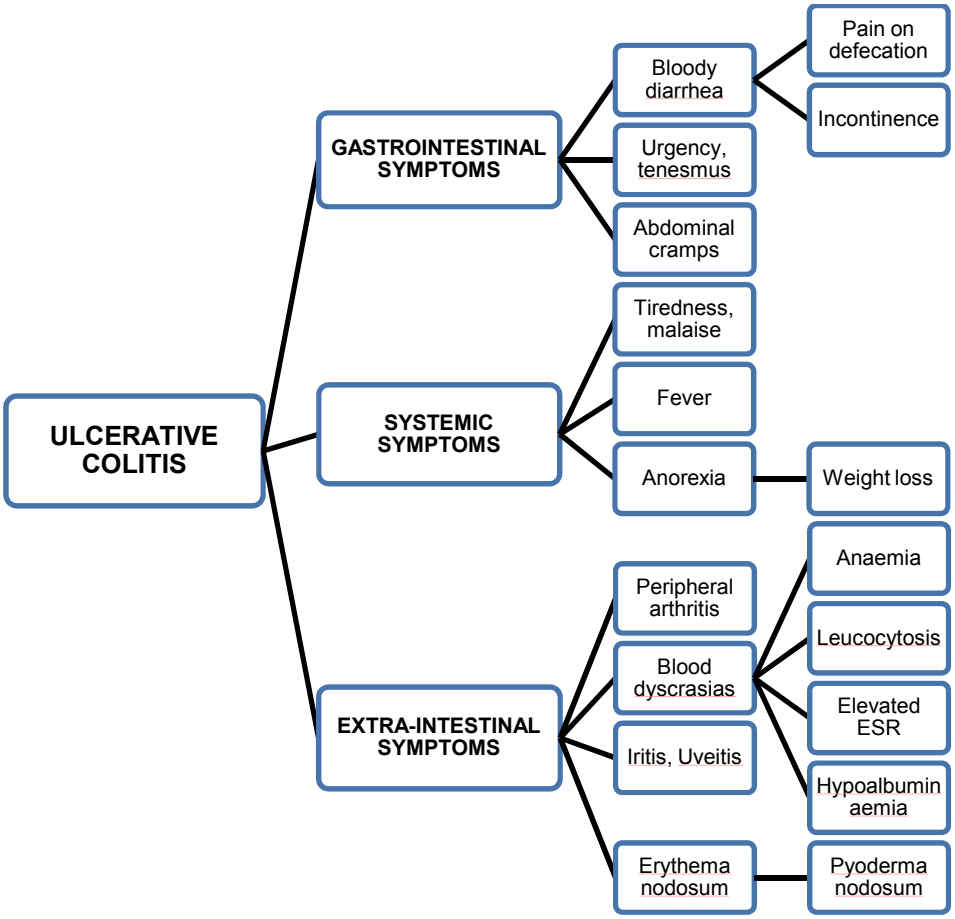



***Figure 1.1:*** *Typical symptomatic manifestations of Ulcerative Colitis.*

Generally, the severity of the symptoms parallels the intensity and extent of the inflammation (Merck Manual, 1997; van Bodegraven and Wijmenga, 2009). An attack may be acute and fulminant, characterized by sudden violent diarrhoea, high fever, signs of peritonitis, and profound toxemia. However more typically, an attack begins insidiously, and may be characterized by an increased urgency to defecate, mild lower abdominal cramps, and blood and mucus in the stools (Merck Manual, 1997).

### 1.2.1 Possible theories explicating the aetiology of UC

Since the emergence of UC in the 1800's the aetiology thereof was but an enigma and remains as such to the present day. Therefore, since no single agent or distinct mechanism can be responsible for all aspects of the disease current hypotheses suggest the involvement of a multitude of factors each with a relatively weak effect acting together to influence the appearance and pathogenesis of UC. Most theories encompass a combination of

environmental influences, genetic predisposition, altered cellular immunity, and inappropriate and excessive responses to dietary triggers (Oliva-Hemker and Fiocchi, 2002).

#### 1.2.1.1 Environmental influences

Seemingly unrelated environmental factors such as smoking, oral contraception, diet, blood transfusions, pollution, hygiene, occupation, breastfeeding, drugs, geographical and social status, stress, microbial agents, intestinal permeability and appendectomy rates have exhibited an association with UC (Koutrobakis *et al.*, 1996; Corrao *et al.*, 1998; Danese *et al.*, 2004; Loftus, 2004; Garcia Rodriguez *et al.*, 2004). However the influence of smoking holds significant prominence since numerous studies have established an intriguing correlation between smoking and increased protection against UC (Harries *et al.*, 1982; Somerville *et al.*, 1984; Reif *et al.*, 1995; Russel and Stockbrugger, 1996; Rubin and Hanauer, 2000; Cosnes, 2004; Danese *et al.*, 2004). Therefore despite the uncertainty as to the components of tobacco responsible for the positive influence, conventional UC therapy has been supplemented with transdermal nicotine patches where an improvement in clinical symptoms in patients with mild to moderate UC had been observed (Pullan *et al.*, 1994). Furthermore, this modulatory effect of nicotine on the immune responses has since also been observed *in vitro* (Madretsma *et al.*, 1996).

Since the colon is the site of nutrient absorption and UC exclusively involves the colon the association between diet and UC is a logical one (Fiocchi, 1998). Nevertheless, research has established that implementation of nutritional therapies in UC provided no beneficial influences whereas an elemental diet substantially improved the condition in CD patients (Teahon *et al.*, 1991). The only evidence of the role of diet in the appearance of UC is apparent in breastfed infants where studies have shown that natural feeding provides protection to the infant GIT and that any damage to the bowels of an infant may lead to the development of UC in adolescence (Whorwell *et al.*, 1979; Koletzko *et al.*, 1989).

#### 1.2.1.2 Genetic influences

The genetic influence of IBD appears to be more pronounced in CD than UC. This conclusion arises from results of twin studies and studies of familial aggregation conducted over several decades (Tysk *et al.*, 1988; Satsangi *et al.*, 1994; Peeters *et al.*, 1996). Most recently, the concordance rates for UC among monozygotic and dizygotic twins were found to be 18.2% among the monozygotic pairs and 4.5% among the dizygotic pairs (Orholm *et al.*, 2000; Halfvarson *et al.*, 2003). In spite of this, UC is not a Mendelian trait and is thus most likely due to a combination of environmental and genetic influences. Thus, the predisposition to UC is inherited, but when exposed to the cause, the development of the disease is promoted (Karlinger *et al.*, 2000).

#### 1.2.1.3 Immune reactivity to microbial antigens

A direct cause-and-effect relationship between a single micro-organism and inflammation still remains one of the most popular triggers for IBD. Despite the identification of numerous bacterial pathogens as suspects for CD, an infectious origin of UC is less definitive. Essentially diplostreptococcus has been suspected of being the causative agent for UC however the presence of *E. coli* in patients with UC in relapse, but not in normal controls suggests the culpability of this organism as well (Burke and Axon, 1987). The traditional approach to the identification of potential micro-organisms linked to IBD has been through the identification of antibodies against known micro-organisms, however since all IBD patients present with elevated titres against bacteria, viruses, and fungi the mere presence of the antibodies are of limited value (Fiocchi, 1998; Targan, 1998). Investigators have also postulated the possibility that components of the normal intestinal flora could act as a trigger to initiate or somehow contribute to UC (Fabia *et al.*, 1993; Bamias *et al.*, 2005; Thompson-Chagoyan *et al.*, 2005).

In the presence of an impaired mucosal barrier or injury to the mucosa, commensal flora are thought to function as modulators of intestinal inflammation (Chadwick and Anderson, 1990; Duchmann *et al.*, 1995; Duchmann *et al.*, 1996). During inflammation there is a counteracting mechanism with which the intestine defends its integrity. The efficacy of this defence mechanism is not only dependent on the strength of the pathogen, but also on the resistance capability of the bowel wall. This is in turn dependent on the composition of the mucus produced from mucosal cells as well as on the turnover capability of the mucosal epithelial. During a typical anti-inflammatory defence, inflammation is overcome with no residual alteration. However if the individual is predisposed to UC this type of down-regulation does not occur and the inflammation is sustained or even increases due to secondary immunological processes, resulting in chronic inflammation and possible tissue damage (Karlinger *et al.*, 2000). The chronicity of inflammation is then not only a result of the continuous antigenic response but also due to the resultant defective epithelial barrier function (Ramage *et al.*, 1988).

### 1.2.2 Pathogenesis and pathophysiologic mechanisms of UC

The characteristic mucosal inflammation of UC is said to be due to a culmination of a series of events and processes which are initiated by an antigen. Despite the postulation that microbial pathogens are the source of these antigens it is now generally accepted that the constant antigenic stimulation is actually provided by the commensal enteric bacteria which results in continuously activated pathogenic T-cells and chronic inflammation (Sartor, 2006; Strober *et al.*, 2007; Xavier and Podolsky, 2007). IBD pathogenesis can thus be explicated

by four broad possible mechanisms that drive the pathogenic immunologic responses to luminal microbial antigens. The four mechanisms include:

1. Induction of intestinal inflammation by microbial pathogens,
2. Dysbiosis of commensal bacteria,
3. Host genetic defects in containing commensal bacteria, and
4. Defective host immunoregulation.

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) was initially thought to be a credible aetiologic agent of CD however a 2-year prospective trial of clarithromycin, rifabutin and ethambutol proved otherwise (Selby *et al.*, 2007). Similarly, the identification of a microbial agent that contributes to UC pathogenesis has also remained elusive. The commensal bacterial population in healthy individuals remains relatively stable, however when even subtle changes in the bacterial composition and function occurs the mucosal barrier function and immune response are profoundly affected. Basically, a larger amount of bacteria was found to be attached to the epithelia of IBD patients compared to healthy individuals however the role of the dysbiosis in IBD remains uncertain (Swidsinski *et al.*, 2002; Teitelbaum and Walker, 2002).

Since the gastrointestinal environment is home to a range of food-derived antigens and externally-derived micro-organisms a mutual interdependence needs to exist between the gut microbiota and host immune system in order to avoid unnecessary inflammation and tissue damage (Thompson-Chagoyan *et al.*, 2005). Generally, a state of 'oral tolerance' or immune non-responsiveness is experienced when a host is exposed to dietary antigens (Hanauer, 2006). This mechanism prevents the development of an immune reaction to intestinal intraluminal antigens (Spiekermann and Walker, 2001). Thus a more plausible pathogenic mechanism for UC may be described by the presence of defects in the homeostatic functions of the host. These homeostatic functions rely on the efficient exclusion of commensal bacteria by the secretion of luminal antimicrobial peptides and complexing IgA/IgM, a relatively impermeable mucosal barrier, extrusion of xenotoxins, rapid repair of epithelial defects, down-regulation of immune responses and secondary phagocytosis of bacteria that translocate across the epithelial barrier (Sartor, 2006; Clavel and Haller, 2007). However defects in these functions result in an enhanced microbial antigenic exposure, induced and perpetuated by the commensal bacteria, which inevitably overwhelms the host's immune tolerance, leading to pathogenic T-cell responses and chronic inflammation (Shanahan, 2004; Tannock, 2005).

#### 1.2.2.1 The typical epithelial immune regulatory mechanism (mucosal homeostasis)

The role of the intestinal epithelium in the initiation of the mucosal immune response can be explained by two broad mechanisms which may involve: (1) the release of cytokines, chemokines and other proinflammatory substances from the epithelium; and (2) the epithelium itself may serve as antigen presenting cells (APC's) (Mayer *et al.*, 1991; Kagnoff and Eckmann, 1997). This latter antigen-driven response may be appropriately directed against an unrecognized pathogen, or may be an inappropriate response to a typically inoffensive antigen (Podolsky, 1991).

Typically, commensal bacteria activate a sequence of homeostatic responses by epithelial cells, macrophages, dendritic cells, T lymphocytes, and B-cells which allows the host to co-exist with the potentially toxic microbial products (Clavel and Haller, 2007; Strober *et al.*, 2007). The basis of this co-existence however relies on the down-regulation of bacterial receptors and the induction of intracellular molecules that terminate the innate and adaptive immune responses, and furthermore the stimulation of protective molecules that mediate mucosal barrier function (Sartor, 2008). In a normal host the mucosal immune system has the ability to mount an immune response to pathogens while remaining tolerant to commensal antigens (Neuman, 2007).

Cell-mediated immune responses typically occur when an antigen is taken up and processed by an APC as illustrated in Figure 1.2. This antigen-APC complex in conjunction with T-cells is then presented in the context of the appropriate major histocompatibility complex (MHC). An antigenic epitope is formed by the binding of the MHC class II antigen on the APC and T-cell receptor-CD4 complex which causes T-cell activation. However a co-stimulatory signal/ligand which is not antigen specific is essential for complete T-cell activation (Lenschow *et al.*, 1993). Activated T-cells then express a second ligand which inhibits interleukin-2 (IL-2) expression and further T-cell proliferation (Boussiotis *et al.*, 1993; Janeway and Bottomly, 1994). Two primary subsets of CD4+ T-cells and their distinct patterns of T-cell cytokine production allow the distinction between the dominance of cell-mediated and humoral immunity. Cells producing large amounts of IL-2 and interferon (IFN)-ɣ are categorized as T helper 1 (Th1) cells, and IL-4, IL-5 and IL-10 are cytokines characteristically produced by Th2 cells (De Carli *et al.*, 1994). IFN-ɣ produced from the Th1 cells lead to the activation of macrophages which subsequently produce IL-12 and IL-18, thus favouring the further differentiation of Th1 cells (Wenner *et al.*, 1996; Dinarello, 1999; Pizarro *et al.*, 1999). IFN-ɣ which is also secreted by the differentiated Th1 cells inhibits the differentiation of Th2 cells and conversely, production of IL-10 by Th2 cells diminishes Th1 responses (Fiorentino *et al.*, 1989; Creery *et al.*, 1996). These activated macrophages are

also responsible for the production of pro-inflammatory cytokines such as IL-1, tumour necrosis factor (TNF), and the chemokine IL-8.

The broad spectrum of pro-inflammatory effects of TNF holds particular significance in IBD. Production of TNF leads to the activation of other macrophages in an autocrine fashion, and when bound to cell surfaces TNF may provide a co-stimulatory signal which further augments T-cell responses (Shreiber *et al.*, 1992). TNF further contributes towards inflammation by (a) permitting the influx of newly recruited inflammatory cells into the mucosa, (b) promoting the release of nitric oxide, platelet-activating factor, and prostacyclins, (c) activating granulocytes and their functions and, (d) enhancing the production of metalloproteinase that participates in local tissue breakdown (Beutler, 1995; MacDonald and Pender, 1998). The influx of newly recruited inflammatory cells via integrins, selectins and members of the immunoglobulin family amplifies the inflammatory response by permitting the migration of leukocytes along a gradient of chemokines and chemo-attractants into the submucosa and mucosa (Springer, 1994).

Once present in the mucosa and submucosa these cells release numerous non-specific inflammatory substances namely, the products of arachidonic acid metabolism which include thromboxanes, leukotrienes, prostaglandins, and free radicals such as reactive oxygen metabolites and nitric oxide. The local release of neuropeptides further modulates the inflammatory response. Finally, once the harmful foreign antigen has been eradicated, the T lymphocytes attenuate the immune response by means of CD4+CD5+ cells (Iellem *et al.*, 2003). More importantly, apoptosis of the aggressively overproliferative T-cells, triggered by a variety of inter- and intracellular signals provide a built-in mechanism for immunosuppression (Jacobson-Brown and Neuman, 2004). The release of the anti-inflammatory or immunosuppressive cytokines such as IL-1 receptor agonist, soluble TNF-α receptor, IL-4, IL-5, IL-10 (released from Tr cells) and transforming growth factor (TGF)-β (released from Th3 cells), also limits the expansion of specific lymphocytes and returns activated macrophages and other inflammatory cells to their normal resting state (Mosmann and Sad, 1996).

#### 1.2.2.2 Immune regulatory mechanism in UC

The loss of tolerance against the autologous commensal bacteria of the colon is believed to be the primary cause of UC (Kanauchi *et al.*, 2003). Toll-like receptors (TLR), responsible for this microbial recognition also induces antimicrobial genes and controls the adaptive immune responses. TLR's are imperative for the discrimination of pathogenic bacteria from commensal bacteria (Cario, 2005). TLR4 expression in particular has been found to be up-

regulated in UC patients, resulting in increased sensitivity/loss of tolerance to the enteric commensal bacteria (Cario and Podolsky, 2000).

This loss of tolerance is evident from the altered patterns of cytokine release compared to healthy individuals. Typically, T-cells are classified as either CD4+ or CD8+ T-cells. CD8+ cells are known as the suppressor cells due to their down-regulatory influence on the immune system and the CD4+ cells are the helper cells (Th1, Th2, Th3, and Tr) which are distinguishable by their pattern of cytokine production. IBD patients experience an increased release of IL-1, IL-2, IFN-ɣ, and TNF-α, all of which mediate the cellular immune response and polarize immune activity towards a Th1 response (Reinecker *et al.*, 1993; Reimund *et al.*, 1996). However UC has been shown to exhibit additional involvement of a defective Th2 response characterized by secretion of IL-4, IL-5 and IL-10 (Camoglio *et al.*, 1998; Sawa *et al.*, 2003). However more importantly there is a substantial decrease in secretion of the immunosuppressive/anti-inflammatory cytokines TGF-β and IL-10 (Thompson-Chagoyan, 2005).

The inhibition of T-cell apoptosis has also been implicated in the development of UC since the failure to regulate T-cells has a direct influence on the failure to regulate pro-inflammatory cytokines resulting in an inappropriate, sustained and detrimental immunologic reaction, ultimately leading to the exaggerated death of enterocytes and pathological inflammation of the colon (Ciccocioppo *et al.*, 2002). Figure 1.2 systematically details the differences in the immunologic processes involved in a typical homeostatic response to commensal enteric bacteria compared to a patient with UC.

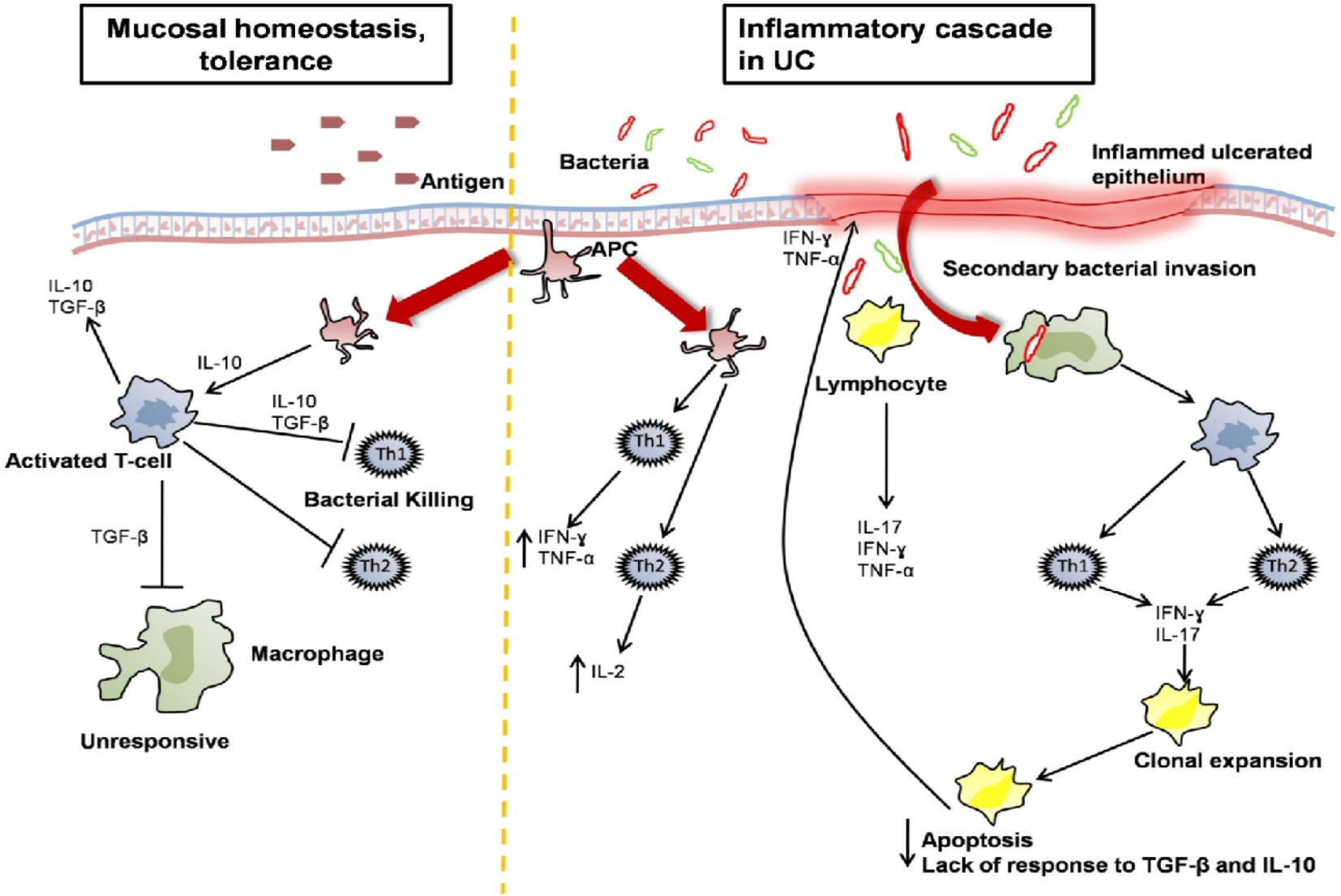



***Figure 1.2:*** *Immunology of mucosal homeostasis and tolerance to commensal bacteria in an undiseased individual compared to the inflammatory cascade experienced by Ulcerative Colitis patients (adapted from Sands, 2000; Sartor, 2008).*

### 1.3 Goals of therapy and common treatment options

Due to the range and extent of disease manifestations the goals of UC therapy are broad and non-specific. The focus of therapy is thus primarily placed on the treatment of active disease by ameliorating the signs and symptoms characteristic of the disease state. However the achievement of this aim is intertwined with the induction and maintenance of states of remission, thus permitting mucosal healing and restoration of well-being and an active lifestyle. Drug therapy can thus be conceptualized as either anti-inflammatory or adjunctive.

#### 1.3.1 Adjunctive therapy

Since various symptomatic manifestations of UC represent persistent and sometimes incapacitating conditions, adjunctive therapy to anti-inflammatory therapy is essential. These conditions/symptoms occur secondary to bowel irritability and hence do not form part of the foundation of UC treatment. Typically, among the acutely ill hospitalized patients attention to adequate fluid volume and electrolyte replacement is imperative. Furthermore, UC patients may occasionally require analgesics, blood transfusions, intravenous nutritional support, chronic iron supplementation, and vitamin B12 therapy (van Rosendaal, 1989; Hartman *et al.*, 2009). However the most predominant and consistent of the experienced symptoms-diarrhoea-almost always requires chronic treatment.

##### 1.3.1.1 Anti-diarrhoeal treatment

It is generally recommended that those with diarrhoea reduce their intake of laxative-acting foods and/or employ anti-diarrhoeal drugs such as loperamide and diphenoxylate (Barrett and Dharmsathaphom, 1988). However, the use of antidiarrheals is recommended with caution (Hanauer, 1993). Despite the apprehension associated with chronic anti-diarrheal use, amongst other resources, the South African Medical Formulary states an approved dosage of 4-8mg of loperamide HCl for the treatment of chronic diarrhea (Galambos *et al.*, 1976; SAMF, 2005).

In 1976 loperamide HCl was introduced and approved as a prescription anti-diarrhoeal agent by the United States Food and Drug Administration (FDA) for the control and symptomatic relief of acute non-specific diarrhoea and chronic diarrhoea commonly associated with IBD (Verhaegen *et al.*, 1974; Nelemans and Zelvelder, 1976; Pelemans and Vantrappen, 1976; Heel *et al.*, 1978; Sandhu *et al.*, 1981; Cann *et al.*, 1984; Johnson *et al.*, 1986; Hovdenak, 1987; Lavö *et al.*, 1987; van Loon *et al.*, 1989; Efskind *et al.*, 1996; Yu *et al.*, 2004). Initially it was listed as a schedule 5 substance however in 1982 it was de-scheduled based on its low potential for abuse and dependence (Federal Register, 1982). Finally in 1988 it was further

deregulated and is currently available without a prescription both in the United States and South Africa (Fletcher *et al.*, 1995).

Loperamide HCl is a synthetic piperidine derivative with structural similarities to opiates such as pethidine, morphine and isopropamide (Heel *et al.*, 1978; Awouters *et al.*, 1983). However the unique benefit of loperamide HCl is attributed to its high affinity for opiate receptors and its pure opiate agonist activity specific to the GIT (Heykants *et al.*, 1974; Mackerer *et al.*, 1976; Wuster and Hers, 1978; Niemegeers *et al.*, 1979; Van Nueten *et al.*, 1979; Yoshida *et al.*, 1979). Despite the numerous mechanisms of action of loperamide, the principle anti-diarrhoeal action is due to the inhibition of intestinal motility which results from the enhancement of the circular segmental intestinal muscle contractions which retards the forward peristaltic motions and consequently increases intestinal transit time (Van Neuten *et al.*, 1974; Mackerer *et al.*, 1976; Schiller *et al.*, 1984; Basilisco *et al.*, 1985; Kachel *et al.*, 1986; Basilisco *et al.*, 1987; Kirby *et al.*, 1989). Apart from reducing intestinal motility loperamide also reduces the daily fecal output volume, inhibits intestinal secretion of fluid and electrolytes, and increases anal sphincter tone (Van Neuten *et al.*, 1974).

### 1.3.2 Anti-inflammatory therapy

#### 1.3.2.1 Aminosalicylates

First-line therapy for patients with UC has focussed on sulfasalazine for decades however the identification of mesalamine, or otherwise known as 5-aminosalicylic acid (5-ASA) as the active moeity responsible for its anti-inflammatory effects, along with patient allergy and intolerance to the sulfa-moeity of sulfasalazine, has led to the development of numerous 5-ASA based conjugates (Azad Khan *et al.*, 1977; Qureshi *et al.*, 2005). Not unlike the aetiology of UC, the precise mechanism of action of 5-ASA remains unknown, but is likely due to a combination of anti-inflammatory effects. Most commonly, the aminosalicylates are known to inhibit the cyclooxygenase pathway thereby inhibiting the production of prostaglandin E2 in inflamed intestinal mucosa. Furthermore, inhibition of the 5-lipoxygenase pathway inhibits arachidonic acid metabolism and blocks the production and chemotactic activity of leukotrienes (Collier *et al.*, 1976; Sharon *et al.*, 1978; Stenson and Lobos, 1982; Stenson, 1990; Grisham, 1994). 5-ASA is also capable of altering the immune response at a more fundamental level by reducing antibody secretion and lymphocyte function by blocking the production of IL-1 and TNF-α (Mahida *et al.*, 1991; Cominelli *et al.*, 1992; Rachmilewitz *et al.*, 1992).

Despite the indistinct mechanism of action of 5-ASA it is well established that the optimal therapeutic effect of 5-ASA is predominantly dependent on its topical action at the site of inflammation within the colon. The clinical aim is thus to achieve minimal systemic absorption

whilst maximizing 5-ASA delivery to the affected regions of the colonic mucosa (Cohen, 2006). The pioneering agent, sulfasalazine, was shown to exhibit its therapeutic effect in UC by its ability to withstand gastric breakdown and systemic absorption until entry to the colon. Sulfasalazine consisted of a sulfapyridine molecule bonded to 5-ASA where on entry to the colon, bacterial azoreductase enzymes initiated the cleavage of the bond between the two permitting the anti-inflammatory action of 5-ASA exclusively on colonic mucosa. However the identification of the sulfa group as the responsible candidate for the dose-related adverse-effects experienced has led to the development of numerous sulfa-free 5-ASA agents with an improved safety and tolerance (Nielsen, 1982; Myers *et al.*, 1987; Sandborn and Hanauer, 2003).

These sulfa-free mesalamine conjugates occur either in the form of inactive prodrugs such as balsalazide and olsalazine, or as special oral controlled release formulations, or further as rectal formulations (Cohen *et al.*, 2000; Klotz, 2000; Marshall and Irvine, 2000; Qasim *et al.*, 2001; Gisbert *et al.*, 2002; Rizzello *et al.*, 2003). The efficacy of these marketed 5-ASA products in UC have been found to be comparable with sulfasalazine (Riley *et al.*, 1988a, b; Willoughby *et al.*, 1988; Rachmilewitz, 1989; Andreoli *et al.*, 1994) however their release characteristics rely on specific predetermined mechanisms that ultimately influence the capability of these products to efficiently target the colon (Table 1.2).

***Table 1.2:*** *Commercially available 5-aminosalicylic acid based products*

| Proprietary name | Generic name | Site of delivery | Mechanism of release | Unit strength | Dosage form | Daily dose | | Limitations | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Induction | Maintenance | | |
| **Asacol®** (Procter & Gamble Pharmaceuticals) **Mesren®** (Teva UK Ltd) **Ipocol®** (Sandoz Ltd) | Mesalamine | Terminal ileum, colon | 5-ASA coated with a gastro-resistant resin film of Eudragit® S-100, a polymethacrylate derivative that exclusively dissolves at pH values exceeding 7. The coating is thus theoretically not compromised in the stomach or small intestine. | 400mg | Tablets | 1.6-4.8g | 0.8-4.8g | Literature suggests that the pH of the colon in healthy individuals is between 5-6.5 and only reaches 7 in the distal colon. In addition, the colonic pH in UC patients is considerably lower than in healthy individuals.<br>Large variations in the position and time of tablet disintegration with the occasional appearance of intact tablets in stools due to the target pH of coating dissolution not being met.<br>Considerable inter- and intra- batch variability in dissolution characteristics.<br>The variations in the gastrointestinal characteristics of UC patients result in inconsistent release of 5-ASA resulting in high variations in the $C_{max}$, $T_{max}$ and AUC of 5-ASA in healthy individuals compared to diseased individuals. | Schroeder *et al.*, 1987; Evans *et al.*, 1988; Norlander *et al.*, 1990; Raimundo *et al.*, 1992a, b; Fallingborg *et al.*, 1993; Klotz and Stracciari, 1993; Sciarretta *et al.*, 1993; Wilding, 1999; Nugent *et al.*, 2001; Mesalazine, 2003; Sandborn and Hanauer, 2003; Sinha *et al.*, 2003; Cohen, 2006. |
| **Claversal®** (GlaxoSmithKline) **Salofalk®** (Dr Falk GmbH & Co) **Mesasal®** (SmithKline Beecham Pharma) | Mesalamine | Distal ileum, colon | 5-ASA coated with Eudragit® L-100 which exclusively dissolves at a pH≥6, typically when the pH is consistently at 6.4. | 250mg, 500mg | Tablets | 1.5-4.0g | 0.75-4.0g | | |
| **Pentasa®** (Shire US, Inc.) | Mesalamine | Stomach, duodenum, ileum, jejunum, colon | Granules of 5-ASA coated with ethylcellulose. pH-independent release occurs through a semi-permeable membrane. | 250mg, 500mg, 1000mg | Tablets, capsules, sachets | 2-4g | 1.5-4g | Release occurs throughout the GIT resulting in systemic absorption.<br>Ineffective targeting of the colon, requiring higher doses to ensure therapeutic efficacy.<br>High doses are associated with adverse effects. | Rasmussen *et al.*, 1983; Goebell *et al.*, 1993; Layer *et al.*, 1995; Keller *et al.*, 1998; Carter *et al.*, 2004; Friend, 2005. |
| **Lialda® (US) Mezavant® (EU)** (Shire Pharmaceuticals, Inc.) | Mesalamine | Colon | Uses MMX™ Multi Matrix System Technology where lipophilic and hydrophilic matrices enclosed within a gastro-resistant, pH-dependent coating facilitate the prolonged exposure of the colonic mucosa to 5-ASA. | 1.2g | Tablets | 1.2-2.4g | 1.2-2.4g | Despite the efficiency of being a once daily dosage form, this product still relies on a pH-dependent coating. This causes the same limitations as that typically found in Asacol®, and Claversal®.<br>However the MMX™ technology allows a more reproducible and consistent release pattern. | Prantera *et al.*, 2005; Sandborn *et al.*, 2007. |
| **Dipentum®** (Celltech Pharmaceuticals, Inc.) | Olsalazine | Colon | A dimer of 5-ASA molecules linked by an azo bond which undergoes azoreduction exclusively in the colon resulting in the release of two 5-ASA molecules. | 250mg | Capsule | 2-3g | 1g | Despite its efficiency in colonic targeting there is a relatively slow release of the active agent from the carrier in the colon.<br>Furthermore, since 5-ASA has a high dose requirement coupling this drug to a polymer substantially increases the mass required to be administered.<br>Increases net luminal water secretion, increases intestinal transit time, and induces diarrhea. | Ryde and Ahnfelt, 1988; Wadworth and Fitton, 1991; Klotz, 2000; Sandborn and Hanauer, 2003; Klotz, 2005; Cohen, 2006. |
| **Salazopyrin®** **Azulfidine®** (Pfizer US Pharmaceuticals) | Sulfasalazine | Colon | 5-ASA linked to a sulfapyridine molecule by an azo bond. Release of 5-ASA occurs as a result of azoreduction. | 500mg (200mg 5-ASA) | Tablets | 2-4g *0.8-1.6g 5-ASA* | 2-4g *0.8-1.6g 5-ASA* | Its fundamental limitation is the release of sulfapyridine along with 5-ASA. The sulfa group is responsible for patient allergy, intolerance and non-compliance. | Azad Khan *et al.*, 1977; Nielsen, 1982; Myers *et al.*, 1987. |
| **Colazal®** (Salix Pharmaceuticals, Inc.) | Balsalazide | Colon | 5-ASA linked to 4-aminobenzoylb-alanine (4ABA) by azo-bond | 750mg (262mg 5-ASA) | Capsule | 2–6.75 g *0.7–2.4g 5-ASA* | 2–6.75 g *0.7–2.4g 5-ASA* | Since the recommended daily dose is significantly high, a large number of capsules are required per dose, ultimately resulting in patient non-compliance due to a high pill burden. | Prakash and Spencer, 1998; Muijsers and Goa, 2002; Klotz, 2005. |

#### 1.3.2.2 Corticosteroids

Despite corticosteroids being amongst the most beneficial and longest used agents in the treatment of IBD and UC, at their therapeutic doses their biologic effects are deleterious and numerous (Truelove and Witts, 1955; Kane *et al.*, 2002). The use of conventional corticosteroids such as prednisone is thus generally reserved for patients with moderate to severe cases of the disease or for patients that have failed to respond to the first-line therapy of 5-ASA (Lichtenstein *et al.*, 2006). Notwithstanding their benefit to risk ratio, these agents are almost exclusively effective in inducing remission and have limited efficacy in the maintenance of remission. Their effectiveness is derived from their profound anti-inflammatory and immunologic effects specifically acting by reducing the production of pro-inflammatory cytokines, inhibiting a variety of leukocytic functions, as well as interfering with the metabolism of arachidonic acid and production of eicosanoids (Goppelt-Struebe, 1997; Goulding *et al.*, 1998; Angeli *et al.*, 1999).

Budesonide, marketed as Entocort® by AstraZeneca Pharmaceuticals (Wilmington, NC, USA), is a highly potent corticosteroid that has been formulated in a capsule containing pellets of budesonide in an ethylcellulose matrix which is then surrounded by an outer acrylic-based resin coating that dissolves at a pH≥5.5 (Nilsson *et al.*, 1995; Edsbacker *et al.*, 2003). Budesonide is thus released slowly as it passes through the small intestine with 69% of the drug being delivered to the ileo-colonic region. Despite its limited bioavailability and thus improved toxicity profile it has yet failed to show benefits in the maintenance of remission in UC patients which may also be attributed to its ineffective ability to target the colon (Truelove and Witts, 1959; Lennard-Jones *et al.*, 1965; Powell-Tuck *et al.*, 1981).

#### 1.3.2.3 Immunomodulators

Due to the recent understanding that the pathophysiology of UC finds derivation from an upregulated mucosal immune system, immunomodulators such as azathioprine (AZA) and 6-mercaptopurine (6-MP) are showing increasing promise especially in cases of steroid-resistance/dependence (Sands, 2000; Hibi *et al.*, 2003). AZA, the prodrug that yields 6-MP, has been speculated to possess additional immunosuppressive properties however these two agents are still used interchangeably (Crawford *et al.*, 1996). Despite their efficacy, the long time to response of these agents (approximately 4 months) limits their use in the induction of remission and furthermore provides a breach in long-term therapy with these agents (Present *et al.*, 1980; Pearson *et al.*, 1995).

Cyclosporine, an important immunosuppressant finds application in severe cases of steroid-refractory UC with responsiveness shown in 82% of patients hospitalized with severe flares resistant to intravenous corticosteroids (Lichtiger *et al.*, 1994). Furthermore, due to its rapid

rate of response it is postulated to provide a bridge to long-term immunomodulatory therapy with AZA (Fernandez-Banares *et al.*, 1996). Tacrolimus, a similar immunosuppressant to cyclosporine has recently also been shown to be successful in cases of refractory UC when used in combination with 6-MP or AZA (Fellermann *et al.*, 1998; Gerber *et al.*, 1998).

Arising from the observation that individuals who quit smoking showed an increased predisposition to UC and UC symptoms, several studies are now focussing on the development of locally targeted nicotine delivery systems that minimize the systemic effects of nicotine whilst maintaining its inhibitory action on the production of TNF and IL-2 and sustaining mucus production (Madretsma *et al.*, 1996; Pullan, 1996; Compton *et al.*, 1997; Green *et al.*, 1997; Sandborn *et al.*, 1997).

#### 1.3.2.4 Biological Response Modifiers

Historically the treatment goals of UC were only to induce and maintain remission of symptoms and mucosal inflammation however these goals have since evolved to include steroid-sparing, endoscopic healing and reduction in hospitalizations and surgery (Sandborn, 2008). As a consequence treatment options are thus dependent on the severity of the disease, patient responsiveness, and the safety-efficacy profile of the treatment employed. According to the UC practice guidelines from the American College of Gastroenterology, the use of biological response modifiers are reserved for patients who have failed therapy with the aminosalicylates, corticosteroids, and immunomodulators (Kornbluth and Sachar, 2004).

Infliximab, the first biological response modifier to gain FDA approval for use in IBD, has since shown a short-term response rate of 60% and remission rate of 30% in UC refractory patients. Furthermore, these patients showed remission rates of 25-30% at 6-12 months (Sands, 2000; Rutgeerts *et al.*, 2005). Numerous other biological agents are however currently being investigated as depicted in Figure 1.3.

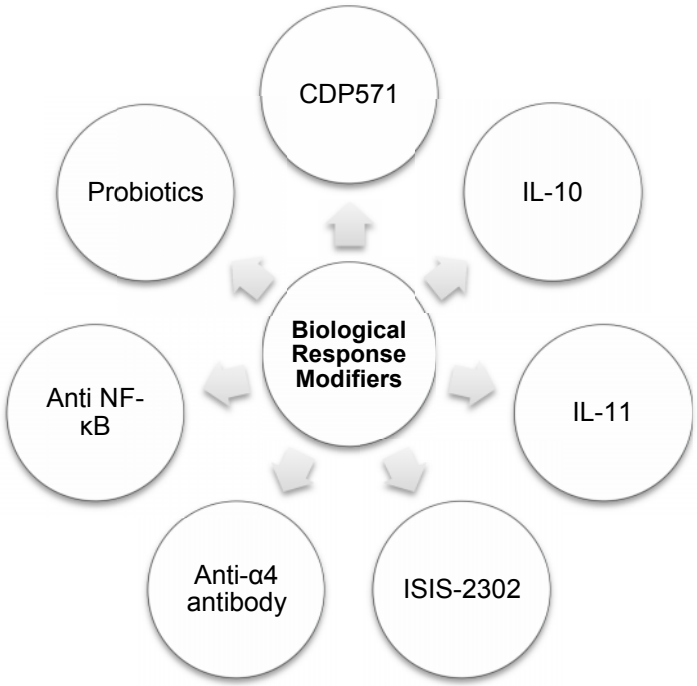



***Figure 1.3:*** *Emerging biological response modifiers currently being investigated.*

**1.4 Statement of the problem**

Despite the existence of a substantial armamentarium of anti-inflammatory and immunoregulatory agents available for use in the induction and maintenance of remission it has been estimated that in North America alone there is approximately one million people affected with IBD (Bamias *et al.*, 2005) and in Europe it is estimated to be 2.2 million (Loftus, 2004). Each of the numerous marketed 5-ASA delivery systems as depicted in Table 1.2, used particularly for the treatment of UC, possess numerous limitations that considerably impacts on patient compliance and ultimately on periods of quiescence and therapeutic success.

The predominant factor for the reduction in patient compliance with 5-ASA therapy is the inconvenience of frequent dosing schedules, as well as the pill burden associated with each dose (Kane *et al.*, 2003; Shale and Riley, 2003). However further reasons for the loss of compliance relies on a complexity of factors such as gender, employment status, marital status and periods of symptomatic remission (Kane *et al.*, 2001). Despite the effectiveness of rectal 5-ASA preparations their method of administration is associated with numerous tribulations not limited to leakage, retention, burning sensations and bloating (Frieri *et al.*, 1999; Gionchetti *et al.*, 1999; Cohen *et al.*, 2000; Lim and Hanauer, 2004). Furthermore, since UC patients typically suffer from concurrent diarrhoea the retention of these formulations for the time required for onset of its therapeutic effect can be challenging.

The oral route thus remains the most common and convenient method of drug administration with between 60-80% of marketed drugs being used orally (Lennernas and Abrahamsson, 2005; Masaoka *et al.*, 2006). The 5-ASA commercially available orally administered delivery systems possess exceptional and comparable safety profiles. In addition, no significant difference exists in terms of their systemic exposure however there is an almost 1000-fold variation in their mucosal 5-ASA concentrations (Sandborn and Hanauer, 2003). This was observed during an assessment of multiple biopsies taken at the same locations through the patients' lower GIT after cessation of a single week of therapy with each 5-ASA formulation (De Vos *et al.*, 1992). The large variations in the mucosal 5-ASA concentrations can thus be postulated to be the cause for the difference in efficacy of each formulation. Therefore, the local delivery of an adequate concentration of 5-ASA to the colonic mucosa is substantially more critical than the systemic delivery of this agent (McLeod *et al.*, 1990; De Vos, 2000; Frieri *et al.*, 2005; Marteau *et al.*, 2005).

The concept of colon-targeted drug delivery via the oral route is a simple one. Ideally, the formulation must inhibit drug release in the gastric and small intestinal environments (upper GIT), and allow drug release in the colon. The simplicity of this concept is however challenged by the exposure of the formulation to a range of conditions and environments along its passage through the GIT. The currently exploited mechanisms for targeting 5-ASA to the colon rely on one or more of the following variables namely, gastrointestinal transit times, pH, enzyme activity, oxidation-reduction potential, and intraluminal pressure (Friend, 2005). Notwithstanding the inconsistency of these variables between each individual, the impact of disease conditions such as UC may have a profound influence on these variables and consequentially on the release characteristics of the delivery system (Duthie *et al.*, 1964; Barrow *et al.*, 1991; Hebden *et al.*, 2000; Nugent *et al.*, 2001; Sinha *et al.*, 2003). There is thus an urgent need to develop an orally administrable colon-targeted drug delivery system where the release characteristics are independent of fluctuating gastrointestinal conditions, and where an effective concentration of the drug can be delivered to the colonic mucosa.

Apart from the pill-burden associated with remission maintenance therapy with 5-ASA, the need for concurrent adjunctive therapy with anti-diarrhoeal agents such as loperamide HCl poses an additional burden to patient compliance. Thus, ideally the delivery system should facilitate a reduction in the pill burden, daily dosing requirements and allow for concurrent adjunctive and anti-inflammatory therapy with a single delivery system administration.

## 1.5 Approach to the problem

Apart from the therapeutic efficacy of colon-targeted drug delivery in the treatment of local colonic conditions such as UC and CD, this form of drug delivery also holds immense potential for the systemic delivery of proteins and therapeutic peptides (Davis, 1990; Van den Mooter and Kinget, 1995). Proteins and peptides are typically degraded and poorly absorbed in the upper GIT attributable to the relatively elevated levels of luminal and mucosal digestive enzymes in this region. Therefore the reduced vigor of the enzymatic behaviour in the colon facilitates an improved bioavailability of these molecules when release is targeted to this region (Saffran *et al.*, 1986; Gibson *et al.*, 1989; Van den Mooter *et al.*, 1998; Chourasia and Jain, 2004). Furthermore, the beneficial influence of colon-targeted drug delivery may be extended to the treatment of diseases that are sensitive to circadian rhythms such as asthma, angina pectoris and arthritis, where an intentional time delay in absorption is required (Youan, 2004).

As stated previously, despite the effectiveness of rectal formulations in targeting the colon they rarely succeed in extending proximally beyond the distal colon, notwithstanding their numerous other disadvantages (Jay *et al.*, 1985; Hardy *et al.*, 1986; Marshall *et al.*, 2010). Orally administered drugs for colon-targeting are thus preferred in spite of the difficulties associated with this route of administration (Basit, 2005). Taking into account the distal location of the colon in the GIT, a colon-targeted drug delivery system should ideally prevent premature drug release in the stomach and small intestine but effect an abrupt onset of drug release upon entry to the colon. Typically, this is achieved by formulating the system such that conditions specific to the colon are utilized to effect release. Similarly, based on the site and mechanism of the therapeutic action of loperamide HCl it is expected that by targeting the drug to the myenteric plexus of the small and/or large intestine a greater therapeutic benefit will be experienced at the same or reduced conventional dosage.

Generally the physiological changes that occur along the GIT is characterized as a continuum with decreases in enzymatic activity, motility and fluid content, and an increase in pH, from the oesophagus to the rectum. By exploiting these GIT characteristics numerous approaches and systems have been explored and developed for colon-targeted drug delivery but the majority of these have never reached the stage of clinical evaluation. Moreover, the impact of disease on the delivery system also poses a challenge in developing therapeutically effective products (Friend, 2005). As an example, the luminal pH of the distal intestine in UC is actually lower than that seen in healthy individuals. This was demonstrated by a study conducted by Fallingborg and co-workers (1993) where the colonic pH of UC

patients was found to be between 2.3 and 7.0. Similarly, intestinal transit time is also variable on account of diarrhoea experienced in UC (Sandborn and Phillips, 1995).

After consideration of the complexity of physiological constraints associated with the development of a colon-targeted drug delivery system, the most appropriate approach to achieving a targeted delivery of a therapeutic concentration of the drug at the colonic mucosa is by the development of an enzyme-responsive system. Colonic microflora have been extensively explored as a mechanism of modulating drug delivery in the colon due to the vast array of bacterial species as well as the large bacterial count (Vandamme *et al.*, 2002; Sinha and Kumria, 2003; Chourasia and Jain, 2004). Moreover, these colonic bacteria are predominantly anaerobic in nature and secrete enzymes capable of metabolizing a range of substrates that escape digestion in the upper GIT (Cummings *et al.*, 1989). Thus the fundamental point is to exploit a specific bacterial population that exists uniquely to the target site. Similarly, targeting of loperamide HCl to the small intestine should ideally also be based on exploiting an enzyme specific to the required site of action.

For this purpose, the employment of polymers that are termed 'stimuli-responsive' or 'smart' are suggested to be the most attractive approach to 'activating' drug release at the desired site in response to a pre-determined reliable stimulus. Thus for colon-targeting, materials that are recalcitrant to conditions in the stomach and small intestine but susceptible to bacterial fermentation in the colon serve as promising carriers for 5-ASA. By implementing such materials a system that ensures minimal drug release in the upper gastrointestinal regions and preferential triggering of drug release upon entry to the colon can be achieved. Consequentially, by ensuring that a maximum concentration of drug contained within the delivery system is released specifically in the colon an improved therapeutic efficacy with smaller doses and a less frequent dosing schedule will be achieved.

### 1.6 Novelty of the Approach

The inherent benefits and innovative nature of the formulation approach can be ascribed to the role of the delivery system in the circumvention of the challenges typically associated with current UC therapy and the relevant treatment options. Essentially, the novelty of the approach is derived from several formulation properties which are listed below.

1. Only naturally occurring polysaccharide polymers are employed.

    *These polymers are available in abundance, are inexpensive and exist in a variety of structures with varying properties. Furthermore, they can be easily chemically modified, and are highly stable, safe, non-toxic, and hydrophilic. However, most*

*importantly, they are biodegradable and thus fall within the category of 'generally regarded as safe' (GRAS) materials (Klein, 2009).*

2. The formulation allows for the targeted delivery of two different drugs to different regions of the GIT with the oral administration of a single delivery system.

*Since the treatment of UC necessitates concurrent adjunctive and anti-inflammatory therapy the targeting of loperamide HCl to the small intestine and 5-ASA to the colon with just a single delivery system would permit not only a reduced dosage required but also an improved therapeutic efficacy and improved patient compliance due to the reduced pill burden.*

3. Physiological stimuli in the relevant gastrointestinal region serve as a trigger for initiating drug release.

*The exploitation of the enzyme pepsin in the stomach and the polysaccharidases in the colon for triggering targeted drug release serve as consistent stimuli that remain unaffected by the disease condition and which ensures reliable and reproducible drug release characteristics. The ability of the formulation to respond to the specific enzyme populations implicates the formulation as an 'intelligent' drug delivery system.*

4. The formulation preparatory process is simple and holds easily scale-up potential.

### 1.7 Aim and Objectives of this study

The aim of this study was to design and develop a novel stimuli-responsive oral tablet (SROT) system for the treatment of UC which enabled the induction of concurrent states of remission and anti-inflammatory action with the administration of a single delivery system that provided targeted drug delivery of 5-ASA to the colon and loperamide HCl to the small intestine.

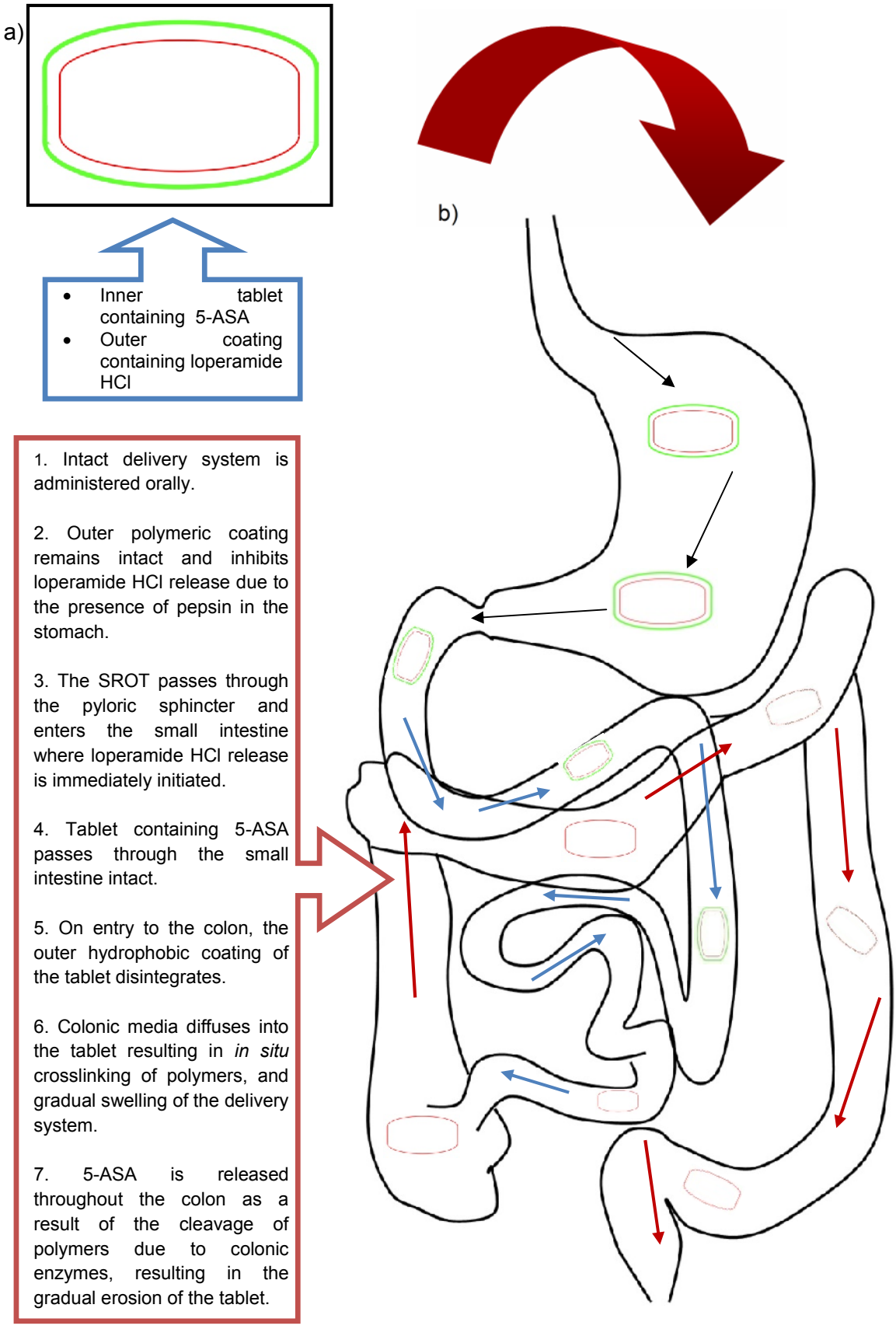



***Figure 1.4:*** *Schematic representation of a) the configuration of the stimuli-responsive oral tablet with the outer coating containing loperamide HCl, and the inner 5-aminosalicylic acid tablet component and; b) the postulated passage of the delivery system through the gastrointestinal tract.*

In order to accomplish this aim the following objectives were outlined:

i. To review the current and novel techniques employed for achieving colon-targeted drug delivery and to assess their limitations and advantages.
ii. To evaluate various stimuli-responsive polymers and assess their applicability in colon-targeting as well as for small intestinal targeting.
iii. To explicate theoretical considerations of the GIT and the influence of such factors in small intestinal and colonic drug delivery.
iv. To identify the most appropriate stimulus to be targeted for colonic delivery whilst avoiding any limitations experienced by other reviewed systems.
v. To identify the most appropriate stimulus to be targeted for small intestinal drug delivery.
vi. To identify a selection of appropriate polymers for application in both components to ensure optimum stimuli-responsiveness.
vii. To determine a suitable method of preparation of each component of the delivery system.
viii. To synthesize variants of each component of the delivery system according to the Box-Benkhen Experimental Design followed by physicomechanical and physicochemical analysis of the variants.
ix. To establish optimum parameters for the formulation of the inner 5-ASA tablet component and the outer loperamide HCl containing shell component.
x. To undertake *in vivo* animal studies on a pig model to determine the *in vitro-in vivo* correlation of the delivery system and to assess the pharmacokinetics of the delivery system in relation to the marketed 'gold-standard' formulations of both drugs.
xi. To investigate alternatives to the formulation of a tablet component and to assess the physicochemical and physicomechanical characteristics of such configurations.

### 1.8 Overview of this dissertation

This dissertation has been structured so as to provide the literature reviewed and data acquired in a logical manner so that the process of the development of the SROT is outlined in a clear, coherent and concise manner as depicted in Figure 1.5 whilst in keeping with the objectives outlined above.

**CHAPTER 1** focuses on IBD with specific attention to UC. This chapter aims to establish a clear understanding of the disease condition including the hypothesized aetiologies thereof, as well as the disease pathogenesis. By understanding the condition in its totality the importance of site-specific delivery of the necessary pharmaceutical agents is stressed. An overview of the current systems available on the market for remission maintenance of UC

and their respective shortcomings are described, thus providing the rationale and motivation for conducting this study.

**CHAPTER 2** outlines the numerous gastrointestinal characteristics that require consideration prior to development of orally administered targeted delivery systems. Furthermore, various novel colon-targeted drug delivery systems are reviewed in terms of their benefits and limitations. Stimuli-responsive polymers and their potential application in drug delivery are described, with particular attention to those employed for targeting of drugs to the colon

**CHAPTER 3** describes the preliminary studies conducted for the development of the inner 5-ASA loaded tablet component for delivery to the colon. Preformulation studies included the determination of the appropriate polymers to be employed, the methods of tablet preparation, optimum tablet coating, as well as the most appropriate *in vitro* dissolution conditions.

**CHAPTER 4** describes the preformulation studies conducted on the outer loperamide HCl containing shell layer such that an ideal responsiveness to pepsin is achieved. Preliminary *in vitro* evaluation was conducted to determine the influence of the type of polymer, tablet-shell configuration and crosslinking on the pepsin-responsiveness of the shell.

**CHAPTER 5** focuses on the preparation of variants of the 5-ASA loaded colon-targeted tablet formulation as constructed according to the Box-Benkhen Experimental Design, followed by optimization. Furthermore, physicomechanical and physicochemical properties were analyzed to determine the influence of colonic enzymes on the formulation's ability to respond to these enzymes *in vitro*.

**CHAPTER 6** focuses on the preparation of variants of the loperamide HCl-loaded pepsin-responsive shell formulation as constructed according to the Box-Benkhen Experimental Design, followed by a constrained statistical optimization process. Physicomechanical and physicochemical studies were performed to establish the mechanistic action of pepsin on the release characteristics of the shell.

**CHAPTER 7** describes the determination of an ideal procedure for dosage administration and blood sampling from a pig model. Furthermore, this chapter places emphasis on the *in vivo* drug release characteristics of the SROT and therefore the determination of the plasma concentration profiles of 5-ASA and loperamide HCl from the SROT in comparison to the relevant marketed 'gold-standard' formulations.

**CHAPTER 8** focuses on an alternative approach to employing polysaccharide polymers for colon-targeted drug delivery applications. The novel formulations consisting of hydrolyzed polyacrylamide and polysaccharide blends were evaluated for the influence of surface area and porositometric influences on the drug release properties. The formulations were also analyzed for the physicomechanical properties.

**CHAPTER 9** outlines the conclusions derived from this work, the recommendations for future work, the potential prospects of the developed delivery system, as well as the principle challenges experienced during the study.

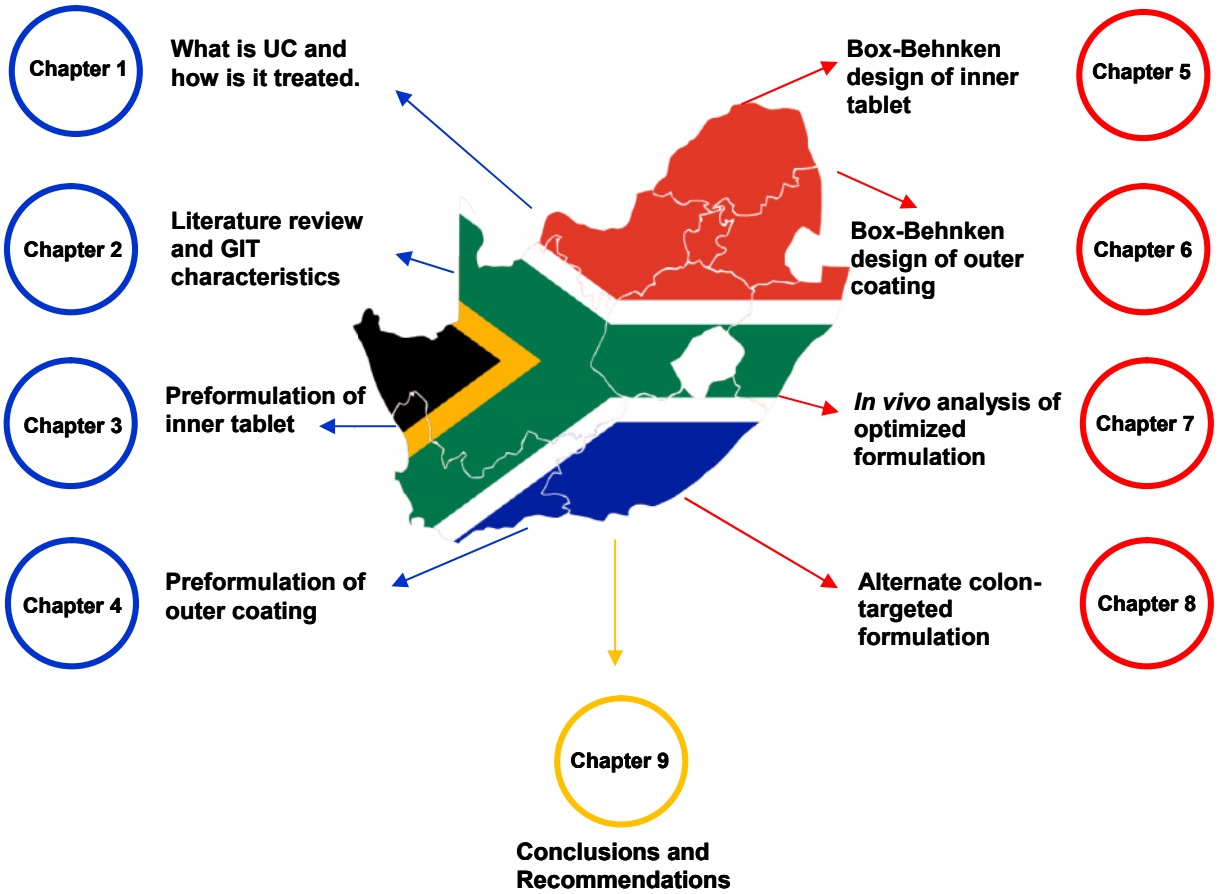



***Figure 1.5:*** *Map of the dissertation.*

# CHAPTER 2

# OVERVIEW OF STIMULI-RESPONSIVE POLYMERS AND THEIR APPLICATION IN GASTROINTESTINAL TARGETED DRUG DELIVERY

## 2.1 Introduction

The paradigm of drug discovery and development has undergone a shift from the serendipitous approach to one that is more rational. Nevertheless, this process still remains costly, time consuming, and risky. The director of economic policy at the world's largest research-based pharmaceutical company, Pfizer, recently estimated the cost of developing a new drug to vary between $800 million to nearly $2 billion per drug (Masia, 2008). As a result, the development of new drug delivery systems have been gaining increasing attention as they can be developed at a fraction of the cost and time, providing a 're-birth' of old drugs, with improved efficacy and patient compliance, whilst maximizing the return on investments for pharmaceutical companies (Speers, 1999).

Initially, drug research emerged with the focus placed on the botanical origin of drugs. This occurred in the early human civilizations, with subsequent progression to a synthetic chemistry phase in the mid 20$^{th}$ century. However currently, focus in drug research has shifted to the biotechnological approach to drug discovery (Sneader, 2005). The foundations of novel drug delivery systems were laid down as early as 1952 with the inception of the pioneering sustained release capsule which subsequently led to the introduction of concepts such as prolonged, time and extended release systems or sophisticated programmable systems (Starr, 2000). Furthermore, drug delivery has also changed from being orientated towards the systemic delivery of drugs to more specific targeting of affected organs and cellular targeting (Pillai *et al.*, 2001).

### 2.1.1 Influence of gastrointestinal physiology on targeted drug delivery system design

The implied simplicity of oral drug delivery is a misconception stemming from the elusive physiology of the gastrointestinal tract (GIT). Despite the strides being made in understanding the physiology and mechanisms of the GIT large gaps remain in our knowledge, resulting in the complexity and dynamicity of the organ being underestimated. For this reason, typically, only one or two variables are taken into account during drug delivery system design and drug targeting. It is thus obvious that the design of functional drug delivery systems which behave in a reproducible manner will remain out of reach as long as a clear understanding of the conditions it will be subjected to remains unknown.

The GIT, otherwise known as the alimentary canal, is basically a hollow muscular digestive tube that winds through the body and essentially functions to acquire nutrients and remove physiological waste products. In other words, it is a selective barrier between the systemic circulation and the environment which digests food, absorbs nutrients, electrolytes and fluid, whilst preventing the absorption of potentially harmful substances (Marieb and Mallatt, 1997; Reed and Wickham, 2009). Thus, based on the complexity of the processes and functions involved the GIT is differentiated into various segments with unique characteristics along its length (Liu *et al.*, 2003a; Basit, 2005). Figure 2.1 illustrates a simplistic view of this organ.

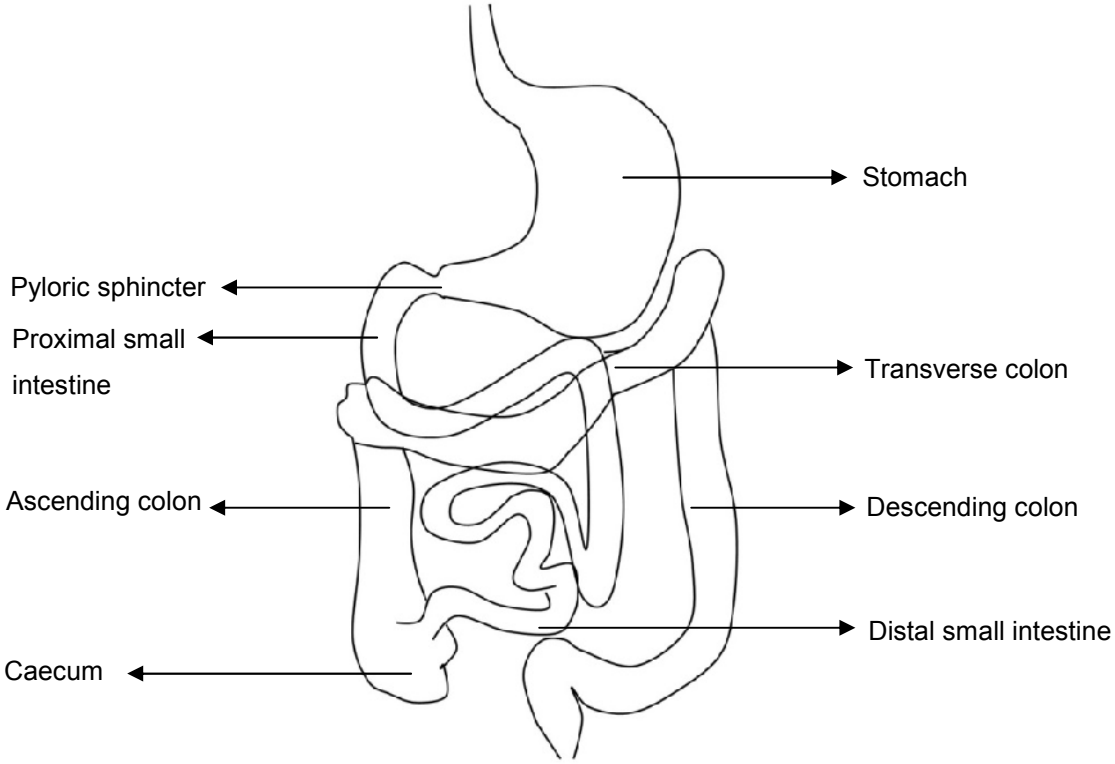



***Figure 2.1:*** *Illustration of a simplified representation of the gastrointestinal tract.*

As presented earlier Ulcerative Colitis (UC) represents a local inflammation of the colon; therefore it would be ideal to target the therapeutic principle namely, 5-aminosalicylic acid (5-ASA) directly at the affected luminal site resulting in an effective drug concentration in the colonic mucosa, a reduction in the systemic availability of the drug and in turn a reduction in the adverse effects (Klotz, 2005). In addition, for the concurrent adjunctive treatment of UC it is preferable to target the loperamide HCl at its site of action namely, to the small intestine. For the most part, the lack of appreciation and understanding of the disease state on the gastrointestinal environment limits the design of efficient targeted drug delivery systems. In addition inter-individual variability with regard to gastrointestinal physiology for example, intra-luminal pH profiles, motility, pressure, and transit times amongst others must be considered. Several of the most prominent of these physiological parameters are explicated

in Table 2.1 with specific reference being made to each major site within the GIT of a healthy individual, as well as the influence of UC on the relevant physiological characteristic.

Based on the reviewed literature, the overall physiological changes along the GIT can generally be regarded as a continuum of decreases in motility and fluid content, and increases in the pH profiles, enzymatic activity, and luminal pressure occurring from the oesophagus to the rectum. The concept of drug targeting can thus be based on the identification and exploitation of a specific characteristic that is unique to the target organ so as to effect drug release at the required site and at the desired rate. This is typically achieved by employing stimuli-responsive polymers that are capable of responding to specific stimuli in the target organ.

Since targeting of drugs to the small intestine does not pose an immense challenge, the impetus of this chapter is placed on colon-targeting technology. Targeting of drugs to the small intestine is almost always performed by employing an enteric coating that disintegrates at a pH≥6. These coatings typically consist of polymethacrylate copolymers but may also consist of cellulose acetate phthalate etc.

***Table 2.1:*** *Physiological characteristics of specific regions of the gastrointestinal tract of a healthy individual compared to an individual with Ulcerative Colitis*

| Physiological parameter | Healthy individual | | | Ulcerative Colitis | References |
|---|---|---|---|---|---|
| | Stomach | Small intestine | Colon | | |
| **Transit and motility** | *"almost everything seems to affect gastric emptying" (Olsson and Holmgren, 2001)*<br><br>The variability of gastric transit times (GTT) and emptying times is dependent on the fed or fasted state of the individual, body posture, food type, concomitant drug administration, as well as the size and density of the delivery system.<br><br>The GTT of single-unit delivery systems is reported to vary from 15 minutes to more than 3 hours. | Small intestinal transit time (SITT) is theorized to be relatively constant at 3-4 hours and independent of external influences. However, more recently Coupe and co-workers determined the SITT variability of single and multiple-unit systems to range between 0.9 to 6.2 hours. Intra-subject variability was also observed as the SITT of a single individual varied between 1.5 to 5.4 hours. Furthermore, intestinal motility is governed by the migrating myoelectric complex (MMC) which dominates in the fasted state. | Like the small intestine, movement of substances through the colon is non-continuous and slow. The total transit time in this region is highly variable due to the dependency on factors such as diet, mobility, stress, disease states and drugs. The colon often has a residence time in excess of 24 hours. | Patients affected by IBD typically experience an accelerated intestinal motility due to the presence of inflammation and diarrhea. Furthermore, patients with UC were shown to have an increased colonic transit compared to controls (51.7 hours vs. 24.3 hours). | Hunter *et al.*, 1982; Kaus et al., 1984; Davis *et al.*, 1986; Metcalf *et al.*, 1987; Devereux *et al.*, 1990; Coupe *et al.*, 1991; Cummings *et al.*, 1992; Vassallo *et al.*, 1992; Price *et al.*, 1993; Meier *et al.*, 1995; Hebden *et al.*, 2000; Olsson and Holmgren, 2001; Weitschies *et al.*, 2005; McConnell *et al.*, 2008a. |
| | Evaluation of a radio-labelled osmotic tablet via gamma-scintigraphy revealed an average gastric transit time of 0.8 hours, average SITT of 3 hours, and highly variable average colonic transit time of 6.6 hours. | | | | |
| **Pressure** | Since there is sufficient fluid present in the stomach, the luminal pressure in this region is not as significant as that of the colon. | The pressure generated by the muscular contractions of intestinal wall required for the grinding and propulsion of luminal contents varies in intensity and duration along the small intestine. | The action of haustral contractions coupled with the increased viscosity of the colonic environment is believed to be the cause of the higher effective luminal pressure of this region. | Since UC is a chronic inflammatory state, the inflammation and injury that occurs results in increased luminal pressures. The altered contractile rhythms, villous motility and mucosal deformation in UC propagates this change in luminal pressures. | Fallingborg *et al.*, 1985; Reddy *et al.*, 1991; Yang *et al.*, 2002; Basit, 2005; Flanigan *et al.*, 2008. |
| **pH profiles** | It is well established that the pH of the GIT increases from the stomach to the distal small intestine. This gradual increase has however since been assumed to continue from the small intestine to the colon. | | | The gastrointestinal pH of UC patients is considerably lower than that of healthy individuals. This effect is most pronounced in the colon for example Raimundo and co-workers observed a pH of 4.7 in the right colon in a case of acute UC, and a subsequent study exposed colonic pH's as low as 2.3. | Evans *et al.*, 1988; Raimundo *et al.*, 1992b; Fallingborg *et al.*, 1993; Nugent *et al.*, 2001; Vandamme *et al.*, 2002; Basit, 2005; Jose *et al.*, 2009. |
| | The pH of the gastric region remains fairly consistent within the pH range of 1 and 3.5, maintaining an acidic environment for the proteolytic action of gastric enzymes. | Intraluminal intestinal pH's demonstrate a substantial inter-individual variability but is generally considered to range between 5.5 and 6.8. The pH is said to increase distally from 6.63±0.53 in the jejunum, reaching a peak of 7.49±0.46 at the ileocaecal junction. | As early as 1988, it was determined that the pH of the distal small intestine was 7.5, and that of the caecum was 6.4. This drop in pH on entry to the colon is attributed to the fermentation of polysaccharides present in the colon resulting in the production of short chain fatty acids. The pH then only rises to 7 in the descending colon. | | |
| **Enzymes and bacteria** | The gastric bacterial and enzyme populations are modest with only $10^2$ CFU/g of contents with pepsin being the most important in this region. | The bacterial population increases along the GIT with $10^4$ CFU/g in the proximal small intestine and $10^7$ CFU/g in the ileum. | The colon contains over 400 distinct species of mostly anaerobic bacteria having a population of $10^{11}$–$10^{12}$ CFU/g with *Bacteroides*, *Bifidobacterium*, *Eubacterium* and *Lactobacillus* being the most prevalent. Furthermore, enzymes responsible for the degradation of polysaccharides such as α-L-arabinofuranosidase, β-D-fucosidase, β-D-galactosidase, β-D-glucosidase, β-xylosidase are also widely active in this region. | It is believed that in conditions where the transit time is altered, a change in the bacterial numbers occurs. In UC in particular the number of facultative anaerobes increases and this altered microbial composition consequently results in increased immune stimulation, epithelial dysfunction, or enhanced mucosal permeability. However, the types and activities of the bacterial glycosidases remain unchanged in UC. | Cooke, 1967; Gorbach *et al.*, 1968; Gorbach, 1971; Van der WielKorstanje and Winkler, 1974; Simon and Gorbach, 1984; Englyst *et al.*, 1987; Bernhardt and Knoke, 1997; Eckburg *et al.*, 2005; McConnell *et al.*, 2008b; Sartor, 2008. |
| **Fluid volumes** | The fluid volume throughout the GIT is highly variable. The free fluid volume of the stomach can range from 118±82mL post-mortem and 45±18mL in the fasted state. | The small intestinal fluid volume can range from 212±110mL post mortem, and 54±41mL and 105±72mL in the fed and fasted states respectively. | In 1990, Cummings and co-workers determined that the colonic fluid volume post mortem was 187mL, however in the fed and fasted states this volume was substantially lower since it only quantified the volume of fluid not bound to digesta. | Chronic diarrhoea which is common during active UC results in an increased loss of fluid and consequent dehydration. As a result colonic contents are less viscous and less solid. | Gotch *et al.*, 1957; Cummings *et al.*, 1990; Schiller *et al.*, 2005. |

## 2.2 Application of stimuli-responsive polymers in targeted drug delivery

With the emergence of more novel and effective drug therapies, increased importance is being placed upon the methods by which these drugs are being delivered to the body. Even though commercially available controlled release systems have been proven to be therapeutically advantageous over conventional systems their shortfall lies in their insensitivity to the changing metabolic states of the body. Mechanisms capable of responding to these physiological variations must be provided in order to synchronize drug release profiles with the changing physiological conditions. Ideally, a drug delivery system should respond to physiological requirements, sense the changes and alter the drug release profile accordingly (Gupta *et al.*, 2002).

Stimuli-responsive polymeric drug delivery is a field of controlled drug delivery whereby the dynamics of the typical physiological process of a particular disease state is closely monitored and the quantity and site of drug release is affected only according to the physiological need (Kost and Langer, 2001). The realization of the site-specific and self-regulated drug delivery is achieved by the incorporation of specific polymers that are distinguishable by their ability to undergo rapid changes in their microstructure from a hydrophilic to a hydrophobic state which is triggered by small changes in the environment. The macroscopic changes that occur are reversible; therefore the system is theoretically capable of returning to its initial state when the trigger is removed (Galaev and Mattiasson, 1999; Kumar *et al.*, 2007).

Common stimuli that drive these changes are represented in Figure 2.2 (Galaev and Mattiasson, 1999; Kost and Langer, 2001; Gupta *et al.*, 2002; Schmaljohann, 2006; Kumar *et al.*, 2007). These stimuli are then further categorized as either external or internal stimuli. Externally controlled systems rely on externally applied stimuli that are produced with the help of different stimuli-generating devices, which ultimately results in pulsed drug delivery. Internally regulated systems are also known as self-regulated devices, where the release rate is controlled by a feedback mechanism that is produced within the body to control the structural changes in the polymer network and to exhibit the desired drug release, without any external intervention (Kost and Langer, 2001; Gupta *et al.*, 2002). Responses to these stimuli may be manifested as changes in shape, surface characteristics, solubility, formation of intricate molecular assemblies or sol-gel transitions (Kumar *et al.*, 2007).

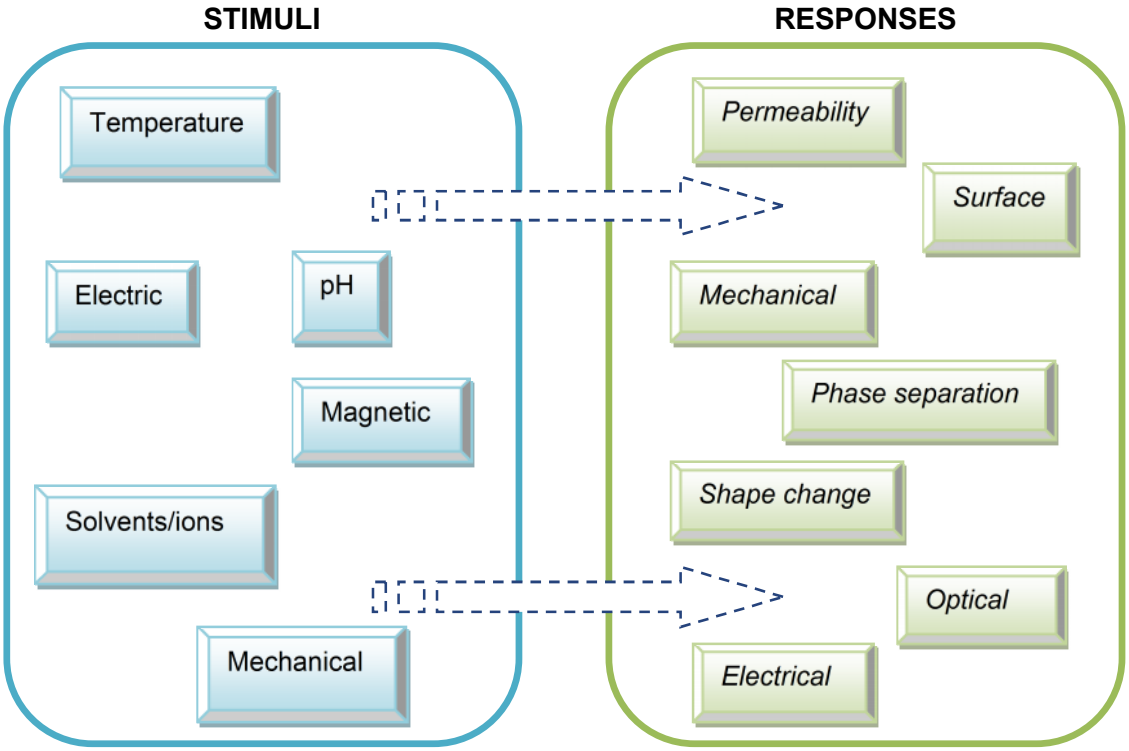



***Figure 2.2:*** *Potential stimuli and responses of synthetic stimuli-responsive polymers (adapted from Schmaljohann, 2006).*

In the last decade a vast majority of pharmaceutical research has been focused on the development of targeted drug delivery systems that allow for the delivery of pharmaceuticals to specific sites, organs, tissues or cells in the body where drug therapy is necessary, for example the specific targeting of chemotherapeutics to only cancer cells (Nakayama *et al.*, 2006). Stimuli-responsive polymers have been explored as a means of achieving such drug targeting by exploiting site-dependent stimuli such as pH and the presence of high quantities of particular enzymes. However numerous other stimuli and polymers have been explored and are explicated further.

### 2.2.1 Thermo-responsive polymers

Temperature may act as both an external and internal stimulus. Physiologically, thermal stimuli show substantial significance for example during a fever when there is an elevation of body temperature due to the presence of pyrogens. This elevation in temperature is mediated by an elevated concentration of prostaglandin E2 within certain areas of the brain thus altering the firing rate of neurons that control thermo-regulation (Aronoff and Neilson, 2001; Chaterji *et al.*, 2007). Changes in temperature that can trigger drug release can be either due to increased body temperature in a diseased state or due to an external modulation for example in the form of heat-triggered subdermal implants amongst others.

Thermo-responsive monolithic hydrogels are being investigated for their potential to obtain 'on-off' drug release profiles in response to stepwise temperature changes (Okano *et al.*, 1990; Bae *et al.*, 1991a, b). In one such case poly(*N*-isopropylacrylamide) crosslinked butyl methacrylate (BMA) hydrogels were loaded with indomethacin and analyzed for their on-off release profile. 'On' was achieved at low temperatures and 'off' at high temperatures. This occurred due to the formation of a skin-type barrier that formed a dense, less permeable gel surface layer when the temperature was suddenly changed. The barrier was formed due to the faster collapse of the gel surface than the interior and was regulated via the length of the methacrylate alkyl side-chain (Okuyama *et al.*, 1993).

The limited therapeutic activity and insolubility of anti-cancer drugs, problems of accessibility and heterogeneity of tumours, and toxicity/immunogenicity of the delivery agent all intensify the need for newer drug delivery modalities that are capable of eradicating these challenges. Despite the development of many diverse chemotherapeutic systems such as low molecular weight prodrugs, liposomes and micro- and nanoparticles, there is yet limited therapeutic efficacy in the available chemotherapeutic drug delivery systems (Fung and Saltzman, 1997; Allen, 1998; Jones and Leroux, 1999; Liu *et al.*, 2003c; Barbe *et al.*, 2004; Lin *et al.*, 2005; Ambruosi *et al.*, 2006; Dong and Feng, 2006; Farokhzad *et al.*, 2006; Vicent and Duncan, 2006; Cheng and Kuhn, 2007).

Soluble polymeric drug carriers are amongst the more popular modes of chemotherapeutic drug delivery. This is owed to its improved drug pharmacokinetic profiles, thus leading to increased tumour accumulation over free drug via passive targeting or also known as the enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986; Iyer *et al.*, 2006). However, a major drawback of these carriers is their inability to intrinsically target a particular physiological site. Since hyperthermia increases the permeability of tumour vasculature compared to normal vasculature a combination of chemo and radiotherapy establishes a synergistic activity that enhances solid tumour cytotoxicity (Engin *et al.*, 1995; Engin, 1996; Issels, 1999).

### 2.2.2 Electro-responsive polymers

Delivery systems that exploit electrical fields as externally regulated stimuli benefit not only from the ready availability of equipment but also from the ability to precisely control the magnitude of the applied current, the duration of electrical pulses and the interval between these pulses (Anal, 2007). These systems are formulated from polymers called polyelectrolytes which contain a relatively high concentration of ionizable groups along the backbone chain, thus bestowing pH-responsive as well as electro-responsive properties upon them (Qiu and Park, 2001; Anal, 2007).

Technologies such as sonophoresis, iontophoresis and infusion pumps have benefited from being electrically controlled (Aoki *et al.*, 2004). A polymer reservoir with a pair of electrodes placed across its rate limiting membrane was developed as a drug delivery device. Controlled and predictable drug release rates were achieved and postulated as being as a result of an alteration of the magnitude of the electrical field between the electrodes (D'Emanuele *et al.*, 1988; D'Emanuele and Stainforth, 1989; Kagatani *et al.*, 1997; Murdan, 2003; Kulkarni *et al.*, 2010).

### 2.2.3 Magnetically-responsive polymers

Magnetic drug targeting is based on the attraction of magnetic micro- and nanoparticles to an external magnetic field source. In principle, in the presence of a magnetic field gradient, a translational force will be exerted on the particle/drug complex. This effectively traps the complex in the field at the target site and pulls it toward the magnet (Pankhurst *et al.*, 2003; Grief and Richardson, 2005). However several factors always require consideration namely, the magnetic properties of the carrier particles, field strength, field geometry, drug/gene binding capacity; as well as physiological parameters such as the depth to the target site, the rate of blood flow, vascular supply and body weight (Neuberger *et al.*, 2005).

Current chemotherapeutic approaches rely on highly non-specific means of delivery. These drugs are generally administered intravenously and the resulting systemic circulation of these agents indefinitely results in numerous adverse effects. In addition, healthy cells are targeted along with the cancer cells. The premise that cytotoxic drugs could be attached to magnetic micro- and nanoparticles was explored by injecting these particles into a subject either via intravenous or intra-arterial injection (Senyei *et al.*, 1978; Mosbach and Schröder, 1979; Alexiou *et al.*, 2000; Zebli *et al.*, 2005; Alexiou *et al.*, 2006; Martina *et al.*, 2008). The drugs were then guided and concentrated at the tumour site by aid of high gradient, external magnetic fields generated by rare earth permanent magnets.

Although theoretically very effective, these carriers are partnered with a range of challenges. In order to retain the magnetic carrier-drug complex at a specific location, the externally applied field must have a relatively strong gradient. Additionally, as soon as the drug is released from the magnetic complex, it is no longer responsive to the applied field. It is then free to resume its normal distribution patterns in the body and if the drug is released while the complex is still within the vasculature, even if they are held at the target site, there will then be some degree of systemic distribution. There is also the potential for embolization as the particles are capable of accumulating and blocking blood flow or they may also concentrate in the liver (Dobson, 2006). There is also the problem as to the depth that the

magnet may function, as is encountered when scaling up from small animals with near-surface targets to larger animals and humans.

### 2.2.4 Ultrasonically-responsive polymers

The application of ultrasound in drug delivery proves to be highly advantageous as it is non-invasive and capable of penetrating deep into the interior of the body allowing targeted drug delivery by controlling parameters such as frequency, power density, duty cycles and time of application (Lavon and Kost, 1998; Rapoport *et al.*, 2004). Its main mechanism of action relies on the generation of thermal energy, perturbation of cell membranes under the influence of micro-convection or inertia cavitation, and enhanced permeability of blood capillaries (Gao *et al.*, 2005).

One of the pioneering approaches of exploiting ultrasound in drug delivery involved directing ultrasonic waves directly at the polymers or the hydrogel matrix (Sershen and West, 2002). Kost and Langer (2001) evaluated various bio-erodible and non-erodible polymers as drug carrier matrices and found that enhanced polymer erosion and drug release was observed when the samples were exposed to ultrasound. The systems responses to the ultrasonic triggering were rapid (within 2 min) and reversible. Enhanced release was also observed in non-erodible systems exposed to ultrasound where the release was diffusion dependent (Kost *et al.*, 1988, 1989).

### 2.2.5 Light-responsive polymers

Since the stimulus of light can be imposed instantaneously and can be delivered in specific quantities with high accuracy, it renders light-responsive hydrogels highly advantageous over others. Also, the capacity for instantaneous delivery of the sol-gel stimulus renders light-responsive polymers potentially applicable in the development of optical switches, display units and ophthalmic drug delivery systems. Light-responsive polymers may be UV or visible light sensitive; however, visible light-responsive polymers and hydrogels are more beneficial in that they are safe, inexpensive, readily available, clean and easily manipulated (Qiu and Park, 2001).

By introducing a light-sensitive chromophore, for example trisodium salt of copper chlorophyllin to poly(*N*-isopropylacrylamide) hydrogels, visible light-responsive hydrogels can be formulated (Suzuki and Tanaka, 1990). When light is applied to the hydrogel, the chromophore absorbs the light which is then dissipated locally as heat by radiationless transitions, increasing the ‘local’ temperature of the hydrogel. This increase in temperature alters the swelling behaviour of the hydrogel. By addition of another functional group, such as an ionizable group of polyacrylic acid, the light-responsive hydrogel can be rendered pH-

sensitive as well and may be activated (i.e. induced to shrink) by visible light and can be deactivated (i.e. induced to swell) by increasing pH (Suzuki *et al.*, 1996). Although the action of the light stimulus is instantaneous, the reaction of the hydrogels to the action is slow since light energy first has to be converted into thermal energy.

### 2.2.6 pH-responsive polymers

All pH-responsive polymers contain pendant acidic or basic groups such as carboxylic and sulfonic acids or ammonium salts, that are capable of either accepting or releasing protons in response to environmental changes in pH (Qiu and Park, 2001; Gupta *et al.*, 2002; Schmaljohann, 2006). These changes thus lead to conformational changes of the soluble polymers and a change in the swelling behaviour of the hydrogels when ionizable groups are linked to the polymer structure. On exposure to aqueous media of appropriate pH and ionic strength, pendant groups ionize and develop fixed charges on the polymer network, causing electrostatic repulsive forces responsible for pH-dependent swelling or deswelling of the hydrogel, which ultimately controls drug release (Gupta *et al.*, 2002). Since variations in pH occur within the body, unlike temperature changes, this property can be exploited to direct a response to a certain tissue or cellular compartment. Furthermore, local pH changes in response to specific substrates can be generated and exploited for modulating drug release.

Poor oxygen perfusion within the interstitial matrix of a tumour results in a different metabolic profile of the tumour to surrounding tissues. This leads to elevated lactic acid levels and consequently a reduction in the pH (Kurisawa and Yiu, 1998). The triggering, targeting and controlling of drug release according to variations in pH can thus be achieved by targeting the extracellular tissue or the lysosomes. Tumour tissue has an extracellular pH between 6.5-7.2, which is slightly lower than the normal pH of 7.4; and after cellular uptake, drug conjugates reach lysosomes which have a pH of 4.5-5.0. In the latter case, hydrolytic enzymes such as cathepsin B may also be exploited for effecting drug release (Duncan, 2003).

### 2.2.7 Ionic strength-responsive polymers

Polymers that contain ionizable groups typically respond to changes in ionic strength which may ultimately manifest as changes in the size of polymer chains, polymer solubility and the fluorescence quenching kinetics of the chromophores bound to the electrolytes (Morrison *et al.*, 1994; Kathmann *et al.*, 1997; Szczubialka *et al.*, 2003). Polyampholytes generally incorporate both anionic and cationic charged moieties into a single polymer chain. This accounts for the unusual rheological behaviour of these polymer systems as a result of the attractive Coulombic interactions between the oppositely charged species (Salamone *et al.*, 1985; Corpart and Candau, 1993; Kathmann *et al.*, 1997).

Different concentrations of salts, which determine the ionic strength, may cause phase transitions in ionic strength-responsive polymers. Cu(II) metal ion was immobilized on poly(NIPAAm-*co*-vinylimidazole) for protein separation using the affinity binding of specific proteins to Cu(II) (Kumar *et al.*, 1998). With an increase in the ionic strength, the polymer chains which bind the proteins precipitate. The high salt concentration reduces the repulsive electrostatic strength of the copolymer, which results in an increase in the hydrophobic interactions and thus leads to precipitation.

### 2.2.8 Glucose-responsive polymers

Glucose-responsive hydrogel systems have the ability to provide self-regulated insulin release in response to the concentration of glucose in the blood, thereby controlling the concentration of insulin within a normal range (Gil and Hudson, 2004). The most common of these hydrogel systems utilize immobilized enzymes or biocatalysts, more specifically glucose oxidase (Kang and Bae, 2003; Ghanem and Ghaly, 2004; Srivastava *et al.*, 2005). The rationale behind this is that generally when an enzyme is covalently coupled to a smart polymer, environmental changes result in drastic changes in the polymer conformation which significantly affects the enzyme activity and substrate access to the enzyme molecule. These biocatalysts act by catalyzing an enzymatic reaction in their soluble state and the products of this enzymatic reaction then triggers the gel phase transition.

In the case of insulin delivery, glucose oxidase exploits the pH sensitivity of the polymer used to immobilize the enzyme. The glucose oxidase oxidizes glucose to form gluconic acid which causes a pH change in the environment (Qiu and Park, 2001). The pH-sensitive hydrogel then exhibits a volume transition in response to the lowered pH, which occurs due to the formation of gluconic acid. Therefore, the swelling ratio of the hydrogel is regulated by the body's glucose levels (Chaterji *et al.*, 2007). Figure 2.3 depicts a schematic of this process.

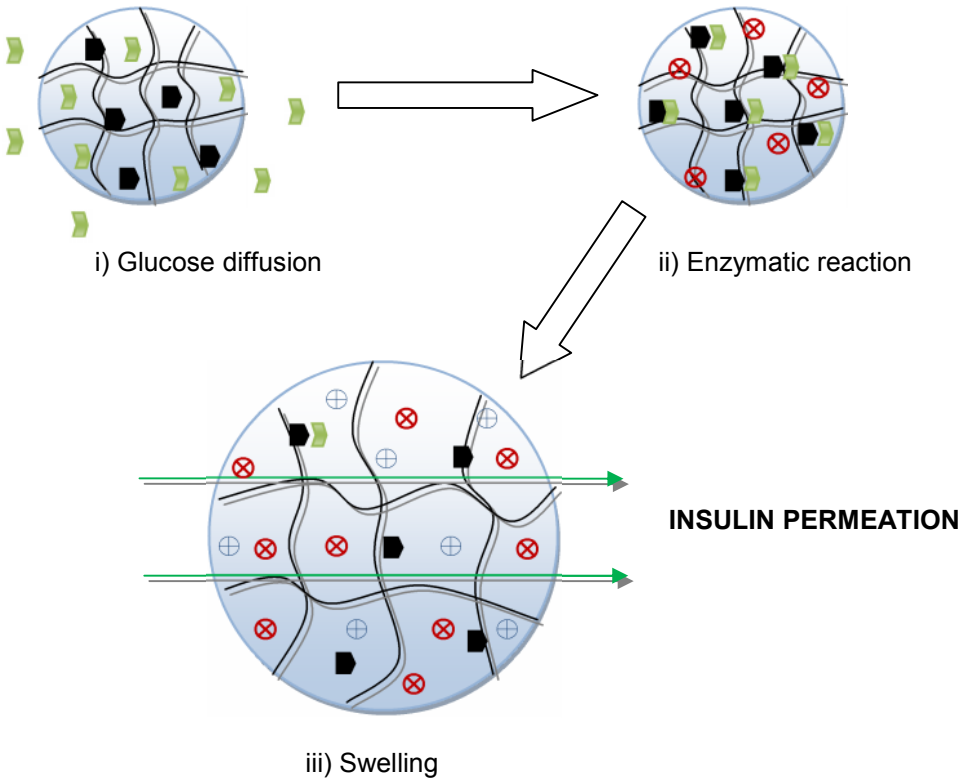



***Figure 2.3:*** *Schematic representation of a glucose-responsive glucose oxidase-loaded membrane. ◗= Glucose oxidase, ❱= Glucose, ⊗= Gluconic acid (adapted from Miyata et al., 2002).*

### 2.2.9 Enzyme-responsive polymers

As described previously, self-regulated or internally-regulated systems allow drug release rates to be adjusted by the system itself in response to feedback information provided by the physiological needs of the body (Heller, 1985; Pitt, 1986; Heller, 1988; Kost, 1990; Cullander, 1992). Enzyme-responsive systems are such that no external intervention is required, however apart from this, these systems provide a high specificity of interactions and a broad range of possibilities attributed to the large number of enzymes known (Goldbart *et al.*, 2002).

It is well documented that the gastrointestinal bacterial population increases continuously from the mouth to the rectum, with a dramatic increase in bacterial numbers in the colon (Simon and Gorbach, 1984; Bernhardt and Knoke, 1997; Eckburg *et al.*, 2005). The flourishing colonic bacterial population exists since the pH and motility of this region is appreciably more conducive to bacterial growth compared to the stomach and small intestine. The unmistakably demarcated difference between the population and type of bacterial growth in the various regions of the GIT, as well as the variety of enzymes produced by colonic bacteria provides a substrate which can effectively be exploited as a trigger for drug release in this region. Thus, this formulation approach has been and currently

is being extensively explored for targeting of drugs and other pharmaceuticals directly to the colon.

### 2.3 Approaches to colon-targeting by exploiting site-specific stimuli

Since the revelation of the effectiveness of 5-ASA in the treatment of UC, as well as the recognition of the sulphonamide component of sulfasalazine as the causative agent responsible for the adverse effects experienced, numerous 5-ASA compounds and delivery systems have emerged to provide a more attractive approach to UC treatment. In general, four primary approaches have been proposed for targeted delivery of 5-ASA and other pharmaceuticals to the colon. These approaches have been implemented in an attempt to meet the following criteria imperative for effectively delivering drugs to the colon:

- the delivery system should remain intact when travelling through the upper GIT;
- the incorporated drugs should be protected from chemical and enzymatic degradation in the stomach and small intestine;
- the delivery system should initiate drug release immediately upon reaching the colon; and
- the relevant drug should be released in a manner such that a consistent and effective rate of drug release is achieved that ensures optimum therapeutic efficacy in a local or systemic capacity.

Based on these criteria various design strategies have been investigated primarily focusing on the exploitation of a characteristic unique to the colonic environment as a means of triggering drug release whilst maintaining the integrity of the delivery system throughout its passage through the upper GIT. The gastrointestinal features that are typically exploited as triggering mechanisms include pH, transit time, pressure and microflora. Table 2.2 provides a detailed description of each of these approaches, as well as examples of the most effective and extensively explored novel delivery systems based on each of the above characteristics.

***Table 2.2:*** *Stimuli-responsive colon-targeted drug delivery approaches*

| Design Approach | Mechanism of Drug Release | Polymers typically employed | Experimental and theoretical limitations | Examples | Conclusion | References |
|---|---|---|---|---|---|---|
| **pH-dependent systems** | Formulations are typically coated with an enteric polymer coating that remains insensitive to the acidic conditions of the stomach by existing in the unionized form, and dissolving at a more neutral pH of 6-7, theoretically found in the colon. The most commonly employed pH-dependent polymers include the methacrylic acid copolymers, Eudragit® S and Eudragit® L. | Methacrylic acid copolymers such as:<br>• Eudragit® S100<br>• Eudragit® L100<br>• Eudragit S<br>• Eudragit FS<br>• Eudragit P4135 F<br>• Eudragit L 30 D-55<br>• Eudragit FS 30 D | • The presence of a disease state such as UC considerably influences the pH characteristics of the GIT.<br>• The concept is based on the **assumption** that the luminal pH continues to rise from the small intestine to the colon.<br>• Time of fragmentation of these systems is highly variable and time of tablet disintegration ranges from 5-15 hours.<br>• Site of disintegration ranges from the small intestine to the distal colon, and in some cases the system passes through the GIT intact. | The pioneering study of pH-dependent systems was conducted in 1982 by Dew and co-workers, where Eudragit® S was employed as a coating over a capsule. The results from this study provided the basis for the commercialization of Asacol®. Other marketed pH-dependent systems are shown in table 1.2.<br>To improve the colon-specificity numerous avenues are being explored such as enteric polymer modification, development of new polymers and combining of existing polymers. The latter is exemplified by the development of TARGIT™-a targeting technology comprised of a mixture of Eudragit® L and S. | The gastrointestinal pH is not a sufficiently reliable mechanism for colon-targeted drug delivery. Despite the efficacy of enteric polymers in targeting the small intestine, their success in targeting the colon is mixed. The erratic behaviour of these pH-dependent colon targeted systems is also evident *in vivo*.<br><br>Basit (2005) made the observation, "*given our current knowledge of the gastrointestinal pH, one can only speculate whether some of these products would have reached the market had this information been available in the early 1980's.*" | Dew *et al.*, 1982; Schroeder *et al.*, 1987; Ashford *et al.*, 1993a ; Fallingborg *et al.*, 1993; Peeters and Kinget, 1993; Sciarretta *et al.*, 1993; Watts and Illum, 1997; Wilding, 1999; Rudolph *et al.*, 2001; Tuleu *et al.*, 2001; Cole *et al.*, 2002; Sinha *et al.*, 2003; Basit, 2005; Safdi, 2005; Asghar and Chandran, 2006; Ibekwe *et al.*, 2006a, b, 2008. |
| **Time-dependent systems** | These formulations are designed so as to ensure release of the drug load after a predetermined lag time. Usually a nominal lag time of 5-6 hours is incorporated into the system. | • Hydroxypropyl methylcellulose (HPMC)<br>• Hydroxyethylcellulose (HEC)<br>• Ethylcellulose (EC)<br>• Microcrystalline cellulose<br>• Hydroxypropyl methylcellulose acetate succinate | • The concept is based on the assumption that the pre-set lag time of 5-6 hours is the time required for the system to reach the colon and that transit times are a consistent parameter.<br>• Gastric emptying and gastrointestinal fluid volumes considerably influences the position of the delivery systems at the time of release.<br>• Factors such as the presence of concurrent disease states, inter- and intra-individual variability, as well as diurnal rhythms significantly influence gastrointestinal transit times. | The earliest device developed based on this principle is known as Pulsincap™, which consists of an impermeable capsule sealed at one end with a hydrogel plug. The plug hydrates and swells in contact with gastrointestinal fluids, and ejects from the capsule body after a pre-set lag time, enabling drug release. However, studies found the position in the GIT at the time of drug release varied from the stomach to the descending colon.<br>A combined pH- and time-dependent mechanism which has been evaluated proved to be plagued by similar variability in the site of drug release. | The fact that time-dependent systems are unable to sense and adapt to an individual's transit time, and merely releases drug after the pre-set lag time, irrespective of whether the system is in the colon or not, significantly limits its use. Therefore, these systems are not ideal to deliver drugs specifically to the colon for the treatment of UC. | Goo *et al.*, 1987; Coupe *et al.*, 1991; Reddy *et al.*, 1991; Wilding *et al.*, 1992; Ishibashi *et al.*, 1998; Hebden *et al.*, 1999a,b; Fukui *et al.*, 2001; Halsas *et al.*, 2001; Yang *et al.*, 2002; Crison *et al.*, 2003; Alvarez-Fuentes *et al.*, 2004; Lee *et al.*, 2004; Sangalli *et al.*, 2004. |
| **Pressure-dependent systems** | Pressure-dependent systems are based on the premise that the muscular contractions of the intestinal wall which are responsible for the grinding and propulsion of luminal contents creates a pressure differential which varies in intensity and duration throughout the GIT. | • Ethylcellulose as the hydrophobic coating<br>• Polyethylene glycols (PEG) as the suppository base. | • There is limited data regarding the various luminal pressures of the GIT, as well as whether these pressures are subject to inter- and intra-individual variability as is the case with pH and transit times. | A pressure-controlled colon delivery capsule (PCDC) composed of a drug dispersed in a suppository base, coated with a water-insoluble polymer was developed. Once swallowed, the suppository base melts and increases volume, resembling a liquid- | The exploitation of the gastrointestinal pressure is an innovative means of targeting drugs to the colon however the absence of sufficient data of the various luminal pressures places the viability of these systems into question. | Digenis and Sandefer, 1991; Ritschel, 1991; Moës, 1993; Takaya *et al.*, 1995; Basit, 2005. |

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|---|---|---|---|---|---|---|
| | Due to the reabsorption of water from the colon the viscosity of its luminal contents increases. As a result the colonic pressure increases and along with the haustral contractions, an exploitable trigger for colon targeting is provided. | | • As a result the *in vitro* evaluation of such systems poses a considerable challenge. | filled balloon. The balloon withstands the pressures of the stomach and small intestine as there is sufficient fluid in the lumen to dissipate the pressure. However, due to the increase in viscosity of colonic contents the balloon is unable to withstand the colonic pressure and consequently ruptures. | | |
| **Prodrugs** | These are pharmacologically inactive drug derivatives where the promoeity (inactive portion of the drug) minimizes absorption until the active component is released at its target site. This typically occurs as a result of enzymatic action. The promoeity thus functions to either increase the hydrophilicity of the parent drug or to increase its molecular size or both, thus minimizing absorption of the drug prior to reaching the colon. | • Sulphapyridine linked to 5-ASA via an azo bond (Sulfasalazine)<br>• p-aminohippurate linked to 5-ASA via an azo bond (Ipsalazine)<br>• 4-amino benzoyl-b-alanine linked to 5-ASA via an azo bond (Balsalazide)<br>• Dimer of 5-ASA linked via an azo bond (Olsalazine) | • Each new prodrug is classified as a new chemical entity and is therefore subjected to stringent regulations prior to market approval being granted.<br>• A universal system of promoeity-drug system is elusive thus thwarting the implementation of various different existing drugs into such a formulation. | The classic example of a prodrug is that of sulfasalazine where 5-ASA is linked to a sulpha group via an azo bond. Despite its efficacy in targeting the colon its adverse effects profile has led to the development of numerous other 5-ASA based prodrugs such as olsalazine, balsalazide and ipsalazine.<br>Other examples of non-commercialized prodrug approaches include biorecognizable HPMA copolymer conjugates of 9-aminocamptothecin (9-AC), where 9-AC is bound via spacers of amino acid residues and aromatic azo bonds or, low molecular weight saccharide-based prodrugs of dexamethasone and budesonide. | Apart from the advantage of prodrugs for targeting specific regions of the GIT, they also provide potential for patent protection as the newly synthesized compound can be patented as a new composition. | Peppercorn and Goldman, 1972; Azad-Khan *et al.*, 1977; Chan *et al.*, 1983; Svartz, 1988; Wadworth and Fitton, 1991; Cui *et al.*, 1994; Fedorak *et al.*, 1995; Sakuma *et al.*, 2001; Sinha and Kumria, 2001; Chourasia and Jain, 2003; Friend, 2005. |
| **Enzyme-dependent systems** | Enzyme-dependent systems primarily involve the fermentation of non-starch polysaccharides by colonic anaerobic bacteria. These polysaccharides are typically incorporated into delivery systems via a film coating or matrix formation, thus providing a substrate for enzymatic cleavage and subsequently the initiation of drug release in the colon. | • pectin<br>• chitosan<br>• guar gum<br>• amylose<br>• alginates<br>• chondroitin sulphate<br>• amylopectin<br>• starch<br>• xanthan gum | • Even though these polysaccharide-based systems provide an ideal substrate for enzymatic cleavage in the colon the hydrophilicity of the polymers employed often result in the swelling and premature dissolution of the polymers in the upper GIT. | In an attempt to restrain the hydrophilic characteristics of the polysaccharide polymers chemical modification or combination of the polymer with a hydrophobic polymer has been explored. This generally limits the swelling of the system in the upper GIT, whilst permitting the bacterial degradation of the system in the colon. Using this approach numerous novel systems have been investigated over two decades. With time these systems have evolved into more effective and complex technologies. | The exploitation of colonic enzymes as a trigger is the most appealing approach for targeting drugs to the colon as it relies solely on the unique enzymatic ability of colonic microflora thus enabling a more specific targeting, independent of the pH variations along the GIT and the presence of a concurrent disease condition. | Rubinstein *et al.*, 1992b; Shun and Ayres, 1992; Rubinstein *et al.*, 1993; Milojevic *et al.*, 1996; Wakerly *et al.*, 1996; Wong *et al.*, 1997; Krishnaiah *et al.*, 1998; Lorenzo-Lamosa *et al.*, 1998; Park *et al.*, 2002; Al-Saidan *et al.*, 2005; Varshosaz *et al.*, 2006; McConnell *et al.*, 2008b; Wei *et al.*, 2008. |

## 2.4 Enzyme-responsive colon-targeting

Taking advantage of the over 400 distinct species of anaerobic bacteria and their corresponding enzymatic activities in the colon a more adaptable means of colon-targeting is achieved by implementing various naturally acquired polymeric materials into various delivery systems. Extensive research in the last two decades has explicated the attractive characteristics that natural polysaccharides possess as potential substrates for enzymatic degradation and consequently for enzyme-responsive colon-targeting. Since many of these polymers are already used as pharmaceutical excipients their safety, toxicity and availability is vastly simplified. Furthermore, despite the immense promise that this formulation approach holds there are yet no marketed enzyme-responsive products of 5-ASA available.

The resistance of polysaccharides to the digestive action of gastrointestinal enzymes allows the retention of the integrity of these polymers in the physiological environments of the stomach and small intestine. However on entry to the colon they are acted upon by bacterial polysaccharidases which ultimately results in the degradation of these materials. Furthermore, these polymers consist of a large number of derivatizable groups, a wide range of molecular weights, and varying chemical compositions which result in the immense appeal in drug delivery (Chourasia and Jain, 2003). Numerous polysaccharide polymers hold promise in enzyme-responsive colon-targeting and the most popular of these are illustrated in Figure 2.4.

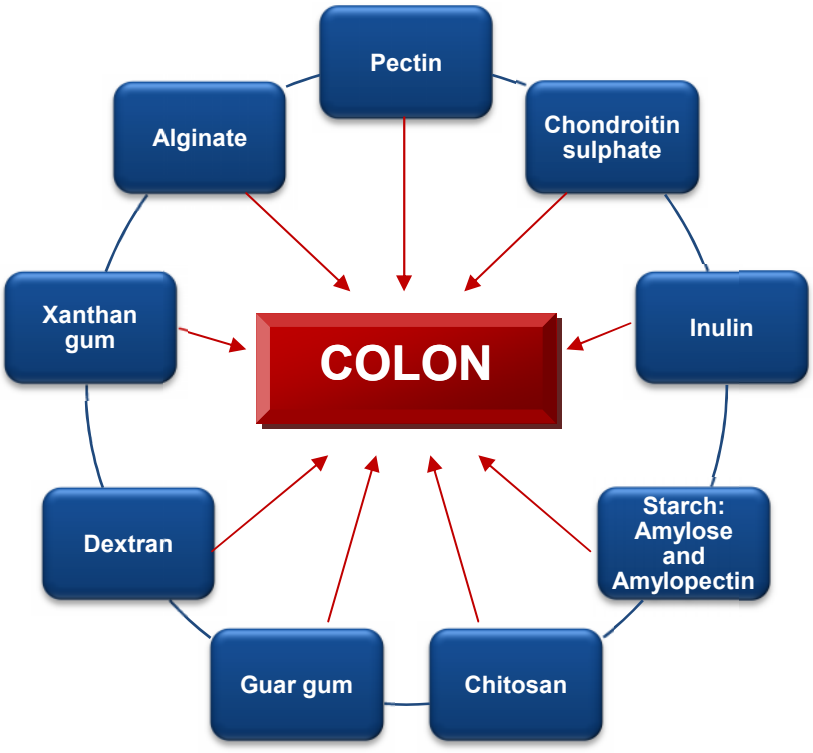



***Figure 2.4:*** *Several polysaccharide polymers that hold applicability in colon-targeting.*

Even though mostly non-starch polysaccharides have been said to be useful in colon-targeting, the components of starch have also been found to be applicable for this purpose. Starch is comprised of two major components namely, amylose and amylopectin. The former of which has been the most exploited for colon-targeted drug delivery (Basit, 2000). Initially it was believed that starch is broken down by pancreatic enzymes in the small intestine. However it was later established that certain forms of starch remain resistant to conditions of this region (Englyst *et al.*, 1992). A form of this resistant starch, retrograded amylose, escapes digestion in the small intestine and is available for fermentation by colonic polysaccharidases. Amylose is fermented by a wide range of colonic bacteria and it is believed that it is susceptible to more than 50% of the colonic bacterial population (Salyers *et al.*, 1977a, b; Macfarlane and Englyst, 1986; Milojevic *et al.*, 1996; Siew *et al.*, 2004). Like other polysaccharides the use of amylose is limited by its premature solubility in the upper GIT and an effective approach to resolving this challenge is by the combination of amylose with the hydrophobic polymer, ethylcellulose, to form a mixed coating (Milojevic *et al.*, 1996; Siew *et al.*, 2000; Leong *et al.*, 2002).

### 2.4.1 Investigated enzyme-responsive colon-targeted drug delivery systems

The inception of this concept dates back to the early 1990s and the evolution of the technology has continued to the present day. Figure 2.5 provides a snapshot of the various enzyme-responsive colon-targeted drug delivery systems that have been explored. The majority of these systems have focused on the implementation of polysaccharides that fulfill the characteristics of remaining resistant to small intestinal pancreatic degradation, whilst being susceptible to bacterial degradation in the colon (Cummings *et al.*, 1996; Englyst *et al.*, 1996). However, despite the numerous polymers explored as well as the extensive exploration into more effective enzyme-responsive colon-targeted drug delivery systems, only a selection of these have undergone *in vivo* studies in man. Furthermore, only one of these has reached large scale clinical trials namely, the COLAL$^{TM}$ system composed of amylose-ethylcellulose coated pellets.

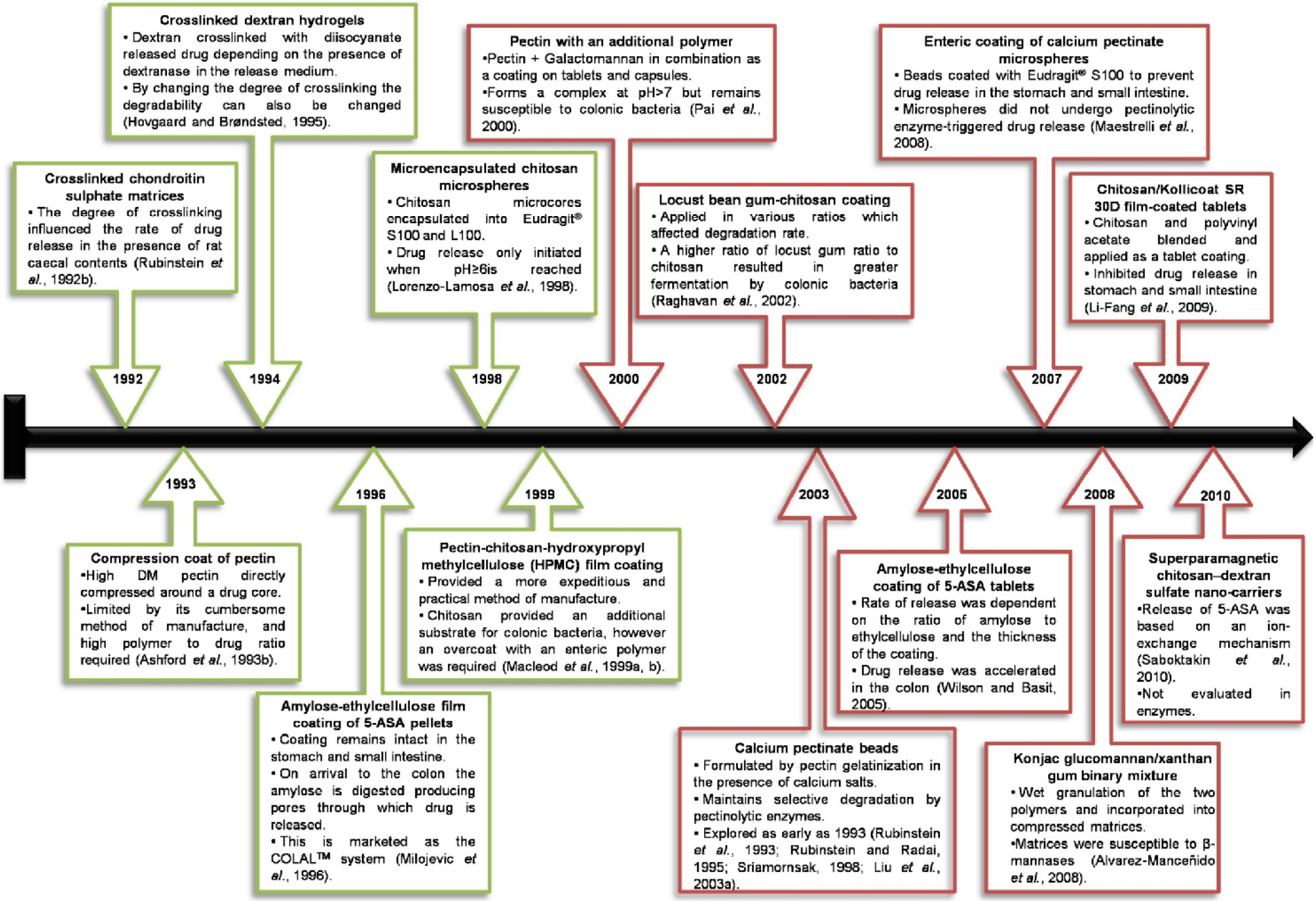



***Figure 2.5:*** *Timeline of the evolution of novel polysaccharide based enzyme-responsive colon-targeted drug delivery systems.*

### 2.4.2 Bio-relevant *in vitro* dissolution testing of enzyme-responsive colon-targeted drug delivery systems

An integral component of pharmaceutical research and the development of novel oral modulated drug delivery systems is the process of *in vitro* dissolution testing. The necessity of the dissolution method to be discriminative, reproducible, scientifically justifiable and most importantly bio-relevant arises from the decisive information that may be acquired from this study. This may include information regarding (Yang *et al.*, 2002; Yang, 2008):

- drug release kinetics;
- implications of formulation changes;
- manufacturing process changes;
- critical processing variables;
- quality assurance during clinical manufacturing;
- *in vitro/in vivo* correlation;
- impact of pH and hydrodynamic conditions on drug release characteristics;
- drug releasing mechanism involved; and
- the possibility of an *in vivo* surrogate.

Therefore, dissolution testing should be performed in physicochemically and hydrodynamically relevant conditions that simulate the environments that the delivery system may encounter. It has been proposed that the Biopharmaceutics Classification System (BCS) correlates *in vitro* product dissolution and *in vivo* systemic bioavailability for immediate release formulations (Amidon *et al.*, 1995). However no such framework exists for drugs acting locally in the GIT, especially for colon-targeted drug delivery systems. Since 5-ASA is fundamentally a locally acting drug it is recognized that its release profile is the determinate factor of an adequate local bioavailability. Furthermore, the systemic absorption of 5-ASA in the small intestine is the cause of increased side effects (Lewis, 2003). Based on these facts a method for determining the dissolution profile of 5-ASA under bio-relevant conditions is essentially the most effective means of evaluating 5-ASA delivery systems (Steed *et al.*, 1997).

Currently, the USP recommends the use of four dissolution methods which includes the basket method, paddle method, reciprocating cylinder method, or flow-through cell method. However, several constraints relating to the dissolution of ‘complex’ delivery systems have necessitated the need for modification of these methods (Pillay and Fassihi, 1999). Currently, there is no prescribed dissolution method in the pharmacopoeia for colon-targeted drug delivery systems (USP 33, NF 28). However the ideal method of dissolution testing for such a delivery system should as closely as possible mimic the *in vivo* gastrointestinal conditions

with regard to pH, bacteria, enzyme population and activity, fluid volume, and motility. Despite the difficulty in the standardization and validation of these complex specifications numerous dissolution methodologies have been reported and evaluated (Larsen *et al.*, 1989; Rubinstein *et al.*, 1992a; Molly *et al.*, 1993; Rubinstein *et al.*, 1993; Van den Mooter *et al.*, 1994; Hovgaard and Brøndsted, 1995; Milojevic *et al.*, 1996; Wong *et al.*, 1997; Prasad *et al.*, 1998; Jung *et al.*, 2000; Katsuma *et al.*, 2002; Raghavan *et al.*, 2002; Yang *et al.*, 2002; Krishnaiah *et al.*, 2003; Fetzner *et al.*, 2004; Shyale *et al.*, 2005; Sinha *et al.*, 2005; Das *et al.*, 2006; Jain *et al.*, 2007; Ji *et al.*, 2007; Pitarresi *et al.*, 2007; Schellekens *et al.*, 2007; Sinha *et al.*, 2007; Ugurlu *et al.*, 2007; Wei *et al.*, 2007).

### 2.5 Concluding Remarks

The focus of this chapter was placed on the factors of the gastrointestinal physiology that influence the design of delivery systems and in particular that of colon-targeted delivery systems. Impetus was placed on colon-targeted drug delivery systems since it essentially formed the more challenging yet beneficial aspect of the SROT. Furthermore, this chapter elaborated on the concept of stimuli-responsive drug delivery with particular emphasis on the typical polymers employed. The most commonly explored approaches to colon-targeted drug delivery were described and the most effective of these approaches identified. Consequently, the evolution of this technology namely, enzyme-responsive colonic drug delivery was illustrated from its inception in the early 1990's to the present day, with particular emphasis being placed on the novel delivery systems developed and the polymers typically employed. Finally, the importance and relevance of a bio-relevant dissolution testing system for enzyme-responsive colon-targeted drug delivery systems was described.

# CHAPTER 3

# PRELIMINARY DESIGN OF THE INNER ENZYME-RESPONSIVE COLON-TARGETED ORAL TABLET COMPONENT OF THE STIMULI-RESPONSIVE ORAL TABLET SYSTEM

## 3.1 Introduction

### 3.1.1 Approach to preliminary formulation design

An extensive range of systems are currently available that ensure the controlled release of drugs to various regions of the gastrointestinal tract (GIT), where the nature of the delivery system is dictated by the properties and dose of the drug, the reason for controlling release of the drug, as well as the interactions of constraining physiological and pathological factors (Davis, 1985). However:

> *"the rational design and evaluation of effective controlled release delivery systems needs to take into account the trinity of drug, delivery and destination",*
>
> -Davis (1985)

Davis further explicated that 'controlled release systems' also encompassed those that were intended for site-specific targeting. Essentially, this means that each factor of the trinity is interrelated and it is therefore imperative to take into consideration each aspect and their corresponding constraints when developing a new drug delivery system. Therefore, variables such as the solubility and stability of drugs, the regions of absorption throughout the GIT, the *in vitro* and *in vivo* release characteristics of the delivery system, as well as the physiological parameters of gastrointestinal transit have to be evaluated during formulation development. Expanding on this concept, Davis and Illum (1998) later proposed a '4D' approach for drug delivery system design and development (Figure 3.1).

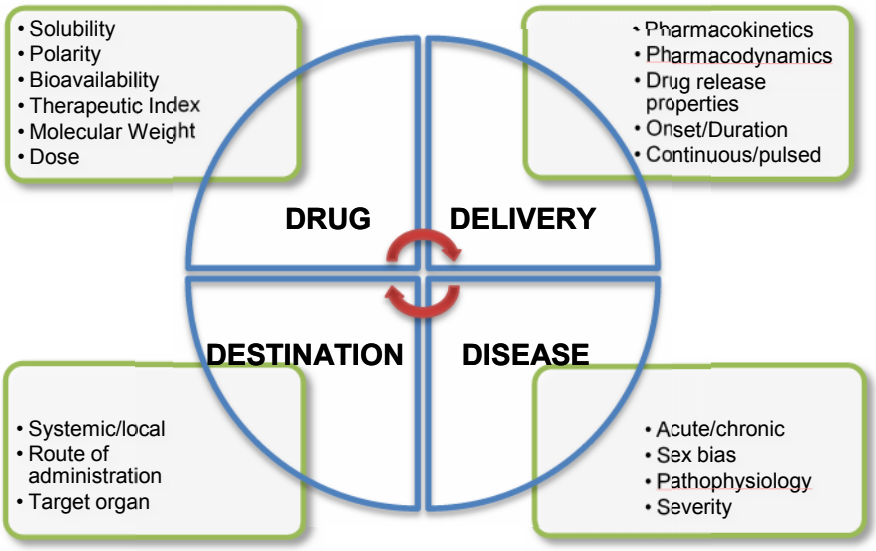



***Figure 3.1:*** *Illustration of the '4D' approach to drug delivery design and development (adapted from Pillai et al., 2001).*

This concept follows a logical approach where the *drug*, the *disease* and the *destination* should be considered first based on where in the body the drug should achieve its effect or the preferred route administration (Davis and Illum, 1998). Once these factors are addressed, only then is it appropriate to consider what the most effective mode of delivery should be. It is therefore preferable to choose a drug delivery system for a drug rather than a drug for a drug delivery system (Pillai *et al.*, 2001).

### 3.1.2 Understanding colon-targeted drug delivery

#### 3.1.2.1 Benefits of targeting drugs to the colon

Colon-targeted drug delivery offers numerous therapeutic advantages ascribed to the near neutral pH and the extended transit time of this region (Basit and Bloor, 2003; Jose *et al.*, 2009). Drugs that are typically destroyed/degraded by the digestive enzymes in the stomach and metabolized by pancreatic enzymes in the small intestine are generally minimally affected in the colon (Kinget *et al.*, 1998; Chourasia and Jain, 2003). Furthermore, due to the diminutive hydrolytic and hostile environment of the colon it exists as a promising site for the absorption of proteins and peptides, and is also a highly responsive site for the absorption of poorly absorbable drugs or for the delivery of diagnostic agents (Bai *et al.*, 1995; Watts and Illum, 1997; Tamara, 2004). In addition, disease states such as nocturnal asthma, arthritis and cardiac arrhythmias are typically affected by circadian rhythms and thus require a delay in drug release for therapeutic efficacy (Jose *et al.*, 2009).

The colon can however also be liable to numerous local pathological conditions such as constipation, Crohn's disease, Ulcerative Colitis (UC), carcinomas and infections. UC in particular is a chronic inflammatory disease that exclusively affects the colonic mucosa. However since the precise underlying cause of the disease remains doubtful, treatment is aimed at alleviating the symptoms of the disease and maintenance of states of remission (Kane, 2006). For this latter purpose 5-aminosalicylic acid (5-ASA) is recognized as the first-line treatment option but due to the mucosal confinement of inflammation this drug should ideally be targeted to the colonic mucosa, with minimization of systemic absorption (Travis, 2002; Klotz, 2005).

#### 3.1.2.2 Is there a need for yet another colon-targeted 5-ASA formulation?

Despite the efficacy of 5-ASA, outside of the clinical trial setting this drug suffers from poor patient adherence attributable to the inconvenient and multiple daily dosing schedule of large doses of the available 5-ASA containing formulations. This patient non-compliance of 5-ASA formulations essentially impacts on the therapeutic outcome which snowballs to an increased risk of symptomatic relapse, increased morbidity, increased risk of colorectal cancer and ultimately an increase in the overall cost of healthcare (Kane, 2006). It has been documented

that the compliance rates for patients suffering from chronic medical conditions that require long-term drug therapy is estimated to be 50%, or may even be as low as 30% for certain specific medical conditions (Rudd, 1995; Morris *et al.*, 2000).

Furthermore, despite the extensive range of 5-ASA containing formulations available on the market, as depicted in Chapter 1 Table 1.2, the popularity of a specific 5-ASA containing formulation varies for example, Asacol® is the choice of 65% of the UK market, Pentasa® in France (72%), Salofalk® in Germany (57%), and Asacol® in Canada (46%) (Travis, 2002). This variability is suggested to be as a direct result of the specific diagnosis and disease location of individual patients as well as the activity, side effect profile, efficacy, and cost associated with each 5-ASA containing formulation. However ultimately, the efficacy is dependent on the ability of the formulations to effectively target the affected region resulting in maximal colonic mucosal concentrations with the lowest dose and dosing schedule. Thus, the continuing research and development of newer 5-ASA formulations suggests that the ideal product has not yet been found and hints towards the need for a more effective and reproducible orally administered colon-targeted formulation (Klotz, 2005).

The ultimate goal of pre-formulation studies is thus to identify a candidate system that achieves a targeted delivery of 5-ASA to the colonic mucosa whilst preventing premature release of the drug in the stomach and small intestine (upper GIT). The anatomical and physiological considerations for the development of such a system were delineated in Chapter 1, with the conclusion in Chapter 2 that the targeting of enzymes specific to the colon would be the most efficient for achieving such site-specific targeting. As a consequence the release of 5-ASA in the colon should be initiated and controlled by the specific enzyme population in the region. Preliminary formulation design on the tablet was thus performed in a sequential manner that considered the formulation constraints whilst acknowledging the '4D' approach as theorized by Davis and Illum (1998). This process is outlined in Figure 3.2. The subsequent identification of the candidate system was then based on coherent comparison of dissolution data through each step of formulation design in order to determine if any one of the preliminary formulations could be considered for further optimization.

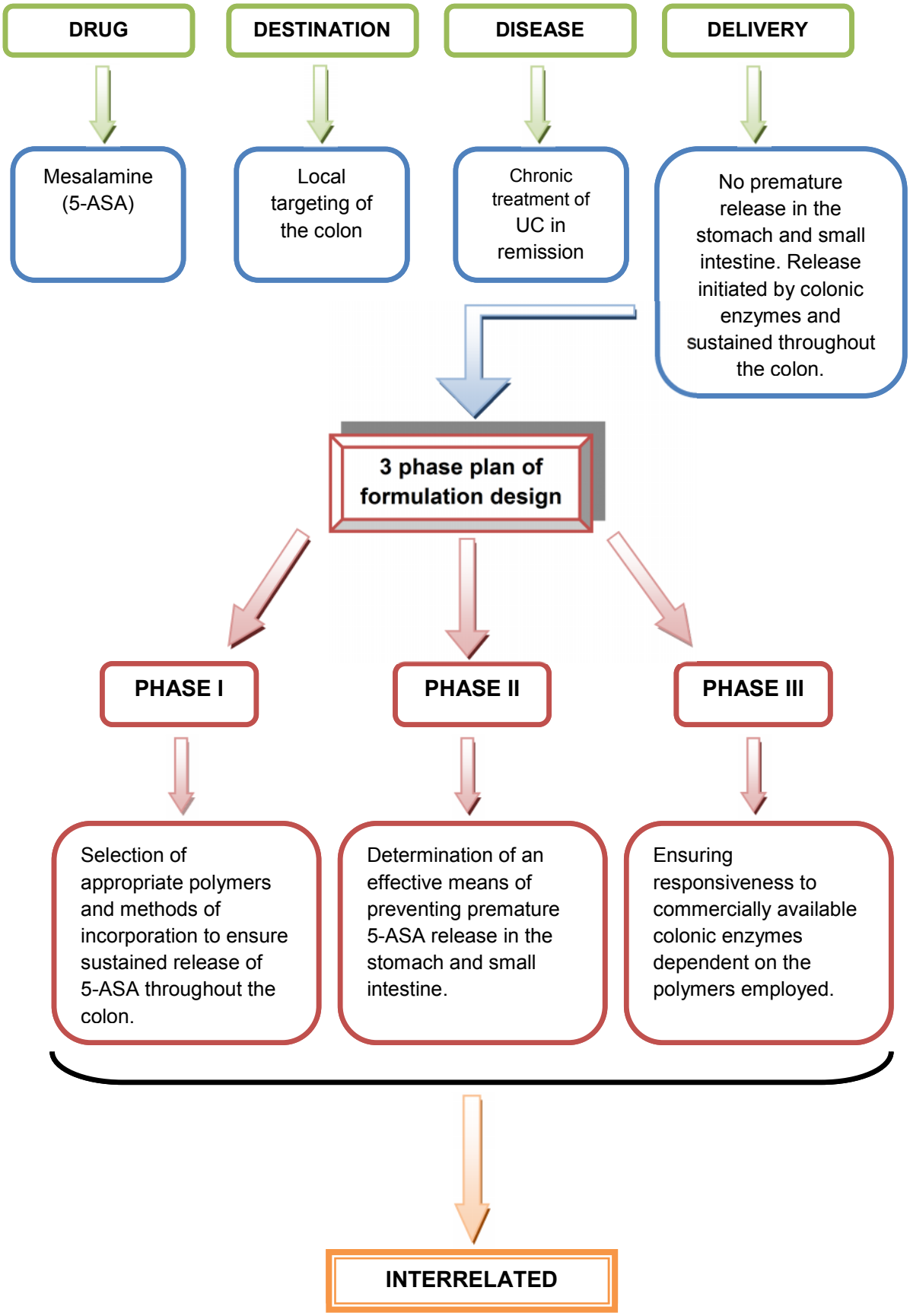



***Figure 3.2:*** *Schematic illustration of the process involved in the design of the oral enzyme-responsive colon-targeted tablet delivery system.*

**Part I: Preliminary formulation development employing DPH**

## 3.2 Materials and Methods

### 3.2.1 Materials

Mesalamine (5-ASA, approximately 99%, Mw=153.14g/mol, solubility=1.7mg/mL at 25°C in water) and diphenhydramine HCl (DPH, Mw=291.82g/mol, solubility=100mg/mL at 21.5°C in water) were obtained from Sigma-Aldrich (St.Louis, MO, USA) with the former employed for its anti-inflammatory action on the colonic mucosa and the latter to establish the flexibility of the system for incorporation of drugs with a high solubility. The polysaccharide polymers employed included chitosan (CHT) (food grade) (Wellable Group Marine Biological & Chemical Co., Ltd., Shishi City, Fujian, China), pectin (LM-105-AS) (CP Kelco, Lille Skensved, Denmark), xanthan gum (XG) from xanthomonas campestris (Fluka, Buchs, AG, Switzerland), carboxymethylcellulose sodium salt (CMC), medium viscosity (Fluka, Buchs, AG, Switzerland), sodium alginate (SA) (Protanal®) from FMC Biopolymer (Brakerøya, Drammen, Norway), and lastly pectin AM 901 (low methoxy, degree of esterification 38-44%), pectin AMID CF 005 (low methoxy, amidated citrus pectin, degree of esterification 33-39%, degree of amidation 11-17%), and pectin AMID CF 020 (low methoxy, amidated citrus pectin, degree of esterification 25-31%, degree of amidation 19-23%) all obtained from Tate & Lyle (Northriding, Gauteng, South Africa). The hydrophobic polymers cellulose acetate phthalate (CAP) and ethylcellulose (EC) were supplied by Sigma-Aldrich (St.Louis, MO, USA). The hydrophilic polymers investigated included hydroxyethylcellulose (HEC) purchased from Merck-Schuchardt (Schuchardt, Hohenbrunn, Germany), hydroxypropylmethyl cellulose (HPMC, Methocel K4M CR Premium) purchased from Colorcon (Dartford, Kent, England) and polyethylene oxide (PEO, Polyox™ WSR-303) purchased from The Dow Chemical Company (Midland, MI, USA).

Sodium tripolyphosphate (TPP) (technical grade, 85%, Mw=367g/mol) and barium chloride 2-hydrate ($BaCl_2$) (minimum 99%) were implemented as crosslinking agents and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Saarchem-Merck (Wadeville, Gauteng, South Africa) respectively. Other crosslinking agents also investigated included zinc sulphate ($ZnSO_4$, Mw=287.54g/mol) and calcium chloride dihydrate ($CaCl_2$, Mw=147.02g/mol) obtained from Rochelle Chemicals (Johannesburg, Gauteng, South Africa) and aluminium sulphate ($Al_2(SO_4)_3$, Mw=342.15g/mol) purchased from Saarchem-Merck Laboratory Supplies (PTY) Ltd. (Midrand, Gauteng, South Africa). The ethylcellulose aqueous dispersion (Surelease®, E-7-19040) was obtained from Colorcon™ (Dartford, Kent, England) and methacrylic acid-methyl methacrylate copolymer (1:2) (Eudragit® S-100) (ES-100) was obtained from Röhm Pharma Polymers (Degussa) (Darmstadt, Germany). The

ammonia solution (25%, $NH_4OH$, Mw=35.05g/mol) was supplied by Rochelle Chemicals (Johannesburg, Gauteng, South Africa) and ethanol rectified (95%) was obtained from Merck Chemicals (PTY) Ltd. (Wadeville, Gauteng, South Africa). The commercial colonic enzymes employed included pectinase from *Aspergillus aculeatus* (aqueous solution, ≥9,500U/mL) purchased from Sigma-Aldrich (Copenhagen, Denmark) and β-glucosidase from almonds (lyophilized powder, 276U/g) purchased from Fluka analytical (Gillingham, Dorset, England). All other reagents employed were of analytical grade and used without further purification. De-ionized water was obtained from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, USA).

### 3.2.2 Formulation and evaluation of the various means of achieving *in situ* crosslinking of various grades of pectin for sustained drug release

Essentially, the distinctive properties of various grades of pectin are directly related to their degree of esterification (DE) and amidation (DA). Low-methoxylated (LM) pectins (<50% esterified) are derived from highly methoxylated (HM) pectins (>50% esterified) after a process of de-esterification and when this process is carried out in the presence of ammonia some carboxylic acid groups present on the galacturonic chain become amidated resulting in amidated LM pectins (Tho *et al.*, 2005). The presence of free carboxylic acid groups in LM pectins enables the crosslinking of this polymer with various divalent cations through a postulated 'egg-box' model. In this model junction zones are created by ordered, side-by-side associations of the pectin chains where specific sequences of the galacturonic acid monomers form cavities where the cations may fit and link the polymer chains together by electrostatic and ionic bonding (Grant *et al.*, 1973; Morris *et al.*, 1982; Powell *et al.*, 1982; Fang *et al.*, 2008).

An investigation into the viability of *in situ* crosslinking as a possible mechanism for sustaining drug release in the colon, whilst employing enzyme-responsive polymers was undertaken by exploring various grades of commercially available pectin in conjunction with various crosslinking agents by the preparatory process of either wet granulation (WG) or direct compression (DC). This was performed in a sequential manner as shown in Table 3.1.

***Table 3.1:*** *Experimental formulations evaluated for the identification of the optimum crosslinking agent and method of preparation for in situ crosslinking various grades of pectin*

| Form. | Grade of Pectin | | | Crosslinking agent | | | Method of Preparation | | [1]Ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | [2]Apple | [3]Citrus | [4]Citrus | $ZnSO_4$ | $Al_2(SO_4)_3$ | $BaCl_2$ | DC | WG | 1:1 | 2:1 |
| 1 | x | | | X | | | x | | | x |
| 2 | | x | | X | | | x | | | x |
| 3 | | | x | X | | | x | | | x |
| 4 | x | | | X | | | | x | x | |
| 5 | x | | | | x | | | x | x | |
| 6 | x | | | | | x | | x | x | |
| 7 | x | | | X | | | x | | x | |
| 8 | x | | | | x | | x | | x | |
| 9 | x | | | | | x | x | | x | |

[1] *Ratio of pectin to crosslinking agent (w/w)*
[2] *Pectin AM 901*
[3] *Pectin AMID CF 005*
[4] *Pectin AMID CF 020*

Briefly, the directly compressed tablets were prepared by initially triturating the crosslinking agent in a mortar and pestle and subsequently blending it with the relevant pectin in the appropriate ratio and with a consistent quantity of model drug, DPH. Blending was performed for 10 minutes using a cube blender (Erweka AR 400, Apparatebau GmbH, Heusenstamm, Germany). Magnesium stearate (MgSt) was subsequently incorporated into the blend as a lubricant in a 0.5%$^{w}/_{w}$ concentration. This was then compressed into flat-surfaced tablets (13mmx5mm) at a compression force of 5N using the Beckmann Hydraulic Press (Beckman Instruments, Inc., Fullerton; USA).

In a similar manner, the wet granulated tablets were prepared by combining the triturated crosslinking agent with DPH and pectin. This was subsequently blended for 20 minutes using the cube blender. An ample quantity of 95% rectified ethanol was then pipetted onto the blend to allow for sufficient wetting of the powder blend so that a wet mass was formed. The wet mass was subsequently granulated through a 500µm mesh sieve (Laboratory Test Sieve, Endecotts Ltd., London, England) and granules were allowed to dry at 40°C for 3 hours in a convection oven (Memmert, Schwabach, Germany). The granules were subsequently dispersed in a 1%$^{w}/_{w}$ concentration of MgSt for lubrication and compressed into 13mmx5mm flat-surfaced tablets using the Beckmann Hydraulic Press. Each formulation was maintained at a consistent weight irrespective of the method of preparation, crosslinking agent and the ratios of such employed.

### 3.2.3 Incorporation and evaluation of various synthetic hydrophilic polymers on the drug release characteristics of an *in situ* crosslinked DPH-loaded tablet

A selection of polymers complying with a range of physicochemical and physicomechanical properties were evaluated for their efficacy in sustaining drug release. Preliminary studies evaluated their ability to sustain drug release in conditions simulating the gastric and small

intestinal environments. Initially, tablets comprising of pectin (Pectin AM 901), $BaCl_2$ and DPH were combined with the pH-sensitive synthetic polymer, ES-100, by either WG or DC. After determination of the most effective means of ES-100 incorporation, the influence of various synthetic hydrophilic polymers on the drug release characteristics of these tablets were investigated. The five formulations comprising of varying constituting polymers and their corresponding quantities are indicated in Table 3.2. The quantities are expressed as a percentage of the total weight of the formulations.

***Table 3.2:*** *Experimental formulations consisting of polymeric constituents with varying hydrophilic properties*

| Form. | ES-100 | Pectin | $BaCl_2$ | HEC | HPMC | PEO | WG | DC | DPH | MgSt |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 28.8 | 40.3 | 3.6 | | | | x | | 25.9 | 1.4 |
| 2 | 28.8 | 40.3 | 3.6 | | | | | x | 25.9 | 1.4 |
| 3 | 28.6 | 14.3 | 1.4 | | 28.6 | | | x | 26.7 | 0.4 |
| 4 | 28.6 | 14.3 | 1.4 | | | 28.6 | | x | 26.7 | 0.4 |
| 5 | 28.6 | 14.3 | 1.4 | 28.6 | | | | x | 26.7 | 0.4 |

When a DC approach of preparation was employed, the combination of polymers were thoroughly blended for 15 minutes with a cube blender which was modified to hold a number of 20mL glass vessels that each enclosed smaller quantities of the blend, thus permitting efficient blending on a smaller scale. Thereafter, each blend was compressed into flat-surfaced tablets (13mmx5mm) at 5N with the Beckmann Hydraulic Press. WG employed an identical approach to that of DC however prior to compression a sufficient quantity of 95% rectified ethanol was pipetted onto the dry blend to form a wet mass. This mass was subsequently passed through a 2mm mesh sieve (Laboratory Test Sieve, Endecotts Ltd., London, England) and the granules were allowed to dry at 40°C for 3 hours in a convection oven. The dried granules were then compressed at 5N using the Beckmann Hydraulic Press into 13mmx5mm flat-surfaced tablets.

### 3.2.4 Fabrication of crosslinked DPH-loaded SA granules dispersed within polymeric matrices for sustained drug release

In an attempt to further sustain drug release in simulated gastric and small intestinal environments investigations were ushered toward the fabrication of crosslinked DPH-loaded granules comprised essentially of an anionic polysaccharide polymer, in particular SA crosslinked with an assortment of crosslinking agents. These granules were subsequently dispersed within matrices comprised of an ideal combination of polymers as determined from previous investigations. The crosslinking agents investigated for incorporation into the granules and/or matrices included zinc gluconate ($C_{12}H_{22}O_{14}Zn$), $ZnSO_4$, $CaCl_2$ and $BaCl_2$. Furthermore, a cationic polysaccharide polymer, namely CHT in conjunction with SA was also evaluated in an attempt to further sustain drug release. More specifically, formulations were scrutinized using each of these crosslinking agents in various combinations, and

subsequent to the identification of the most effective crosslinking agent for this purpose, variations of the ratio of polymer to the specific crosslinking agent was evaluated as depicted in Table 3.3.

***Table 3.3:*** *Polymer and crosslinking agent variations in preparation of granules and matrices*

| Form. | Granules | | | Matrix | | |
|---|---|---|---|---|---|---|
| | **Polymer** | **[1]CA** | **[2]Ratio** | **Polymer** | **[1]CA** | **[2]Ratio** |
| 1 | SA | $C_{12}H_{22}O_{14}Zn$ | 2:1 | Pectin | $BaCl_2$ | 2:1 |
| 2 | SA | $BaCl_2$ | 2:1 | Pectin | $BaCl_2$ | 2:1 |
| 3 | SA | $C_{12}H_{22}O_{14}Zn$ | 2:1 | Pectin | $C_{12}H_{22}O_{14}Zn$ | 2:1 |
| 4 | SA | $CaCl_2$ | 2:1 | Pectin | $CaCl_2$ | 2:1 |
| 5 | SA | $ZnSO_4$ | 2:1 | Pectin | $BaCl_2$ | 2:1 |
| 6 | SA/CHT | $ZnSO_4$ | 3:1:1.5 | Pectin | $BaCl_2$ | 2:1 |
| 7 | SA/CHT | $ZnSO_4$ | 3:1:1 | Pectin | $BaCl_2$ | 2:1 |
| 8 | SA/CHT | $ZnSO_4$ | 4:1:1 | Pectin | $BaCl_2$ | 2:1 |
| 9 | SA/CHT | $ZnSO_4$ | 5:1:1 | Pectin | $BaCl_2$ | 2:1 |

*[1] Crosslinking agent*
*[2] Ratio of polymer to crosslinking agent (w/w) or sodium alginate:chitosan:salt (w/w/w)*

Thus, in preparation of these experimental formulations, SA or the combination of SA and CHT was thoroughly blended with the relevant triturated crosslinking agent, in the relevant polymer to crosslinking agent ratio with DPH, using the previously described modified cube blender. De-ionized water was pipetted directly onto the dry blend and was employed as a solvent to facilitate the formation of a wet mass. The wet mass was then passed through a 2mm mesh sieve and the resultant granules were allowed to dry at 40°C for 12 hours in a convection oven. Once dried, the granules were dispersed within the relevant matrix determined according to Table 3.3. All experimental formulations also included PEO, ES-100, and MgSt in quantities as determined previously. The combinations were subsequently compressed into 13mmx5mm flat-surfaced tablets at a compression force of 5N. Tablets from each formulation were maintained at a consistent weight whilst accounting for the different crosslinking agents investigated as well as the change in ratios with the inclusion of CHT in the granules.

### 3.2.5 The influence of various hydrophobic polymers and their method of incorporation into the tablet for sustaining drug release

Following the identification of the most effective crosslinking agent for forming SA-CHT granules, as well as the identification of an appropriate SA and CHT combinatory ratio, studies were undertaken to elucidate the influence of the incorporation of three hydrophobic polymers namely, EC, CAP and ES-100 for further sustaining drug release. The incorporation of these polymers were essentially performed by either 1) dry blending of the relevant polymer with the components of the DPH-loaded granules prior to granulation, or 2) ‘misting’ of the formed granules with a prescribed quantity of the relevant polymer solution, in the appropriate concentration, or 3) by employing the relevant polymer solution as the

solvent for WG. The method of incorporation of the hydrophobic polymers with the appropriate polymer ratio is depicted in Table 3.4.

***Table 3.4:*** *Methods of incorporation of hydrophobic polymers with diphenhydramine HCl-loaded granules*

| Form. | Hydrophobic polymer | Method of Incorporation | [1]Ratio of SA:CHT:$ZnSO_4$ |
|---|---|---|---|
| 1 | ES-100 | [2]ES-100 latex employed as the solvent for WG. | 4:1:1 |
| 2 | | De-ionized water employed as the solvent for WG. ES-100 latex was then 'misted' onto the moist granules and allowed to dry at 40°C for 12 hours. | 4:1:1 |
| 3 | EC | EC was dry-blended with SA, CHT and | 4:1:1 |
| 4 | | $ZnSO_4$ prior to granulation and de-ionized water employed as the solvent. | 2:2:1 |
| 5 | | Granules were prepared with de-ionized | 2:2:1 |
| 6 | | water as a solvent. [3]EC solution was misted | 1:1:1 |
| 7 | | onto the moist granules and allowed to dry at 40°C for 12 hours. | 3:2:1 |
| 8 | CAP | Granules were prepared with de-ionized | 3:2:1 |
| 9 | | water as a solvent. [4]CAP solution was 'misted' onto the moist granules and allowed to dry at 40°C for 12 hours. | 1:1:1 |

*[1] Ratio of sodium alginate:chitosan:zinc sulphate (w/w/w)*
*[2]ES-100 latex was prepared by adding 6.63g of ES-100 powder to 36.7mL of de-ionized water. The solution was stirred for 5 minutes with subsequent addition of 3.38g of a 1M ammonia solution in a drop-wise fashion to the periphery of the vortex whilst being stirred. This solution was agitated for a further 60 minutes at 25°C. The plasticizer, triethyl citrate (3.32g), was then added to the solution in the same manner as the ammonia solution and again allowed to stir for 60 minutes.*
*[3]EC solution was prepared by slowly dissolving 10g of EC in 100mL of acetone at 25°C to result in a 10%$^{w}/_{v}$ concentration.*
*[4]A 10%$^{w}/_{v}$ CAP solution was prepared by dissolving 10g of the polymer in small quantities in 100mL of acetone maintained at 25°C.*

Granules consisting of SA, CHT, $ZnSO_4$ and DPH were prepared as described previously in section 3.2.4 and in accordance with the ratios depicted in Table 3.4. This briefly involved the blending of the components in their relevant ratios with subsequent addition of the appropriate solvent for wet mass formation. The wet mass was then passed through a 2mm mesh sieve. If required, the relevant hydrophobic solution was 'misted' over the formed moist granules which were then allowed to dry at 40°C for a further 12 hours in a convection oven. The dried granules were then dispersed within matrices consisting of pectin and $BaCl_2$ (2:1 w/w ratio), PEO, ES-100 and MgSt, which were then compressed into 13mmx5mm flat-surfaced tablets at a compression force of 5N. Irrespective of the variations in the combinations of SA, CHT and $ZnSO_4$ the weight of the tablets were kept constant.

### 3.2.6 Construction of calibration curves by spectroscopic determination of DPH in simulated fluids

Calibration curves for DPH were conducted in both simulated gastric fluid (SGF) (pH 1.2; 37°C) and simulated intestinal fluid (SIF) (pH 6.8; 37°C) using a known series of concentrations of DPH (0-0.7mg/mL) in both simulated fluids. Linear curves were plotted such that the observed absorbances from spectroscopic analysis, analyzed at a wavelength of 254nm were plotted on the y-axis and the concentrations (mg/mL) on the x-axis. With the intercept set at 0, the $R^2$ values were determined to be 0.996 in SGF and 0.997 in SIF.

### 3.2.7 *In vitro* release studies of preliminary formulations containing DPH

Characterisation of DPH release from the various investigated formulations was assessed employing the USP 33 rotating paddle apparatus (Caleva Dissolution Apparatus, Model 7ST) (Caleva Ltd., Sturminster Newton, Dorset, England). The parameters employed included 900mL of the relevant test media maintained at 37°C and a paddle speed of 50rpm. The test media included either SGF (pH 1.2) or SIF (pH 6.8). The duration of dissolution studies varied for each formulation and the relevant simulated medium employed. Formulations were placed on a steel mesh assembly within each vessel to facilitate an even distribution of test medium around the formulations. Sampling was performed by manually withdrawing 5mL aliquots at the relevant time periods and sink conditions maintained by replacing the fluid withdrawn with an equivalent quantity of the relevant drug-free simulated fluid. Samples were subsequently analyzed by UV spectroscopy (Lambda 25, UV/VIS Spectrometer, PerkinElmer®, Waltham, MA, USA) at a maximum excitation wavelength of 254nm. The fractional drug release achieved at each time point was then quantified based on the calibration curves constructed in the relevant simulated fluid.

## 3.3 Results and Discussion

### 3.3.1 Calibration curves for the determination of DPH concentration in SGF and SIF

Figure 3.3 displays the calibration curves obtained for DPH in SGF (pH 1.2; 37°C) and SIF (pH 6.8; 37°C) at a maximum excitation wavelength of 254nm.

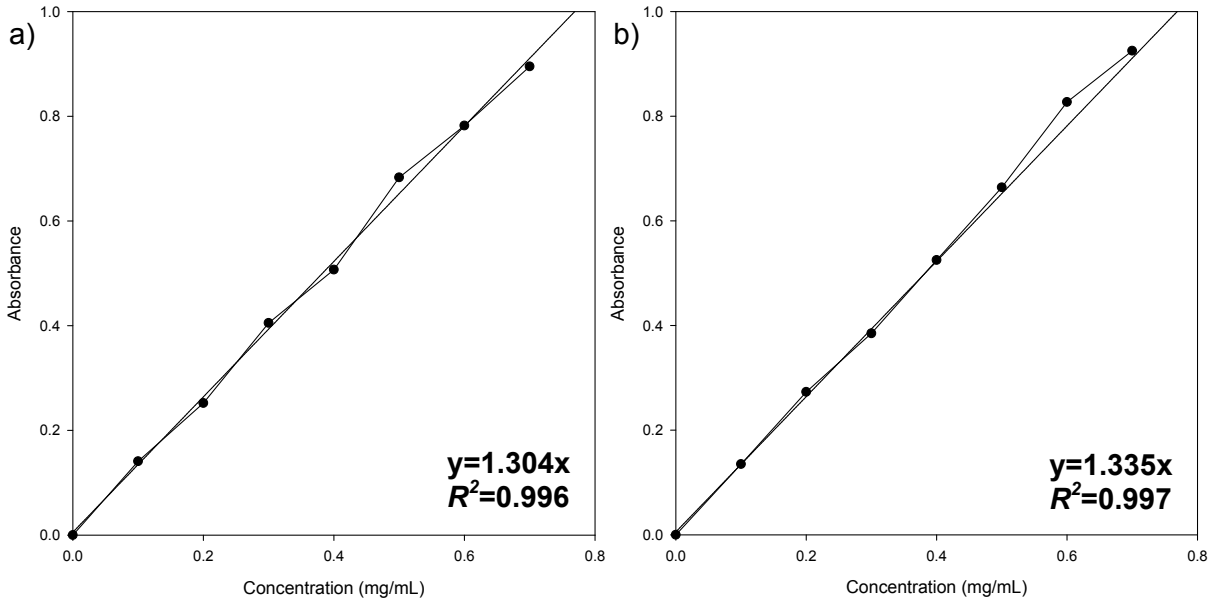



***Figure 3.3:*** *Diphenhydramine HCl calibration curves in a) simulated gastric fluid (pH 1.2; 37°C) and b) simulated intestinal fluid (pH 6.8; 37°C).*

### 3.3.2 Most appropriate grade of pectin for *in situ* crosslinking

$ZnSO_4$ was initially employed as a 'model' crosslinking agent carrying divalent cations that were capable of crosslinking the various amidated and non-amidated pectins. Release studies conducted over a 24 hour period in SGF and SIF provided vastly different release characteristics dependent on the type of pectin employed. The LM apple-derived non-amidated pectin showed a reduced DPH release over the 24 hour period in both SGF and SIF when incorporated with $ZnSO_4$ (Figure 3.4a). However this effect was more pronounced in SIF where the fractional release of DPH was only 0.616 (with $ZnSO_4$) compared to 0.975 (without $ZnSO_4$) after 24 hours.

However, the incorporation of $ZnSO_4$ into tablets comprised of amidated citrus pectin (DA 11-17%, DE 33-39%) resulted in an elevated rate of fractional drug release in both SIF and SGF. A fractional release between 0.873-0.971 (with and without $ZnSO_4$) was achieved in both SGF and SIF after 24 hours (Figure 3.4b). In contrast, tablets prepared with amidated pectin (DA 19-23%, DE 25-31%) showed an elevated rate of drug release in SGF when $ZnSO_4$ was included. However after 24 hours >0.931 fractional release was achieved from both formulations in SGF. In SIF, a reduction in the release rate in the formulations

containing $ZnSO_4$ is only evident after the first 3 hours of release studies where both formulations exhibited a ±0.263 fractional release of DPH (Figure 3.4c).

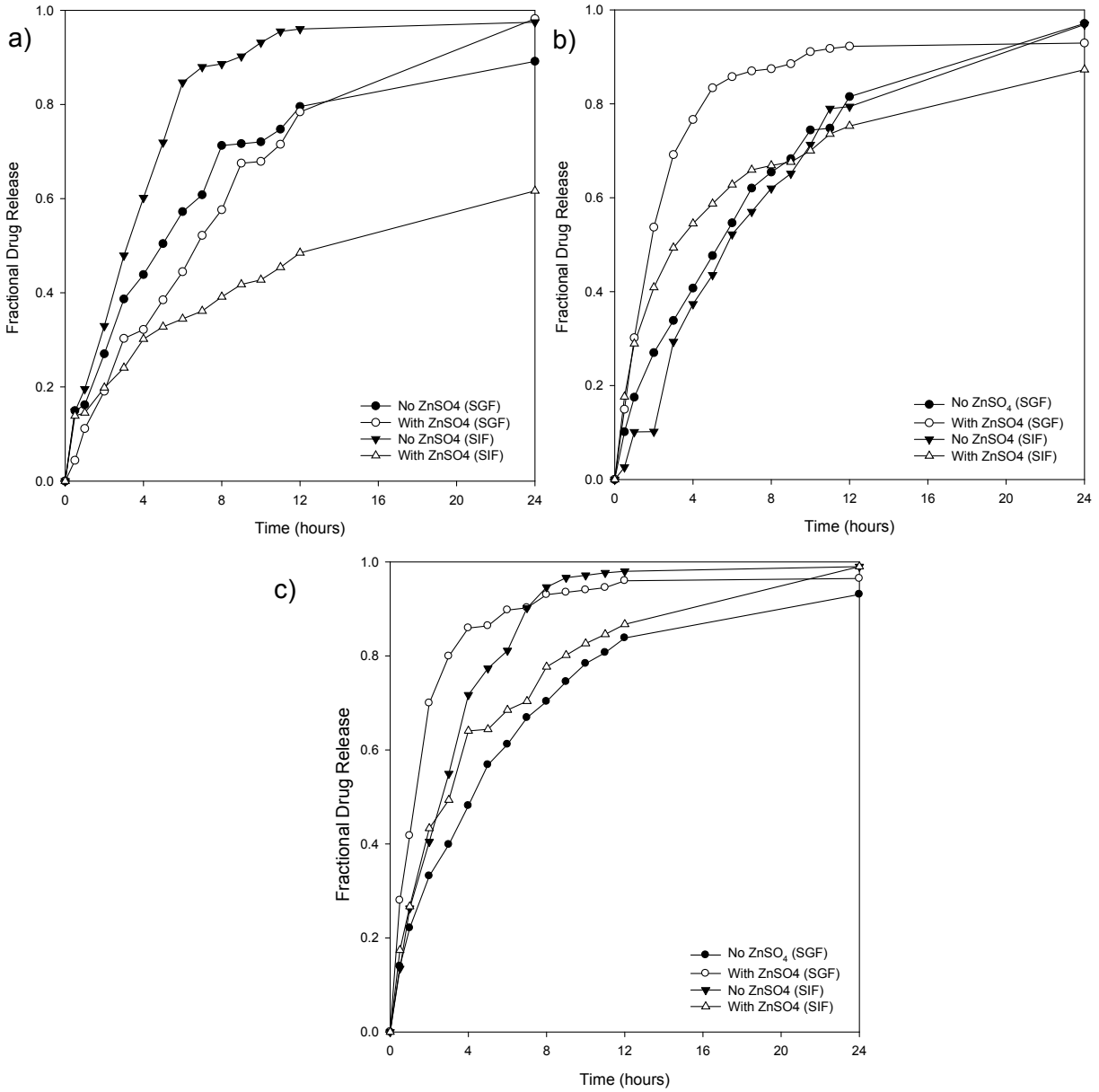



***Figure 3.4:*** *Composite release profiles of diphenhydramine HCl release from in situ crosslinked tablets of a) apple pectin (DE 38-44%), b) amidated citrus pectin (DE 33-39%, DA 11-17%), and c) amidated citrus pectin (DE 25-31%, DA 19-23%) (N=3, SD<0.08 in all cases).*

Basically, the ultimate result of crosslinking is the gelation of pectin. Crosslinking however is influenced by various intrinsic and extrinsic factors such as the degree of methoxylation, charge distribution along the backbone chain, average molecular weight, ionic strength, pH, temperature etc. (Axelos and Thibault, 1991; Clark and Farrer, 1996; Lootens *et al.*, 2003). Thus the release data acquired from the *in situ* crosslinked DPH-loaded tablets of various grades of pectin can thus be explained based on the crosslinking ability founded on two key principles namely, the influence of DA and DE, as well as the influence of solution pH on crosslinking (pH of the simulated fluid).

It is speculated that the amide groups present on the amidated pectin chain contributes to the higher formation of intra- and inter-molecular hydrogen bonds. Therefore an increase in the degree of amidation of pectin should theoretically result in an increase in the formation of strong inter- and intra-molecular hydrogen bonds, resulting in a more hydrophobic character and a consequently lower solubility (Tho *et al.*, 2005). Conversely, an increase in the DE results in an increase in the solubility of pectin. Even though crosslinking of pectin typically occurs when completely dissolved in an aqueous solution forming entanglements that result in a co-operative network at gel point, the situation for an *in situ* crosslinkable matrix should be evaluated on a more macroscopic level. Basically, on exposure of the formulation to simulated fluids, only the surface of the pectin particles are exposed and hydrated. Thus, the surface molecules of this highly swellable polymer swells, allowing the introduction of cations and the consequent crosslinking between the relevant pectin and cations on the surface of the powder particles with entrapped drug, thereby prohibiting the total dissolution of the particle and complete dissolution of the drug.

Since the apple pectin had a greater solubility in the simulated fluids and a greater degree of swelling it resulted in improved crosslinking and retardation of drug release. The two amidated pectins likely exhibited a lower solubility in the simulated fluids resulting in their ineffectual crosslinking and accelerated drug release profiles. The ability of the amidated pectin (DE 25-31%, DA 19-23%) to crosslink and thus retard drug release in SIF but not in SGF can be explained by the reduced presence of $H^+$ ions in SIF. Thus there is less interaction of the carboxyl groups of pectin with free $H^+$ facilitating the interaction of pectin with the free $Zn^{2+}$ cations from the incorporated $ZnSO_4$.

### 3.3.3 Most effective crosslinking agent for facilitating the *in situ* crosslinking of the apple pectin

Irrespective of the valence and type of salts incorporated, the method of WG was ineffective in reducing the release rate of DPH as >80% of the drug was released within the first hour in all cases (Figure 3.5a). Similarly, the DC method achieved >90% of DPH release in the first hour from the formulations containing $ZnSO_4$ and $Al_2(SO_4)_3$. A more sustained rate of fractional DPH release was achieved with the incorporation of $BaCl_2$. In the latter case, only 0.868 fractional release of DPH was achieved in 3 hours, with 0.706 fractional release in the first hour (Figure 3.5b). Apart from the initial 'burst' release of DPH within the first hour, the incorporation of $BaCl_2$ in the formulation proved to be the most promising for sustaining drug release in SGF. In addition, based on previous studies it was established that the *in situ* crosslinked apple pectin provided a reduction in the release rate in both SGF and SIF, therefore the ability of $BaCl_2$ to crosslink with pectin *in situ* in SGF may be extrapolated to its ability to crosslink in SIF.

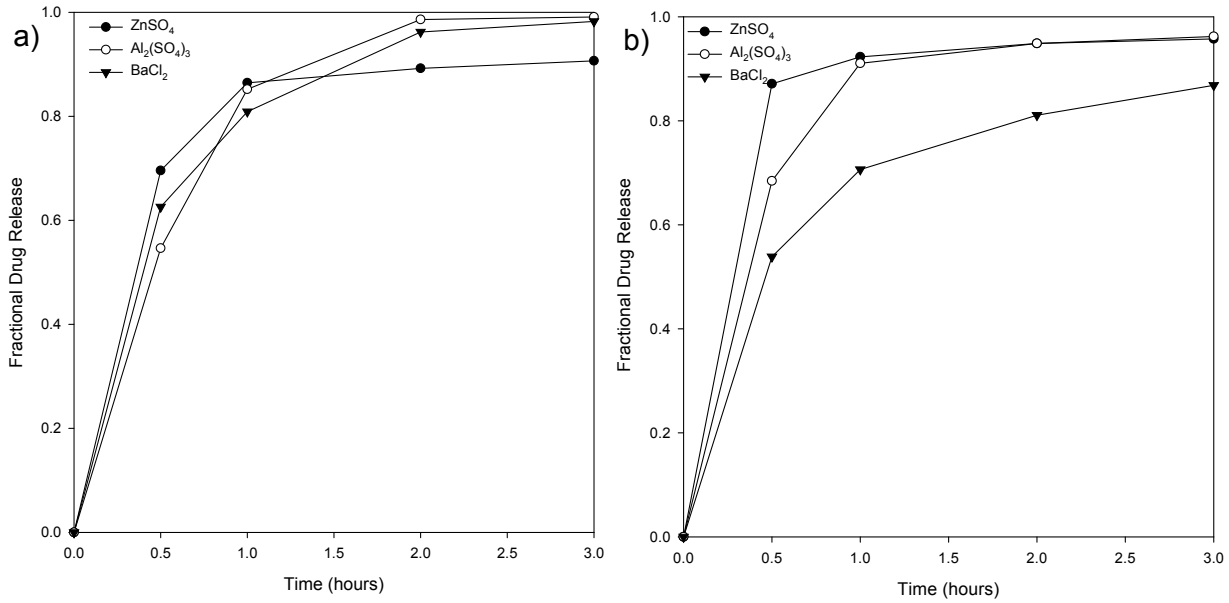



***Figure 3.5:*** *Fractional drug release profiles of in situ crosslinked diphenhydramine HCl-loaded tablets with either zinc sulphate, aluminium sulphate or barium chloride prepared by a) wet granulation and b) direct compression (N=3, SD<0.05 in all cases).*

Furthermore, the ratio of the crosslinking agent to pectin also contributes to the efficiency of *in situ* crosslinking and ultimately on the ability to sustain drug release. This is evident from the release profiles of the apple pectin *in situ* crosslinked with $ZnSO_4$ in a 2:1 (w/w) ratio (Figure 3.4a) compared to the same pectin grade crosslinked with $ZnSO_4$ in a 1:1 (w/w) ratio (Figure 3.5b). After 1 hour of dissolution studies in SGF a 0.923 fractional DPH release was achieved from tablets in a 1:1 (w/w) ratio compared to only 0.111 fractional DPH release from tablets in a 2:1 (w/w) ratio. Therefore an optimal ratio of the polymer to crosslinking agent existed and once this optimum ratio was exceeded an accelerated rate of release was achieved. Therefore, further studies evaluated the influence of varying ratios of pectin to $BaCl_2$ on the drug release characteristics, even though based on this study a 2:1 (w/w) ratio exhibited the most promise.

### 3.3.4 Effect of incorporating a pH-sensitive synthetic polymer and a synthetic hydrophilic polymer in the *in situ* crosslinked DPH-loaded tablet

Prior to the inclusion of the synthetic hydrophilic polymers, the two preparatory methods of ES-100 incorporation exhibited vastly different release profiles. WG resulted in complete DPH release being achieved in only 3 hours whereas in this same time only 0.340 fractional release of DPH was achieved when prepared by DC (Figure 3.6a). ES-100 is a random copolymer of methacrylic acid and methylmethacrylate with a distinctive property of being resistant to dissolution in pH's less than 7 (Arasaratnam *et al.*, 2000). By implementation of this polymer a drastic reduction in the release rate in SGF was expected. Even though a sustained release of DPH over the 24 hour period was achieved, the initial 'burst' release in the first 3 hours provided the basis for incorporation of a highly swellable hydrophilic polymer

that was capable of further reducing the premature release of DPH whilst maintaining the sustained release character over the 24 hour period.

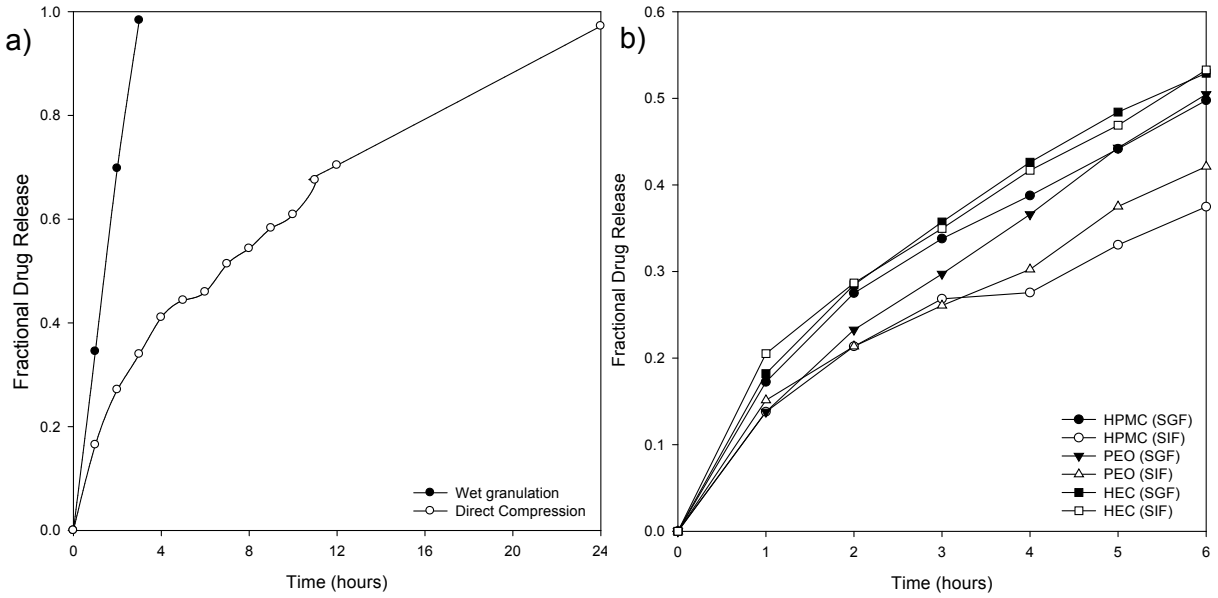



***Figure 3.6:*** *Release profiles of a) in situ crosslinked tablets with Eudragit® S-100 prepared by wet granulation and direct compression and b) in situ crosslinked tablets with Eudragit® S-100 prepared by direct compression with the incorporation of various hydrophilic polymers (N=3, SD<0.086 in all cases).*

Furthermore, even though the 11:1 (w/w) ratio of pectin to $BaCl_2$ proved to be effective in controlling drug release when in the presence of ES-100 it was uncertain whether this effect was as a result of the ES-100 or the change in the pectin to $BaCl_2$ ratio. Nevertheless, for the quantitative and qualitative comparative analysis of the influence of the hydrophilic polymer on release data a similar ratio (10:1 w/w) of pectin to $BaCl_2$ was employed.

The grade of HPMC employed in the study was of a high viscosity, fast hydrating non-ionic type typically employed for controlling the release of medium to high solubility drugs from hydrophilic matrix systems. Nevertheless, *in vitro* release studies conducted over a 6 hour period in SGF and SIF showed that HPMC provided no additional barrier to drug release in SGF (Figure 3.6b) compared to when no HPMC was present (Figure 3.6a). This is obvious based on the 0.338 fractional release from tablets with HPMC compared to a 0.340 fractional release from tablets without HPMC after 3 hours. The influence of HPMC was only seen in SIF where a 0.268 fractional release was achieved in this same time. Tablets that contained HEC showed no significant difference in the rate of drug release in both SGF and SIF with a fractional release of 0.529 and 0.533 respectively after 6 hours. The most attractive of the hydrophilic polymers employed was PEO which exhibited a 0.297 and 0.261 fractional release in SGF and SIF respectively after 3 hours (Figure 3.6b).

### 3.3.5 Effect of crosslinked DPH-loaded SA granules and crosslinked DPH-loaded SA-CHT granules on the drug release characteristics in SGF

Investigations into the most appropriate crosslinking agent for implementation in DPH-loaded SA granules led to the identification of $ZnSO_4$ as the most appropriate salt that effectively reduced the fractional release of DPH in the first 2 hours of release studies. The initial 'burst' release of DPH during this period was limited to 0.111 fractional release compared to a fractional release ranging from 0.152 to 0.268 with the other investigated salts.

Therefore, for the evaluation of SA-CHT DPH-loaded granules only $ZnSO_4$ was employed and since the mean gastric residence time of formulations typically range from 1 hour to a maximum of 3 hours, *in vitro* release studies were conducted exclusively in SGF for a period of only 2 hours (Cheng *et al.*, 2004). Based on the release characteristics of the various formulations (Figure 3.7) it is evident that incorporation of $BaCl_2$ in the matrix as well as the granules of the formulations provided the most accelerated fractional release compared to when $ZnSO_4$ was employed in the granules. The most effective reduction in the premature release of DPH was achieved from granules composed of SA, CHT and $ZnSO_4$ in a 4:1:1 (w/w/w) ratio, with only 0.106 fractional release in 2 hours compared to 0.158 fractional release from the granules in a 3:1:1 (w/w/w) ratio in the same time.

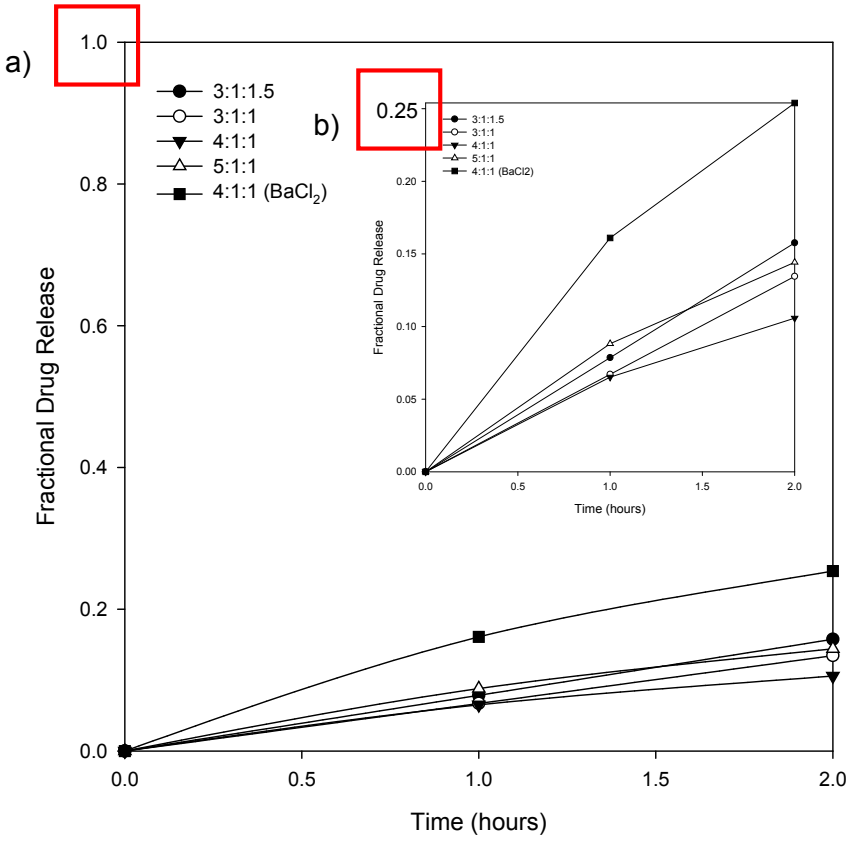



***Figure 3.7:*** *Release profiles from a) diphenhydramine HCl-loaded sodium alginate, chitosan and zinc sulphate granules dispersed within matrices of pectin, barium chloride, polyethylene oxide and Eudragit®S-100 with inset b) indicating the identical release profile except with the maximum fractional drug release scaled down (N=3, SD<0.045 in all cases).*

Furthermore, the potential formation of a polyelectrolyte complex (PEC) between the anionic SA and cationic CHT may provide a further means of reducing premature drug release (Lee *et al.*, 1998). Even though this phenomenon typically only occurs in aqueous solutions, the permeation of the acidic SGF medium into the tablet is theorized to cause solubilisation of the CHT and SA facilitating this PEC formation. Thus, a 4:1:1 (w/w/w) ratio of SA:CHT:$ZnSO_4$ is believed to strike an optimal balance between PEC formation and crosslinking of the anionic sites on the SA molecule with $ZnSO_4$.

### 3.3.6 Effect of the hydrophobic polymer and its method of incorporation on the ability of the tablet to minimize premature drug release in SGF

Since the purpose of hydrophobic polymer incorporation was to prevent/inhibit the premature release of DPH from the tablet in the upper GIT, data was collected based exclusively on the *in vitro* release behaviour of the various formulations in SGF for a period of 2 hours. The data obtained is shown in Table 3.5.

***Table 3.5:*** *Results acquired from in vitro drug release studies on the formulations incorporating the various hydrophobic polymers*

| Polymer | [1]Ratio (w/w/w) | Method of Incorporation | [2]Fractional Drug Release |
|---|---|---|---|
| **ES-100** | 4:1:1 | ES-100 latex was employed as the solvent for facilitation of wet mass formation prior to granulation. | 0.088 |
| | 4:1:1 | De-ionized water was employed as the solvent for wet granulation. ES-100 latex solution was then 'misted' onto the moist granules and allowed to dry at 40°C for 12 hours. | 0.061 |
| **EC** | 4:1:1 | EC was dry-blended with SA, CHT and $ZnSO_4$ prior to granulation and de-ionized water employed as the solvent. | 0.212 |
| | 3:2:1 | | 0.141 |
| | 2:2:1 | Granules were prepared with de-ionized as a solvent. EC solution was misted onto the moist granules and allowed to dry at 40°C for 12 hours. | 0.149 |
| | 1:1:1 | | 0.110 |
| | 3:2:1 | | 0.103 |
| **CAP** | 3:2:1 | Granules were prepared with de-ionized as a solvent. CAP solution was 'misted' onto the moist granules and allowed to dry at 40°C for 12 hours. | 0.157 |
| | 1:1:1 | | 0.243 |

[1] *Ratio of SA:CHT:$ZnSO_4$ (w/w/w)*
[2] *Fractional drug release of DPH achieved after 2 hours in SGF (N=3, SD<0.024 in all cases)*

The most effective inhibition of premature DPH release in SGF was exhibited by the tablets composed of crosslinked DPH-loaded granules which were subsequently 'misted' with the ES-100 latex. These tablets achieved a fractional DPH release of only 0.061 in 2 hours. Despite the efficacy of this formulation the method of preparation presented with numerous manufacturing challenges that resulted in an erratic and irreproducible formulation, which necessitated the identification of an alternative means of preventing premature drug release.

**Part II: Preliminary formulation development employing 5-ASA**

## 3.4 Materials and Methods

### 3.4.1 Materials

Materials used were as described in section 3.2.1.

### 3.4.2 Formulation and evaluation of novel tablet coatings comprising various polymeric combinations that prevent premature drug release in the upper GIT

Crosslinked drug-loaded granules were prepared by combining SA and CHT in a 4:1 (w/w) ratio with 200mg of 5-ASA. These components were thoroughly blended in a mortar and pestle. Instead of incorporating the crosslinking agent in a triturated, non-hydrated form, a 20%$^{w}/_{v}$ aqueous solution of $ZnSO_4$ was directly pipetted onto the dry blend to facilitate the formation of a wet mass and an even distribution of the crosslinking agent. This coagulation was subsequently manually granulated through a 2mm mesh sieve and the resultant granules were allowed to dry at 30°C for 24 hours in a convection oven. An ES-100 latex solution was subsequently 'misted' onto the dry granules and these were allowed to dry for a further 6 hours. The final dehydrated granules were then dispersed within a matrix of pectin and $BaCl_2$ in a 2:1 (w/w) ratio, PEO, XG and MgSt, which was then compressed into 13mmx5mm flat-surfaced tablets. The total weight of granules per tablet was 700mg, with a final tablet weight of 950mg. XG and PEO featured in equal quantities. The schematic illustrated in Figure 3.8 indicates the 5 different coating combinations investigated, the solution preparatory process, as well as the total weight gain (TWG) achieved after coating.

It must be noted that the contents of the tablets were consistent for all formulations and each tablet was coated by a manual dip-coating process. The basic premise of the dip-coating process essentially involved the dipping/submerging of the tablets in the coating solution, immediate removal of the tablets from the solution and subsequent drying of the tablets under a fume hood. The drying time was dependent on the concentration of the coating solutions however the volatility of the solvent expedited this process substantially. The total weight gain achieved post-coating was calculated based on Equation 3.1.

$$TWG\,(\%) = \left(\frac{x}{y} - 1\right) \times 100 \qquad \text{Equation 3.1}$$

*Where x is the weight of coated tablets and y the weight of uncoated tablets.*

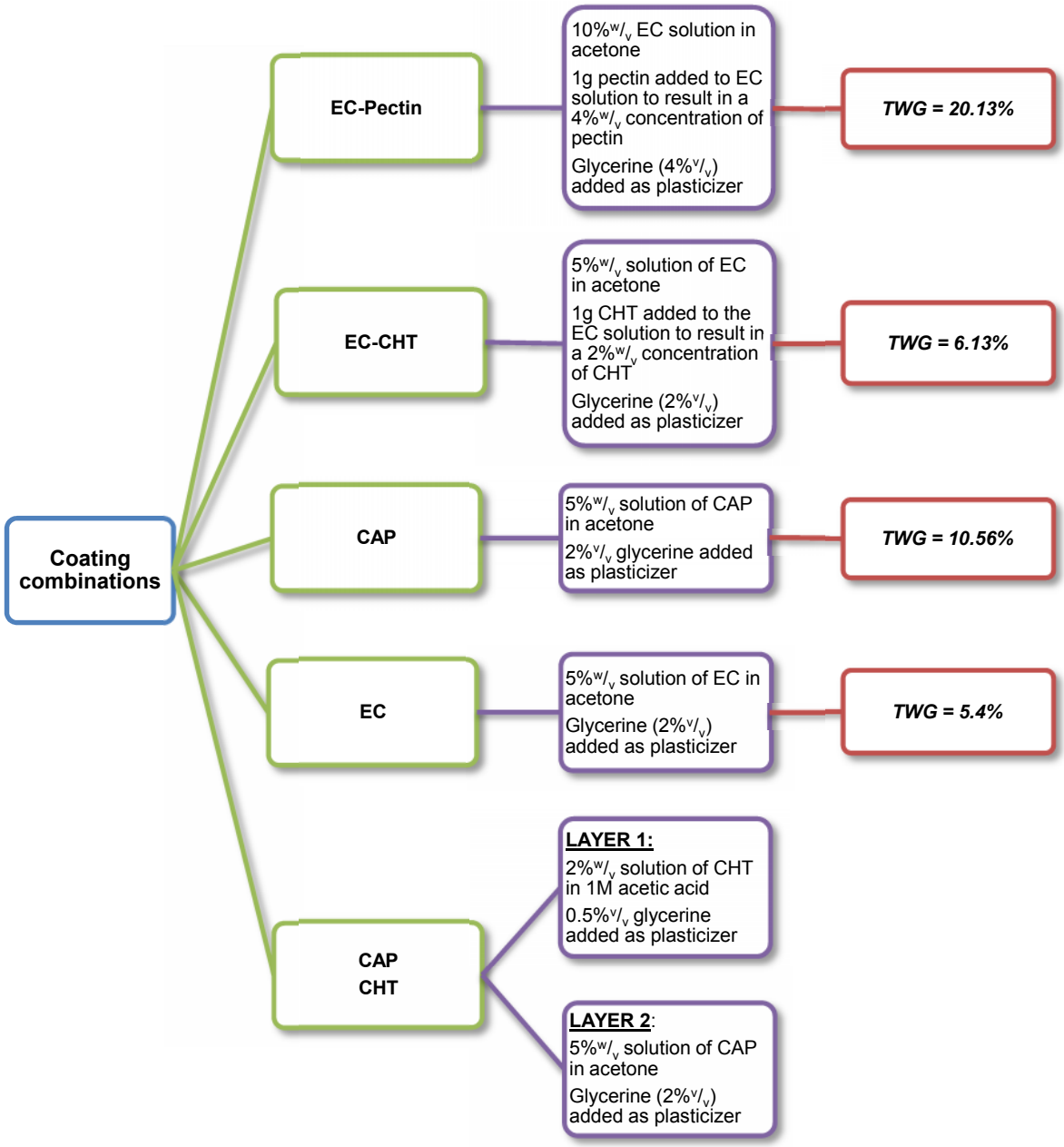



***Figure 3.8:*** *Outline of the coating combinations evaluated.*

### 3.4.3 Formulation and evaluation of crosslinked 5-ASA loaded CHT granules dispersed within a polymeric matrix and dip-coated with a bi-layer of EC and CAP

In order to increase the susceptibility of the tablet formulation to colonic enzymes, specifically to β-glucosidase, CHT drug-loaded crosslinked granules were formulated. This was performed by thoroughly blending 5-ASA with CHT using a mortar and pestle. However, prior to this process a 1M acetic acid solution was pipetted directly onto the CHT powder, in order to acidify the CHT thus forming $NH_3^+$ sites available for crosslinking. A 10%$^w/_v$ aqueous TPP solution was pipetted onto the blend and a wet coagulation was formed. This was subsequently passed through a 1mm mesh sieve and allowed to dry at 30°C for 24 hours in a convection oven. The dried granules were then dispersed within a matrix consisting of

pectin and $BaCl_2$ (2:1 w/w ratio), XG and PEO (in equal quantities) and MgSt all with a combined weight of 600mg. The total tablet weight was 1200mg, with 200mg of 5-ASA per tablet, and the granules employing 600mg of the tablet weight. The components (matrix+granules) were subsequently compressed into shallow bi-concave tablets with a diameter of 13mm and a thickness of 5mm using a mini rotary tablet press (Karnavati Mini Press II, Rimek Products, Gujarat, India). The process of dip-coating was performed as described in section 3.4.2 and the bi-layer coating consisted of an inner layer of 10%$^{w}/_{v}$ EC and an outer layer of 10%$^{w}/_{v}$ CAP.

### 3.4.4 Formulation and evaluation of novel coating solutions of a combination of pectin or CHT with an EC aqueous dispersion

Crosslinked 5-ASA loaded granules were fabricated as described in section 3.4.3. However the dried granules were dispersed within a matrix of pectin and $BaCl_2$ (2:1 w/w ratio), XG, CMC and MgSt, so that the final tablet weight was 1200mg and contained 200mg of 5-ASA. PEO was excluded from the formulation and the quantity made up with XG. The matrix and granules were then compressed into shallow bi-concave tablets using the mini rotary tablet press. An investigation was then undertaken to determine the capability of two separate polymeric solutions namely 1) a pectin-EC aqueous dispersion and 2) a CHT-EC aqueous dispersion to form an efficient tablet coating that ensured the prevention of premature drug release in the upper GIT. These coatings were applied using a pan coater apparatus.

#### 3.4.4.1 CHT-EC aqueous coating dispersion

Briefly, a 10%$^{w}/_{v}$ solution of CHT was prepared in 1M acetic acid. The EC aqueous dispersion was reconstituted by creating a dispersion of 60%$^{w}/_{w}$ of the initial solution and water (40%$^{w}/_{w}$). The water was added to the dispersion and mixed with a low shear mixer for approximately 15 minutes and was maintained at 25°C. The CHT solution (100mL) was then added to the dispersion and stirring was continued for a further 15 minutes to ensure a uniform dispersion. Gentle agitation was continued throughout the coating process to prevent sedimentation. Coating was performed according to the parameters in Table 3.6.

***Table 3.6:*** *Pan coater parameters for coating with various ethylcellulose combinations*

| Parameter | Setting |
|---|---|
| **Pump** | |
| Pump speed | 3 |
| Spray rate | 1.5g/min |
| **Coating parameters** | |
| Drum speed | 4.5 |
| Temp set point | 45°C |
| Actual Bed temp | 40°C |
| Atomizing pressure | 250kPa |

### 3.4.4.2 Pectin-EC aqueous coating dispersion with an HPMC pre-coat

An Opadry® HPMC pre-coat was investigated as a means of preventing any possible interactions between the EC aqueous dispersion and 5-ASA. The HPMC coating solution was prepared by rapidly adding 125g (10%) of Opadry® to 1125g (90%) of de-ionized water. The solution was stirred for 45 minutes prior to coating and agitation was maintained for the duration of the coating process to prevent coagulation of the solution. Coating was conducted in the pan coater apparatus where tablets were individually weighed prior to heating and combined with 100g of placebo tablets as a means of bulking up the coating batch. Opadry® was applied until a 1% TWG (based on Equation 3.1) was achieved. The coating parameters are shown in Table 3.7.

***Table 3.7:*** *Pan coater parameters employed for the Opadry® pre-coat*

| Parameter | Setting |
|---|---|
| **Pump** | |
| Pump speed | 5 |
| Spray rate | 2.8g/min |
| **Coating parameters** | |
| Drum speed | 6.5 |
| Temp set point | 45°C |
| Actual Bed temp | 40°C |
| Atomizing pressure | 250kPa |

The pectin-EC aqueous coating dispersion was prepared by blending 500g of the initial EC dispersion with 250g of a 2%$^{w}/_{v}$ aqueous pectin (LM-105-AS) solution. 375g of de-ionized water was added to this blend and stirred for 15 minutes prior to coating and was maintained at 25°C for the duration of coating. After the achievement of the 1% TWG with the pre-coat, the tablets were removed from the pan coater, re-weighed and subsequently replaced into the pan. The pectin-EC aqueous coating dispersion was then applied for a period of 2 hours based on the parameters in Table 3.6.

### 3.4.5 Development and optimization of the Wurster coating process parameters and determination of the optimal TWG of the tablets

The Wurster coating process, also known as the Fluid Bed Dryer (FBD) coating process is such that innumerable processing parameters such as the atomizing pressure, bed temperature, pump speed, spray rate, spray pattern, distance between the orifice plate and the partition, amongst others, have to be strategically optimized prior to an optimally coated tablet being achieved. The basic design of the commercially available FBD is comprised essentially of a slightly conical coating chamber (Wurster chamber) with a cylindrical partition at the bottom which regulates the flow pattern of particles within this chamber. Fundamentally, the optimization of the regions of flow within this chamber forms the foundation for the effective and uniform coating of granules, pellets and tablets. The purpose

of the air flow in the down bed region (region outside of the partition) is to maintain the substrate in a near weightless suspension with the primary goal being to have the tablet travel rapidly downward, and then be drawn horizontally toward, and ultimately into the gap at the base of the partition and orifice plate (Jones, 2008). A simplistic illustration of the flow pattern within the Wurster chamber is provided in Figure 3.9.

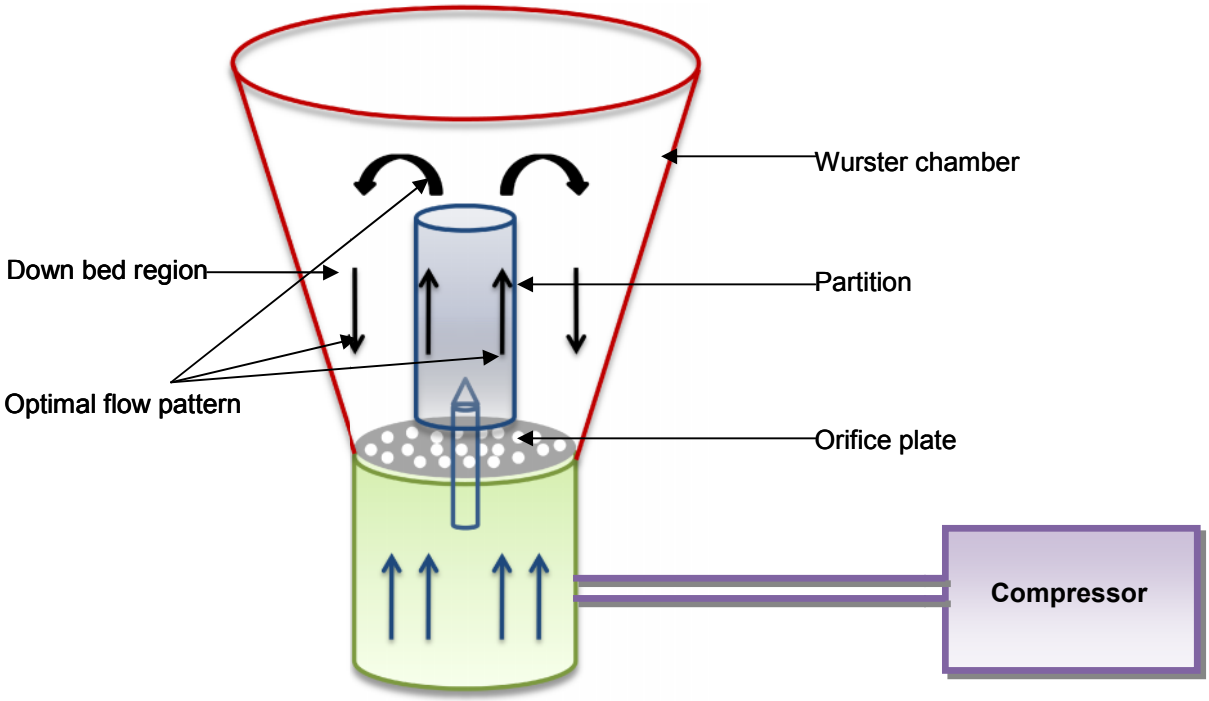



***Figure 3.9:*** *Illustration of the optimal flow pattern within the Wurster chamber.*

The pectin-EC aqueous coating dispersion was prepared as described in section 3.4.4 and the 5-ASA loaded enzyme-responsive tablets were fabricated as described in section 3.4.3. Optimization of the coating process parameters based on this coating dispersion (excluding the HPMC pre-coat) identified the following parameters: constant inlet temperature of 40°C, a flow rate of 2.54mL/min for the first hour and 3mL/min for the subsequent 3 hours, constant spray pressure of 0.1kPa and blower speed between 1700-2000 rpm.

The optimal TWG of the tablets was then determined based on the ability of the coating to prevent/minimize premature release of 5-ASA in conditions simulating the upper GIT (stomach and small intestine) whilst permitting an initiation of drug release on entry into the colon. This process was conducted based on a trial and error basis where a minimum (3.8%), maximum (22.9%) and a mid-point (8.4%) TWG was investigated.

### 3.4.6 Construction of calibration curves by spectroscopic determination of 5-ASA

Calibration curves were constructed in all the relevant dissolution media using initial stock solutions of known concentrations of 5-ASA. Six-level dilutions were performed to result in

various standard concentrations. Linear curves of the observed absorbances at the relevant wavelengths from spectroscopic analysis were then plotted against the known concentrations (mg/mL). All intercepts were set at 0 and the corresponding correlation coefficients were all greater than 0.99. A detailed explication of the concentrations, simulated fluids, wavelengths and corresponding $R^2$ values are shown in Table 3.8.

***Table 3.8:*** *Method of preparation of the various simulated media as well as the corresponding $R^2$ values on construction of the corresponding calibration curves*

| Simulated Fluid | pH | Method of preparation | Wavelength | Standard concentrations | $R^2$ |
|---|---|---|---|---|---|
| [1]Gastric fluid (SGF) without pepsin | 1.2 | 2g sodium chloride (NaCl) was dissolved in 1000mL de-ionized water. 7mL of concentrated HCl was then added to the solution to result in a solution pH of 1.2. | 301.5nm | 0-0.0313mg/mL | 0.999 |
| [1]Gastric fluid (SGF) with pepsin | 1.2 | SGF was prepared as above with the inclusion of 3.2g of pepsin (from porcine gastric mucosa) to 1000mL of the solution. | 301.5nm | 0-0.0417mg/mL | 0.997 |
| [2]Intestinal fluid (SIF) without pancreatin | 6.8 | A solution of sodium hydroxide was prepared by dissolving 4g NaOH in 1000mL de-ionized water and a separate potassium dihydrogen phosphate solution was prepared by dissolving 13.6g of the salt in de-ionized water. The two solutions were combined until a pH of 6.8 was obtained. | 334.5nm | 0-0.0313mg/mL | 0.999 |
| [3]Colonic fluid (SCFa) without enzymes | 7.4 | SCFa was prepared from identical solutions as that of SIF however the solution was made up to a pH of 7.4. | 330nm | 0-0.0313mg/mL | 0.999 |
| [3]Colonic fluid (SCFa) with pectinase and β-glucosidase | 7.4 | SCFa was prepared as above with the inclusion of 40mg of β-glucosidase and 12mL of pectinase per 1000mL of SCF. | 330nm | 0-0.0313mg/mL | 0.999 |
| [4]Colonic fluid (SCFb) without enzymes | 5.9 | SCFb consisted of an acetate buffer prepared by dissolving 8.2g of sodium acetate anhydrous in 1000mL de-ionized water. 280μL of glacial acetic acid was then added to result in a solution pH of 5.9. | 331nm | 0-0.055mg/mL | 1.000 |
| [4]Colonic fluid (SCFb) with pectinase and β-glucosidase | 5.9 | SCFb was prepared as above with the inclusion of 40mg of β-glucosidase and 12mL of pectinase per 1000mL of SCF. | 331nm | 0-0.04mg/mL | 0.999 |

*[1] SGF with and without pepsin was prepared according to USP 33, NF 28 specifications.*
*[2] SIF without pancreatin was prepared according to USP 33, NF 28 specifications.*
*[3] SCFa without enzymes was prepared according to USP 33, NF 28 specifications, and the quantity of β-glucosidase in SCFa was prescribed by Nunthanid et al., 2008, and the quantity of pectinase by Turkoglu and Ugurlu, 2002.*
*[4] SCFb without enzymes was prepared based on a modified method described Klein et al., 2008, and the quantity of β-glucosidase and pectinase was determined by Nunthanid et al., 2008 and Turkoglu and Ugurlu, 2002 respectively.*

### 3.4.7 *In vitro* release studies of the preliminary formulations containing 5-ASA

*In vitro* release studies were performed using the reciprocating cylinder apparatus (Bio-Dis® CALEVA RRT8, Caleva Ltd., Sturminster Newton, Dorset, England) which enabled a more realistic approach to the environments and conditions that the delivery system may encounter along the passage through the GIT. This apparatus employed a consistent test medium volume of 220mL, maintained at 37±0.5°C, with mesh sizes of 420µm for both the top and bottom of the glass cylinders and a dip rate of 10dpm in all experiments (Rohrs *et al.*, 1995). Formulations were subjected to SGF for a period of 2 hours, SIF for the subsequent 4 hours, and finally SCFa or SCFb for the final 18 hours. The preparatory process and pH of the simulated fluids are explicated in Table 3.8. SCF with a pH of 7.4 and 5.9 were employed since initially it was believed that the pH of the colon was slightly alkaline, with more recent evidence suggesting that the colonic pH is actually acidic due to the production of fatty acids in this region.

Sampling was conducted by manually withdrawing 5mL aliquots at the relevant time periods and sink conditions maintained by replacing the fluid withdrawn with an equivalent quantity of the relevant drug-free simulated fluid. Samples were subsequently analyzed by UV spectroscopy and drug content quantified based on the relevant standard linear curves analyzed at a $\lambda_{max}$ of 301.5nm for 5-ASA in SGF ($R^2$=0.999), 334.5nm in SIF ($R^2$=0.999) and 330nm in SCF ($R^2$=1 no enzymes, and $R^2$=0.999 with enzymes) (Nunthanid *et al.*, 2008). The fractional drug release achieved at each time point was then quantified based on the calibration curves constructed in all the necessary simulated fluids.

## 3.5 Results and Discussion

### 3.5.1 Effect of various hydrophobic polymeric coatings and their combination with pectin or CHT for inhibiting premature drug release

XG was included in formulations consisting of SA-CHT granules loaded with 5-ASA as a means of enhancing enzymatic degradation by colonic enzymes. Since it was previously established that the release characteristics of matrices with either XG or PEO were identical half of the PEO quantity in the formulation was substituted with an equivalent quantity of XG.

Due to the low TWG of the EC coated formulations (TWG=5.4%) the fractional 5-ASA release in the first 6 hours was between 0.434 and 0.481 (Figure 3.10a). This signified >40% of the loaded 5-ASA being released in the upper GIT, resulting in less than 60% of drug available for release in the colonic region. Nevertheless, the enzyme-responsiveness of the system was evident from the slight elevation in the fractional release of 5-ASA when in the presence of colonic enzymes. Formulations coated with CAP to a TWG of 10.56% showed a

slight improvement in the minimization of drug release in the upper GIT. However, most significantly was the clear evidence of enzyme-responsiveness as only 0.248 fractional release was achieved in SCFa without enzymes after 24 hours compared to complete drug release in the presence of enzymes after the same time (Figure 3.10b). Formulations coated with pectin-EC showed minimal drug release over the 24 hour period in the presence and absence of enzymes where only 0.143 fractional release of 5-ASA occurred in SCFa without enzymes and 0.129 in SCFa with enzymes. This phenomenon was likely due to the 'over-coating' of the formulations to a TWG of 20.13% which resulted in the inhibition of the permeation of colonic media into the formulation thus preventing polymeric degradation by colonic enzymes (Figure 3.10c). Formulations coated with a CHT-EC combination released 5-ASA immediately on initiation of drug release studies due to its TWG of only 6.13% thus preventing the determination of its enzyme-responsiveness (Figure 3.10d).

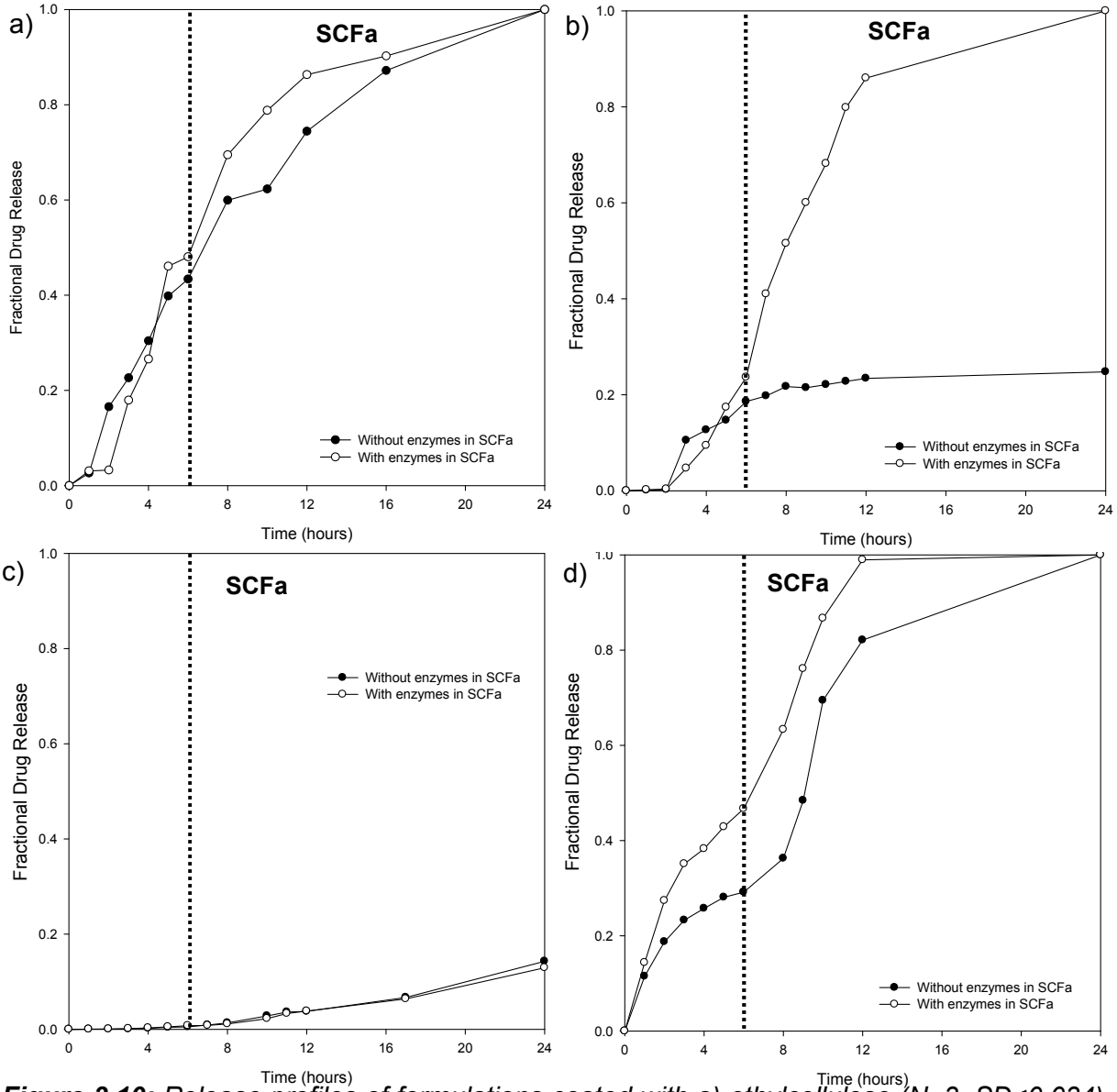



***Figure 3.10:*** *Release profiles of formulations coated with a) ethylcellulose (N=3, SD<0.034), b) cellulose acetate phthalate (N=3, SD<0.013), c) pectin-ethylcellulose (N=3, SD<0.005), and d) chitosan-ethylcellulose (N=3, SD<0.046)*.

Lastly, the bi-layer CHT-CAP coated formulations showed identical release profiles for the first 12 hours of dissolution studies irrespective of the presence of enzymes however after the 24 hour period a 0.902 fractional release was achieved in the presence of colonic enzymes compared to only 0.245 in the absence of colonic enzymes. This can be explained by the inhibition of entry of colonic media into the formulation by the outer CAP layer thus preventing the action of β-glucosidase on the CHT layer for the first 6 hours in SCFa. Eventually, when a sufficient volume of colonic media permeated through the outer CAP layer, degradation of the CHT layer was initiated resulting in the elevated drug release after the 6$^{th}$ hour in SCFa with enzymes. Despite the efficacy of this formulation as well as the CAP coated formulation in both preventing premature drug release in the upper GIT as well as showing responsiveness to colonic enzymes, these formulations could not be reproducibly manufactured. This was attributable to the flat-surfaced nature of the tablets. The 'sharp' edges of the tablets were not coated to the same degree as the rest of the tablet, forming several regions of the tablet that were unevenly coated resulting in irreproducible drug release characteristics. Furthermore, the process of dip-coating augmented the irreproducibility of the formulations.

### 3.5.2 Effect of crosslinked CHT drug-loaded granules in tablets coated with a bi-layer of EC and CAP

As a means of increasing the responsiveness of the formulation to β-glucosidase present in the colon, whilst minimizing the number of preparatory steps, SA was excluded from the granules and CHT was crosslinked with TPP. Despite the irreproducible manufacturing process of dip-coating flat-surfaced tablets, this process was nevertheless implemented on the shallow bi-concave tablets which proved to be substantially more successful due to the more rounded and less defined edges of the tablets. The improved enzyme-responsiveness of the crosslinked CHT granules is evident when comparing the release profiles attained from the tablets exposed to colonic enzymes to those that were unexposed. For the first 4.5 hours the formulations exhibited an identical release profile with less than 1% of 5-ASA being released. However from 4.5 to 11.5 hours only 0.085 fractional release of 5-ASA was achieved in the absence of enzymes compared to 0.569 fractional release in the presence of colonic enzymes (Figure 3.11).

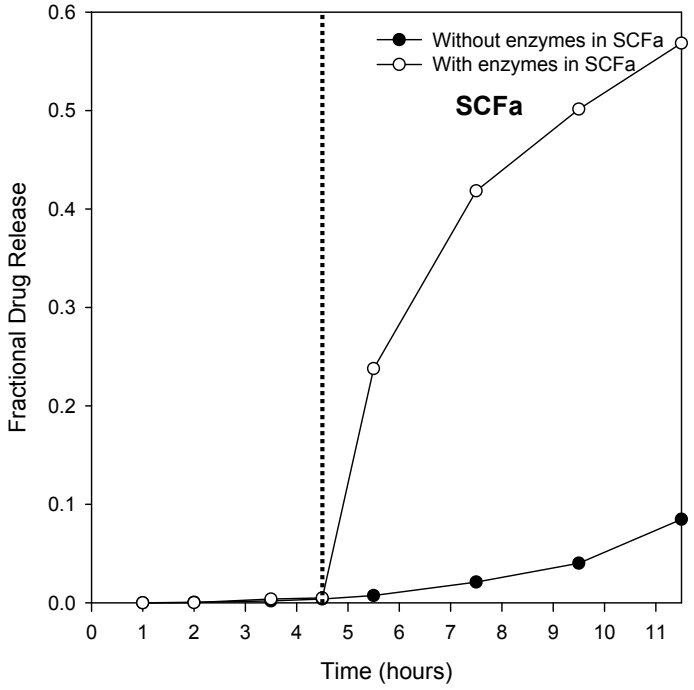



***Figure 3.11:*** *Release profiles of chitosan crosslinked granules compressed into bi-concave tablets and coated with a bi-layer of ethylcellulose and cellulose acetate phthalate (N=3, SD<0.104 in all cases).*

Despite evidence of the enzyme-responsiveness of this formulation, the tablets behaved in an erratic manner with the large standard deviations attributable to the irreproducible erosion of the coating of the tablets due to the inconsistent coating procedure. In addition, even though the 'misting' of the granules with ES-100 latex was excluded, a sustained release of 5-ASA was still achieved.

### 3.5.3 The effect of combination coatings consisting of pectin or CHT with an EC aqueous coating dispersion on the drug release profiles of tablets

The solubility of CHT in exclusively dilute acidic solutions necessitated the need to dissolve the CHT in a 1M acetic acid solution prior to its incorporation with the aqueous EC dispersion. This was imperative since the EC dispersion was essentially composed of a concentrated solution of ammonium hydroxide. Tablets undergoing coating with this solution were periodically visually inspected to determine the quality and degree of coating achieved after certain time points. During this time it was established that the coating was insufficiently adhering to the surface of the tablets, irrespective of the duration of coating application as depicted in Figure 3.12. This non-adherence is primarily attributed to the incompatibility between the acidic CHT solution and the alkaline EC aqueous dispersion which results in an unstable dispersion (Wei *et al.*, 2009). However, other possible causes for the ineffective adherence of the coating and the consequent 'chipping' of the tablets may be explained by a possible interaction between 5-ASA in the tablet and the ammonium hydroxide, or even due to the highly hydrophilic nature of the tablet core absorbing the coating solution.

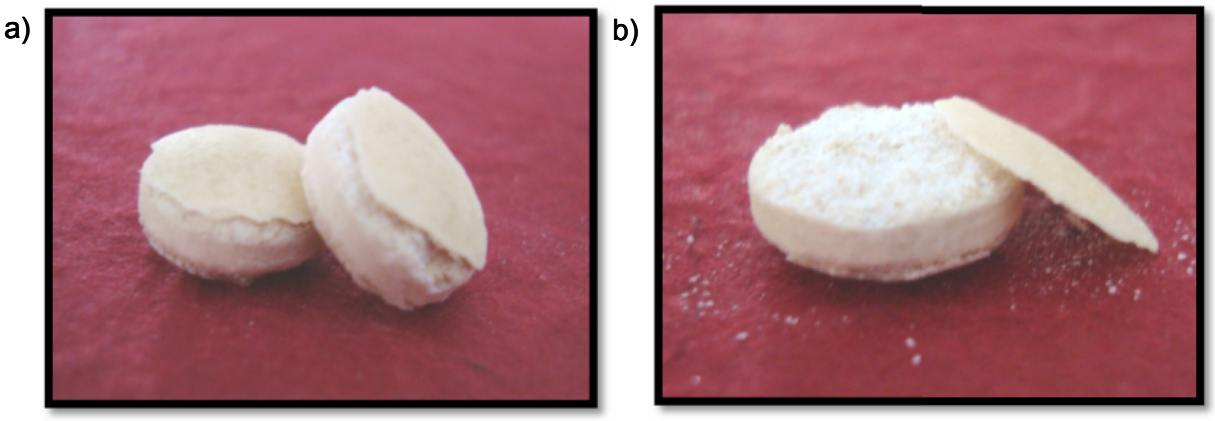



***Figure 3.12:*** *Digital images of a) chitosan-ethylcellulose coated formulations with the chipped edges, and b) with capping.*

Therefore, since the latter two possible reasons for the non-adherence of the coating could not be ruled out subsequent studies substituted the CHT solution for an aqueous pectin solution however a pre-coat of a hydrophilic polymeric solution (Opadry®) was applied to avoid the possible interaction between 5-ASA and the ammonium hydroxide. After achieving a 1% TWG with the pre-coat of Opadry® it was established that this coating layer effectively adhered to the surface of the tablets however the edges of the tablets showed evidence of chipping. Nevertheless, the pectin-EC coating solution was subsequently applied to the tablets based on identical parameters as that of the CHT-EC coating process. After 3 hours of coating, the tablets were visually inspected and showed evidence of not only coating over the pre-coat but also on the previously chipped tablet edges (Figure 3.13). The ability of this solution to effectively coat the 'naked' edges of the tablet led to the conclusion that an incompatibility between CHT and the EC aqueous dispersion definitely existed. Furthermore, the chipping of the tablets occurred as a result of the high friability of the tablets, which could be overcome by introducing a binder into the formulation.



***Figure 3.13:*** *Digital image of pectin-ethylcellulose coated formulations with chipped edges.*

### 3.5.4 Optimization of FBD coating process parameters and determination of an optimal TWG

Subsequent to the identification of the optimized coating process parameters the optimal TWG of the tablets was evaluated at 3 levels namely, a minimum, maximum and mid-point TWG. The optimal TWG was then identified based on the achievement of the ideal *in vitro* release characteristics when performed in the Bio-Dis® release rate tester and subjected to SGF for 2 hours, SIF for 3 hours and SCFb (pH 5.9) with and without commercial colonic enzymes for a further 19 hours. The tablets were coated to weight gains of 3.8%, 8.4% and 22.9% which employed coating durations of 1 hour, 3 hours and 5 hours respectively.

Essentially, the maximum TWG of 22.9% revealed no drug release in the entire 24 hour period. These tablets remained physically intact and showed no evidence of coating erosion. The minimum TWG of 3.8% revealed a maximum fractional release of 0.116 in conditions simulating the upper GIT (Figure 3.14a) and tablets coated to a weight gain of 8.4% revealed a maximum fractional release of 0.038 in identical conditions (Figure 3.14b). Furthermore, along with the reduction in the premature 5-ASA release in the first 6 hours, the tablets coated to 8.4% TWG still ensured enzyme-responsiveness of the delivery system in simulated colonic conditions. Based on these results, an optimal TWG was identified to be slightly greater (10%) than the evaluated 8.4% to ensure no drug release in SGF and SIF whilst enabling an initiation of drug release on entry to simulated colonic conditions.

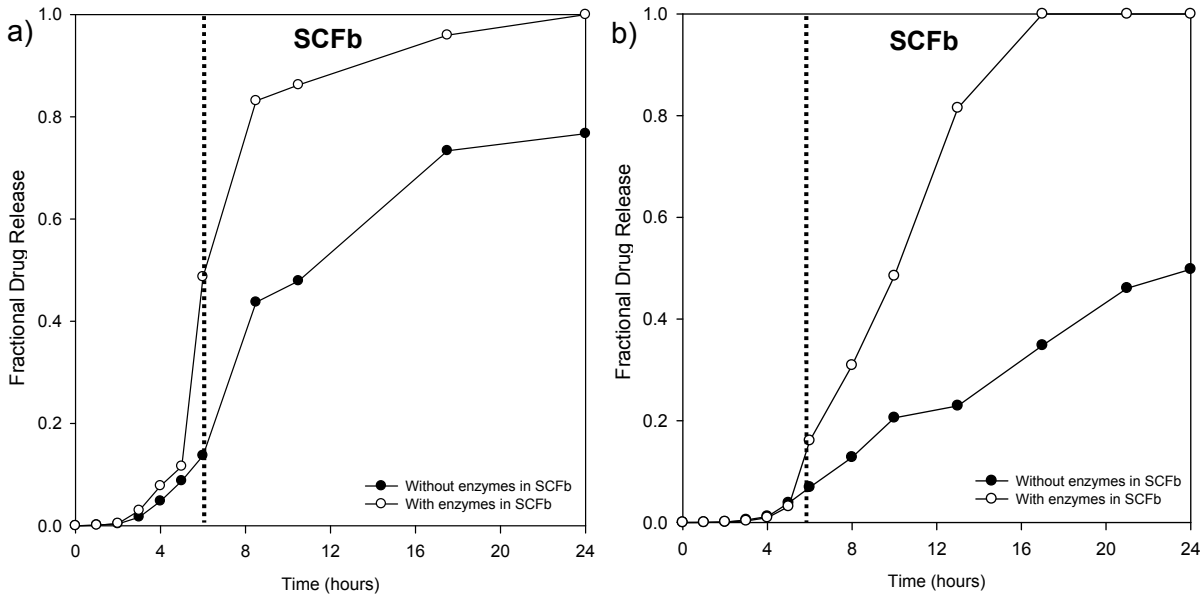



***Figure 3.14:*** *Drug release profiles of identical tablets coated to weight gains of a) 3.8% (N=3, SD<0.052 in all cases) and b) 8.4% (N=3, SD<0.016 in all cases).*

### 3.6 Concluding Remarks

Part I of preliminary formulation studies was performed with the incorporation of a highly soluble drug namely, DPH. The results obtained from these preliminary studies identified numerous crucial formulation constituents which included:

- the efficacy of apple pectin for *in situ* crosslinking;
- identification of $BaCl_2$ as the most appropriate crosslinking agent for this purpose; and
- determination of the optimal ratio of the pectin and $BaCl_2$ to be 2:1 (w/w).

Furthermore, these studies also identified numerous formulation preparatory processes and polymers that were ineffective in achieving the desired formulation characteristics by a process of elimination. As a consequence, subsequent pre-formulation studies (Part II) employed these previously identified formulation variables with the incorporation of 5-ASA. The hydrophilic polysaccharide polymers such as XG and CMC proved to be beneficial in improving not only the responsiveness of the formulations to colonic enzymes but also in providing a sustained release of 5-ASA in simulated colonic conditions. Furthermore, based on the constraining influences of a successful colon-targeted drug delivery system the prerequisite of the tablet was to achieve a minimization of 5-ASA release in the stomach and small intestine. Of the numerous investigated approaches for achieving this aim, the combinatory coating solution of pectin and EC aqueous dispersion proved to be the most promising. The optimum TWG of this coating solution was also determined to be 10%. Thus preliminary formulation development enabled the identification of a candidate system that could be further optimized to achieve the ideal enzyme-responsive colon-targeted drug release characteristics.

# CHAPTER 4

# PRELIMINARY DESIGN OF THE OUTER PEPSIN-RESPONSIVE POLYMERIC COATING COMPONENT OF THE STIMULI-RESPONSIVE ORAL TABLET SYSTEM

## 4.1 Introduction

Characteristically, chronic diarrhoea is reported in 66-92% of IBD sufferers, the severity of which is dependent on the degree of intestinal inflammation (Ciancio and Chang, 1992). Diarrhoea is essentially defined as an intestinal disorder that is characterised by an abnormal or increased frequency (>3/day) and liquidity of faecal evacuations (Talley *et al.*, 1991; Talley *et al.*, 1994; Wenzl *et al.*, 1995; Fine and Schiller, 1999; Shah *et al.*, 2001; Farthing, 2007). However there is still no consensus as to the definition of chronic diarrhoea, even though it has been suggested that the definition encompass a period long enough to allow most cases of acute diarrhoea, mostly attributed to a self-limited infection, to run its course. Generally for clinical purposes diarrhoea occurring for a period of 4 weeks can be considered as chronic (mostly due to non-infectious etiologies), with a duration of 6-8 weeks providing a greater distinction (Stanton and Clemens, 1989; Fine and Schiller, 1999).

In developed countries chronic diarrhoea has been attributed to conditions such as Irritable Bowel Syndrome (IBS), IBD, malabsorption syndrome, chronic infections and idiopathic secretory diarrhoea (Read *et al.*, 1980; Bayless, 1989; Bytzer *et al.*, 1990; Bertomeu *et al.*, 1991; Afzalpurkar *et al.*, 1992; Schiller *et al.*, 1994; Ravikumara, 2008) whereas in lesser developed countries chronic bacterial, mycobacterial and parasitic infections are the predominant causes of chronic diarrhoea. However, chronic diarrhoea derived from functional disorders, IBD and malabsorption are also common in these countries (Tandon *et al.*, 1966; Awori *et al.*, 1972; Ahmed *et al.*, 1976; Chatterjee, 1977; Kotwal *et al.*, 1978; Manatsathit *et al.*, 1985; Kent and Banks, 2010).

Essentially, the 4D approach to drug delivery system development is founded on the notion that in order to develop an effective drug delivery system, the drug, disease, destination and route of delivery must be taken into consideration as an entirety, and not each factor exclusively (Pillai *et al.*, 2001). Basically, this leads to the general tenet that a drug delivery system may be chosen for a specific drug, but a drug may not be chosen for a drug delivery system. As described in Chapter 1, section 1.7, the aim of the study was to formulate a single-unit orally administered tablet that facilitated the targeted delivery of 2 drugs to different regions of the gastrointestinal tract (GIT). Furthermore, the structure of the tablet should essentially comprise of a compressed tablet containing one of the drugs, and an outer

coating that surrounds the tablet and which contains the second drug. For the simultaneous symptomatic and remission-inducing treatment of Ulcerative Colitis (UC), it was established that 5-aminosalicylic acid (5-ASA) be preferentially targeted to the colon in response to the unique enzyme and bacterial population of the region. In addition, loperamide HCl should be targeted to the stomach and release thereof initiated and controlled in response to pepsin found exclusively in the region. Targeting of the stomach was based on the incorrect notion that the site of the therapeutic effect of loperamide HCl existed in the stomach. Nevertheless, the site-specific delivery of numerous drugs to the stomach could be beneficial for example, in the treatment of gastric ulcers using antibiotic and/or acid-suppressing agents, or further yet for drugs with narrow absorption windows.

Therefore, initial preliminary formulation development attempted to establish a suitable pepsin-responsive outer formulation that targeted and controlled the release of loperamide HCl in the stomach. However, it was subsequently established that loperamide HCl showed a high affinity for the opioid receptors (μ, δ, κ) present in the myenteric plexus and submucosal plexus of the small and large intestine (Bueno, 2005). Furthermore, it is poorly absorbed from the GIT and does not exert any systemic opioid agonist influences. Thus its the therapeutic action is dependent on the site and rate of drug delivery (Heykents *et al.*, 1974; Wuster and Herz, 1978; Niemegeers *et al.*, 1979; Van Nueten *et al.*, 1979; Reynolds *et al.*, 1984). Ideally, loperamide HCl should be targeted to the small intestine and release thereof sustained throughout this region.

Pre-formulation studies on the outer pepsin-responsive shell was thus conducted based on the two methods of exploiting pepsin present in the stomach namely, by being positively responsive to pepsin and increasing drug release in its presence (Figure 4.1a) or by being negatively responsive where drug release is inhibited in its presence (Figure 4.1b). The subsequent identification of the candidate system/s was then performed through the coherent comparison of dissolution data through each step of preliminary formulation design.

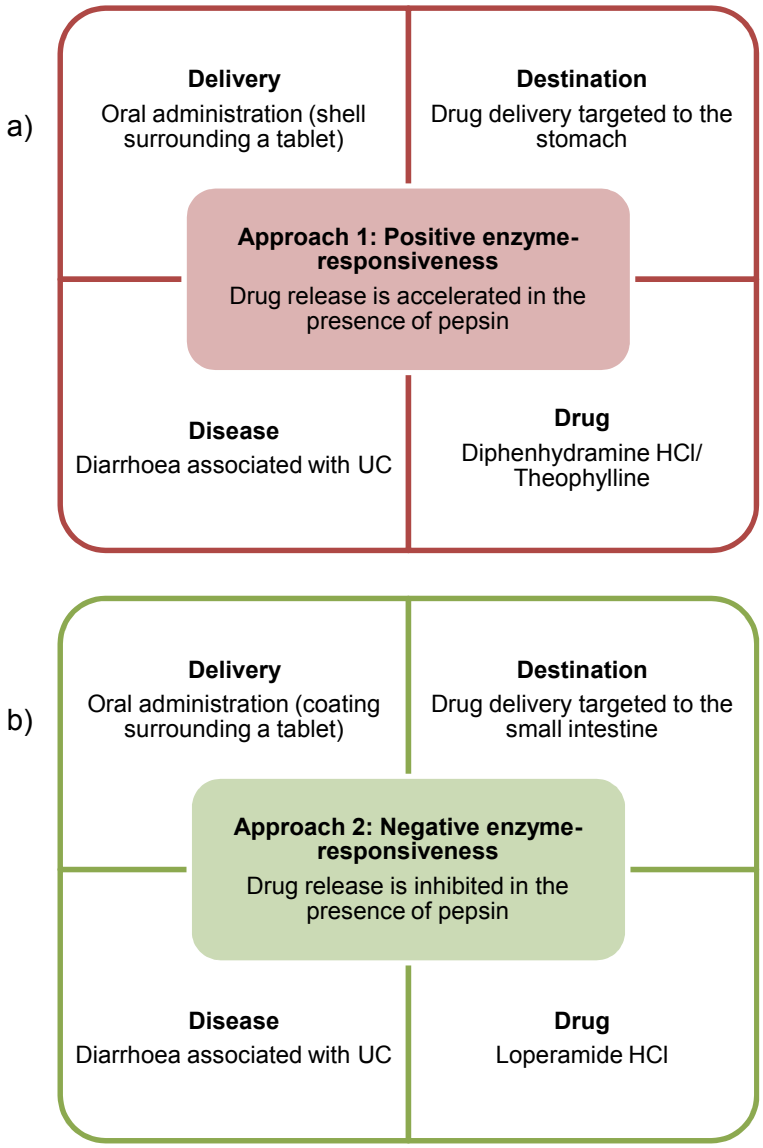



***Figure 4.1:*** *The differing 4D approaches applied in the pre-formulation of the outer pepsin-responsive shell with a) depicting the approach employed using a model drug showing positive pepsin-responsiveness, and b) depicting the approach using loperamide HCl for small intestinal drug delivery by being negatively responsive to pepsin.*

## Part I: Preliminary formulation development of positive pepsin-responsive shells

### 4.2 Materials and Methods

#### 4.2.1 Materials

Chitosan (CHT) (food grade) was purchased from Wellable Group Marine Biological & Chemical Co. (Shishi City, Fujian, China) and chitosan (medium molecular weight) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The model drugs, diphenhydramine HCl (DPH, Mw=291.82g/mol, solubility=100mg/mL at 25°C in water) and theophylline (THP, Mw=108.16g/mol, solubility=5.5-8mg/mL at 25°C in water) were obtained from Sigma-Aldrich (St.Louis, MO, USA) and Fluka, Biochemika (Buchs, AG, Switzerland) respectively. Tri-

sodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) was purchased from Saarchem-Merck (Wadeville, Gauteng, South Africa) and sodium bicarbonate ($NaHCO_3$) from Rochelle Chemicals (Johannesburg, Gauteng, South Africa). Gelatine (gel strength: 160 bloom) and glutaraldehyde (GA) (50% solution in water) were purchased from Sigma-Aldrich (Munich, Bavaria, Germany), and lactose was supplied by Saarchem-Merck (UniLAB®, Wadeville, Gauteng, South Africa). De-ionized was obtained from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, USA) and all other reagents were of analytical grade and used without further purification.

### 4.2.2 Formulation of pepsin-responsive crosslinked and non-crosslinked CHT shells

The chitosanolytic activity of pepsin had been previously established (Yalpani and Pantaleone, 1994) however more recently a study conducted on the degradation characteristics of CHT by pepsin revealed that initially this degradation reaction occurs considerably fast then gradually slows down. Furthermore, maximal chitosanolytic activity occurs when the pH of pepsin is maintained between pH 4-5 and the temperature is elevated to 55°C (Tao *et al.*, 2005). Since the pH of the stomach is between pH 1-3 and thus the pH of simulated gastric fluid (SGF) cannot be altered, an attempt was made to alter the micro-environmental pH of the CHT shells to a pH of 5.5 with the addition of $NaHCO_3$. Essentially, the CHT shell structure was formulated according to a casting and solvent evaporation technique and the resultant shells were incorporated with the inner colon-targeted tablet as depicted in Figure 4.2.

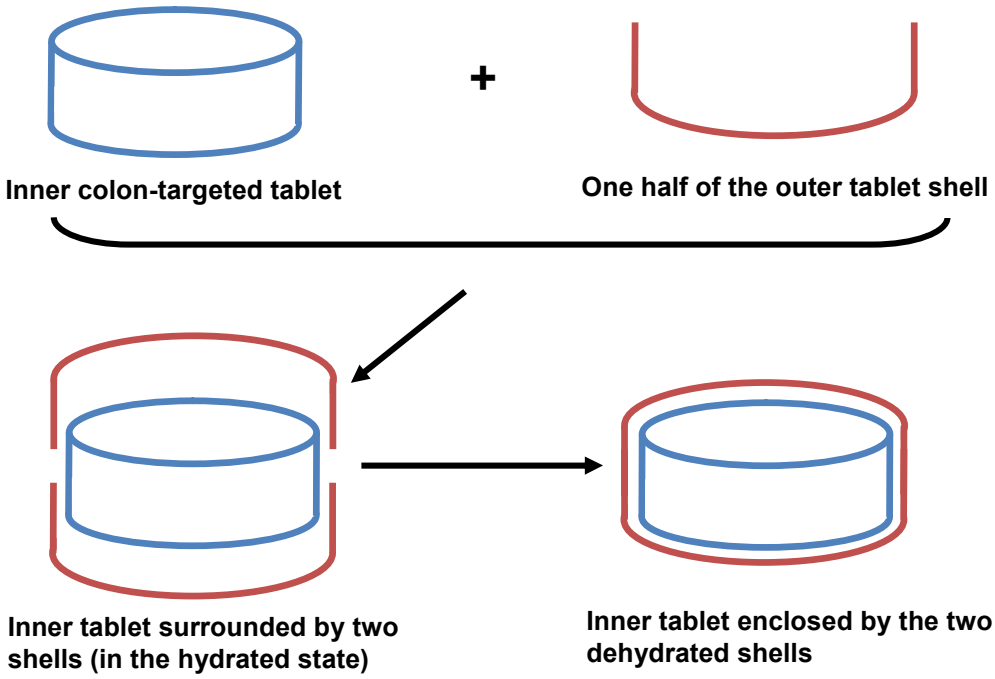



***Figure 4.2:*** *Schematic illustration of the incorporation of the outer shell with the inner colon-targeted tablet of the stimuli-responsive oral tablet system.*

The rationale for the fabrication of a shell (from 2 half shells) surrounding the inner tablet was dependent on the intended site of targeting of the drug within the GIT. Essentially, this was based on the following observation: once the shells were hydrated by the gastric medium (SGF), they swelled and detached from the inner colon-targeted tablet, these shells then remained buoyant on the gastric contents and therefore would be retained in the stomach for a longer period of time. Consequently, the increased gastric retention time of the shells would allow for a sustained release of the entrapped drug in the stomach over a longer period of time and thus an improved formulation efficacy.

These shells were fabricated by preparing a 0.1g/mL CHT solution in 1M acetic acid. The model drug DPH was then added to this solution (0.02g/mL) and agitation was permitted for 20 minutes to ensure a homogenous distribution of the drug. Subsequently, glycerol serving as a plasticizer was added to the solution in a 2:1 w/w ratio to CHT and again allowed to stir for a further 20 minutes. A sufficient quantity of $NaHCO_3$ was gradually added to this solution until a solution pH of 5.5 was achieved. 1mL aliquots of this solution were then pipetted into pre-lubricated cylindrical polystyrene moulds with individual wells with inner diameters of 13.5mm. The moulds were then placed to dry in a convection oven (Memmert, Schwabach, Germany) maintained at 40°C for 24 hours. After 24 hours in the oven, the moist shells were gently removed from each well. Essentially, each well contained one half of the shell (13.5mm diameter and 2.5mm height) to be incorporated with the tablet. The moulds were such that it allowed the CHT solution to adhere to the edges of each well resulting in the shell structure (hollow inside) (Figure 4.2).

Crosslinking of the shells was performed by submerging the shells in a $10\%^{w}/_{v}$ aqueous $BaCl_2$ solution maintained at 25°C for 1 hour. The shells were then removed from the crosslinking solution, dried under a fume-hood for 24 hours and subsequently washed with de-ionized water to remove excess surface salt. These shells were subsequently allowed to dry for an additional 6 hours. The drying time of 6 hours facilitated the formation of slightly hydrated and enlarged shells that were capable of encapsulating the flat-surfaced placebo tablets (13mmx5mm). Subsequent to the incorporation of the shells with the inner tablets, the formulations were allowed to dry under a fume-hood for a further 18 hours. This drying time enabled the sufficient drying of the shells as well as effective encapsulation and adherence of the shells to the inner tablet. The non-crosslinked shells were incorporated with the tablets in the same manner (excluding the crosslinking procedure only). The pepsin-responsiveness of these formulations was then assessed according to *in vitro* drug release specifications as described in section 4.2.8.

### 4.2.3 Formulation and evaluation of the pH-responsiveness of crosslinked CHT shells

As previously described, the concept of positive pepsin-responsiveness essentially means that there is an acceleration of drug release in the presence of the enzyme. Thus, by ensuring concurrent pH-responsiveness to the acidic environment of the stomach there will effectively be a greater acceleration in the rate of drug release. Therefore, a study was conducted to determine the pH-responsiveness of the crosslinked CHT shells to simulated gastric fluid (SGF) (pH 1.2; 37°C) and simulated intestinal fluid (SIF) (pH 6.8; 37°C). CHT shells were prepared identical to the procedure described in section 4.2.2 with the exclusion of $NaHCO_3$ from the CHT solution. Crosslinking of these shells was then conducted by submerging each shell in a 10%$^{w}/_{v}$ aqueous solution of tri-sodium citrate for 1 hour at 25°C. Thereafter the shells were removed from the crosslinking solution and underwent an identical washing procedure as well as an identical procedure for incorporation with the flat-surfaced placebo tablets as described previously. These formulations were then evaluated in SGF and SIF based on the conditions described in section 4.2.8.

### 4.2.4 Formulation and evaluation of a combination of chitosan and gelatine crosslinked and non-crosslinked shells

In an attempt to ascertain the applicability of the protein polymer, gelatine, in a pepsin-responsive formulation, two formulations of different concentrations of gelatine with the incorporation of either DPH or THP were prepared. Combinations of chitosan or CHT and gelatine in various concentrations and ratios were also formulated. DPH (high water solubility) and THP (low water solubility) were incorporated in the formulations as model drugs to determine the influence of the solubility of the drug on the release characteristics of the formulation. The formulations were prepared according to Table 4.1.

***Table 4.1:*** *Experimental formulations investigated for potential responsiveness to pepsin*

| Form. | Chitosan | | Gelatine | [1]Ratio | Drug | Crosslinked |
|---|---|---|---|---|---|---|
| | Grade | [2]Conc. | [2]Conc. | | | |
| 1 | - | - | 21% | - | DPH | No |
| 2 | - | - | 15% | - | THP | No |
| 3 | Food grade (CHT) | 20% | 21% | 76:20 | DPH | No |
| 4 | Medium Mw | 3% | 15% | 50:50 | THP | Yes |

[1] *Ratio of chitosan or CHT to gelatine (v/v)*
[2] *Concentration ($^{w}/_{v}$)*

Each formulation was prepared according to the casting and solvent evaporation technique described previously. However, prior to the casting procedure, the relevant chitosan solutions were prepared in a 1M acetic acid solution and the relevant gelatine concentrations were prepared in de-ionized water maintained at 35°C. All solutions were agitated until the relevant polymers were observed to be completely dissolved. The polymer solutions were then blended, if required, in the ratios as depicted in Table 4.1 and the relevant drug was

subsequently incorporated and the solution agitated for 30 minutes to ensure an even distribution. The subsequent casting and solvent evaporation process, as well as the incorporation of the shells with the placebo tablets was performed as in section 4.2.2.

Crosslinking of experimental formulation 4 was performed by submerging the resultant dehydrated shells in an aqueous solution of GA (5.263%$^{v}/_{v}$) maintained at 25°C for a period of 10 minutes. The shells were subsequently washed with de-ionized water and allowed to dry encapsulated over the flat-surfaced placebo tablets over 24 hours under a fume hood. The formulations were then evaluated for responsiveness to pepsin according to conditions described in section 4.2.8.

**4.2.5 Construction of calibration curves by spectroscopic determination of DPH**

The calibration curves for DPH in SGF (pH 1.2; 37°C) without pepsin as well as in SIF (pH 6.8; 37°C) were constructed as described in Chapter 3, section 3.2.6. However the calibration curve for DPH in SGF (pH 1.2; 37°C) with pepsin (3.2g pepsin per 1000mL SGF) ($R^2$=0.969) was constructed using serial dilutions of DPH in concentrations of 0-1mg/mL which were analyzed at a maximum excitation wavelength of 254nm. The linear curve was then plotted such that the observed absorbances from spectroscopic analysis were shown on the y-axis and the concentrations (mg/mL) on the x-axis.

**4.2.6 Construction of calibration curves by spectroscopic determination of THP in SGF**

Calibration curves for THP were conducted in SGF (pH 1.2; 37°C) with pepsin (3.2g pepsin per 1000mL SGF) ($R^2$=0.987) and without ($R^2$=0.996) using a known series of concentrations of THP (0-0.016mg/mL) analyzed at a maximum excitation wavelength of 280nm. Linear curves were plotted such that the observed absorbances from spectroscopic analysis were shown on the y-axis and the concentrations (mg/mL) on the x-axis.

**4.2.7 Determination of the quantity of DPH or THP present in each shell**

With the aim of ascertaining the quantity of DPH or THP entrapped within each shell, individual shells were dissolved in 100mL of SGF. After complete dissolution, the solutions were filtered through a hydrophilic 0.45µm Millipore membrane filter (Millipore® Millex-HV) (Millipore Corporation, Billerica, MA, USA) and analyzed spectroscopically (Lambda 25, UV/VIS Spectrometer, PerkinElmer®, Waltham, MA, USA) at the relevant wavelength. The absorbances attained were then fitted into the relevant calibration curve.

### 4.2.8 Assessment of the pH- and/or pepsin-responsiveness of the investigated experimental formulations by *in vitro* drug release studies

Characterisation of DPH and THP release from the various investigated formulations was assessed employing the USP 33 rotating paddle apparatus (Caleva Dissolution Apparatus, Model 7ST) (Caleva Ltd., Sturminster Newton, Dorset, England). The dissolution parameters and sampling procedure were delineated in Chapter 3, section 3.2.7 and the test media consisted of either SGF with and without pepsin and/or SIF. Sample analysis and quantification of drug release at each time point was also performed as described in section 3.2.7.

## 4.3 Results and Discussion

### 4.3.1 Evaluation of the pepsin-responsiveness of crosslinked and non-crosslinked DPH-loaded CHT shells

*In vitro* drug release studies conducted on the non-crosslinked shells revealed that in the presence of pepsin complete drug release was achieved in the first 30 minutes, however in the absence of pepsin only 0.851 fractional DPH release was achieved in this same time. Complete drug release was only achieved after 1.5 hours in the absence of pepsin (Figure 4.3). Furthermore, the degradation of CHT by pepsin is immediate and rapid with 0.937 fractional release of DPH in the first 15 minutes of release studies compared to 0.590 fractional DPH release in the absence of pepsin.

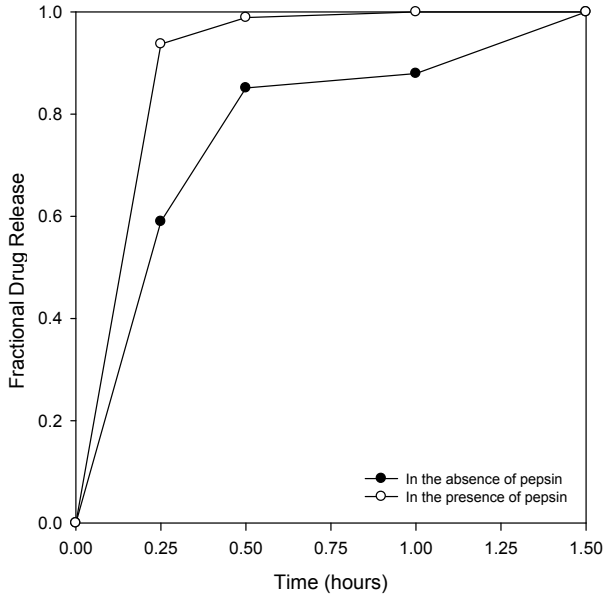



***Figure 4.3:*** *Fractional release profiles diphenhydramine HCl from non-crosslinked chitosan (food grade) shells in simulated gastric fluid with and without pepsin (N=3, SD<0.043 in all cases).*

In an attempt to sustain the release of DPH over the entire 2 hour period in SGF, the CHT shells were crosslinked with $BaCl_2$. However Figure 4.4 depicts the identical release profiles of the crosslinked shells both in the presence and absence of pepsin. These profiles serve as proof of the inefficacy of crosslinking CHT with $BaCl_2$ for ensuring sustained drug release. On the contrary the crosslinked shells became non-responsive to pepsin as is evidenced from the identical release profiles in SGF with and without pepsin.

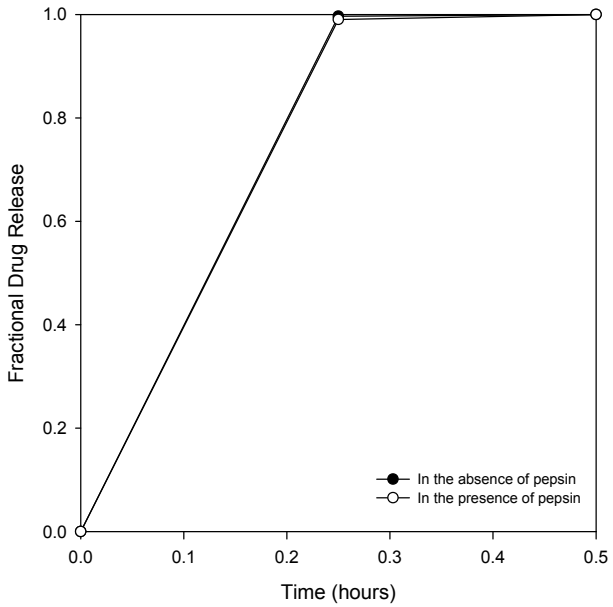



***Figure 4.4:*** *Fractional drug release profiles of diphenhydramine HCl from crosslinked chitosan (food grade) shells in simulated gastric fluid with and without pepsin (N=3, SD<0.004 in all cases).*

### 4.3.2 Effect of the pH of simulated media on the drug release characteristics of the crosslinked CHT shells

The previous study established the inefficacy of $BaCl_2$ in crosslinking the CHT shells, thus tri-sodium citrate was evaluated instead. Figure 4.5 provides evidence of the improved efficacy of this crosslinking agent since 0.974 fractional release of DPH was achieved from these shells after 2 hours in SGF compared to 0.997 fractional DPH release from the $BaCl_2$ crosslinked shells in only 15 minutes (no pepsin) (Figure 4.4). Furthermore, the shells crosslinked with tri-sodium citrate were also found to possess pH-responsive characteristics since after 4 hours in SIF only 0.535 fractional DPH release was achieved compared to 0.974 fractional release in SGF after only 2 hours.

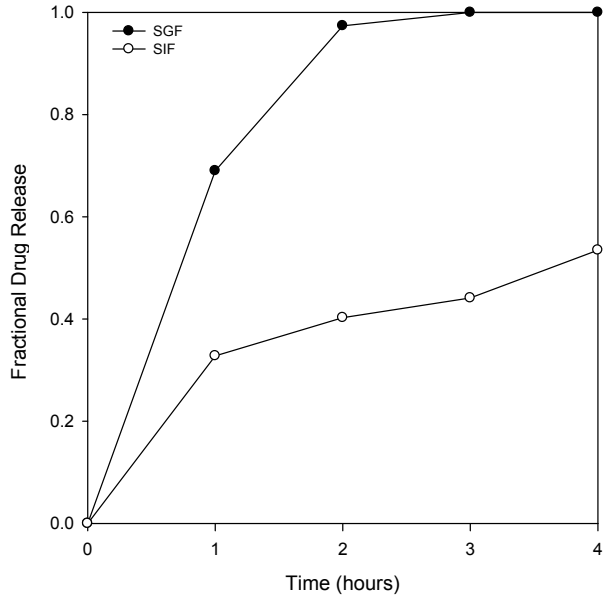



***Figure 4.5:*** *Fractional release profile of diphenhydramine HCl from crosslinked shells in simulated gastric fluid (N=3, SD<0.052 in all cases) and simulated intestinal fluid (N=3, SD<0.078 in all cases).*

It is known that the charge densities of citrate and CHT are controlled by the pH of the solutions. Essentially, at neutral and weakly acidic conditions there is a decrease in the degree of ionization of citrate and a decrease in the number of ionized carboxyl groups. At pH>6 there is a reduction in the ionization of the amine groups of CHT. Therefore the pH-responsiveness of these shells can be attributed to the ability of the shells to swell in weakly acidic conditions, resulting in an increased permeation of dissolution media into the shells and thus an increased rate of DPH release. This phenomenon is however not evident in SIF.

### 4.3.3 Effect of chitosan (medium Mw/food grade) and gelatine on the responsiveness of the crosslinked and non-crosslinked shells to pepsin

Release studies of the non-crosslinked CHT (food grade)-gelatine shells containing DPH showed that in the absence of pepsin complete drug release was achieved in 45 minutes, whereas in the presence of pepsin complete drug release was achieved in 1 hour. Despite the reduced drug release rate over 1 hour in the presence of pepsin there was a sharp spike in the fractional DPH released in the first 30 minutes (Figure 4.6).

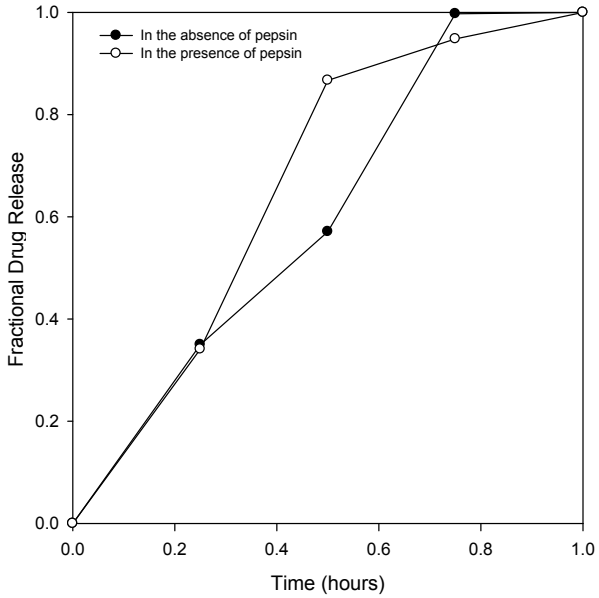



***Figure 4.6:*** *Release profiles of chitosan (food grade)-gelatine shells containing diphenhydramine HCl in simulated gastric fluid with and without pepsin (N=3, SD<0.034 in all cases).*

However, to more conclusively determine the influence of gelatine in the shells, non-crosslinked DPH-loaded gelatine shells excluding CHT were also formulated and evaluated. The release profiles indicated a distinct decrease in the rate of DPH release when in the presence of pepsin with 0.200 fractional DPH release in 15 minutes compared to 0.470 fractional release in the absence of pepsin (Figure 4.7). Therefore, the spike in DPH release from the CHT-gelatine shells can be attributed to the chitosanolytic action of pepsin on CHT whilst the reduced overall rate in drug release in the presence of pepsin can be attributed to the conflicting action of pepsin on gelatine.

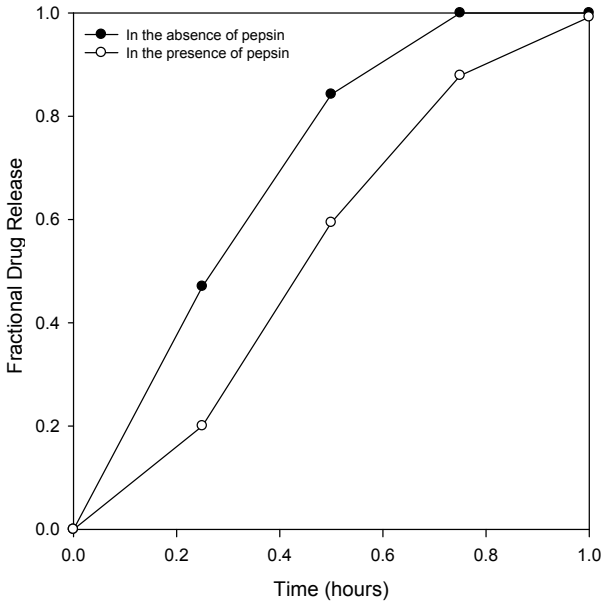



***Figure 4.7:*** *Release profiles of gelatine shells containing diphenhydramine HCl in simulated gastric fluid with and without pepsin (N=3, SD<0.016 in all cases).*

### 4.3.4 The effect of drug solubility on the pepsin-responsiveness of the shells

In the presence of pepsin, only 0.455 fractional release of THP was achieved in 1 hour compared to 0.804 in the absence of enzymes (Figure 4.8a). Furthermore, the chitosan (medium Mw)-gelatine shells containing THP were crosslinked for a short period to enable further sustaining of drug release both in the presence and absence of pepsin. Consequently, in the presence of pepsin these crosslinked shells exhibited 0.362 fractional THP release after 1 hour compared to 0.605 fractional THP release from the non-crosslinked shells (Figure 4.8b). The influence of the grade and concentration of chitosan employed on the release characteristics was not determined.

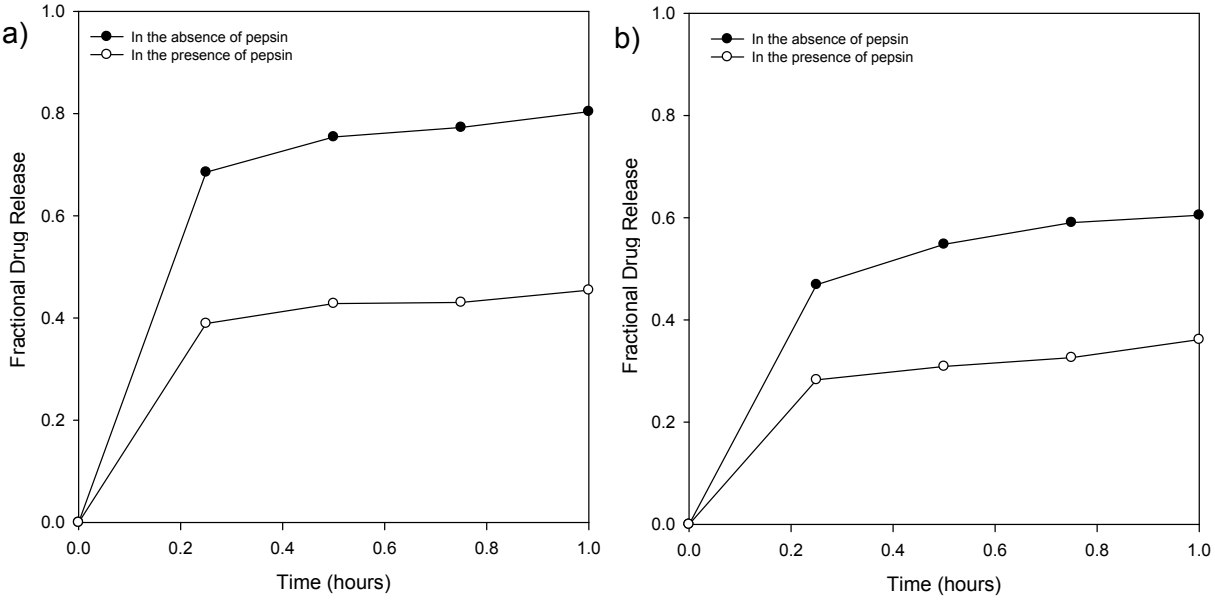



***Figure 4.8:*** *Drug release profiles of a) gelatine shells containing theophylline and b) chitosan-gelatine crosslinked shells containing theophylline in simulated gastric fluid with and without pepsin (N=3, SD<0.023 in all cases).*

## Part II: Preliminary formulation development of the negatively pepsin-responsive outer coating

Previously conducted studies based on approach 1 identified the attractive property of gelatine being negatively responsive to pepsin. This characteristic behaviour promoted the use of gelatine for achieving targeted small intestinal drug delivery by the inhibition of drug release in gastric contents containing pepsin whilst ensuring drug release in simulated small intestinal conditions. The fundamental drawback of the preparation of shells by casting polymer solutions in polystyrene moulds resulted in inconsistent shells due to the formation of bubbles in the polymer solutions after casting. Thus a more appropriate approach to forming these shells was investigated. Essentially, numerous investigations established that directly dip-coating the inner tablets with the gelatine solutions provided a more reproducible as well as aesthetically attractive formulation. Moreover this manufacturing process significantly reduced the formulation preparatory steps.

## 4.4 Materials and Methods

### 4.4.1 Materials

Gelatine, glutaraldehyde (GA), and de-ionized water were obtained as described in section 4.2.1. The additional chemicals employed included loperamide HCl (Mw=513.50g/mol) purchased from Sigma-Aldrich (St. Louis, MO, USA) and lactose supplied by Saarchem-Merck (UniLAB®, Wadeville, Gauteng, South Africa).

### 4.4.2 Formulation and evaluation of crosslinked pepsin-responsive loperamide HCl-loaded gelatine coatings for targeted small intestinal delivery

Loperamide HCl-loaded gelatine solutions were prepared by initially dissolving the water-insoluble drug, loperamide HCl (25mg), in 3mL of ethanol. Thereafter, 50mL of de-ionized water was added to the ethanol-drug solution and stirred for 30 seconds at room temperature. The relevant quantity of gelatine, to produce a 20%$^{w}/_{v}$ and 30%$^{w}/_{v}$ gelatine solution, was then gradually added to the solution and allowed to stir at 35°C for 3 hours (until a clear amber-coloured solution was attained). Shallow bi-concave placebo tablets consisting of lactose were compressed using the mini rotary tablet press (Karnavati Mini Press II, Rimek Products, Gujarat, India) and subsequently dip-coated with the gelatine solutions maintained at 35°C. The tablets were placed on a petri dish and allowed to dry under a fume hood. The drying time varied depending on the gelatine concentration employed. Each tablet was dip-coated thrice with the relevant gelatine solution.

Crosslinking of the dried tablet coating was performed in a GA-lactose mixture consisting of 20g of lactose and 5mL of GA. The 20%$^{w}/_{v}$ tablet shells were enveloped within this dry mixture maintained at 35°C for 15 hours, and the 30%$^{w}/_{v}$ tablet shells for 2, 4 and 6 hours.

### 4.4.3 Construction of calibration curves by spectroscopic determination of loperamide HCl in SGF

Calibration curves for loperamide HCl were conducted in SGF (pH 1.2; 37°C) with (3.2g pepsin per 1000mL SGF) and without pepsin using a known series of concentrations of loperamide HCl (0-0.008mg/mL) in both simulated fluids. Linear curves were plotted such that the observed absorbances from spectroscopic analysis were plotted on the y-axis and the concentrations (mg/mL) on the x-axis. The regression coefficients for the calibration curves were 0.982 in the presence of pepsin and 0.996 in the absence of pepsin.

### 4.4.4 Determination of the quantity of drug within each non-crosslinked gelatine coating

With the aim of ascertaining the quantity of loperamide HCl entrapped within each gelatine coating, the non-crosslinked 20%$^{w}/_{v}$ and 30%$^{w}/_{v}$ shells were dissolved in 100mL of SGF. After complete dissolution the solutions were filtered through a hydrophilic 0.45µm Millipore membrane filter and analyzed spectroscopically at a maximum excitation wavelength of 260nm. The absorbances attained were then fitted into the constructed calibration curve.

### 4.4.5 Assessment of the pepsin-responsiveness of the crosslinked gelatine shells

*In vitro* release studies were performed using the reciprocating cylinder apparatus (Bio-Dis® CALEVA RRT8, Caleva Ltd., Sturminster Newton, Dorset, England) and according to parameters specified in Chapter 3, section 3.4.7. However, the experimental formulations were only subjected to SGF with and without pepsin for a period of 2 hours. Sample withdrawal, UV analysis at a wavelength of 260nm and drug release quantification was performed as described in section 3.4.7.

## 4.5 Results and Discussion

### 4.5.1 The effect of gelatine concentration and duration of crosslinking on the pepsin-responsiveness of the outer coating

*In vitro* release studies of the crosslinked tablet coatings consisting of 20%$^{w}/_{v}$ gelatine showed complete drug release within 30 minutes in the absence of pepsin however at this same time in the presence of pepsin only 0.207 fractional release of loperamide HCl was achieved. After the 2 hours in SGF containing pepsin only 0.346 fractional release of loperamide HCl was attained (Figure 4.9).

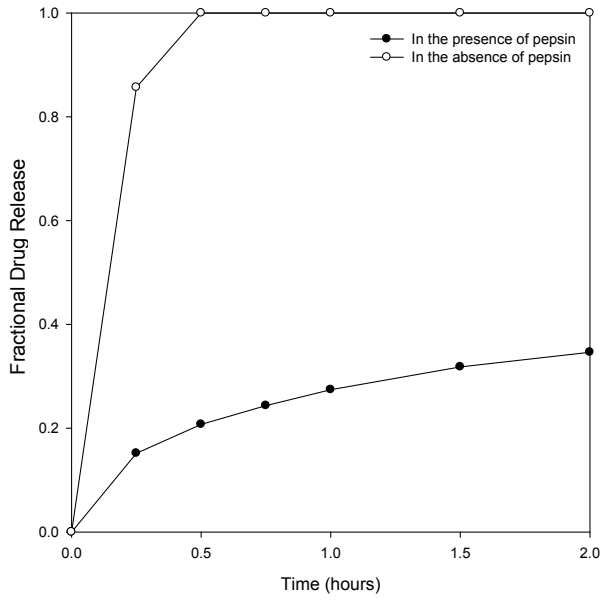



***Figure 4.9:*** *Release profiles in simulated gastric fluid with and without pepsin of 20%$^{w}/_{v}$ gelatine coatings crosslinked for 15 hours (N=3, SD<0.093 in all cases).*

The minimization of drug release in the presence of pepsin is attributable to the duration of crosslinking which is substantiated by the fractional release attained from the 30%$^{w}/_{v}$ shells that underwent crosslinking for a period of 2, 4 and 6 hours. With an increase in the duration of crosslinking there is a reduction in the fractional release of loperamide HCl after 2 hours in SGF containing pepsin for example, 0.634 (2 hours crosslinked), 0.454 (4 hours crosslinked), and 0.228 (6 hours crosslinked) (Figure 4.10a). The correlation between the duration of crosslinking and the rate of fractional loperamide HCl release in SGF containing pepsin may be depicted as 2 hours>4 hours>6 hours. The duration of crosslinking also influenced the time at which complete drug release was achieved in the absence of pepsin for example, 1.5 hours (2 hours crosslinked), and 2 hours (4 hours crosslinked) (Figure 4.10b).

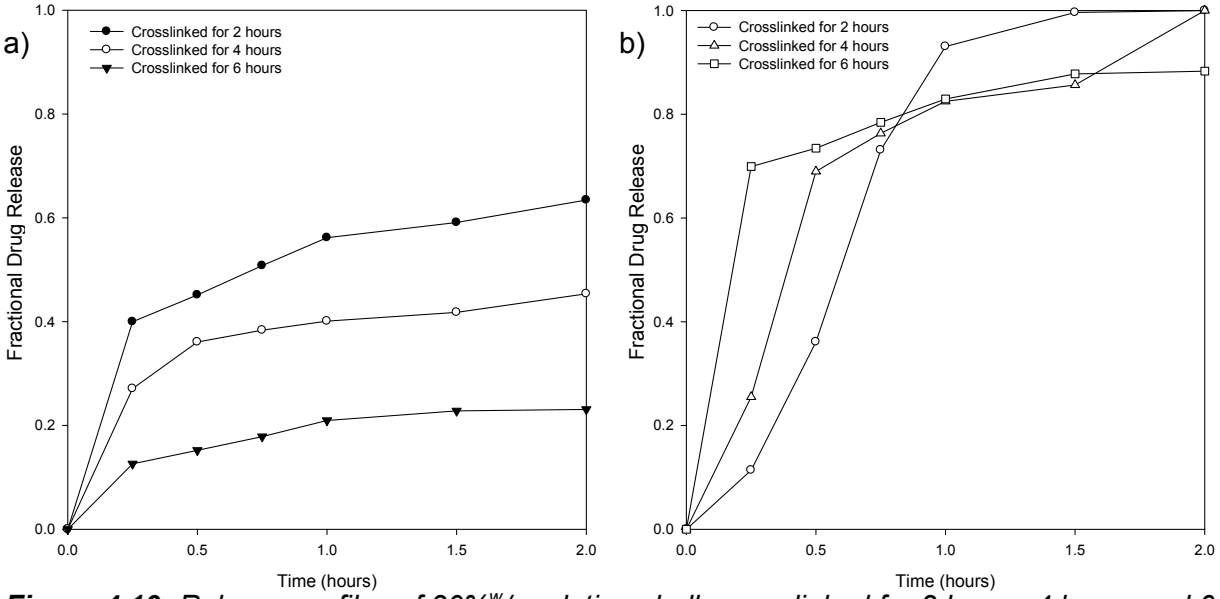



***Figure 4.10:*** *Release profiles of 30%$^{w}/_{v}$ gelatine shells crosslinked for 2 hours, 4 hours and 6 hours a) in the presence of pepsin (N=3, SD<0.007 in all cases), and b) in the absence of pepsin (N=3, SD<0.035 in all cases).*

### 4.6 Concluding Remarks

The initial preliminary formulation development studies attempted to develop a pepsin-responsive outer shell that responded to pepsin positively so that drug release was accelerated in its presence. However, after definitively establishing the actual site of action of loperamide HCl it was determined that drug release should be preferably targeted to the small intestine. Nevertheless, preliminary studies identified an attractive polymer, namely gelatine that exhibited an unusual response to pepsin present in SGF. In the presence of pepsin gelatine minimized the release of loperamide HCl from the shells. Furthermore, by varying the concentration of the gelatine composition of the shells as well as altering the duration of crosslinking the ideal release characteristics could be achieved. Thus preliminary formulation development enabled the identification of a candidate system that could be further optimized to achieve pepsin-responsive small intestinal targeted drug delivery.

# CHAPTER 5

# FABRICATION AND STATISTICAL OPTIMIZATION OF THE ENZYME-RESPONSIVE COLON-TARGETED TABLET THROUGH AN EXPERIMENTAL DESIGN STRATEGY

## 5.1 Introduction

Naturally derived polysaccharide polymers obtained from plants (pectin, guar gum or inulin), animals (chitosan, chondroitin sulphate), algae (alginates) or even microbes (dextran) have been extensively explored for colon-targeting applications. Irrespective of the source, these polysaccharides are hydrolyzed by colonic microflora to simple saccharides. Essentially, the principle of exploiting colonic microflora for colon-targeted drug delivery is based on the notion that on arrival to the colon hydrolysis of the glycosidic linkages of the polysaccharide chains occur, resulting in the release of the entrapped drug (Jose *et al.*, 2009).

Preliminary studies on the development of an enzyme-responsive colon-targeted drug delivery system yielded a comprehensive database that facilitated the establishment of a prototype quality formulation that acknowledged and overcame the constraints associated with typical colon-targeted drug delivery systems. The optimum formulation characteristics relied primarily on the incorporation of polymers that were sensitive to colonic enzymes resulting in the initiation and control of drug release exclusively in the presence of these enzymes.

### 5.1.1 Polysaccharide polymers identified for application in the colon-targeted tablet

#### 5.1.1.1 Pectin

Pectin is a linear non-starch heterogenous polysaccharide polymer composed of α-(1→4)-linked D-galacturonic acid groups with some 1→2 linked L-rhamnose groups. The application of pectin in colon-targeted drug delivery systems had emerged in the early 1990's, primarily due to the stability of the polymer in the stomach and small intestine as well as its susceptibility to colonic enzyme degradation (Ashford *et al.*, 1993b; Rubinstein *et al.*, 1993). The degree of methoxylation of pectin (DM) is the distinguishing factor that determines many of its properties for example, pectins with a high DM are poorly soluble and those with a low DM are highly soluble but hold the advantage for their capability to crosslink with cations (Ashford *et al.*, 1994). In addition to reducing the solubility of pectin in the upper gastrointestinal tract (GIT), crosslinking also controls the drug release rate from crosslinked matrices (Friend, 2005).

Various other means of delivering an effective pectin substrate to the colon have been explored for example, compression coating around a drug core, film coating of a combination of pectin and a hydrophobic polymer or combination of pectin with a second polysaccharide polymer or pH-sensitive polymer (Rubinstein *et al.*, 1993; Rubinstein, 1995; Rubinstein and Radai, 1995; Wakerly *et al.*, 1996; Wakerly *et al.*, 1997; Fernandez-Hervas *et al.*, 1998; Semdé *et al.*, 1998; Semdé *et al.*, 2000; Liu *et al.*, 2003a; Ahmed, 2005; He *et al.*, 2007). Film coatings of pectin-based solutions appear as one of the most promising approaches for colon-targeted drug delivery (Macleod *et al.*, 1999a, b; Ofori-Kwakye and Fell, 2001; Marianne *et al.*, 2003; Ofori-Kwakye and Fell, 2003a, b; Ofori-Kwakye *et al.*, 2004). Even though the combinatory coating solution of pectin and ethylcellulose has been extensively evaluated, the appropriate balance between the inhibition of premature drug release in the upper GIT and the activation of drug release on exposure to colonic enzymes has not yet been achieved.

#### 5.1.1.2 Chitosan

Similarly, chitosan also undergoes enzymatic degradation by the action of colonic bacteria. Chemically, it is a poly(*N*-glucosamine) with a high molecular weight and is unique in the sense that it is polycationic in character (Chourasia and Jain, 2003). Furthermore, the presence of amine groups renders chitosan soluble in weakly acidic conditions but insoluble in higher pH conditions due to protonation of the amine groups (Ravindra *et al.*, 1998). Thus the limitation of its hydrophilicity has led to various avenues being explored with the aim of reducing this characteristic in the upper GIT. Some such formulation approaches include amongst others, the microencapsulation of chitosan in a pH-responsive coating, dispersing of chitosan in a hydrophobic polymer or combining chitosan with an additional polymer to function as a coating surrounding a drug core (Lorenzo-Lamosa *et al.*, 1998; Shimono *et al.*, 2002; Kaur and Kim, 2009).

#### 5.1.1.3 Xanthan Gum

Xanthan gum is an anionic microbial exopolysaccharide that consists of a cellulose backbone and trisaccharide side chains that are composed of a glucuronic acid residue between two mannose units (Alvarez-Manceñido *et al.*, 2008). Apart from its effectiveness as an excipient in sustained release formulations, xanthan gum offers the further benefit of achieving near zero-order drug release kinetics (Talukdar and Kinget, 1995). Along with its high intrinsic viscosity and drug release kinetics the presence of mannose units in xanthan gum renders it susceptible to enzymatic degradation by β-mannanases present in colonic microflora thus making it ideal for incorporation in an enzyme-responsive colon-targeted drug delivery system.

### 5.1.2 Multiparticulate vs. single-unit colon-targeted drug delivery systems

Single-unit colon-targeted drug delivery systems pose the burden of possible premature dose-dumping prior to entry into the colon and/or dose-dumping in the colon resulting in the possible 'flooding' of absorption/affected sites causing ineffectual therapy and evacuation of 'unused' drug (Sinha and Kumria, 2001). In contrast, multiparticulate colon-targeted systems offer the advantage of being less likely to be affected by food and demonstrate a more consistent absorption compared to single-unit systems. In addition, these systems are particularly advantageous for the topical therapy of inflammatory bowel diseases, in particular Ulcerative Colitis (UC) due to the greater potential of providing a uniform distribution of drug to the inflamed parts of the GIT (Davis *et al.*, 1991). Therefore multiparticulate systems have gained popularity over the single-unit systems however the cumbersome manufacturing process and limitations in drug-loading capacity remain the major drawbacks. The novel *in situ* crosslinked enzyme-responsive colon-targeted drug delivery system aims to amalgamate the benefits of multiparticulates and tablets into a single-unit tablet system, whilst avoiding the drawbacks of each. The system and its components are illustrated in Figure 5.1.

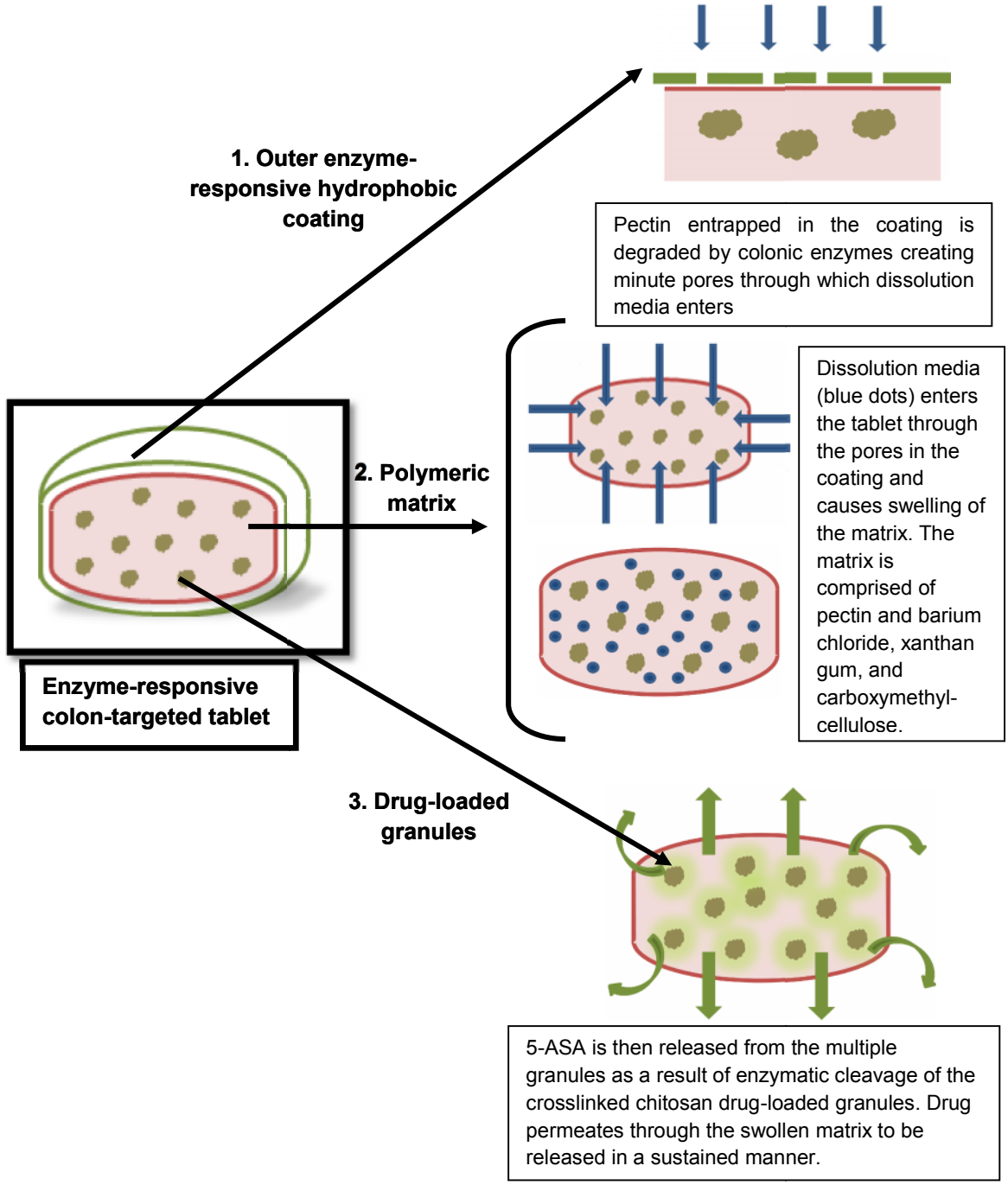



***Figure 5.1:*** *Schematic illustration of the individual components of the novel enzyme-responsive colon-targeted system and the method by which the benefits of multiparticulates are incorporated into a single-unit tablet.*

Therefore, this chapter focussed on the development of the enzyme-responsive colon-targeted single-unit tablet in a systematic and sequential manner guided through a high performance statistically robust experimental design approach, with the fundamental aim of identifying an optimal formulation that achieved an initiation of drug release in conditions simulating the colon, whilst avoiding drug release in the upper GIT.

## 5.2 Materials and Methods

### 5.2.1 Materials

The materials including mesalamine (5-ASA), chitosan (CHT), pectin, xanthan gum (XG), carboxymethylcellulose sodium salt (CMC), sodium tripolyphosphate (TPP), barium chloride 2-hydrate ($BaCl_2$), the ethylcellulose aqueous coating dispersion (EC), the commercial colonic enzymes, pectinase and β-glucosidase were all obtained as described in Chapter 3, section 3.2.1. Asacol® was supplied by Aventis Pharma (Pty) Ltd. (Midrand, Johannesburg, South Africa). De-ionized water was used from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, USA). All other reagents employed were of analytical grade and used as purchased without further purification.

### 5.2.2 Preparation of the enzyme-responsive colon-targeted oral tablet in accordance with a randomized Box-Behnken experimental design template

Tablets were comprised of CHT and 5-ASA granules crosslinked with an aqueous solution of TPP, which were then dispersed within a polymeric matrix comprising of pectin, $BaCl_2$, XG and CMC. The granule-polymer dispersion was then compressed into shallow bi-concave tablets and coated with an enzyme-responsive hydrophobic polymeric coating as depicted in Figure 5.1. The tablets were prepared according to the Box-Behnken statistical experimental design (Minitab® V15, Minitab Inc., PA, USA) and the outer hydrophobic coating layer of the tablet remained consistent for all formulations. The three factor three-tier statistical experimental design had a single central point with three replicates and was used to determine the concentrations and ratios of the tablet components that ensured maximal responsiveness to colonic enzymes whilst ensuring a sustained release of 5-ASA throughout the colon (Table 5.1). The formulation input factors that were evaluated at the three levels included:

- CHT concentration: 200-600mg of total tablet weight
- TPP aqueous solution: 2-10%$^{w}/_{v}$
- XG concentration: 1-10%$^{w}/_{w}$ of tablet matrix

These factors and the corresponding experimental levels were selected based on results from preliminary screening studies which showed that only CHT, TPP and XG had any significant influence on the drug release characteristics of the tablets. Quadratic response surfaces and second order polynominal models were generated to facilitate the prediction of the tablet response (drug release characteristics in the presence and absence of colonic enzymes) in terms of the independent variables (CHT, TPP and XG concentration) investigated. A time-point approach to analyzing the drug release data was employed by

determining the mean dissolution time of the formulations in the presence ($MDT_E$) and absence (MDT) of colonic enzymes over a 24 hour period. MDT and $MDT_E$ were selected as the only formulation responses to be investigated since the mean dissolution times enabled the assessment of the enzyme-responsiveness of the tablet whilst the corresponding drug release profiles established the colon-targeting ability of the formulations.

***Table 5.1:*** *Box-Behnken design template of the 15 statistically derived formulations for the fabrication of the enzyme-responsive colon-targeted tablet*

| Experimental Formulation | Granules | | Matrix |
|---|---|---|---|
| | CHT (mg) | TPP ($\%^w/_v$) | XG ($\%^w/_w$) |
| 1 | 200 | 10 | 5.5 |
| 2 | 600 | 6 | 10.0 |
| 3 | 200 | 6 | 10.0 |
| 4 | 400 | 6 | 5.5 |
| 5 | 400 | 10 | 1.0 |
| 6 | 600 | 10 | 5.5 |
| 7 | 200 | 6 | 1.0 |
| 8 | 400 | 6 | 5.5 |
| 9 | 400 | 2 | 1.0 |
| 10 | 600 | 2 | 5.5 |
| 11 | 400 | 2 | 10.0 |
| 12 | 400 | 6 | 5.5 |
| 13 | 400 | 10 | 10.0 |
| 14 | 200 | 2 | 5.5 |
| 15 | 600 | 6 | 1.0 |

### 5.2.3 Preparation of the enzyme-responsive colon-targeted oral tablet excluding the outer enzyme-responsive hydrophobic coating

Drug-loaded crosslinked granules of CHT and 5-ASA were prepared by blending the required quantity of CHT, determined in accordance with the randomized Box-Behnken experimental design (Table 5.1), with a constant quantity of 5-ASA (200mg per tablet). Prior to the incorporation of 5-ASA with CHT, a 1M acetic acid solution was pipetted directly onto the CHT powder to facilitate the ionization of CHT. The relevant concentration of TPP was then added to the blend in a consistent ratio of 1:800 (v/w) TPP:CHT and blended with a mortar and pestle for 5 minutes to ensure an even distribution of the crosslinking agent. The moist coagulation formed was then passed through a 1mm stainless steel mesh sieve (Laboratory Test Sieve, Endecotts Ltd., London, UK) and the resultant granules were subsequently allowed to dry at 30°C for 24 hours in a convection oven (Memmert, Schwabach, Germany).

The dehydrated 5-ASA loaded granules were then incorporated and dispersed within a matrix of polymers that included pectin, ground $BaCl_2$, XG and CMC. The concentration of XG, an independent formulation variable, was based on a percentage of the matrix components and was prescribed by the Box-Behnken experimental design (Table 5.1). This concentration was dependent on a tablet weight of 1200mg (excluding binder and lubricant)

and the quantity of granules to be included. From preliminary screening studies it was determined that the most effective ratio of pectin to crosslinking agent ($BaCl_2$) that ensured *in situ* crosslinking was 2:1 (w/w). However, the quantity for incorporation was dependent on the quantity of XG included in the matrix as per the Box-Behnken experimental design. CMC was subsequently added in a concentration of 12%$^{w}/_{w}$ of a tablet weight of 1200mg to act as a binder, and magnesium stearate (MgSt) was added in a 0.5%$^{w}/_{w}$ concentration as a lubricant. The matrix components were thoroughly blended with the relevant quantity of 5-ASA loaded granules using a cube blender (Erweka Apparatebau, Heusenstamm, Germany) and compressed into shallow bi-concave tablets with a final weight before coating of 1350mg and 13.6x4.5mm dimensions using a mini rotary tablet press (Karnavati Mini Press II, Rimek Products, Gujarat, India).

### 5.2.4 In-process validation of the enzyme-responsive colon-targeted oral tablet prior to coating

The elucidation of the mechanical properties of the tablets prior to coating holds substantial significance as a critical formulation characteristic since the ability of the tablets to withstand the vigorous coating process is dependent on the successful manufacture of a physically robust tablet. Textural profile analysis was therefore conducted with the intention of characterizing the compressibility of the polymers employed via determination of the matrix indentation hardness of the uncoated tablets, using a *TA.XTplus* Texture Analyzer (Stable MicroSystems, England) fitted with a probe having a spherical indenter of 3.125mm diameter and a 5kg load cell. Essentially, the indentation hardness of the tablets was represented by conversion of the maximum force withstand by the tablet to the Brinell Hardness Number (BHN) as dictated by Equation 5.1.

$$BHN = \frac{2F}{\pi D\left[D - (D^2 - d^2)^{\frac{1}{2}}\right]} \qquad \text{Equation 5.1}$$

*Where F is the force applied to the tablet, D is the diameter of the probe and d the indentation diameter.*

Typically, the ball-probe which is connected to a force transducer in the analyzer measures the force of resistance encountered by the probe during advancement into the sample. This probe is advanced into the sample at a predetermined velocity in accordance with the following textural parameters listed in Table 5.2.

***Table 5.2:*** *Textural settings employed for the calculation of matrix indentation hardness*

| Textural parameter | Setting |
|---|---|
| Test mode | Compression |
| Pre-test speed | 1mm/sec |
| Test speed | 0.5mm/sec |
| Post-test speed | 1mm/sec |
| Distance | 0.25mm |
| Trigger type | Auto (force) |
| Trigger force | 0.05N |

The matrix indentation hardness was subsequently determined based on data acquired through the generation of Force-Distance profiles. A typical profile acquired is depicted in Figure 5.2.

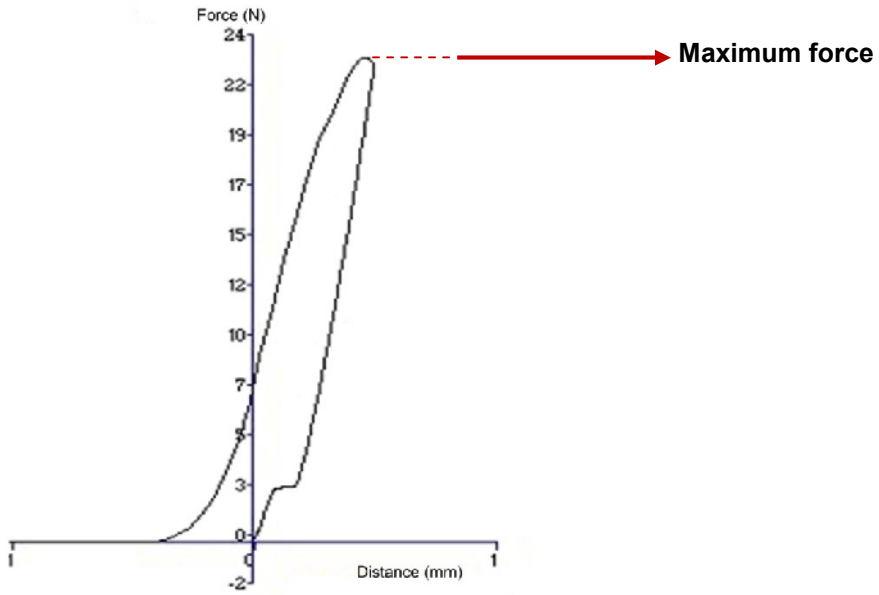



***Figure 5.2:*** *Typical Force-Distance profile acquired for the determination of the matrix indentation hardness of tablets before coating.*

In addition to ensuring the reproducibility of the tablet manufacturing process friability studies were conducted on the tablets prior to coating as a means of establishing the ability of the tablets to withstand the vigorous conditions implemented during the coating process. Thus, friability studies were conducted on 10 samples of uncoated tablets from each formulation prepared according to the Box-Behnken experimental design using a Friabilator (Pharma Test Friabilator PTF, Pharma Test, Hainburg, Austria). This was conducted according to USP test parameters for tablets of weight ≥1g (USP 33, NF 28). Briefly, 10 pre-weighed tablets were subjected to a 100 revolutions at 25rpm for 4 minutes, and friability was set at an upper limit of 1%. The tablets were subsequently removed from the friabilator, dusted and re-weighed. The percentage difference between the initial and the final weights were recorded as the percentage friability based on Equation 5.2.

$$WL_t = \frac{(W_0 - W_t)}{W_0} \times 100$$ Equation 5.2

*Where $WL_t$ is the total weight loss expressed as a percentage, $W_0$ is the initial weight of the tablets, and $W_t$ is the final weight of the tablets.*

Mass uniformity analyses and determination of the uniformity of tablet dimensions prior to coating were also performed to ensure the reproducibility of the tablet manufacturing process as well as the intra- and inter-batch consistency.

### 5.2.5 Coating of the enzyme-responsive colon-targeted oral tablet with the enzyme-responsive hydrophobic solution to ensure site-specific colonic delivery

An enzyme-responsive coating solution was prepared by blending a 4%$^{w}/_{v}$ aqueous pectin solution with an EC aqueous coating dispersion in a 1:2 proportion (w/w). The process of the preparation of this coating solution was elaborated in Chapter 3, section 3.4.4. Furthermore, the optimized coating process parameters were maintained as prescribed in section 3.4.5. The subsequent degree of coating achieved was then expressed as the theoretical percentage of the total weight gain (TWG) according to Equation 3.1.




***Figure 5.3:*** *Three-dimensional illustration of the fluid bed dryer (FBD) and its components.*

### 5.2.6 Construction of calibration curves of 5-ASA in simulated media

The construction of calibration curves of 5-ASA in simulated gastric fluid (SGF) (pH 1.2; 37°C), simulated intestinal fluid (SIF) (pH 6.8; 37°C) and simulated colonic fluid (SCF) (pH 5.9; 37°C) with and without pectinase (3mL per 250mL SCF) and β-glucosidase (10mg per 250mL of SCF) were performed as described in Chapter 3, section 3.4.6.

### 5.2.7 *In vitro* drug release characteristics of the coated enzyme-responsive colon-targeted oral tablet in response to commercially available colonic enzymes

In principle, the use of the USP apparatus III is the most attractive when evaluating modified and modulated release formulations since the effect of the continuous and dynamic pH change that occurs with time along the passage through the GIT can be easily and reproducibly simulated. *In vitro* drug release studies were thus performed based on the parameters and protocol outlined in Chapter 3, section 3.4.7.

The pH gradient and residence times evaluated were based on the mean physiological pH values in each segment, as well as the mean residence times of delivery systems in each segment (Davis *et al.*, 1986; Klein *et al.*, 2002; Basit, 2005). This entailed a gastric residence time of 2 hours in SGF, small intestinal residence time of 4 hours in SIF, and finally SCF with a residence time of 18 hours (Nunthanid *et al.*, 2008). Commercially available colonic enzymes, specifically pectinase (3mL per 250mL SCF) and β-glucosidase (10mg per 250mL of SCF) were included in SCF. The upper gastrointestinal enzymes, in particular pepsin and pancreatin, were excluded from SGF and SIF respectively since the outer coating layer consisting of only pectin and EC was shown, from preliminary studies, to be insensitive to these enzymes. For comparative purposes release studies were conducted in SCF with and without colonic enzymes. Furthermore, the gold-standard marketed 5-ASA formulation, Asacol®, was also subjected to *in vitro* release studies based on identical *in vitro* release parameters. Sample withdrawal, analysis and 5-ASA quantification were performed as described in section 3.4.7.

Computation of the release data was subsequently performed in a manner compliant with a model-independent comparison of all formulations in terms of their respective 5-ASA release behaviour. This was conducted by determining the mean dissolution time ($MDT_{24}$) values of all formulations and in each dissolution medium calculated at 24 hours.

### 5.2.8 Constrained statistical optimization of the enzyme-responsive colon-targeted oral tablet

Surface response methodology is an empirical modelization technique that is devoted to the evaluation of the relationship of a set of controlled experimental factors and the observed

results (Annadurai and Sheeja, 1998). Thus the primary aim of the statistical process was to develop an optimized enzyme-responsive colon-targeted oral tablet that is preferentially targeted to the colon and upon 'activation' by colonic enzymes drug is released in a sustained manner in the colon. Computation of results was performed using multiple regression analysis using the least squares method, where in a system involving three significant independent variables, the mathematical relationship of the response on these variables was approximated by a quadratic (second order) polynomial equation. For optimization purposes, the response parameters evaluated were the mean dissolution time after 24 hours ($MDT_{24}$) in the presence ($MDT_E$) and absence (MDT) of colonic enzymes. Therefore for optimization purposes the MDT was minimized in the absence of colonic enzymes and maximized in the presence of enzymes.

### 5.2.9 Evaluation of the simulated fluid uptake ability of the enzyme-responsive colon-targeted oral tablet in response to colonic enzymes

The presence of pectinase and β-glucosidase in SCF may influence the degree of imbibition of media into the delivery system and can be correlated to the *in vitro* release data obtained. This supposition provided the motivation for establishing the SCF uptake ability of the tablet as a consequence of colonic enzymes. Essentially this process was conducted gravimetrically where the tablets were subjected to conditions identical to those employed during drug release studies however at predetermined time points (9, 12, 15, 18, 21, and 24 hours) each tablet was removed from the relevant SCF and allowed to drip-dry for a period of 2 minutes at room temperature to remove excess SCF. The drip-drying process was conducted in a manner that ensured no introduction of bias since the tablets were held on the bottom mesh of the glass cylinder of the Bio-Dis® apparatus and no physical 'blotting' of the tablets was performed. As a control, this process was replicated in SCF void of enzymes. Fresh samples were used for each individual time point. The degree of SCF uptake at each time point, expressed as a percentage, was then determined based on the increase in weight of the formulation due to imbibed SCF and was calculated according to Equation 5.3.

$$SCF_{uptake}(\%) = \frac{(W_t - W_0)}{W_0} \times 100 \qquad \text{Equation 5.3}$$

*Where $SCF_{uptake}$ is the percentage of SCF uptake by the tablet at the relevant time point, $W_t$ is the weight of the tablet at the relevant time point, and $W_0$ is the initial weight of the tablet.*

The physical dimensions of the tablets could not be determined since with an increase in exposure time to SCF there was an increase in SCF uptake by the tablets which resulted in the rupturing of the coating causing the tablets to have non-consistent dimensions.

Subsequent to establishing the amount of imbibed fluid in the tablet, the hydrated samples were dried in a convection oven at 40°C for 48 hours, and allowed to cool under a fume hood for a further 30 minutes after which they were re-weighed. Since the hydrophilic polymers employed in the tablet possessed the ability to absorb a substantial volume of SCF masking the simultaneous erosion of polymer at that point, this process was conducted to establish the degree of polymer loss/erosion at each time point after the samples were completely dehydrated. Thus the degree of erosion, expressed as a percentage, at each time point was calculated according to Equation 5.4.

$$Erosion\ (\%) = \frac{(W_0 - W_t)}{W_0} \times 100 \qquad \text{Equation 5.4}$$

*Where $W_t$ is the weight of the dried tablet at the relevant time point, and $W_0$ is the initial weight of the tablet.*

### 5.2.10 Evaluation of the polymeric structural and vibrational frequency variations of the tablet and its components relative to its exposure to colonic enzymes

The structure of the native polymers CHT, pectin, XG, and CMC, as well as that of 5-ASA were assessed by Fourier Transmission Infrared Spectroscopy (FTIR) using a Spectrum 2000 FTIR spectrometer with a MIRTGS detector (PerkinElmer Spectrum 100, Lliantrisant, Wales, UK). Furthermore, the drug-loaded crosslinked CHT granules were also evaluated to assess any variations in the vibrational frequencies as a result of crosslinking during granule formation. To categorically elucidate the influence of colonic enzymes on the structural polymeric backbone of the tablet as well as on the coating of the tablet, samples acquired through erosion studies (each time point) were also analyzed by FTIR. Samples were placed on a diamond crystal and processed by a universal ATR polarization accessory for the FT-IR spectrum series at a resolution of 4$cm^{-1}$. Samples were analyzed at wavenumbers ranging from 400-4000$cm^{-1}$.

### 5.2.11 Molecular mechanic simulations of the influence of colonic enzymes on the crosslinked chitosan granules

Molecular Mechanics Computations in vacuum, which included the model building of the energy-minimized structures of multi-polymer complexes, were performed using the HyperChem™ 8.0.8 Molecular Modeling System (Hypercube Inc., Gainesville, Florida, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK) on an HP Pavilion dv5 Pentium Dual CPU T3200 workstation. The structure of TPP was generated as a 3D model from standard bond lengths and angles employing ChemBio3D Ultra whereas the structures of CHT and pectin (ten oligosaccharide units each) were generated using sugar

builder module on HyperChem 8.0.8. The structure of pectinase, polygalacturonase and β-glucosidase peptide analogues were downloaded using GetNetFile online structure retrieval option on ChemBio3DUltra. However, the representative recognition sequences of the respective peptide i.e., polygalacturonase and β-glucosidase were generated using sequence editor module on HyperChem 8.0.8. The generation of the overall steric energy associated with the energy-minimized structures was initially executed initially via energy-minimization using MM+ force field and the resulting structures were again energy-minimized using the Amber 3 (Assisted Model Building and Energy Refinements) force field. The conformer having the lowest energy was used to create the polysaccharide-crosslinker and polysaccharide-peptide complexes. A complex of one molecule with another was assembled by disposing them in a parallel way, and the same procedure of energy-minimization was repeated to generate the final models: CHT-TPP crosslinked granules, β-glucosidase acting on CHT and polygalacturonase acting on pectin. Full geometry optimizations were carried out in vacuum employing the Polak–Ribiere conjugate gradient method until an RMS gradient of 0.001kcal/mol was reached. Force field options in the AMBER (with all hydrogen atoms explicitly included) and MM+ (extended to incorporate non-bonded cut-offs and restraints) methods were the HyperChem 8.0.8 defaults. For calculations of energy attributes, the force fields were utilized with a distance-dependent dielectric constant scaled by a factor of 1. The 1-4 scale factors are following: electrostatic 0.5 and van der Waals 0.5.

Molecular mechanics energy relationship (MMER), a method for analytico-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, non-covalent Coulombic terms, and non-covalent van der Waals interactions for solute partitioning from the bulk aqueous/organic phase. The MMER model for potential energy factor in various molecular complexes can be written as depicted in Equation 5.5.

$$E_{molecule/complex} = V_{\sum} = V_b + V_\theta + V_\varphi + V_{ij} + V_{hb} + V_{el} \qquad \text{Equation 5.5}$$

*Where $V_{\sum}$ is related to total steric energy for an optimized structure, $V_b$ corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), $V_\theta$ denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), $V_\varphi$ represents torsional contribution arising from deviations from optimum dihedral angles, $V_{ij}$ incorporates van der Waals interactions due to non-bonded interatomic distances, $V_{hb}$ symbolizes hydrogen-bond energy function and $V_{el}$ stands for electrostatic energy.*

### 5.2.12 Surface morphological characterization of the enzyme-responsive colon-targeted oral tablet after exposure to colonic enzymes

Surface morphological characterization was undertaken as a means of qualitatively evaluating the influence of colonic enzymes on the tablet. Scanning electron microscopy (SEM) was performed on the coated and uncoated tablet prior to exposure to colonic enzymes as well as on the eroded samples at each time point obtained from SCF uptake studies after exposure to SCF with and without colonic enzymes. This allowed an illustration of pore presence, pore distribution, polymer cleavage or other anomalies that occurred as a result of exposure to colonic enzymes and in relation to the duration of such exposure. Samples were firmly mounted on aluminium stubbs with carbon tape and gold sputter coated with argon gas whilst under a vacuum of 0.1Torr using a SPI-MODULE$^{TM}$ Sputter Coater and SPI-MODULE$^{TM}$ Control (SPI Supplies, Division of Structure Probe Inc., West Chester, PA, USA). Each sample required 4.5 minutes of coating at 90 second intervals to ensure complete coverage of the sample which minimized sample activation during viewing. Subsequent viewing was performed at various magnifications under the Phenom$^{TM}$ desktop scanning electron microscope (FEI Company$^{TM}$, Hillsboro, OR, USA).

## 5.3 Results and Discussion

### 5.3.1 Evaluation of the physicomechanical characteristics of the enzyme-responsive colon-targeted oral tablet prior to coating

Textural analysis of all 15 formulations (N=3) revealed an average BHN of 58.17±2.80N/mm$^2$ (Figure 5.4). The BHN of each formulation showed no significant evidence of being dependent on the concentrations of the polymers and crosslinking agents employed. The results also suggest that the variation in the indentation force withstood by the formulations and thus the corresponding BHN values were likely due to slight inconsistencies that occurred during the manufacturing process. However these inconsistencies were not substantially significant to drastically alter the hardness of the tablets. Figure 5.4 indicates the absence of any discernable pattern with regards to the influence of the concentrations of the polymers and crosslinking agents employed and the corresponding tablet hardness.

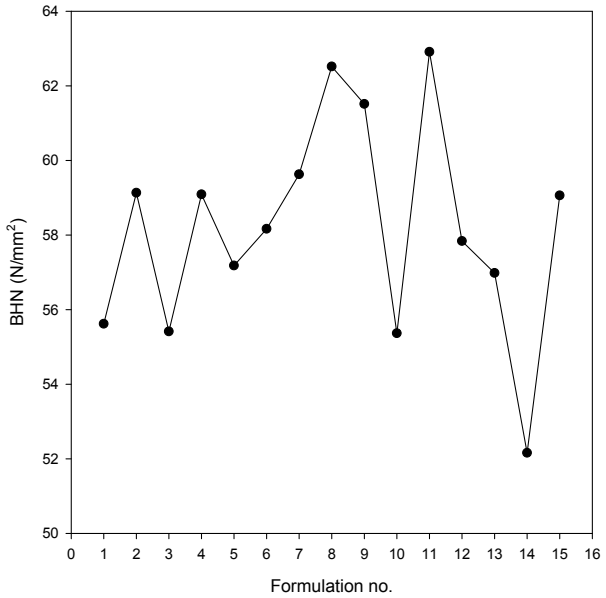



***Figure 5.4:*** *Graphical illustration of the Brinell Hardness Number of all 15 formulations (SD: 0.591-7.633).*

The USP (USP 33, NF 28) states that a friability of <1% is acceptable for tablets ≥1g after undergoing 100 revolutions in a friabilator. Figure 5.5 shows the friability of each formulation and provides evidence that all formulations were compliant with USP specifications. Therefore, theoretically each formulation would be capable of withstanding the vigorous fluidization conditions implemented in the Wurster chamber of the FBD.

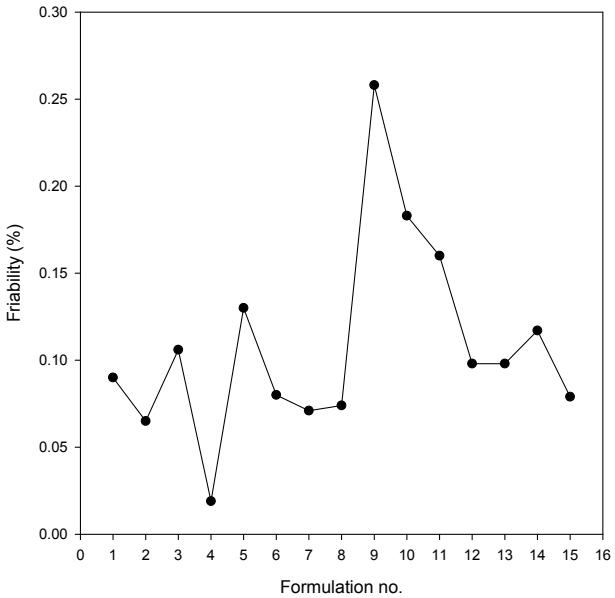



***Figure 5.5:*** *Graphical illustration of the variations in the friability of all 15 formulations (SD: 0.002-0.106).*

The average tablet weight for all 15 formulations (N=20) was 1329.51±2.99mg per tablet. Since the theoretical final tablet weight was 1350mg this represented a loss of 1.52%. However, the dimensions of the tablets (13.6x4.5mm) remained consistent throughout.

### 5.3.2 Box-Behnken Experimental Design for optimization of the enzyme-responsive colon-targeted oral tablet

#### 5.3.2.1 Measured responses for experimental optimization

Diverse drug release patterns were observed with all 15 formulations as illustrated in Figure 5.6a-f. However, based on these profiles it was evident that an elevated fractional drug release was observed from all formulations exposed to colonic enzymes in SCF as opposed to when the enzymes were absent. Furthermore, despite the acceptable friability limits of all formulations, three separate attempts at coating of formulations 3 and 14 proved unsuccessful preventing the evaluation of 5-ASA release from these formulations. This may be attributable to the specific concentrations/quantities of the independent formulation variables incorporated which influenced the compressibility of the combination of polymers and thus the ability of the tablets to withstand not only the fluidization conditions within the FBD but also the temperature change therein.

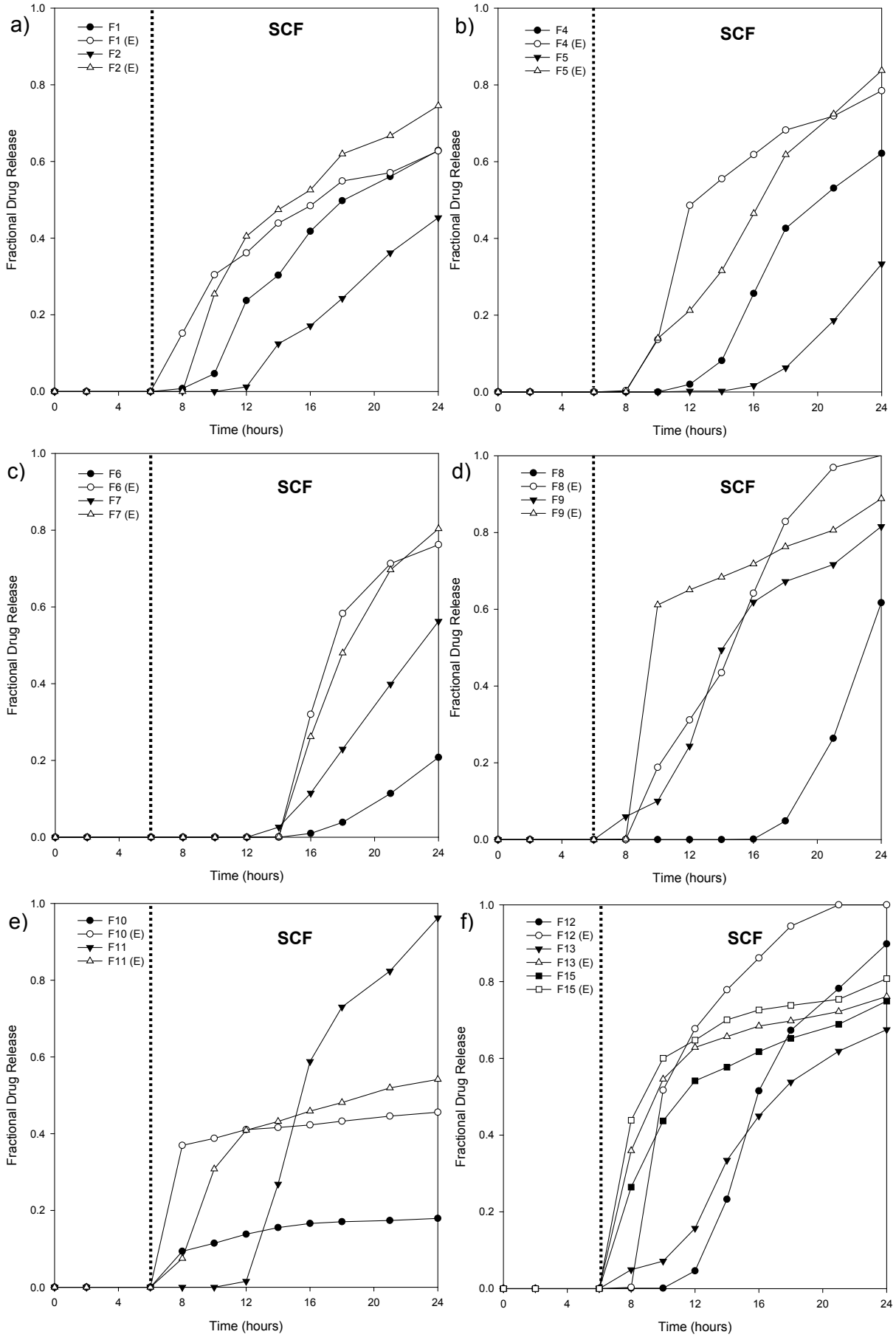



***Figure 5.6:*** *Composite fractional drug release profiles of formulations a) 1 and 2, b) 4 and 5, c) 6 and 7, d) 8 and 9, e) 10 and 11, and f) 12-15; where 'E' denotes simulated colonic fluid containing pectinase and β-glucosidase.*

Due to the diversity of the dissolution patterns acquired a model-independent time-point approach was selected as a means of analyzing and substantiating the release behaviour of all formulations from the Box-Behnken experimental design template. The MDT, defined as the sum of the different fractions of drug release obtained per period of time divided by the initial dose of drug loaded into the system, was calculated for each formulation in the presence and absence of colonic enzymes after 24 hours employing Equation 5.6 (Pillay and Fassihi, 1998).

$$MDT = \sum_{t=1}^{n} ti \frac{Mt}{M\infty} \qquad \text{Equation 5.6}$$

*Where Mt is the fraction of dose released in time ti and ti=(ti+ti-1)/2, M∞ corresponds to the loading dose.*

Since all 15 formulations were identically coated it was expected that until exposure to colonic enzymes all formulations would not release any 5-ASA. This was evident in Figure 5.6a-f where all formulations exhibited no drug release for the first 6 hours (in SGF and SIF). Therefore $MDT_{24}$ was actually based on the fractional release from the 6$^{th}$ hour onwards ($T_6$-$T_{24}$ hours). The MDT values of each formulation (in the absence and presence of colonic enzymes) are shown in Table 5.3.

***Table 5.3:*** *Mean dissolution time ($MDT_{6-24}$) of all 15 formulations in the presence and absence of colonic enzymes*

| Experimental Formulation | $MDT_{6-24}$ (hours) | $MDT_{6-24}$ [1](E) (hours) |
|---|---|---|
| 1 | 32.58 | 36.74 |
| 2 | 18.60 | 41.51 |
| 3 | | [2]N/A |
| 4 | 26.64 | 45.20 |
| 5 | 9.45 | 40.70 |
| 6 | 5.83 | 33.43 |
| 7 | 19.56 | 32.18 |
| 8 | 15.19 | 52.83 |
| 9 | 43.76 | 53.67 |
| 10 | 11.67 | 30.72 |
| 11 | 44.86 | 55.17 |
| 12 | 41.70 | 67.76 |
| 13 | 34.95 | 49.14 |
| 14 | | [2]N/A |
| 15 | 45.92 | 52.03 |

[1] *Pectinase and β-glucosidase are present in SCF*
[2] *Data is unavailable since the formulations disintegrated during the coating process*

Subsequent to the first 6 hours of drug release studies, all formulations not exposed to colonic enzymes showed a variable maximum fractional drug release ranging from 0.179 (formulation 10) to 0.749 (formulation 15) resulting in $MDT_{6-24}$ values ranging from 5.83 to

45.92. In contrast, in the presence of colonic enzymes the maximum fractional drug release of all formulations was substantially elevated ranging from 0.456 (formulation 10) to 1.0 (formulation 8 and 12) resulting in higher $MDT_{6-24}$ values. Despite the presence of this phenomenon in all formulations, the fundamental objective of the study design was to achieve a formulation that showed distinct responsiveness to colonic enzymes by having an elevated rate of drug release in SCF containing enzymes and minimal drug release in the absence of enzymes.

#### 5.3.2.2 Correlation of the experimental and predicted response values

A comparative analysis of the experimental vs. the predicted response values, in particular the MDT in the presence and absence of colonic enzymes, was plotted to establish the applicability of the regression models and the robustness of the Box-Behnken design employed for the optimization of the enzyme-responsive system. The plot depicting the MDT (in the absence of enzymes) of the experimental vs. the predicted values of each formulation show a general conformity with a correlation coefficient ($R^2$) of 0.897 (Figure 5.7a). Similarly the $MDT_E$ (in colonic enzymes) of the correlating experimental and predicted response values showed an $R^2$ value of 0.877 (Figure 5.7b). These $R^2$ values provide evidence of the success of the Box-Behnken experimental design and thus the efficiency of the design in aiding the determination of an optimized enzyme-responsive colon-targeted oral tablet.

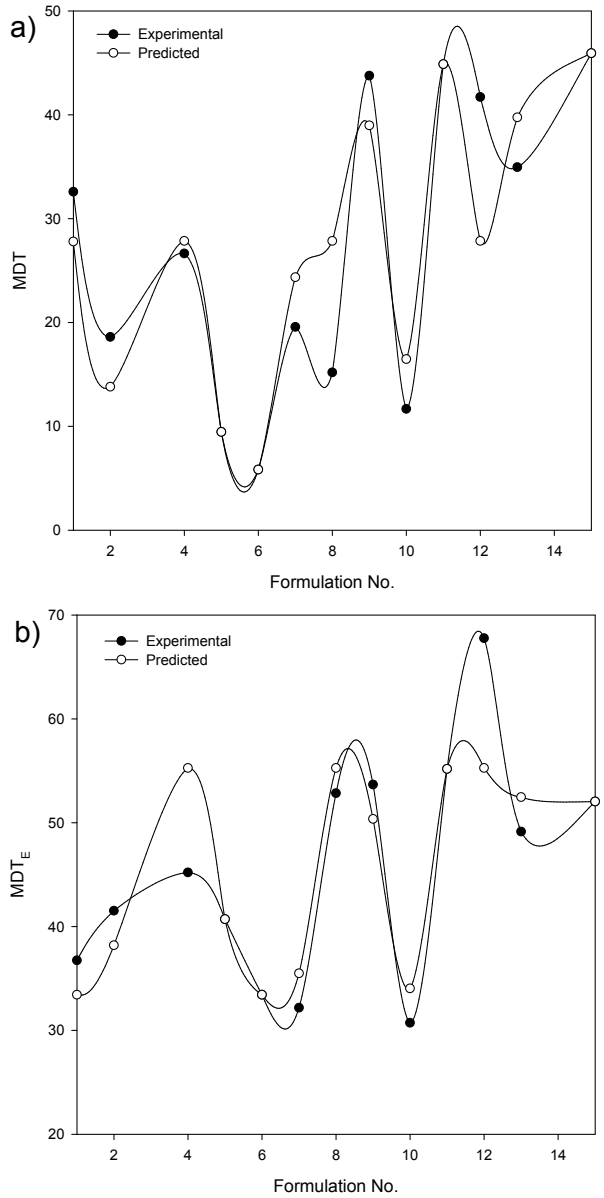



***Figure 5.7:*** *Regression plots of a) the mean dissolution time in the absence of colonic enzymes, and b) mean dissolution time in the presence of colonic enzymes ($MDT_E$) for the determination of the correlation between the experimental vs. the predicted response values.*

### 5.3.2.3 Analysis of the Box-Behnken Response Surface Design

#### 5.3.2.3.1 Response analysis for MDT

Response surface plots of the MDT (no enzymes in SCF) of the enzyme-responsive colon-targeted oral tablet showed that a desirable reduction in the MDT (MDT is minimized) is achievable with a gradual increase in the quantity of CHT incorporated in the formulation with either a minimum ($2\%^{w}/_{v}$) or maximum ($10\%^{w}/_{v}$) concentration of aqueous TPP as the crosslinking agent, when a XG hold value of $5.5\%^{w}/_{w}$ is implemented (Figure 5.8a). This desirable reduced MDT is also evident when the CHT quantity is 600mg and XG is present in a $5\text{-}10\%^{w}/_{w}$ concentration. This was relatively the most significant interaction (*p=0.083*). When XG is excluded from the formulation or exists in a low concentration ($2\%^{w}/_{w}$), a

reduction in MDT is only achievable with a TPP concentration of 10%$^{w}/_{v}$ and a CHT quantity of 400mg (Figure 5.8b).

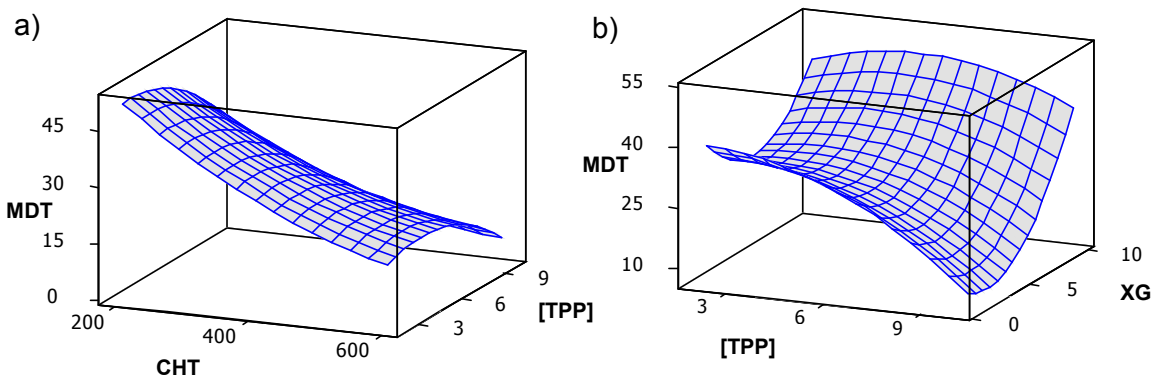



***Figure 5.8:*** *Response surface plots correlating mean dissolution time in simulated colonic fluid (no enzymes) with a) chitosan quantity and tripolyphosphate concentration (xanthan gum hold value of 5.5%$^{w}/_{w}$), and b) xanthan gum quantity and tripolyphosphate concentration (chitosan hold value of 400mg).*

#### 5.3.2.3.2 Response analysis for $MDT_E$

In contrast to the MDT (in the absence of colonic enzymes) a desirable increase in $MDT_E$ is achievable at a CHT quantity of 350-450mg and TPP concentration of 5-7%$^{w}/_{v}$ when a XG hold value of 5.5%$^{w}/_{w}$ is implemented. In order to achieve a maximum MDT in the presence of colonic enzymes the TPP concentration should be maintained between 5-7%$^{w}/_{v}$ and XG between 9-10%$^{w}/_{w}$, when 400mg of CHT is employed (Figure 5.9a). Furthermore, a significant increase in $MDT_E$ is evidenced when 6%$^{w}/_{v}$ TPP is employed with a minimum CHT quantity (200mg) and a maximum XG concentration (10%$^{w}/_{w}$) (Figure 5.9b). It is thus evident that achieving the optimum balance of CHT quantity, and TPP and XG combinations so that there is a reduction in the MDT (when no enzymes are present) and an increase in $MDT_E$ (when enzymes are present) from the enzyme-responsive colon-targeted oral tablet would prove challenging primarily due to the conflicting influences the polymers exhibit when exposed to SCF with colonic enzymes and SCF without enzymes.

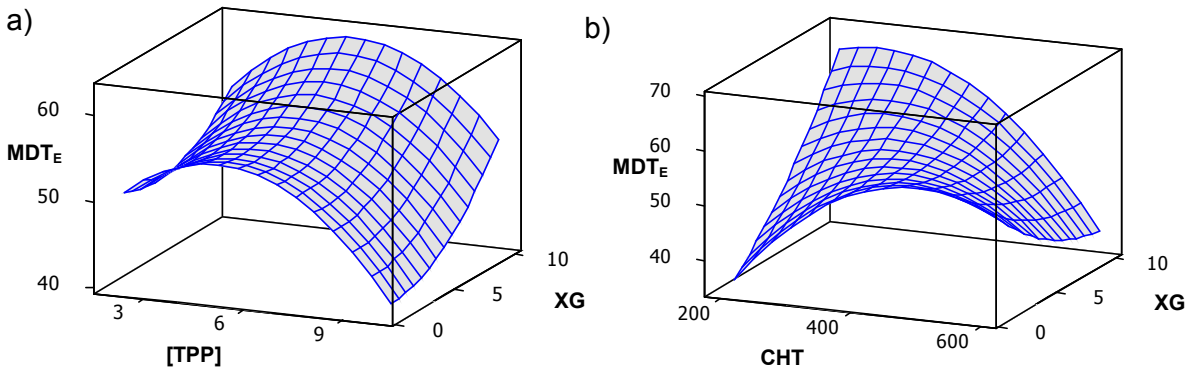



***Figure 5.9:*** *Response surface plots correlating mean dissolution time in simulated colonic fluid with enzymes ($MDT_E$) with a) xanthan gum quantity and tripolyphosphate concentration (chitosan hold value of 400mg) and b) chitosan quantity and xanthan gum concentration (tripolyphosphate hold value of 6%$^{w}/_{v}$).*

#### 5.3.2.4 Residual Analysis of the Box-Behnken Experimental Design for the optimization of the enzyme-responsive colon-targeted oral tablet

Residual analysis is performed to assess the suitability of the multiple regression model and to essentially obtain a linear comparison between the observed values and model predictions. The assumptions of a multiple linear regression model is that the response variables are independent and normally distributed random variables with constant variance and means depending linearly on the explanatory variables (Larsen and McCleary, 1972). The normal probability plots of the residuals of MDT and $MDT_E$ showed clusters of residuals however these clusters fell on a straight line, signifying the normal distribution of data with no evidence of unidentified variables. Furthermore, a residual plot of the standardized residuals of MDT and $MDT_E$ show a random distribution of points fluctuating around zero, with no discernable pattern, indicating a non-violation of the assumptions of zero means and constant variance of the regression model despite the presence of two outliers in these plots. Evidence of this non-violation was provided by histograms of the residuals of MDT and $MDT_E$. The residuals versus the order of data showed a random distribution, with rapid changes in signs (+/-) between consecutive residuals for both MDT and $MDT_E$ (Figure 5.10).

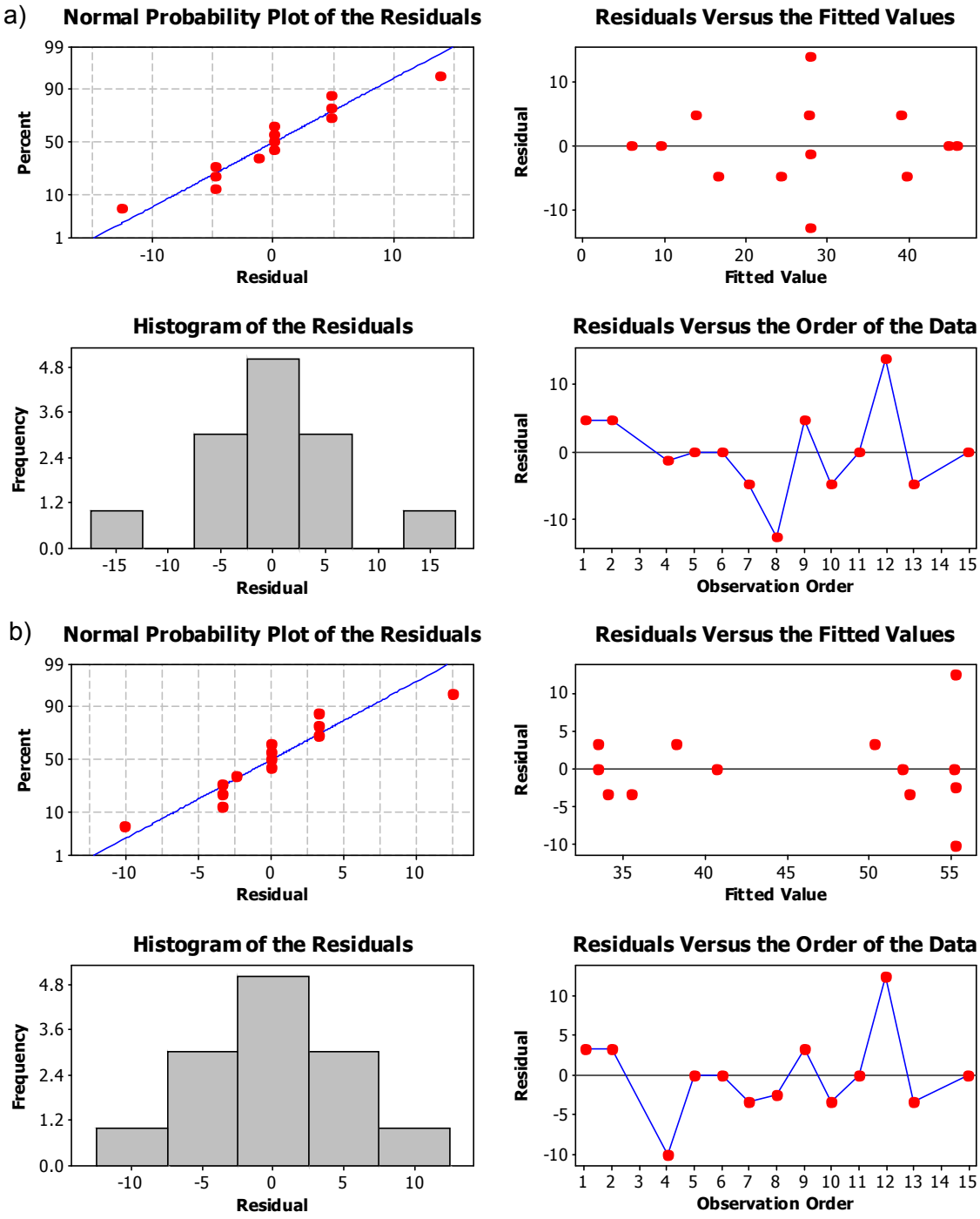



***Figure 5.10:*** *Residual plots of a) mean dissolution time in simulated colonic fluid without enzymes and b) mean dissolution time in simulated colonic fluid with enzymes.*

Based on a full ANOVA analysis of the measured formulation responses it was determined that none of the factors exhibited any significant influence ($p \leq 0.05$). Only the CHT*XG ($p=0.083$) term of the MDT response possessed an almost significant effect, even though $p \geq 0.05$ (Table 5.4).

***Table 5.4:*** *Estimated p-values for mean dissolution time in simulated colonic fluid with and without enzymes*

| Term | p-value | |
|---|---|---|
| | **MDT** | **$MDT_E$** |
| **CHT** | 0.165 | 0.693 |
| **[TPP]** | 0.235 | 0.564 |
| **XG** | 0.219 | 0.450 |
| **CHT*CHT** | 0.675 | 0.268 |
| **[TPP]*[TPP]** | 0.447 | 0.267 |
| **XG*XG** | 0.211 | 0.674 |
| **CHT*[TPP]** | 0.755 | 0.750 |
| **CHT*XG** | 0.083 | 0.261 |
| **[TPP]*XG** | 0.411 | 0.762 |

The complete regression equations generated for mean dissolution time in the absence (MDT) and presence of enzymes ($MDT_E$) are depicted in Equations 5.7 and 5.8 respectively.

$$\begin{aligned} MDT = {} & 38.4154 - 0.0287699[CHT] - 0.709931[TPP] + 4.58070[XG] + 0.00010689\,6[CHT * CHT] \\ & - 0.415781[TPP * TPP] + 0.595761[XG * XG] + 0.00417708\,[CHT * TPP] \\ & - 0.0278926[CHT * XG] + 0.338889[TPP * XG] \end{aligned}$$

Equation 5.7

$$\begin{aligned} MDT_E = {} & -10.8445 + 0.237775[CHT] + 3.66632[TPP] + 3.68471[XG] - 2.56354E-04[CHT * CHT] \\ & - 0.530469[TPP * TPP] + 0.142922[XG * XG] + 0.00348958\,[CHT * TPP] \\ & - 0.0122870[CHT * XG] + 0.0963889[TPP * XG] \end{aligned}$$

Equation 5.8

**5.3.2.5 Constrained optimization of the enzyme-responsive colon-targeted oral tablet**

Optimization of the enzyme-responsive colon-targeted oral tablet was performed employing Minitab® V15 statistical software (Minitab® Inc., PA, USA) to determine the optimum level for each variable. The optimization approach resulted in the attainment of a formulation with a desirability of 0.94 and the fabrication of this formulation included the employment of 403.837mg of CHT per tablet, a TPP concentration of 8.253%$^w/_v$ and a XG concentration of 4.087%$^w/_w$ of the tablet matrix as depicted in Figure 5.11.

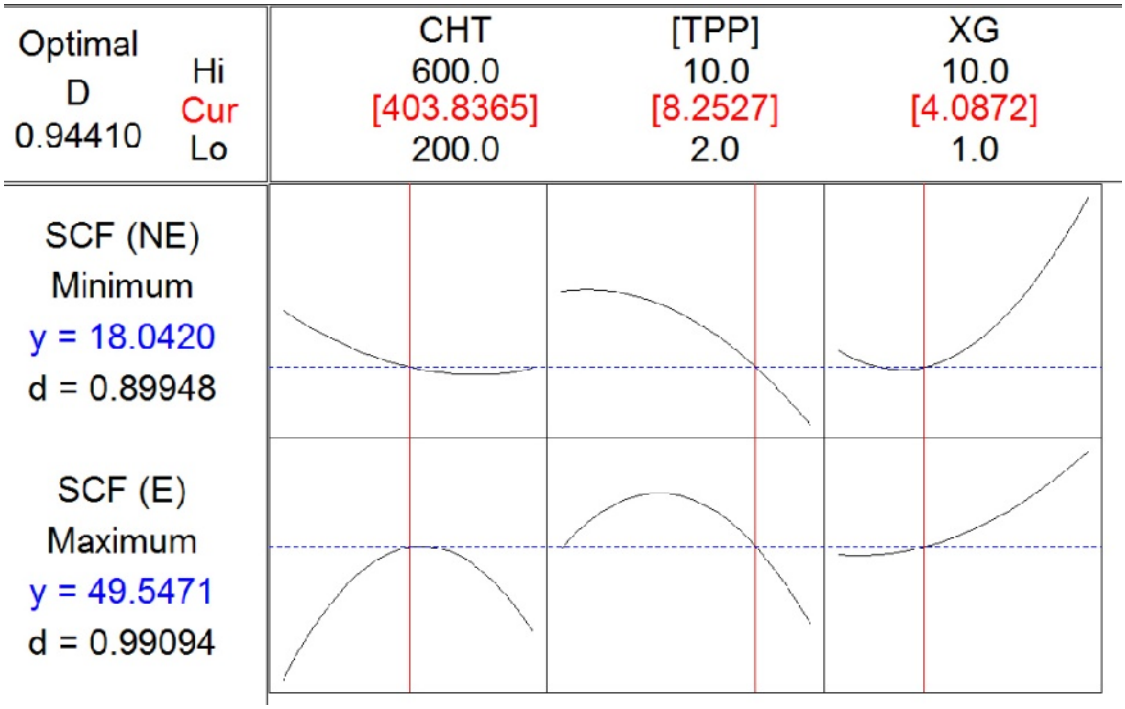



***Figure 5.11:*** *Optimization plots displaying factor levels and desirability values for the optimized formulation.*

***Table 5.5:*** *Comparison of the predicted and experimental values of the response parameter*

| Response parameter | Predicted | [1]Desirability (%) | Experimental | [2]Deviation (%) |
|---|---|---|---|---|
| **Mean Dissolution Time (MDT) (no enzymes)** | 18.0420 | 89.95 | 25.91725 | 5.569 |
| **Mean Dissolution Time ($MDT_E$) (with enzymes)** | 49.5471 | 99.09 | 47.67721 | 1.322 |

[1] *Statistical desirability values of the predicted optimized formulation (%)*
[2] *Deviation of the experimental response values from the predicted*

The predicted optimized formulation had a statistical desirability of ≥90% for both response parameters. Minimization of MDT and maximization of $MDT_E$ was achievable with a single optimized formulation (Table 5.5). The experimental response parameters achieved with the constrained settings for the optimized formulation were in close agreement with the predicted response values. This identified the reliability of the optimization process in predicting the dissolution behaviour in the presence of colonic enzymes more so than when enzymes are absent. *In vitro* release studies of the optimized formulation exhibited no drug release for the first 6 hours in SGF and SIF and a substantial elevation in the 5-ASA release rate was evident when in the presence of colonic enzymes (Figure 5.12). Furthermore, the observable zero-order-like release behaviour would in theory result in a more targeted and uniform distribution of the drug throughout the colon with standard deviations of only 0-0.26317.

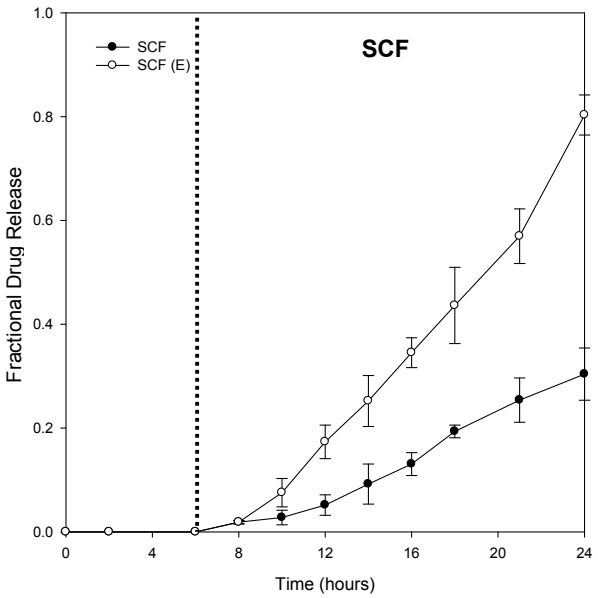



***Figure 5.12:*** *Release profile of 5-aminosalicylic acid from the optimized formulation in the presence (E) and absence of colonic enzymes.*

The optimized formulation achieved a fractional drug release of 0.8030±0.039 in the 24 hour period in the presence of colonic enzymes, however in the absence of the enzymes the same formulation achieved 0.304±0.050 fractional release in the same time. Essentially, this release profile provided the fundamental evidence of the enzyme-responsiveness of the colon-targeted tablet.

In contrast, the conventionally employed 5-ASA loaded system used in the treatment of UC, namely Asacol® is based on the technology of pH-responsive drug delivery where 5-ASA is coated by a copolymer of methacrylic acid and methyl methacrylate that dissolves above a pH of 7 (Basit, 2005). However based on the data obtained from drug release studies of these tablets (N=3) it was established that 5-ASA began releasing from as low as pH 1.2 (SGF), and showed significant deviations from the average (SD: 0.001-0.5495) as depicted in Figure 5.13. Furthermore irrespective of the presence of pectinase and β-glucosidase in SCF, the first 6 hours of release studies should be identical in both cases, which did not occur. The variation in the release profiles over the entire 24 hour period was attributed to inter-batch variability of the conventional system and a non-uniform coating surrounding the tablets. This postulation was confirmed by the observable formation of fissures/fractures along the edges of the tablets through which the 5-ASA was released (the points of least resistance) (Figure 5.14).

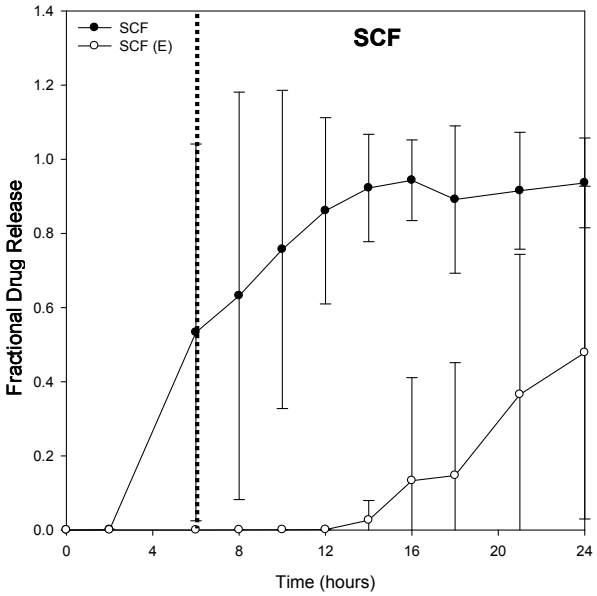



***Figure 5.13:*** *Release profiles of 5-aminosalicylic acid from the conventional commercially available 5-aminosalicylic acid-loaded system (Asacol®) in the presence and absence of colonic enzymes.*

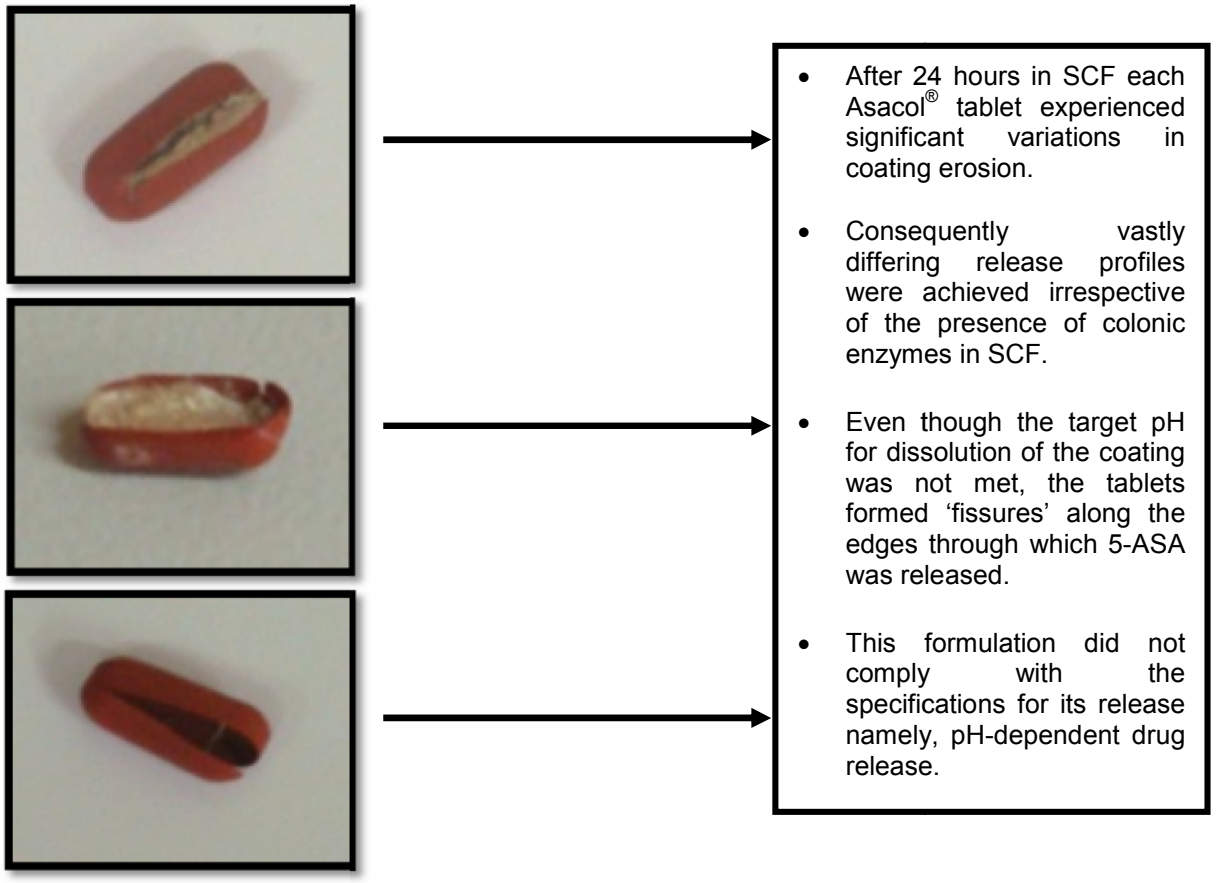



***Figure 5.14:*** *Digital images of the varying extents of fracturing/fissuring of Asacol® in simulated colonic fluid with and without enzymes after 24 hours.*

### 5.3.3 Influence of colonic enzymes on the uptake of SCF and erosion of the optimized enzyme-responsive colon-targeted oral tablet

Gravimetrical data revealed a gradual increase in the weight of the optimized tablet with an increase in exposure time to SCF. Despite the occurrence of this trend both in the presence and absence of enzymes, the extent of SCF uptake in the presence of colonic enzymes was substantially greater, for example 54.57% in the absence of colonic enzymes compared to 101.68% swelling in the presence of enzymes after 12 hours (Figure 5.15). Therefore the greater rate of fractional drug release in SCF with colonic enzymes can be correlated to the greater degree of SCF permeation into the tablet at each time point specifically due to the influence of β-glucosidase and pectinase in the medium. Furthermore, it is evident that this elevated release rate can also be correlated to the greater degree of erosion of the formulation in the presence of colonic enzymes, which in turn is due to the enzymatic cleavage of the crosslinked polymeric chains.

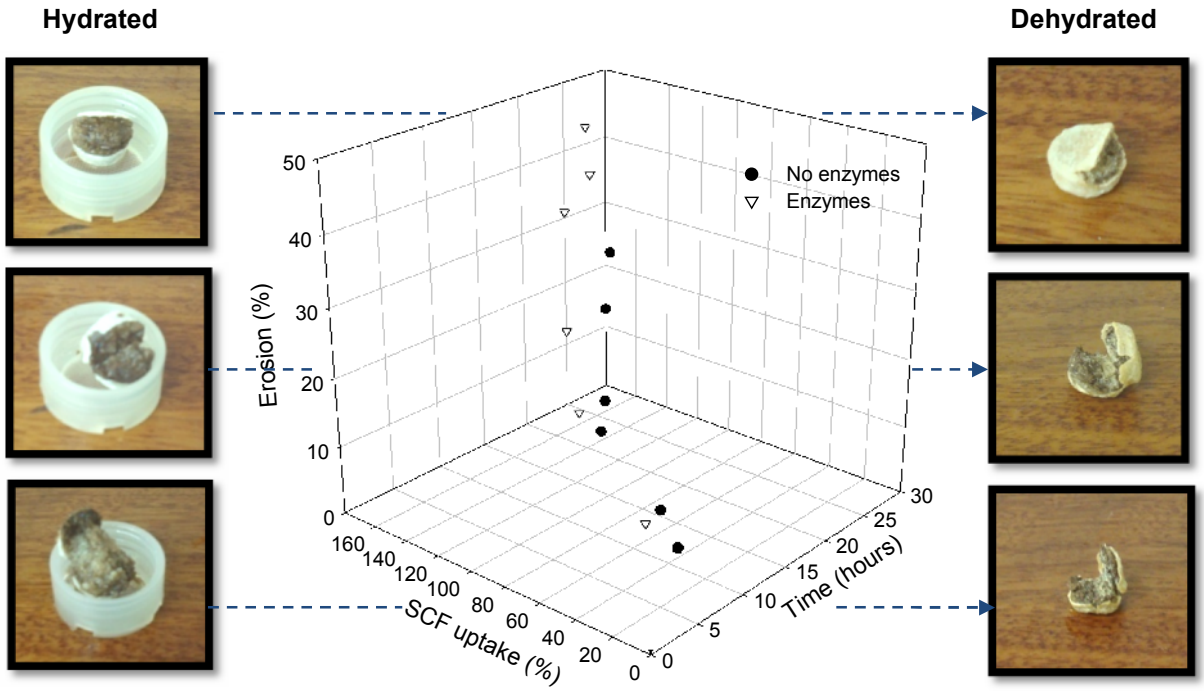



***Figure 5.15:*** *3D-scatter plot of the time after initiation of drug release studies vs. the percentage of simulated colonic fluid uptake vs. the percentage erosion with accompanying digital images of the formulation in its hydrated and dehydrated states.*

### 5.3.4 The influence of colonic enzymes on the structural and polymeric vibrations of the coating and tablet of the enzyme-responsive colon-targeted oral tablet

FTIR spectra of the native polymers employed in the crosslinked 5-ASA-loaded CHT granules were analyzed in relation to the spectrum attained of the optimized granules. CHT showed a distinctive band at 3357cm$^{-1}$ indicative of primary amine NH- stretching and a peak at 1583cm$^{-1}$ indicative of $NH_2$ deformation. A peak at 1025cm$^{-1}$ indicated the presence of an alcohol group with C-OH stretching. 5-ASA has numerous characteristic peaks such as the

two distinct peaks at 2775cm$^{-1}$ and 2521cm$^{-1}$ which are representative of carboxylic acid groups with H- bonded OH- stretching. Furthermore, peaks at 684cm$^{-1}$, 772cm$^{-1}$, 883cm$^{-1}$, 1484cm$^{-1}$, and 1617cm$^{-1}$ can all be related to the basic structure of 5-ASA being a 1,3-substituted aromatic compound. The structure of 5-ASA can also be confirmed by the presence of peaks at 1572cm$^{-1}$, 1190cm$^{-1}$, 1238cm$^{-1}$, 1262cm$^{-1}$, 883cm$^{-1}$, 931cm$^{-1}$ which are representative of an arylamino compound.

The exploitation of CHT in the field of drug delivery is derived from various factors based on its non-toxicity, biodegradability, biocompatibility, bioadhesiveness and the fact that it is the second most abundant polysaccharide in nature. Its abundance originates from its derivation from the deacetylation of chitin which forms a major component of shells of crustaceans, the exoskeletons of insects and the cell walls of fungi, to form a copolymer of glucosamine units linked by β-(1→4)-glycosidic linkages (Illum, 1998; Lin and Lin, 2003; Krajewska, 2004).

Analysis of the crosslinked optimized granules showed that all characteristic peaks present in the spectrum of 5-ASA were unaltered proving no covalent interactions between 5-ASA and CHT. The principle between CHT crosslinking with TPP is essentially based on the fact that when an acid is added to CHT $–NH^{+}_{3}$ sites on the CHT chain become available. In addition, an aqueous solution of TPP ($Na_5P_3O_{10}$) dissociates to give both hydroxyl and phosphoric ions. Therefore, the concurrent presence of the oppositely charges ions allow the interaction of CHT with the negatively charged species of TPP. Bearing this in mind, the preparatory process of the crosslinked 5-ASA-loaded CHT granules involved the incorporation of a 1M solution of acetic acid solution directly onto the CHT prior to TPP addition. The disappearance of the peak at 3357cm$^{-1}$ indicative of primary amine NH- stretching was substantiated by Figure 5.16 which indicates the formation of an $NH_3^+$ bond (peaks at 1573cm$^{-1}$ and 2540cm$^{-1}$) resulting in an ionized compound (amine hydrohalide). In addition, the peak at 1082cm$^{-1}$ indicates the presence of P-O-C stretching due to the TPP.

Since CHT is enzymatically degradable by chitinases and chitosanases the presence of an existing chitinase in β-glucosidase from almond emulsion permits the depolymerization of CHT (Grassmann *et al.*, 1934; Tracey, 1955; Wadsworth and Zikakis, 1984; Il'ina *et al.*, 2001; Zhang and Neau, 2001). However, any substitution on the carbon atoms 2, 3 and 4 such as the amine group on the second carbon atom of CHT completely prevents hydrolysis by β-glucosidase (Dixon and Webb, 1964; Dale *et al.*, 1986). The chitosanolytic activity of β-glucosidase is substantiated by the elevated fractional drug release rate, and degree of SCF uptake and erosion in the presence of this enzyme. A simplistic analysis of the postulated mechanism behind the effect of β-glucosidase on the crosslinked 5-ASA-loaded granules is illustrated in Figure 5.16.

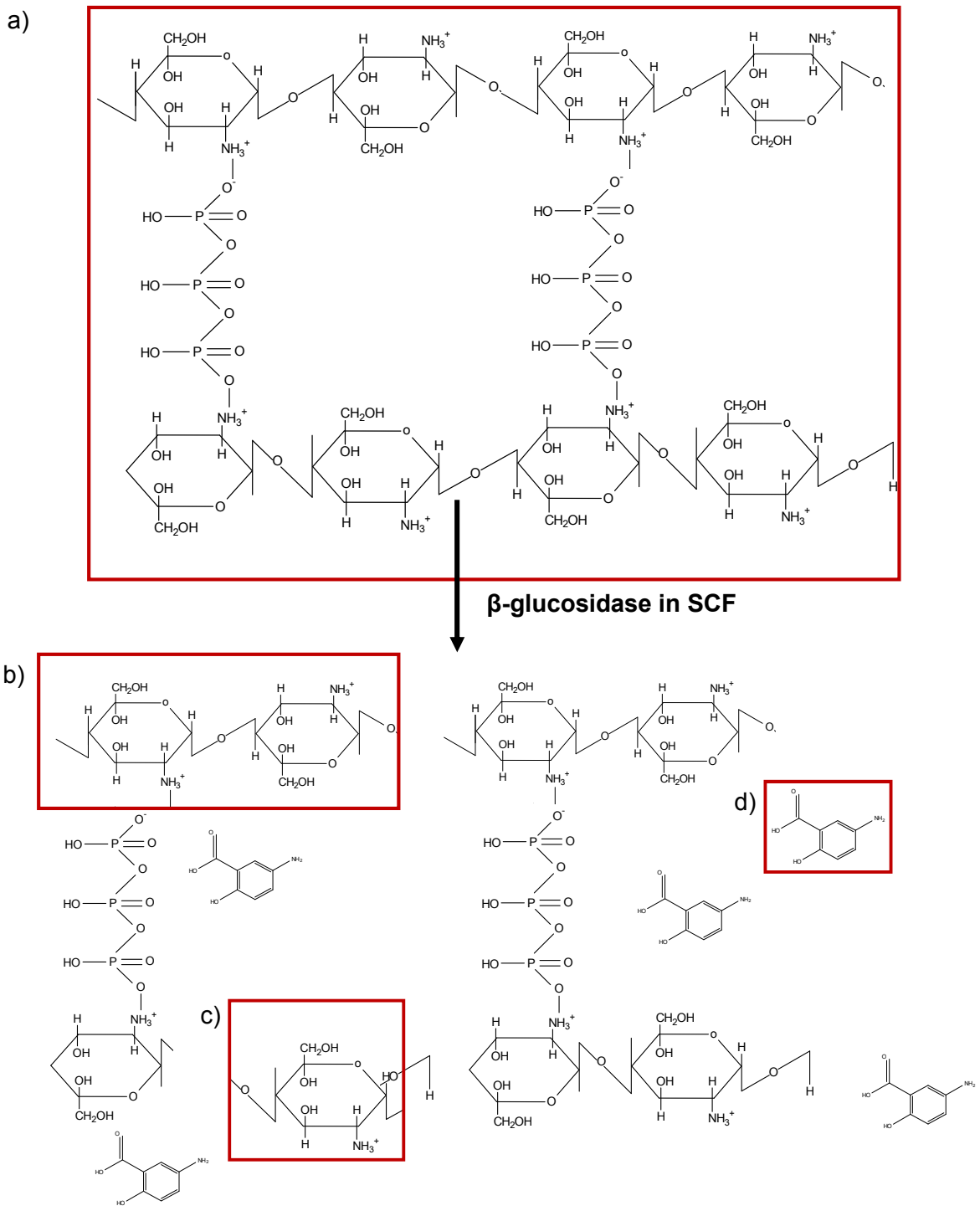



***Figure 5.16:*** *Mechanistic illustration of the action of β-glucosidase on 5-aminosalicylic acid-loaded granules with a) representative of 5-aminosalicylic acid entrapped within the tripolyphosphate-crosslinked chitosan granules, b) the resultant oligosaccharides, and c) glucosamine units that result from depolymerization of chitosan, d) release of 5-aminosalicylic acid.*

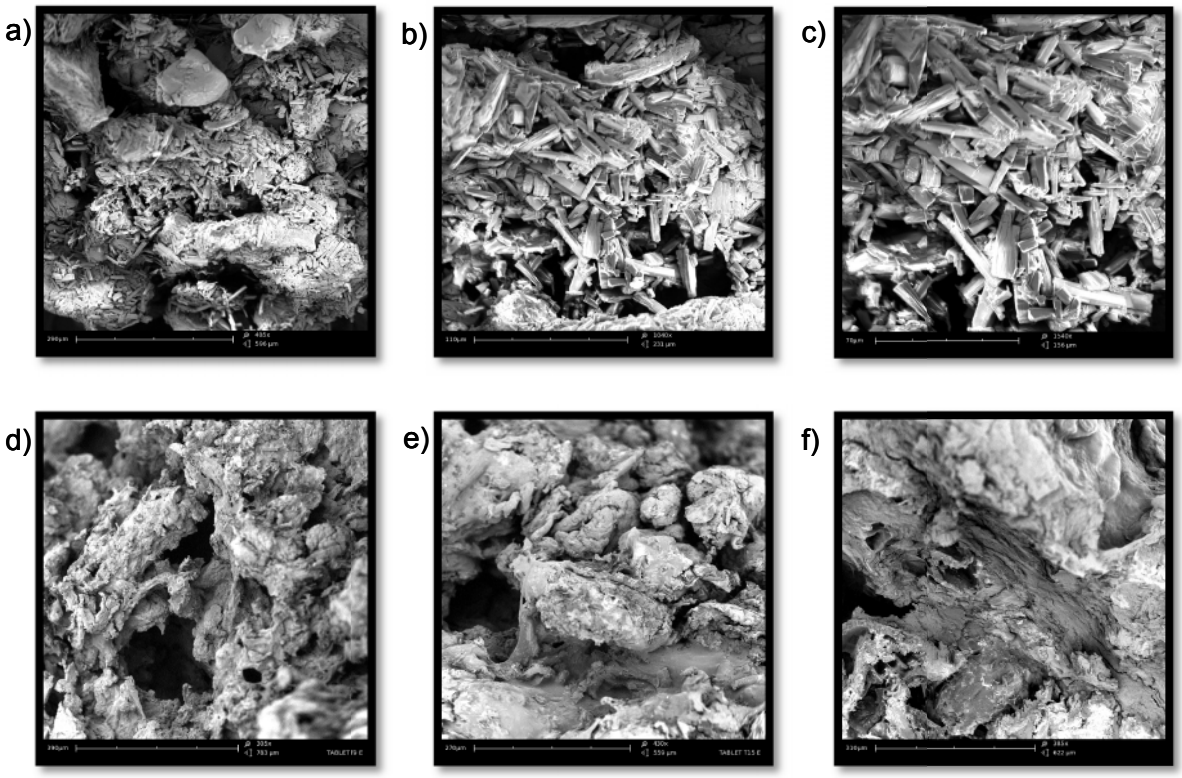



***Figure 5.17:*** *SEM images of the a-c) crosslinked granules at magnifications of x405, x1040, and x1540 respectively, and d-f) granules after exposure to colonic enzymes at T=9, T=15 and T=21 hours respectively at magnifications between x305 to x430.*

In addition to the influence of β-glucosidase on the release of 5-ASA from crosslinked CHT granules, the initiation of drug release is considerably influenced by the presence of pectinase in SCF. Pectinase is comprised of pectin methylesterase and two types of pectin depolymerases. The methylesterase is responsible for removing the methoxy groups from the pectin while the polygalacturonase hydrolyzes the glycosidic linkages next to the free carboxyl groups in the galacturonan chain (Sriamornsak, 1998).

The influence of pectinase on the enzyme-responsive coating is evident from FTIR spectra of the coating of the optimized formulation after exposure to SCF with enzymes after predetermined time points. The distinguishing characteristic of these spectra is the disappearance of peaks from 1589-1512cm$^{-1}$ when exposed to colonic enzymes for 9 hours (T=9, 12 and 15) as indicated in Figure 5.18.

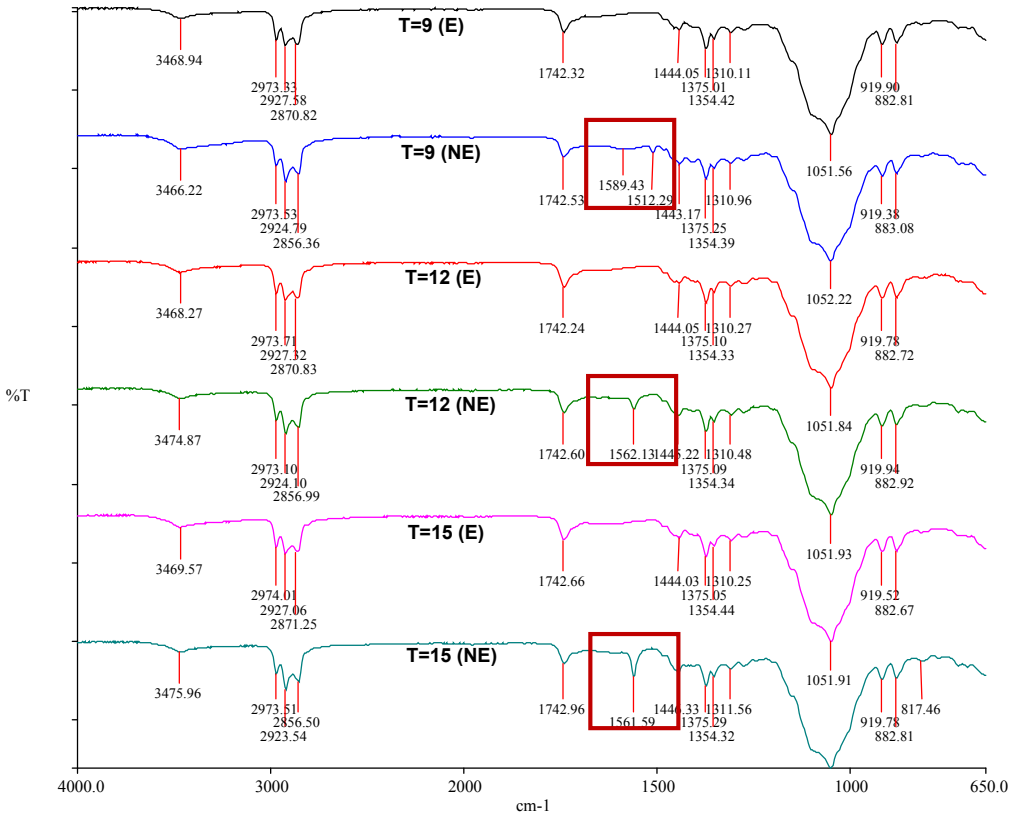



***Figure 5.18:*** *FTIR spectra of the coating of the optimized formulation after 9, 12 and 15 hours of release studies, where 'E' denotes simulated colonic enzymes containing pectinase and β-glucosidase. The red blocks highlight the peaks that disappear when exposed to colonic enzymes.*

Based on the mechanism of action of pectinase on pectin the disappearance of these peaks is postulated to be due to the hydrolization of the glycosidic linkages of the pectin backbone chain resulting in the formation of minute pores through which dissolution media was capable of entering. However after 15 hours of exposure to SCF no difference was evident in the FTIR spectra of the coating exposed to enzymes compared to those that were not exposed to enzymes. Since pectin is a highly hydrophilic polymer it is believed that after 15 hours the majority of the pectin present in the coating has dissolved in the release media and is no longer susceptible to the enzymatic activity of pectinase.

FTIR spectra of the inner component of the oral tablet (enclosed by the coating) after specific time-points in SCF with and without enzymes is illustrated in Figure 5.19. Based on these spectra it is evident that at T=9, where the tablets had been exposed to SCF for a period of only 3 hours no significant difference is observable between the formulations in the presence of enzymes and those in the absence of enzymes. However with an increase in exposure time to SCF containing enzymes there is a gradual disappearance of peaks between 1646cm$^{-1}$ and 1132cm$^{-1}$. This is due to the steady increase in the rate of SCF permeation

into the tablet, which consequently results in an increased quantity of colonic enzymes entering the delivery system causing cleavage of the relevant bonds as well as the gradual release of 5-ASA.

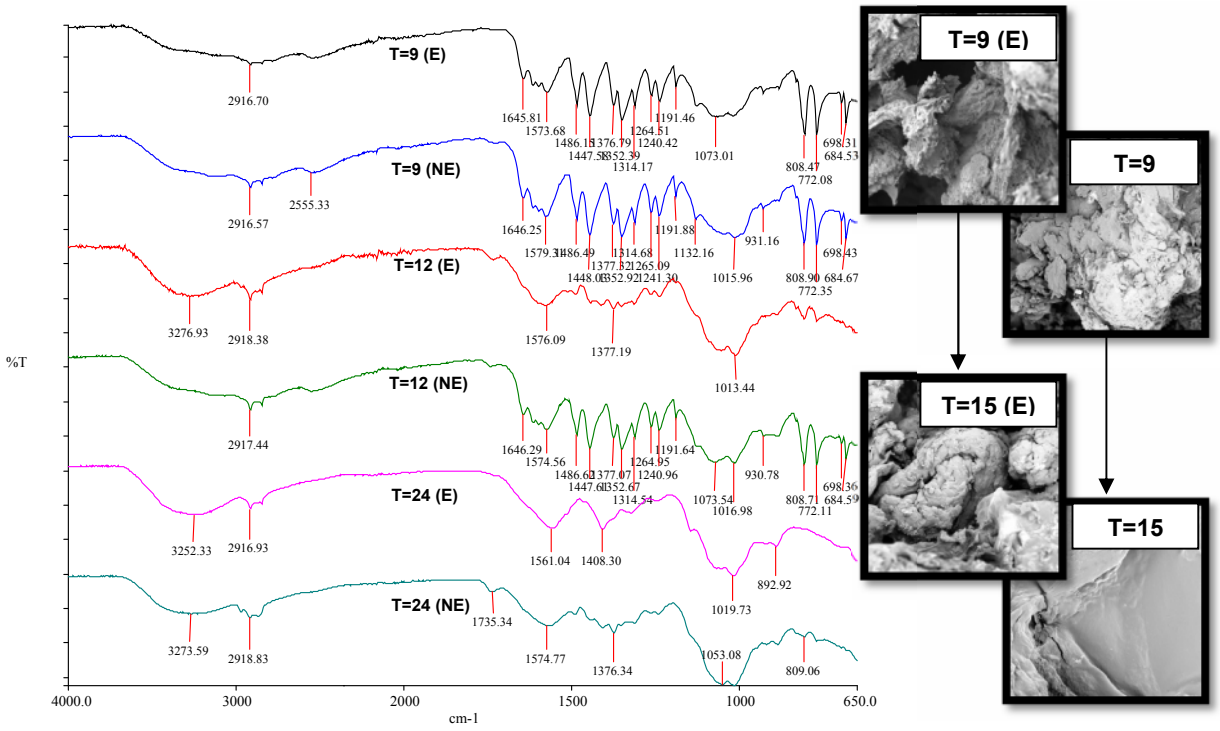



***Figure 5.19:*** *FTIR spectra of the tablet at various time points in the presence and absence of colonic enzymes with accompanying SEM images (x1120) elucidating the basic morphological differences that occur as a result of increasing exposure time to simulated colonic fluid with and without colonic enzymes.*

Surface morphological evaluation of the 5-ASA-loaded crosslinked CHT granules revealed the presence of distinct twig-like structures with sizes ranging from less than 70µm up to 250µm (Figure 5.17a-c). This provides a visual indication of crosslinking between CHT and TPP. However after exposure of the formulation to SCF a vivid morphological difference is evident from the tablet exposed to colonic enzymes to the tablet that is exposed to enzyme-free SCF. In the presence of colonic enzymes the tablets initially appear almost fibre-like with several porous regions. However with an increase in exposure time these tablets generate a less fibrous appearance with more sites of 'polymeric clumping' (Figure 5.17d-f). In contrast, in the absence of colonic enzymes the tablets present with a gradually increasing uniform and smooth morphology with fewer porous sites (Figure 5.19).

### 5.3.5 Molecular mechanics energy relationship (MMER) analysis of the enzyme-responsive colon-targeted oral tablet

It is evident form Figure 5.20 that TPP can crosslink the chitosan by forming both intra- and inter-molecular bonding. The formation of highly stabilized structure of CHT-TPP with $\Delta E_{BINDING}$ = -132.867kcal/mol (equation 5.9, 5.10, and 5.11) also favours the crosslinking

action of TPP. The complex is mainly stabilized by non-bonding interactions in terms of London dispersion forces, H-H bonding and ion pair-ion pair electrostatic interactions.

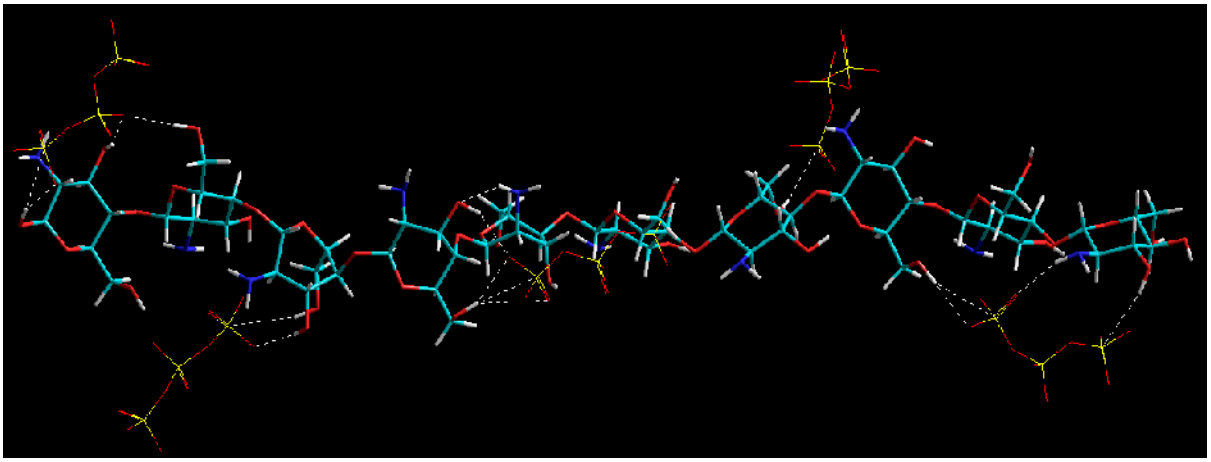


***Figure 5.20:*** *Visualization of geometrical preferences of tripolyphosphate (stick rendering) in complexation with chitosan (tube rendering) after molecular mechanics simulations. Color codes: C (cyan), O (red), N (blue), P (yellow) and H (white).*

$$E_{CHT} = 35.555V_{\sum} = 3.120V_b + 18.035V_\theta + 25.774V_\varphi + 13.323V_{ij} - 24.697V_{el} \quad \text{Equation 5.9}$$

$$E_{TPP} = 199.744V_{\sum} = 1.927V_b + 93.088V_\theta + 1.599V_{\varphi +} 0.046V_{ij} + 103.082V_{el} \quad \text{Equation 5.10}$$

$$E_{CHT\text{-}TPP} = 901.408V_{\sum} = 18.542V_b + 514.621V_\theta + 54.501V_\varphi + 28.920V_{ij} - 1.065V_{hb} + 285.889V_{el}$$
$$[\Delta E_{BINDING} = -132.867 \text{kcal/mol}]$$

Equation 5.11

Therefore, the controlled release behaviour of the enzyme-responsive colon-targeted oral tablet may be attributed to the dense polymeric matrix formed due to this very crosslinking mechanism of TPP. However, the enzymatic stimuli-responsive behaviour of this formulation can be accredited to the action of the enzymes on specific bonding/functional sites on the polysaccharides as shown in Figure 5.21 and Figure 5.22.

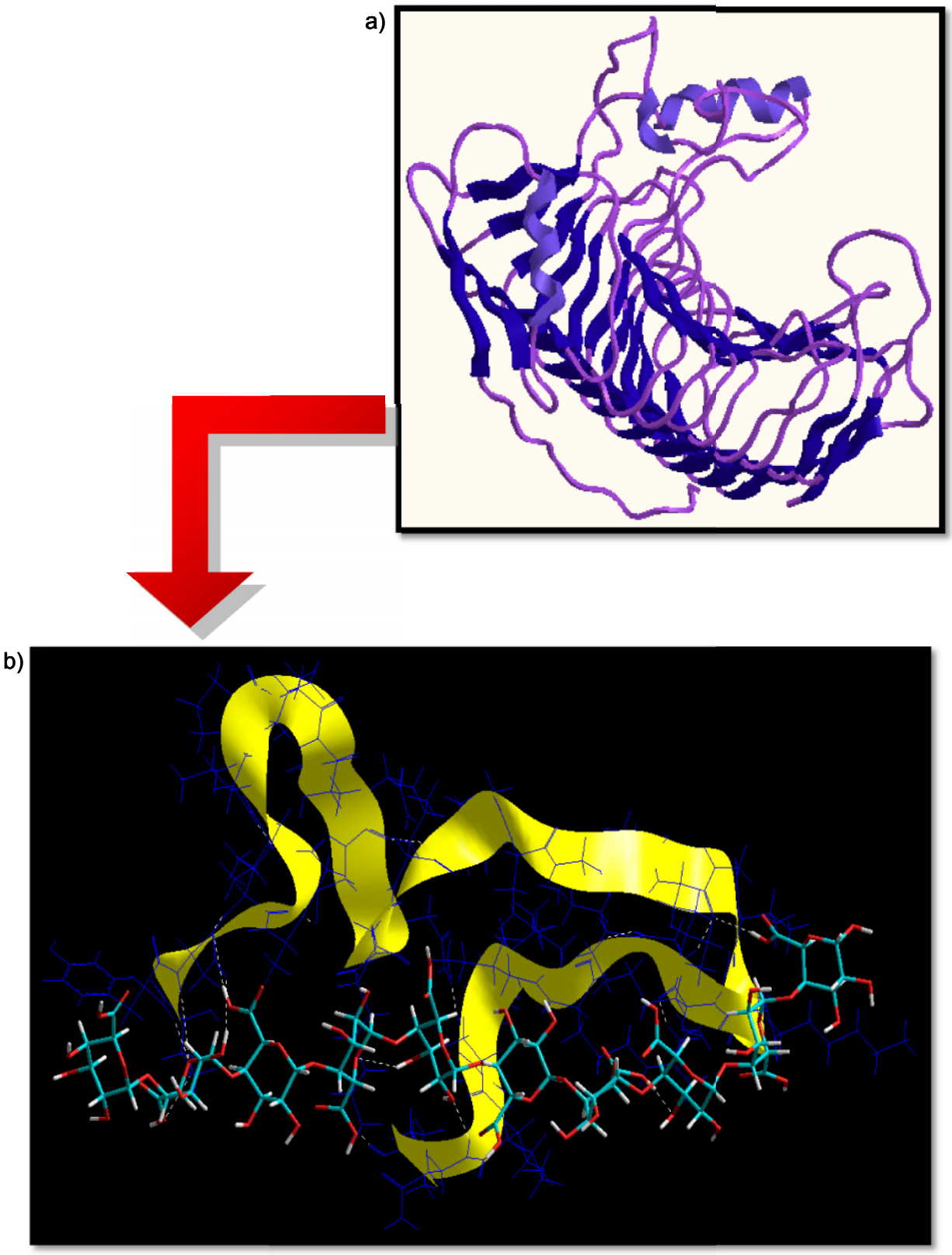



***Figure 5.21:*** *Visualization of a) the polygalacturonase (PGU) (A Chain) and b) geometrical preference of polygalacturonase (blue stick rendering and yellow secondary structure) in complexation with pectin (tube rendering) after molecular mechanics simulations. Color codes: C (cyan), O (red) and H (white).*

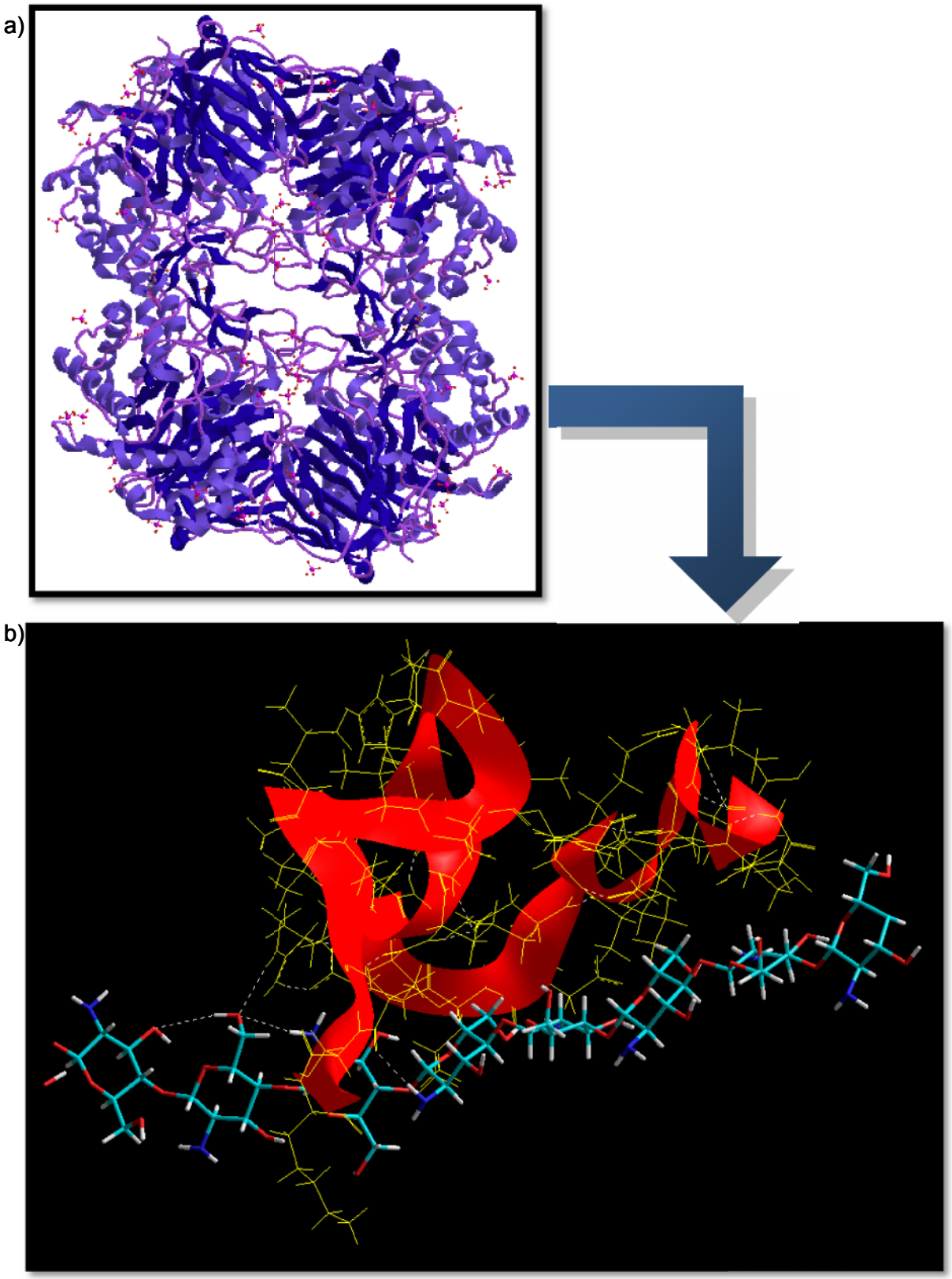



***Figure 5.22:*** *Visualization of a) β-glucosidase (βGD) (A,B,C,D Chains), and b) geometrical preference of β-glucosidase (yellow stick rendering and red secondary structure) in complexation with chitosan (tube rendering) after molecular mechanics simulations. Color codes: C (cyan), O (red), N (blue) and H (white).*

Equations 5.12 and 5.13 also confirmed the interaction potential of the enzymes with polysaccharides accounting for stabilized negative binding energies of -146.042kcal/mol and -64.283kcal/mol for PEC-PGU and CHT-βGD molecular complexes, respectively. The complexes are mainly stabilized by London dispersion forces and ion pair-ion pair electrostatic nonbonding interactions.

$$\mathbf{E_{PEC\text{-}PGU}} = -658.584V_{\sum} = 13.458V_b + 78.405V_\theta + 76.139V_\varphi - 80.142V_{ij} - 21.833V_{hb} - 724.613V_{el}$$
$$[\Delta E_{BINDING} = -146.042\text{kcal/mol}]$$

Equation 5.12

$$\mathbf{E_{CHT\text{-}\beta GD}} = -518.293V_{\sum} = 11.187V_b + 90.6291V_\theta + 80.424V_\varphi - 86.47V_{ij} - 12.503V_{hb} - 601.561V_{el}$$
$$[\Delta E_{BINDING} = -64.283\text{kcal/mol}]$$

Equation 5.13

### 5.4 Concluding Remarks

A novel enzyme-responsive colon-targeted oral tablet has been successfully formulated. The tablet comprised of a range of naturally-derived polysaccharide polymers that imparted a distinct responsiveness of the formulation to colonic enzymes. This responsiveness was evidenced by the initiation and acceleration of 5-ASA release in the presence of these enzymes as opposed to when the enzymes were absent from the simulated media. The achievement of the desirable drug release characteristics were accompanied with evidence relating the drug release to the effect of pectinase and β-glucosidase on the chemical structure of the incorporated polymers, and consequently the degree of SCF uptake and erosion by the tablet. In addition, SEM images further augmented the magnitude of the effect of the enzymes on tablet. Moreover, the promise of the developed system is further pronounced by evidence of the erratic, irreproducible and non-dependable 5-ASA release from the conventional commercially available marketed 5-ASA containing product, Asacol®. Therefore, the developed enzyme-responsive colon-targeted oral tablet imparted the benefits of a multiparticulate system into a single-unit tablet.

# CHAPTER 6

# FABRICATION AND STATISTICAL OPTIMIZATION OF THE PEPSIN-RESPONSIVE POLYMERIC COATING THROUGH AN EXPERIMENTAL DESIGN STRATEGY

## 6.1 Introduction

### 6.1.1 Optimization of loperamide HCl treatment of chronic diarrhoea associated with Ulcerative Colitis

Since its introduction in 1973, loperamide HCl has been widely employed for the effective control and symptomatic relief of acute non-specific diarrhoea as well as chronic diarrhoea associated with inflammatory bowel diseases such as Crohn's Disease (CD) and Ulcerative Colitis (UC) (Lavrijsen *et al.*, 1995; Chen *et al.*, 2000). Even though loperamide HCl exhibits a strong affinity for opiate receptors in both the brain and myenteric plexus, in contrast to opioids such as diphenoxylate, oral doses of loperamide HCl do not reach the blood-brain barrier to any significant extent. Therefore, it does not cause opiate-like central nervous system side-effects (Niemegeers *et al.*, 1974; Schuermans *et al.*, 1974; Wuster and Herz, 1978; Ooms *et al.*, 1984; Ruppin, 1987; Schinkel *et al.*, 1996; Baker and Meert, 2002).

The myenteric and submucosal plexuses of the enteric nervous system essentially comprise of 3 types of opiate receptors namely, mu (μ), delta (δ) and kappa (κ) receptors. However loperamide HCl exhibits preferential selectivity for the μ subtype of the opioid receptor (Ruoff *et al.*, 1991; DeHaven-Hudkins *et al.*, 1999; Chen *et al.*, 2000; Bohn and Raehal, 2006). This μ-receptor resides within the myenteric plexus and through its binding with loperamide the anti-motility effect is exhibited.

A study conducted by Verhaegen and co-workers established that the optimal therapeutic dose of loperamide HCl for the treatment of chronic diarrhea due to IBD was as high as 18mg/day (Verhaegen *et al.*, 1974). Thus based on the site and mechanism of the therapeutic action of loperamide it is expected that by targeting the drug to the myenteric plexus of the small and/or large intestine a greater therapeutic benefit will be experienced at the same or reduced dosage. Therefore, the novel enzyme-responsive outer crosslinked polymeric coating aims to exploit the stomach-specific enzyme, pepsin, to prevent/minimize the premature release of loperamide HCl in the stomach, thus allowing a greater quantity of the drug to reach the small intestine where its therapeutic action is achieved.

### 6.1.2 Polymer vs. Pepsin

Unlike the activity of the proteolytic enzymes of the pancreas and small intestine the behaviour of pepsin is peculiar. Essentially, pepsin is capable of accomplishing extensive degradation of proteins without significantly resulting in an increase in titratable groups. Thus it has been postulated that the specific character of peptic digestion by pepsin is due to the disintegration of protein molecules into smaller fragments which are then united in the original molecule by forces other than those of primary valence. Generally, the proteolytic activity of pepsin results in a considerable excess of carboxyl groups released in relation to the simultaneously released amino groups, leading to the impression that the –COOH groups may form linkages within the protein molecule with some other group other than the primary $–NH_2$ groups. However, this postulation is difficult to definitively prove (Cannan and Muntwyler, 1930).

The biomacromolecule, gelatine, is obtained through the hydrolysis of collagen which exists as the most abundant protein in the skin, connective tissue, bone and cartilage of animals. This biopolymer is typically readily and extensively digested by pepsin. Apart from the biocompatibility, biodegradability and low toxicity of gelatine, its natural origin also offers so-called social advantages such as reduced waste disposal management (Farris *et al.*, 2010). Therefore, gelatine has typically been employed as a wall material for microcapsules and microspheres, as a sealant for vascular prostheses and wound dressing, or furthermore as an adsorbent pad for surgical purposes in clinical applications (Schrieber and Gareis, 2007; Farris *et al.*, 2010). More recently however it has been investigated for potential application in tissue engineering and has also been used in conjunction with synthetic, biological or inorganic molecules (Kang *et al.*, 2006; Heydarkhan-Hagvall *et al.*, 2008; Lien *et al.*, 2008; Farris *et al.*, 2010).

Despite the numerous benefits of gelatine as a biopolymer its poor mechanical properties and water sensitivity are recognized as the major hindrances that limit its more extensive use (Lee and Mooney, 2001; Lee *et al.*, 2004; Yasuda *et al.*, 2005). Among the several investigated approaches of overcoming these hurdles, chemical crosslinking remains the most attractive and widely used technique that improves the thermal, mechanical and water-sensitivity properties of gelatine (Liu *et al.*, 2007; Farris *et al.*, 2010). Several crosslinking agents such as glyoxal, epoxides, isocyanates, formaldehyde and carbodiimides have been investigated however glutaraldehyde is suggested to be the most effective due to its low cost and excellent efficiency in stabilizing collagenous materials which ultimately improves the strength and water resistance of gelatine (Sung *et al.*, 1996; Khor, 1997; Sung *et al.*, 1999; Liang *et al.*, 2004; Carvalho and Grosso, 2006; Bertoldo *et al.*, 2007; Liu *et al.*, 2007).

Therefore, the outer pepsin-responsive intestinally-targeted polymeric coating aims to exploit selected characteristic behaviours of pepsin on the protein molecule, gelatine, to achieve a minimization of loperamide HCl release in the stomach and therefore a targeted delivery of a greater quantity of the drug at the site of action in the small intestine. The loperamide HCl containing coating, its passage through the gastrointestinal tract (GIT) as well as its site of action is depicted in Figure 6.1.

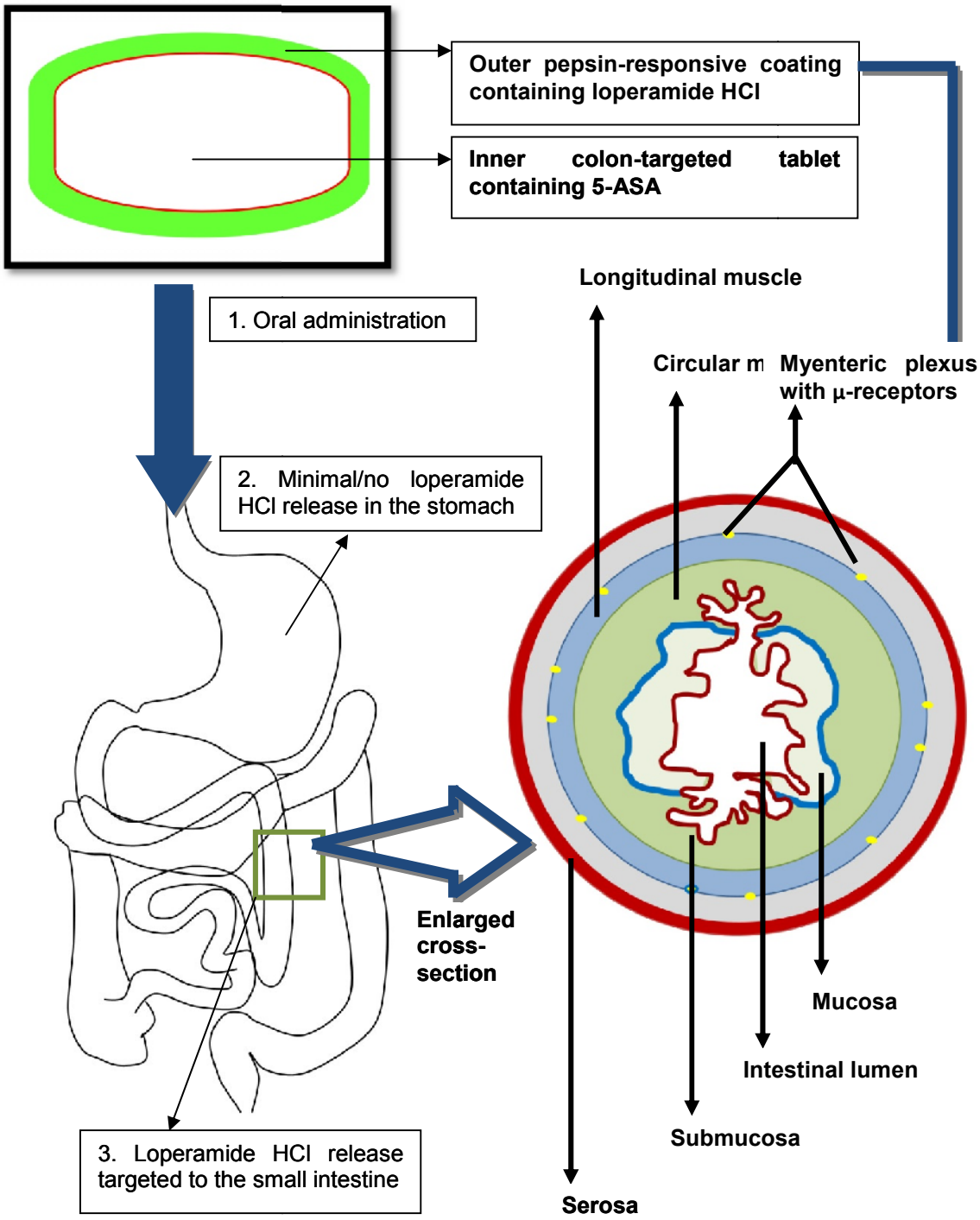



***Figure 6.1:*** *Illustration of the stimuli-responsive oral tablet system (SROT) with the outer pepsin-responsive coating and the site of action of encapsulated loperamide HCl in the myenteric plexus of the small intestinal wall.*

Thus this chapter focussed on the development of an optimized outer pepsin-responsive intestinally-targeted polymeric coating, through a statistically robust experimental design approach.

## 6.2 Materials and Methods

### 6.2.1 Materials

Gelatine, glutaraldehyde (GA), loperamide HCl, and ethanol were obtained as described in Chapter 4, sections 4.2.1 and 4.4.1. Lactose was supplied by Saarchem-Merck (UniLAB®, Wadeville, Gauteng, South Africa). Imodium® was supplied by Janssen Pharmaceutica (Pty) Ltd. (Woodmead, Johannesburg, South Africa). De-ionized water was obtained from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, USA) and all other reagents were of analytical grade and used without further purification.

### 6.2.2 Preparation of the outer pepsin-responsive intestinally-targeted polymeric coating in accordance with a randomized Box-Behnken experimental design template

The outer pepsin-responsive loperamide HCl-loaded coating was prepared according to a 3 factor, 3 tier Box-Behnken experimental design (Minitab® V15, Minitab Inc., PA, USA) that investigated 15 experimental formulations. The 15 formulations were derived from a set of points lying at the mid-point of each edge and the replicated centre point of a multi-dimensional cube (Patel *et al.*, 2010). The coatings encapsulated the colon-targeted 5-ASA loaded tablet as illustrated in Figure 6.1 and were prepared by a dip-coating process that involved submerging the tablets in the aqueous drug-loaded gelatine solution. The dried gelatine coatings were subsequently crosslinked in a moist mixture of GA and lactose for various time periods. The response surface methods of the employed Box-Behnken design enabled the examination of the relationship between the response variables and the set experimental parameters which ultimately allowed the prediction of an optimized formulation. The independent formulation variables were selected based on their influence in ensuring minimal loperamide HCl release in the presence of pepsin, thus ensuring improved drug targeting to the small intestine. The formulation input factors and the dependent measured responses are shown in Table 6.1.

***Table 6.1:*** *Independent variables and dependent measured responses of the outer pepsin-responsive intestinally-targeted coating based on a 3 factor, 3 tier Box-Behnken experimental design*

| **Independent variables** | **Values** | |
|---|---|---|
| | **Minimum** | **Maximum** |
| Gelatine ($\%^{w}/_{v}$) | 10 | 40 |
| Crosslinking ratio (GA:lactose) | 0.1250 | 0.2500 |
| Crosslinking time (hours) | 3 | 15 |
| **Dependent variables** | | **Objective** |
| MDT (no pepsin) | | Maximize |
| MDT (pepsin) | | Minimize |

- Gelatine concentration ($\%^{w}/_{v}$) - the gelatine concentration range of 10-40$\%^{w}/_{v}$ was selected based on the ability of the gelatine solution to form a uniform and reproducible coating in preliminary screening studies. Concentrations <10$\%^{w}/_{v}$ and >40$\%^{w}/_{v}$ were ineffective in forming the coating due to the solution viscosities encountered at each extremity.
- Crosslinking ratio of GA to lactose - the crosslinking ratios of 0.1250, 0.1875 and 0.2500 denoted a GA:lactose ratio (mL/g) of 1:8, 1:6 and 1:4 respectively.
- Crosslinking time (hours) - the crosslinking ratios and durations were selected based on preliminary screening studies which identified a relationship between the concentration of the gelatine solution, the crosslinking ratio and crosslinking time with the ability of the coating to respond to pepsin for example, a shorter duration of crosslinking of a higher concentration of gelatine provided an equivalent/improved pepsin-responsiveness compared to a low concentration of gelatine and longer duration of crosslinking. In addition, logistically it was also more suitable to investigate the shorter crosslinking durations.

Quadratic response surfaces and second order polynomial models were generated to predict the *in vitro* drug release characteristics of loperamide HCl in SGF with and without pepsin in terms of the independent variables investigated. The drug release data and thus the response parameters were analyzed by establishing the mean dissolution time of the formulations in the presence ($MDT_P$) and absence (MDT) of pepsin in SGF. By determining the ability of the formulations to prevent drug release in the presence of pepsin the intestinal targeting ability of the formulations can be indirectly established. The 15 formulations investigated are depicted in Table 6.2.

***Table 6.2:*** *Box-Behnken design template of the 15 statistically derived formulations for the fabrication of the outer pepsin-responsive coating*

| Experimental Formulation | Gelatin ($\%^{w}/_{v}$) | Crosslinking ratio (GA:lactose) | Crosslinking time (hours) |
|---|---|---|---|
| 1 | 10 | 0.1875 | 3 |
| 2 | 10 | 0.2500 | 9 |
| 3 | 25 | 0.1250 | 15 |
| 4 | 40 | 0.2500 | 9 |
| 5 | 25 | 0.2500 | 15 |
| 6 | 25 | 0.2500 | 3 |
| 7 | 25 | 0.1875 | 9 |
| 8 | 10 | 0.1875 | 15 |
| 9 | 25 | 0.1875 | 9 |
| 10 | 10 | 0.1250 | 9 |
| 11 | 40 | 0.1875 | 3 |
| 12 | 25 | 0.1875 | 9 |
| 13 | 40 | 0.1250 | 9 |
| 14 | 25 | 0.1250 | 3 |
| 15 | 40 | 0.1875 | 15 |

### 6.2.3 Preparation of the outer pepsin-responsive intestinally targeted polymeric coating

#### 6.2.3.1 Preparation of the crosslinked gelatine coatings prior to crosslinking

The fabrication process of the gelatine coatings prior to crosslinking is outlined in detail in Chapter 4, section 4.4.2 with the exception that the concentrations of gelatine solutions, crosslinking concentrations and crosslinking durations are prescribed according to the Box-Behnken experimental design as depicted in Table 6.2. This preparatory process is schematically illustrated in Figure 6.2. For the purpose of preparation of the experimental design formulations the inner tablet was a placebo tablet comprising of lactose. The placebo tablets were compressed into tablets of 13.6x4.5mm dimensions using a mini rotary tablet press (Karnavati Mini Press II, Rimek Products, Gujarat, India) that ensured the identical dimensions and shape of the 5-ASA loaded colon-targeted tablet as described in Chapter 5.

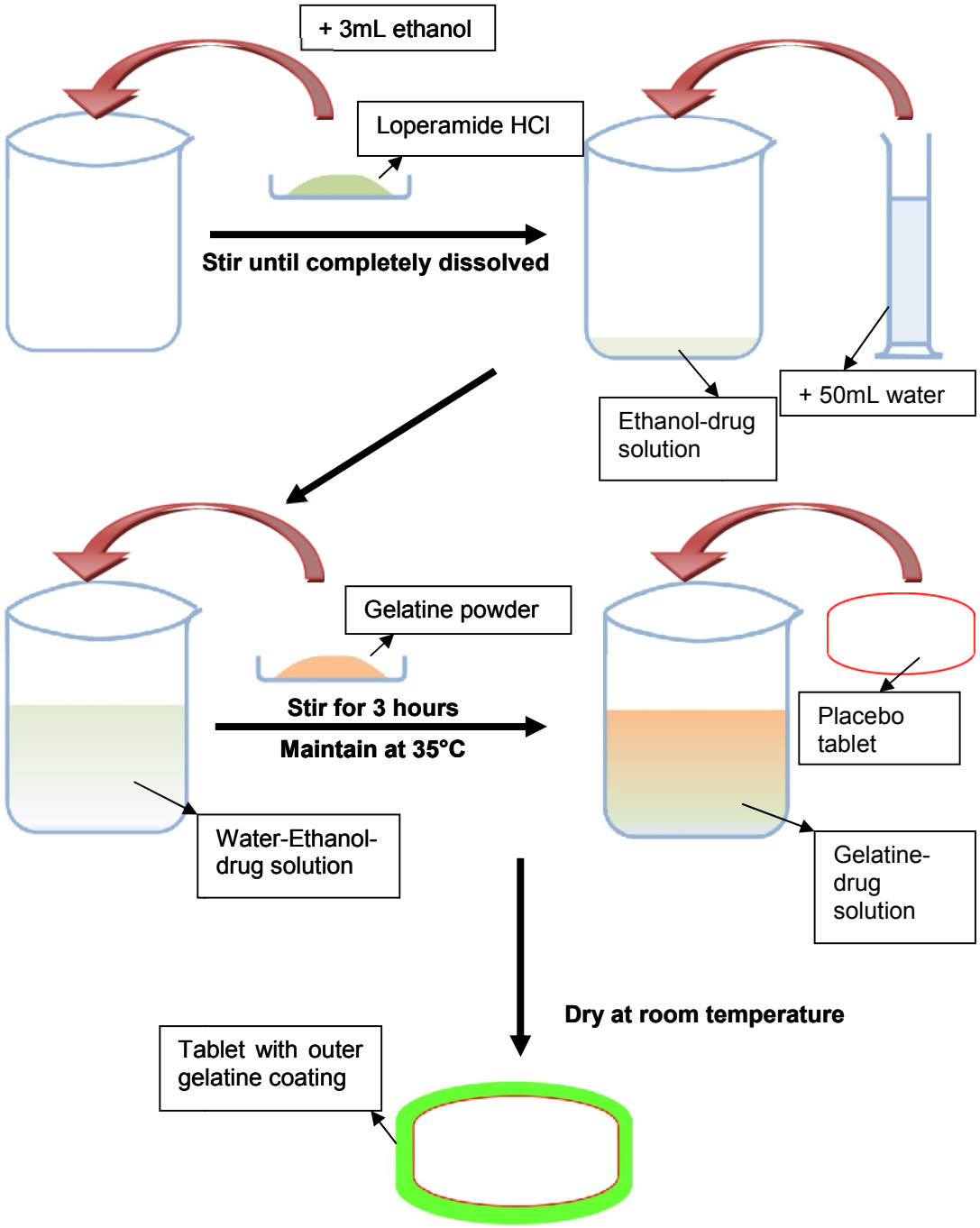



***Figure 6.2:*** *Schematic illustration of the preparation of the outer gelatine coating surrounding the inner tablet prior to crosslinking.*

### 6.2.3.2 Crosslinking of the gelatine coatings

Crosslinking of the dried gelatine coatings surrounding the tablets was performed in the relevant GA-lactose (v/w) concentrations. The crosslinking blend was prepared by thoroughly combining the GA with lactose until 'loose' moist granules were obtained. The sealed mixture was then placed in a convection oven (Memmert, Schwabach, Germany) maintained at 35°C for 3 hours. Thereafter the tablets were submerged within the blend and allowed to crosslink in the oven maintained at 35°C for the required period of time.

### 6.2.4 Elucidation of the influence of the gelatine concentrations and crosslinking parameters on the gravimetrical and dimensional properties of the formulations

In order to establish the influence of the concentration of the gelatine solution and the crosslinking concentrations and durations on the physical properties of the tablets a gravimetrical and dimensional study was conducted on all 15 experimental formulations before and after coating with the gelatine solution, as well as after crosslinking. The relevant weights were recorded and the weight increases calculated according to Equation 6.1.

$$Wt\ (\%) = \frac{Wt_f - Wt_i}{Wt_i} \times 100 \qquad \text{Equation 6.1}$$

*Where Wt (%) is the percentage weight increase of each tablet, $Wt_f$ is the final weight after coating or crosslinking and $Wt_i$ is the initial weight of the tablets before coating and crosslinking.*

The dimensions of the formulations before and after coating with the gelatine solution, as well as after crosslinking, were recorded in terms of the height (mm) and diametrical (mm) changes using an electronic digital calliper. The points at which the height and diameter were measured in each formulation were kept consistent and are depicted in Figure 6.3.

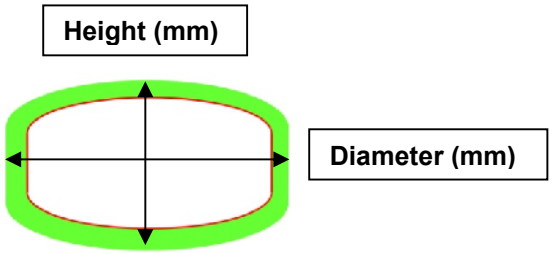



***Figure 6.3:*** *Points of dimensional measurements of each formulation.*

The change in dimensions, expressed as a percentage, was then expressed based on Equation 6.2.

$$D\ (\%) = \frac{D_f - D_i}{D_i} \times 100 \qquad \text{Equation 6.2}$$

*Where D (%) is the percentage increase in the dimensions, $D_f$ is the final dimensions after coating and crosslinking in $mm^2$ and $D_i$ is the initial dimensions before coating and crosslinking in $mm^2$. Note: $D_i$ and $D_f$ = height x diameter.*

### 6.2.5 Construction of calibration curves by spectroscopic determination of loperamide HCl in simulated gastric and intestinal fluid

Calibration curves for loperamide HCl were conducted in simulated gastric fluid (SGF) (pH 1.2; 37°C) with and without pepsin as described in Chapter 4, section 4.4.3. A calibration curve was also generated for loperamide HCl in simulated intestinal fluid (SIF) (pH 6.8; 37°C) at a concentration range of 0-0.051mg/mL analyzed at a wavelength of 260nm. Linear curves were plotted such that the observed absorbances from spectroscopic analysis were plotted on the y-axis and the concentrations (mg/mL) on the x-axis. In SIF a regression coefficient of 0.997 was attained.

### 6.2.6 Determination of the quantity of drug entrapped within each non-crosslinked gelatine coating

The determination of the quantity of loperamide HCl contained in each coating was performed on the non-crosslinked coatings as these were capable of undergoing complete dissolution thus ensuring release of all entrapped drug in the simulated medium. Furthermore, since crosslinking of the gelatine coatings was not conducted in solution, the possibility of 'leaching out' of drug during the crosslinking procedure bore no influence. Thus determination of the quantity of loperamide HCl contained in each coating prepared according to the Box-Behnken experimental design was conducted as described in Chapter 4, section 4.4.4.

### 6.2.7 *In vitro* drug release assessment of the outer loperamide HCl-loaded crosslinked coatings in response to pepsin present in SGF

*In vitro* drug release studies were conducted according to parameters and conditions specified in Chapter 4, section 4.4.5. In addition to subjecting the formulations to SGF (with and without pepsin) for a period of 2 hours, these were also exposed to SIF for a period of 4 hours following the 2 hours in SGF. The dip rate, fluid temperature, mesh sizes and fluid volume were kept consistent and sink conditions maintained throughout the study. Samples were withdrawn at 30 minute intervals for the 2 hours in SGF, and every 2 hours in SIF. Sample withdrawal, analysis and loperamide HCl quantification were performed as described in Chapter 3, section 3.4.7. Samples were however analyzed at a maximum excitation wavelength of 260nm in both SGF and SIF. Data was subsequently computed in a model-independent manner. This involved the determination of the mean dissolution time of all formulations after the 2 hours in SGF ($MDT_2$), thus permitting a quantitative comparison of the drug release behaviour of the formulations in the presence and absence of the gastric enzyme pepsin and consequently establishing the pepsin-responsiveness of the coatings. Furthermore, the gold-standard marketed loperamide HCl formulation, Imodium® was also subjected to *in vitro* release studies based on identical parameters.

### 6.2.8 Constrained statistical optimization of the outer pepsin-responsive intestinally-targeted polymeric coating

Optimization of the outer pepsin-responsive intestinally-targeted coating was performed by generating polynomial equations that related the dependent formulation responses and independent variables. This statistical model was performed under constrained conditions and incorporated the interactive and polynomial terms that enabled the establishment of a formulation that possessed the desirable formulation characteristics. Therefore, for optimization purposes the mean dissolution time (MDT) at 2 hours in the presence ($MDT_P$) of pepsin was minimized and MDT in the absence of pepsin was maximized.

### 6.2.9 Evaluation of the influence of pepsin on the SGF uptake ability and erosion of the gelatine coatings

Since gelatine is primarily derived from collagen, it essentially consists of a mixture of water soluble proteins that are readily and extensively digested by pepsin (Cannan and Muntwyler, 1930; Gold *et al.*, 1997). However, it has been noted that crosslinking of gelatine results in a polymer structure with improved strength and water resistance (Liu *et al.*, 2007). Therefore to elucidate the influence of pepsin on the crosslinked gelatine coating a simulated fluid uptake study was conducted where the optimized formulation was subjected to identical conditions as those employed during drug release studies however at 30 minute intervals for the first 2 hours in SGF and every 2 hours in SIF each formulation was removed from the dissolution medium and allowed to drip-dry for a period of 2 minutes at room temperature to remove excess SGF. The drip-drying process was conducted in a manner that ensured no introduction of bias since the formulations were left remaining on the bottom mesh of the glass cylinder of the Bio-Dis® apparatus and no physical 'blotting' of the formulations was performed. As a control, this process was replicated in SGF without pepsin. Fresh samples were used for each individual time point.

The degree of SGF and SIF uptake at each time point, expressed as a percentage, was then determined based on the increase in weight of the formulations calculated according to Equation 6.3. The weight of the hydrated coatings could be distinguished from the weight of the tablets since once subjected to simulated media the coatings were detachable from the tablets.

$$SGF(SIF)_{uptake}(\%) = \frac{Wt_f - Wt_i}{Wt_i} \times 100 \qquad \text{Equation 6.3}$$

*Where, $Wt_f$ is the weight of the hydrated coating at the relevant time point and $Wt_i$ is calculated by Equation 6.4.*

$Wt$ = *Weight of tablet after crosslinking – weight of tablet before gelatine coating*

Equation 6.4

The physical dimensions of the formulations could not be determined after exposure to SGF since the outer coatings detached from the inner tablet structure.

Subsequent to establishing the influence of pepsin on the gravimetrical changes of the formulations, the hydrated samples were dried in a convection oven at 40°C for 48 hours, and allowed to cool under a fume hood for a further 30 minutes after which they were re-weighed. The influence of pepsin on the erosion of the coating was then determined based on Equation 5.4 (in Chapter 5), where $Wt_i$ was calculated according to Equation 6.4.

### 6.2.10 Evaluation of the polymeric structural and vibrational frequency variations of the outer pepsin-responsive coating and its components

The structures of gelatine, loperamide HCl, GA and the crosslinked gelatine coating were assessed based on a FTIR method described in Chapter 5 section 5.2.10. Furthermore, the samples acquired at each time point from the previously described erosion study (in the presence and absence of pepsin) were also assessed by FTIR. This was performed to categorically elucidate the influence of pepsin on the polymeric backbone structure of the crosslinked gelatine coatings and to divulge critical mechanistic characteristics of the action of pepsin on the structure of the crosslinked coatings and to relate this information to the relevant drug release characteristics.

## 6.3 Results and Discussion

### 6.3.1 Processing conditions for preparation of the outer pepsin-responsive crosslinked gelatine coatings

#### 6.3.1.1 Temperature of gelatine solutions during coating

Gelatine exhibits gel-forming properties at ±35°C. Below this temperature aqueous gelatine solutions, irrespective of the concentration, solidify to form a semi-solid gel. Consequently, this solidification makes it impossible to apply the gelatine as a coating surrounding the tablets. Therefore, in preparation of the gelatine coatings, each solution in concentrations prescribed by the Box-Behnken experimental design was maintained at 35°C for the duration of the coating procedure.

#### 6.3.1.2 Crosslinking method and process parameters

The method of crosslinking the gelatine coatings was based on a method of stressing hard gelatine capsules established by Colé and colleagues in 1997. However this method was

substantially altered in that instead of contaminating the lactose with the vapour of GA, a known volume of GA with a known concentration was added to the lactose. The GA-lactose combinations invariably resulted in the formation of slightly moist granules. Crosslinking of the gelatine coatings was then performed by submerging each tablet within the moist mixture and allowing crosslinking to occur at 35°C. The gel-forming property of gelatine at this temperature ensured that the GA from the GA-lactose combination was capable of permeating the gelatine coating to ensure crosslinking.

### 6.3.2 The influence of gelatine concentrations and crosslinking parameters on the dimensional and gravimetrical properties of the formulations

Dimensional analysis of the 15 experimental formulations revealed a correlation between the concentration of the gelatine solution employed for preparation of the coating and the percentage increase in the dimensions of the tablet inclusive of the coating and the average thickness of the coatings alone. This relationship is depicted in Figure 6.4 where the values above each bar represent the concentration of gelatine ($\%^{w}/_{v}$) employed in that formulation. Based on the histogram it is evident that with increasing gelatine concentrations there is a complementary increase in the average thickness of the coatings and consequently an increase in the overall dimensions of the tablets. Conversely, it is also established that the tablets coated with the lowest concentration of gelatine ($10\%^{w}/_{v}$) underwent the greatest increase in the dimensions of the tablets (%) after crosslinking of the gelatine coatings, irrespective of the crosslinking concentrations and durations.

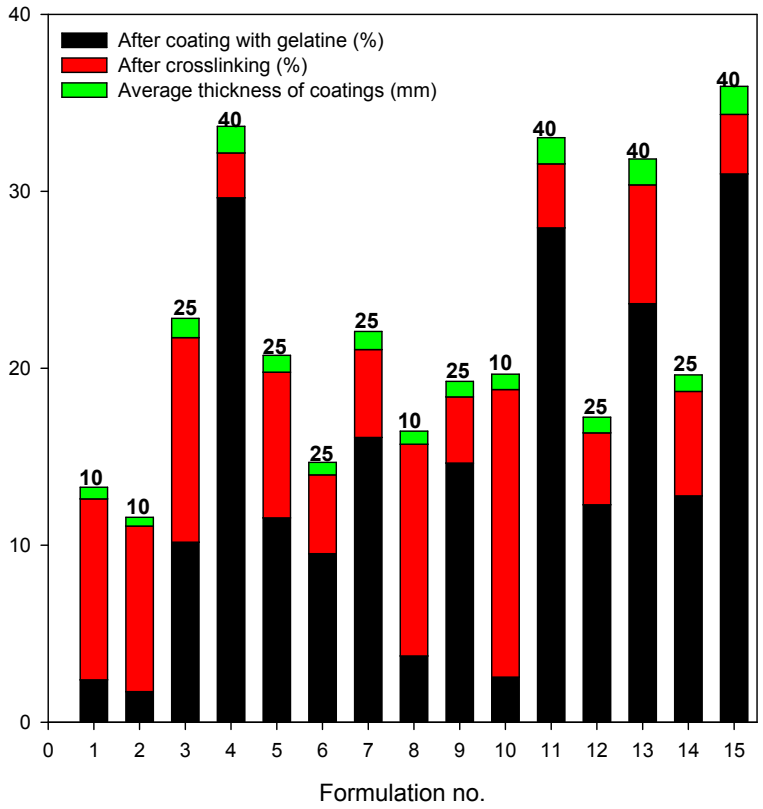



***Figure 6.4:*** *Stacked histogram of all 15 formulations depicting the relationship between the concentrations of gelatine employed, the increase in dimensions of the tablets and the thickness of the coatings.*

Furthermore a comprehensive assessment of the changes of various aspects of the dimensions of the formulations is also provided in Table 6.3. Despite the manual dip-coating process, all formulations exhibited a relatively consistent thickness. This is evident from the thickness of the coatings when determined from the height and diametrical aspect of the tablet which exhibited a standard deviation of 0.03-0.3, dependent on the concentration of gelatine employed.

***Table 6.3:*** *Increase in dimensions of the tablets after each preparatory step and the average thickness of the coatings after crosslinking*

| Form. | Increase in dimensions of tablets (%) | | | Thickness of coating (mm) | | | |
|---|---|---|---|---|---|---|---|
| | [1]After gelatine | [2]After crosslinking | [3]Total increase | [4]Height | [5]Diameter | Average | Difference |
| 1 | 2.39 | 10.23 | 14.32 | 0.76 | 0.56 | 0.66 | 0.14 |
| 2 | 1.73 | 9.35 | 11.24 | 0.69 | 0.31 | 0.50 | 0.27 |
| 3 | 10.17 | 11.56 | 22.90 | 1.12 | 1.06 | 1.09 | 0.04 |
| 4 | 29.63 | 2.54 | 32.92 | 1.58 | 1.41 | 1.50 | 0.12 |
| 5 | 11.55 | 8.23 | 20.74 | 1.09 | 0.81 | 0.95 | 0.20 |
| 6 | 9.52 | 4.46 | 14.41 | 0.73 | 0.66 | 0.70 | 0.05 |
| 7 | 16.09 | 4.97 | 21.85 | 1.09 | 0.94 | 1.02 | 0.12 |
| 8 | 3.74 | 11.97 | 16.16 | 0.86 | 0.62 | 0.74 | 0.16 |
| 9 | 14.64 | 3.74 | 18.93 | 0.96 | 0.80 | 0.88 | 0.11 |
| 10 | 2.55 | 16.25 | 19.21 | 1.03 | 0.71 | 0.87 | 0.23 |
| 11 | 27.93 | 3.62 | 32.57 | 1.57 | 1.38 | 1.48 | 0.14 |
| 12 | 12.29 | 4.06 | 16.84 | 0.92 | 0.86 | 0.89 | 0.04 |
| 13 | 23.65 | 6.71 | 31.94 | 1.49 | 1.45 | 1.47 | 0.03 |
| 14 | 12.79 | 5.90 | 19.45 | 0.85 | 1.03 | 0.94 | 0.13 |
| 15 | 30.98 | 3.37 | 35.41 | 1.79 | 1.37 | 1.58 | 0.30 |

*[1] $D_f$=dimensions after gelatine coating, $D_i$=dimensions of the tablet before coating*
*[2] $D_f$=dimensions after crosslinking of gelatine coating, $D_i$=dimensions after gelatine coating*
*[3] $D_f$=dimensions after crosslinking of gelatine coating, $D_i$=dimensions of tablet before coating*
*[4] Average height of tablet after crosslinking - average height of tablet before gelatine coating*
*[5] Average diameter of tablet after crosslinking - average diameter of tablet before gelatine coating*

The 3D-scatter plot depicted in Figure 6.5 revealed that formulations 5, 6, 7, 9, and 12 showed ‘clumping’ since they all exhibited similar increases in weight after gelatine coating and crosslinking. These formulations possessed a commonality of being coated with a 25%$^{w}/_{v}$ gelatine solution, with a crosslinking concentration of either 0.1875 or 0.2500. Furthermore, the formulations coated with a 10%$^{w}/_{v}$ concentration of gelatine exhibited a greater percentage increase in weight after crosslinking compared to after coating with the gelatine solution.

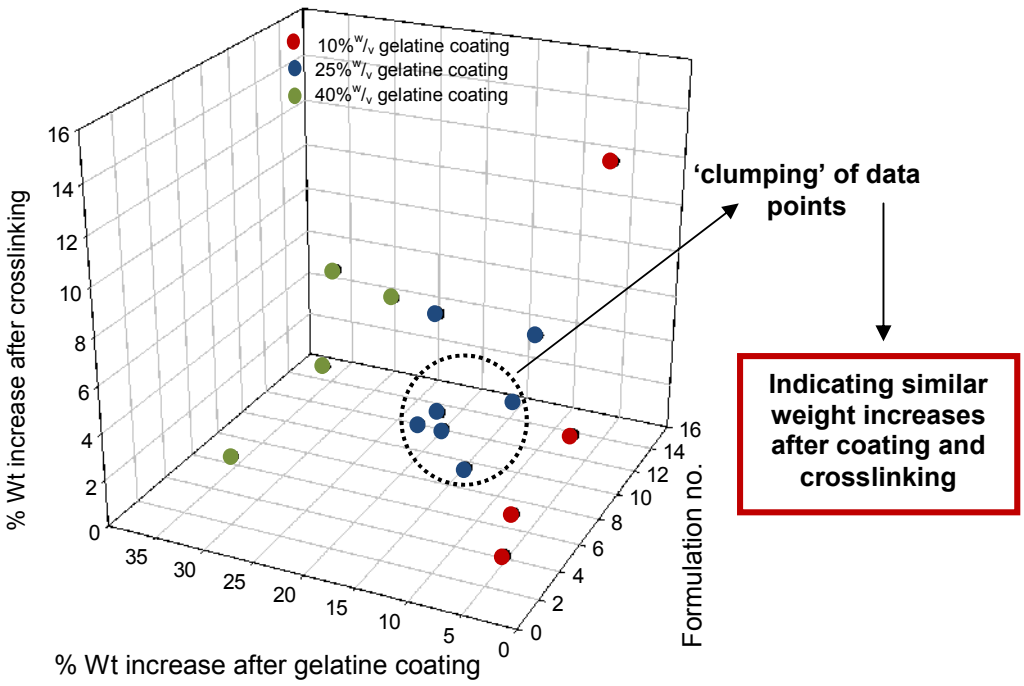



***Figure 6.5:*** *3D-scatter plot of the experimental formulations vs. the percent increase in weight after coating with gelatine vs. the percent increase in weight after crosslinking.*

Furthermore, irrespective of the gelatine concentration, a crosslinking ratio of 0.1250 resulted in the highest percentage of increase in weight after crosslinking as depicted in Figure 6.6.

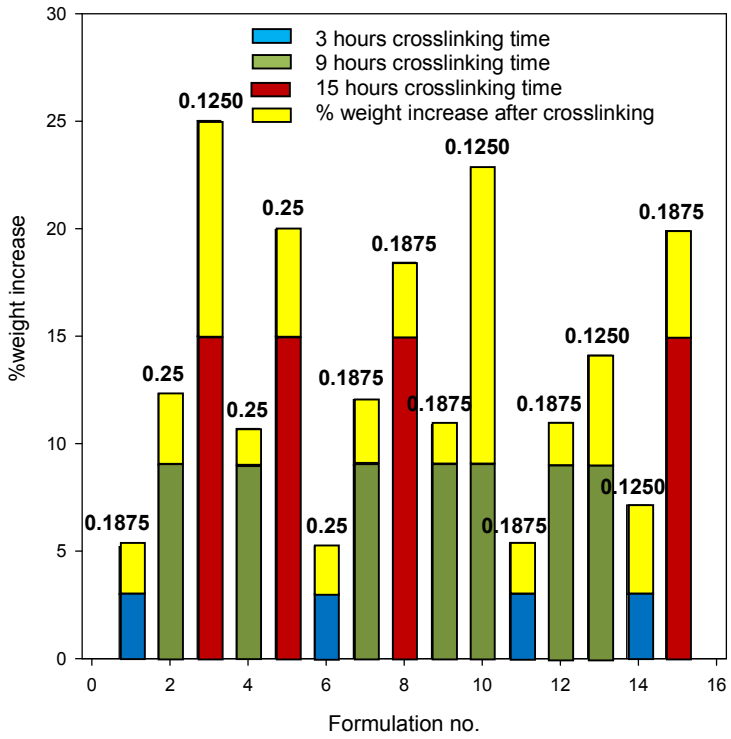



***Figure 6.6:*** *Stacked histogram of the influence of crosslinking durations and concentrations on the percent increase in weight of the formulations after crosslinking.*

### 6.3.3 Drug content per coating in relation to the concentration of gelatine employed

The quantity of drug contained within each coating appeared to increase with increasing concentrations of the gelatine solutions employed, as depicted in Figure 6.7. Furthermore, the variation in drug content between different formulations prepared with a 10%$^{w}/_{v}$ solution of gelatine was substantially greater than the formulations prepared with the 25%$^{w}/_{v}$ and 40%$^{w}/_{v}$ concentrations.

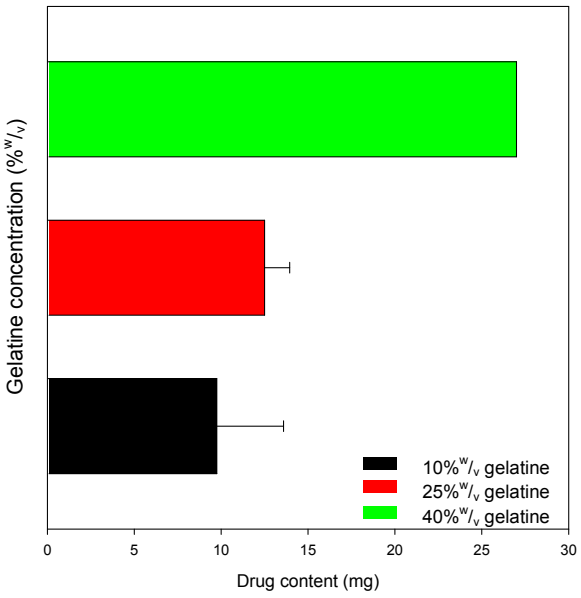


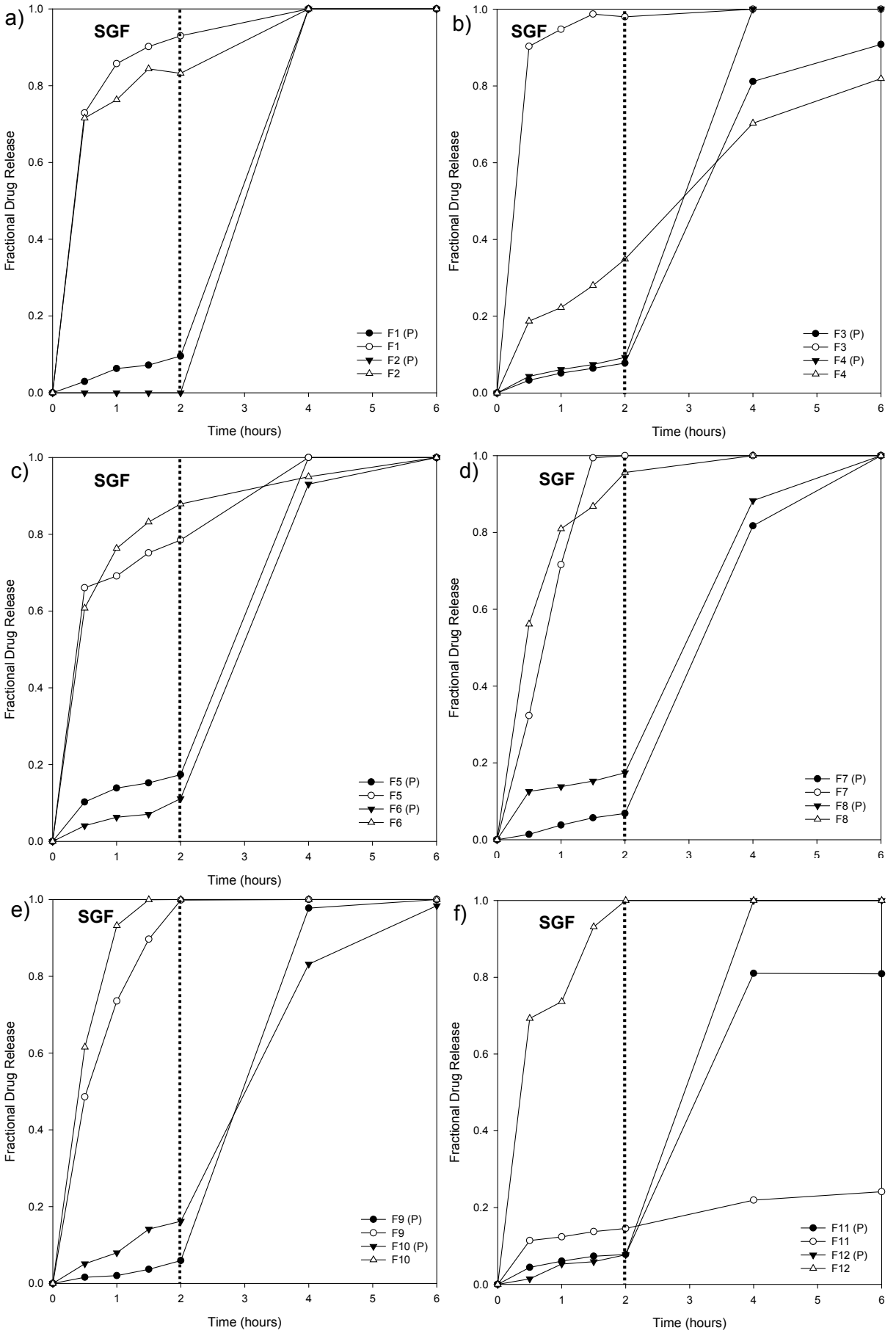

***Figure 6.7:*** *Drug content within the coatings based on the concentration of the gelatine employed.*

### 6.3.4 Box-Behnken experimental design for the optimization of the outer pepsin-responsive intestinally-targeted coating

#### 6.3.4.1 Measured responses for experimental optimization

The *in vitro* drug release profiles of all 15 experimental formulations revealed that irrespective of the concentration of gelatine employed, the crosslinking concentration or the crosslinking duration all formulations exhibited a distinct responsiveness to the enzyme pepsin present in the simulated gastric medium. However the degree of this responsiveness varied between all formulations. Figure 6.8 shows that formulations 1-5 exhibit less than 0.2 fractional release of loperamide HCl in the 2 hours in SGF with pepsin, however only formulations 1, 2, 3, and 5 have between 0.8-1.0 fractional release in SIF. Even though formulation 4 only releases 0.0928 of loperamide HCl in the presence of pepsin, in the absence of pepsin only a relatively small quantity of the contained drug is released thus making the degree of pepsin-responsiveness less prominent. Formulations 6-10 exhibited similar responsiveness to pepsin with less than 0.2 fractional release in the presence of pepsin compared to 100% release in the absence of pepsin. Furthermore, these formulations exhibited complete drug release in SIF. Figure 6.8c provides evidence that formulations coated with 40%$^{w}/_{v}$ gelatine solutions showed the least distinct pepsin-responsiveness since

for example in formulation 11, 0.0778 fractional release was observed in the presence of pepsin compared to 0.1456 in the absence of pepsin.



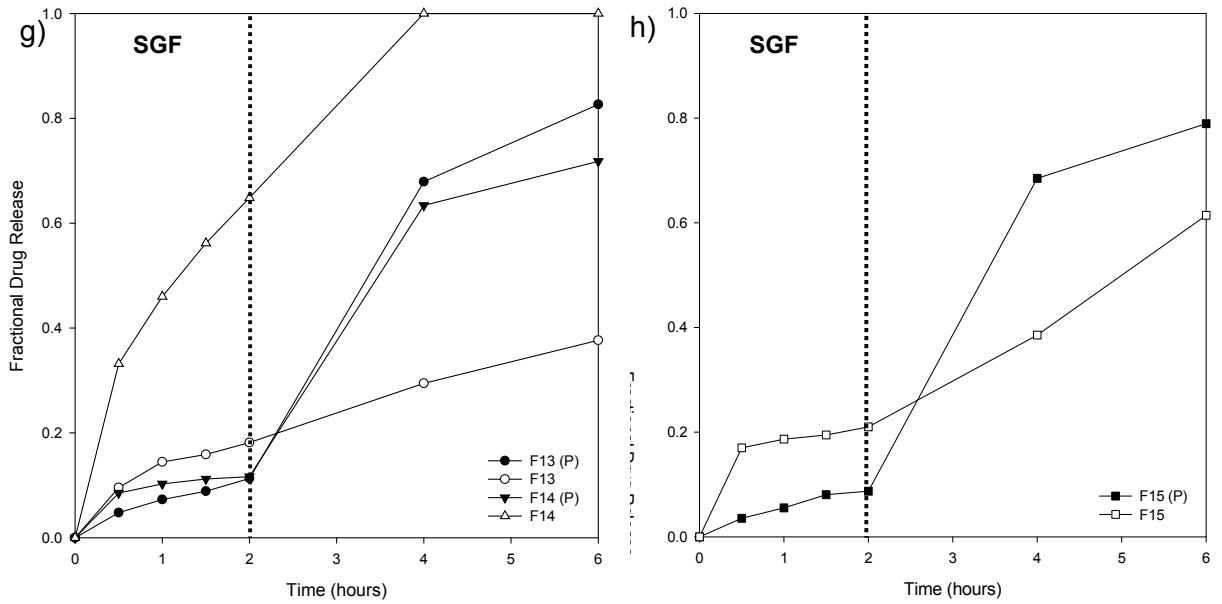



***Figure 6.8:*** *Composite fractional drug release profiles of formulations a) 1 and 2, b) 3 and 4, c) 5 and 6, d) 7 and 8, e) 9 and 10, f) 11 and 12, g) 13 and 14, and h) 15, where 'P' denotes simulated gastric fluid containing pepsin.*

A model independent time-point approach was employed as a means of effectively assessing the pepsin-responsiveness of all 15 experimental formulations by essentially determining the mean dissolution time (MDT) of all formulations in the presence and absence of pepsin in SGF after 2 hours. MDT was calculated by Equation 5.5 in Chapter 5. As previously described all 15 experimental formulations exhibited responsiveness to pepsin, however the optimum degree of responsiveness had to be established by identifying a formulation that provided minimal release in SGF containing pepsin whilst ensuring maximum release when SGF is void of pepsin. Based on the tabulated MDT values depicted in Table 6.4 it is evident that formulations 4, 11, 13 and 15 provided the least preferable MDT with MDT and $MDT_P$ both<1. This correlates with the *in vitro* drug release profiles which previously identified the formulations coated with a 40%$^{w}/_{v}$ gelatine solution as the least pepsin-responsive.

***Table 6.4:*** *Mean dissolution time ($MDT_2$) of all 15 experimental formulations in the presence and absence of pepsin in simulated gastric fluid*

| Experimental Formulation | $MDT_2$ (hours) | [1]$MDT_2$ (P) (hours) |
|---|---|---|
| 1 | 2.72 | 0.25 |
| 2 | 2.47 | 0.00 |
| 3 | 2.93 | 0.21 |
| 4 | 0.91 | 0.24 |
| 5 | 2.27 | 0.48 |
| 6 | 2.53 | 0.27 |
| 7 | 2.88 | 0.18 |
| 8 | 3.16 | 0.57 |
| 9 | 2.76 | 0.14 |
| 10 | 3.01 | 0.42 |
| 11 | 0.42 | 0.22 |
| 12 | 2.81 | 0.20 |
| 13 | 0.50 | 0.29 |
| 14 | 1.76 | 0.34 |
| 15 | 0.60 | 0.24 |

[1] *MDT calculated in SGF containing pepsin*

### 6.3.4.2 Correlation of the experimental and predicted response values

A comparison of the experimental and predicted MDT values of the outer pepsin-responsive coating in SGF revealed the suitability of the regression model of the Box-Behnken experimental design. This was deduced based on the close correlation of the predicted and experimental MDT values (in the absence of pepsin) as depicted in Figure 6.9 which presented with an $R^2$ value of 0.996.

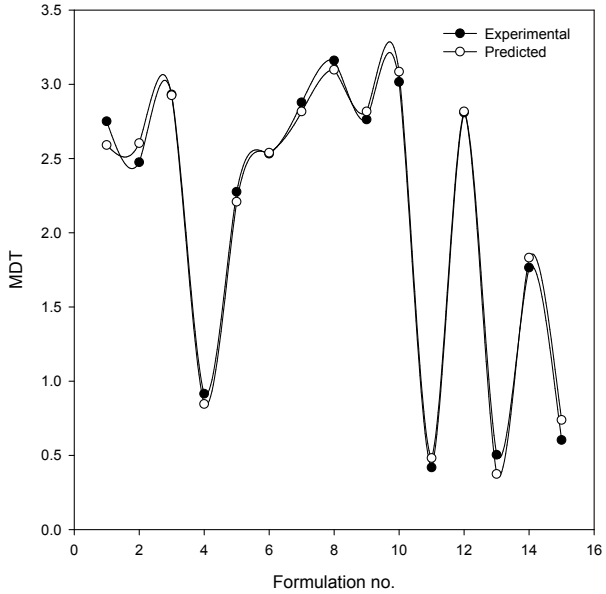



***Figure 6.9:*** *Regression plot of the experimental vs. predicted mean dissolution time values in simulated gastric fluid void of pepsin.*

Similarly, the regression plot of the predicted vs. experimental MDT values in the presence of pepsin also presented with an overall close correlation, however several formulations deviated substantially from the predicted MDT values resulting in a $R^2$ of 0.806 (Figure 6.10).

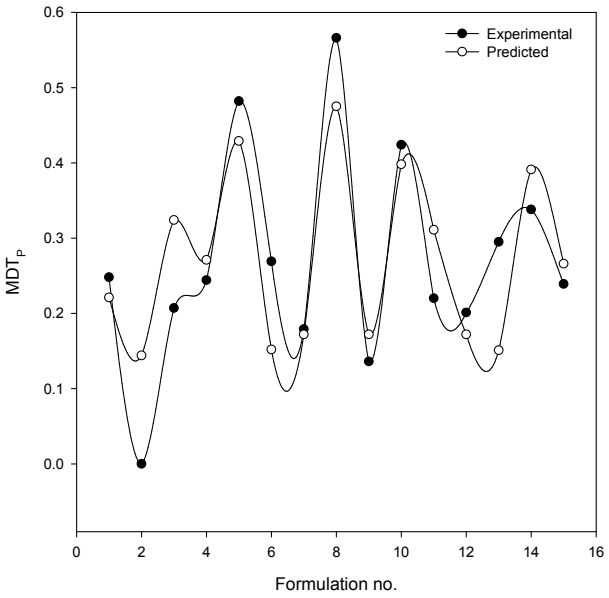



***Figure 6.10:*** *Regression plot of the experimental vs. predicted mean dissolution time values in simulated gastric fluid containing pepsin.*

### 6.3.4.3 Analysis of the Box-Behnken Response Surface Design

#### 6.3.4.3.1 Response analysis for MDT

Response surface plots of the mean dissolution time (MDT) of the experimental formulations in the absence of pepsin identified a maximization of the MDT principally with a 10-20%$^{w}/_{v}$ gelatine concentration, at crosslinking times ranging from 3-15 hours, when a GA-lactose ratio of 0.1875 is implemented. Conversely, a reduced MDT is observed with a 40%$^{w}/_{v}$ gelatine solution across the 3-15 hour crosslinking time periods (Figure 6.11a). A similar relationship is observed between the gelatine concentration and GA-lactose concentration ratios where an increased MDT is evident from formulations with gelatine concentrations between 10-20%$^{w}/_{v}$, across all GA-lactose ratios (Figure 6.11b). Furthermore, with a gelatine concentration hold value of 25%$^{w}/_{v}$ a maximal MDT is only achievable at a GA-lactose ratio of 0.15 or 1:4, when crosslinking is performed for 12-15 hours (Figure 6.11c).

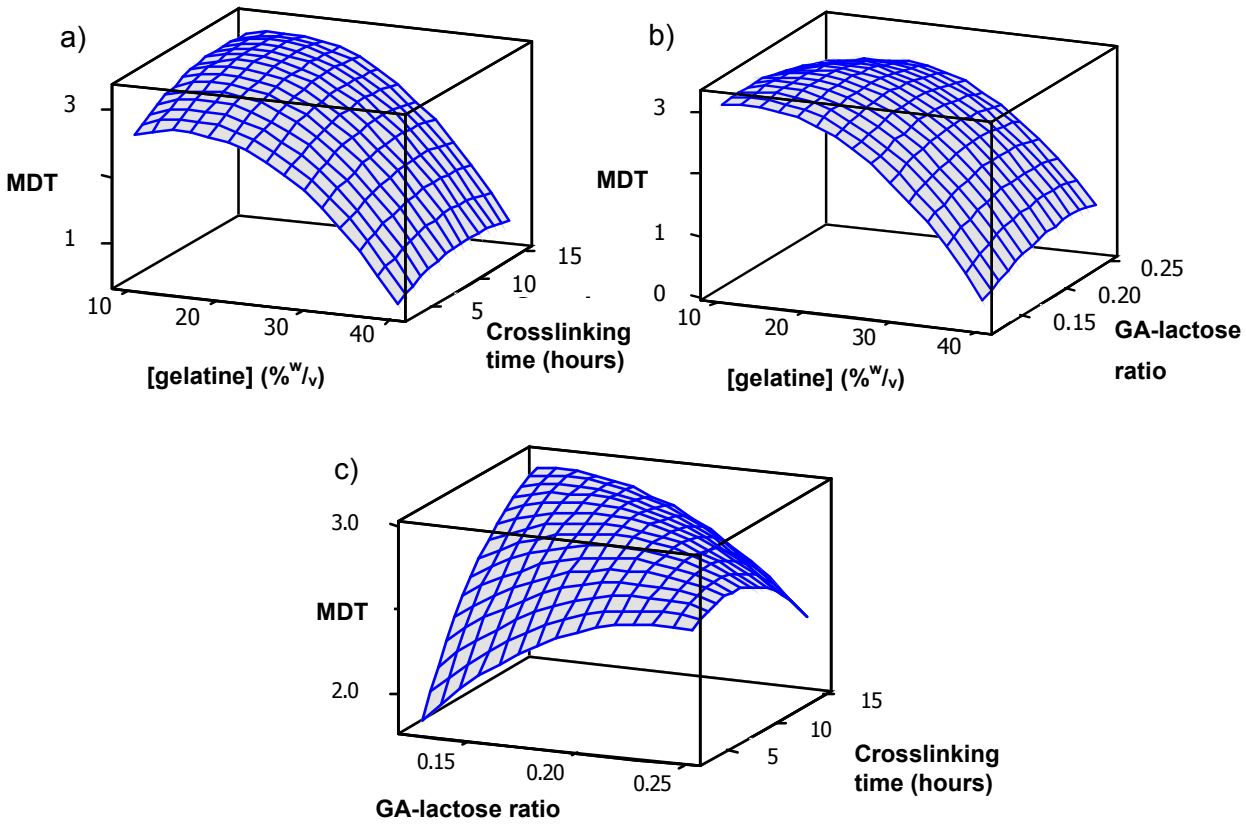



***Figure 6.11:*** *Response surface plots correlating mean dissolution time in simulated gastric fluid without pepsin with a) gelatine concentration and crosslinking time (glutaraldehyde-lactose hold value of 0.1875), b) gelatine concentration and glutaraldehyde-lactose ratio (crosslinking time hold value of 9 hours), and c) glutaraldehyde-lactose ratio and crosslinking time (gelatine concentration hold value of 25%$^{w}/_{v}$).*

#### 6.3.4.3.2 Response analysis for $MDT_P$

To analyse the efficacy of the outer pepsin-responsive coating, the mean dissolution time in the presence of pepsin ($MDT_P$) should be minimized thus enabling the determination of the intestinal targeting ability of the formulation. Thus based on response surface plots a minimal $MDT_P$ is achieved at a gelatine concentration of 10-20%$^{w}/_{v}$ and crosslinking duration of 4-6 hours. However, this reduced $MDT_P$ is also achievable across the entire gelatine concentration range (10-40%$^{w}/_{v}$) when crosslinking is performed for between 6-12 hours (Figure 6.12a). Figure 6.12b illustrates the trend where with an increasing concentration of gelatine, a higher GA-lactose ratio is required to obtain a minimal $MDT_P$, for example at a gelatine concentration of 10%$^{w}/_{v}$ a GA-lactose ratio of 0.25 is necessary for a reduced $MDT_P$, whereas at a concentration of 40%$^{w}/_{v}$ a GA-lactose ratio of 0.15 is required. Furthermore, with a 25%$^{w}/_{v}$ gelatine concentration hold value, a GA-lactose ratio between 0.1875-0.25 and crosslinking time of 3-6 hours is required for minimization of $MDT_P$ (Figure 6.12c).

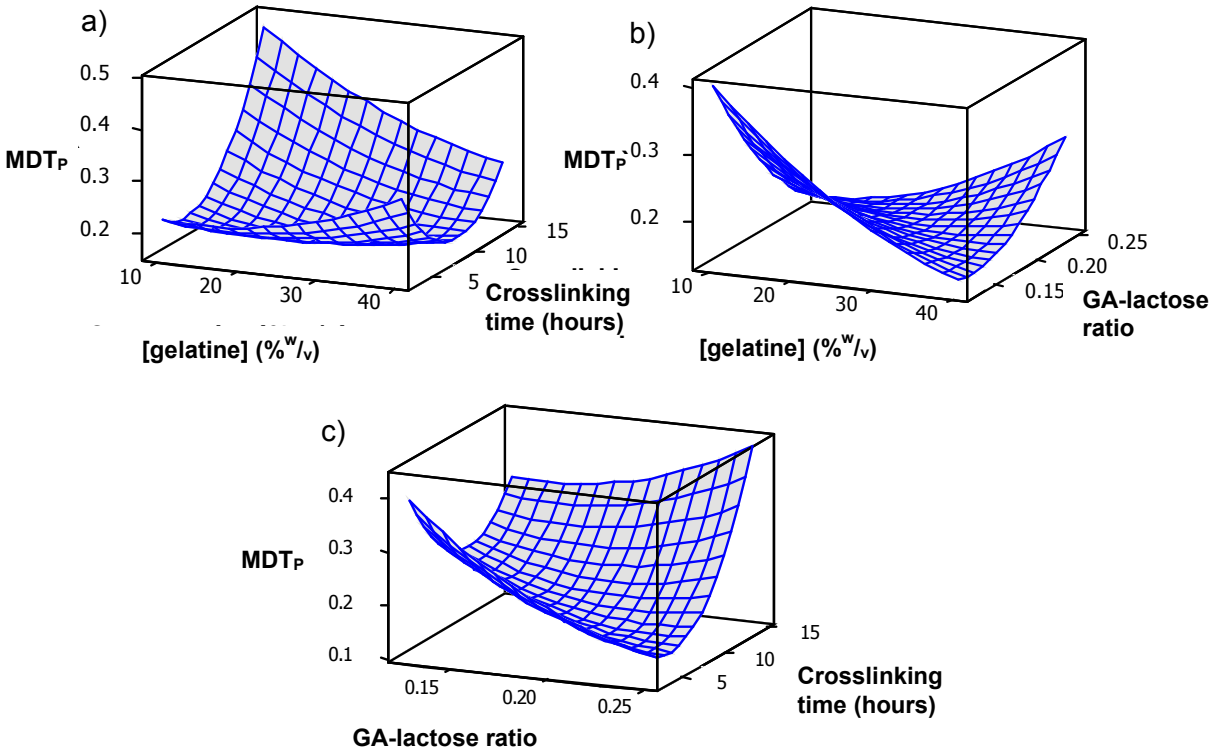



***Figure 6.12:*** *Response surface plots correlating mean dissolution time in simulated gastric fluid with pepsin with a) gelatine concentration and crosslinking time (glutaraldehyde-lactose hold value of 0.1875), b) gelatine concentration and glutaraldehyde-lactose ratio (crosslinking time hold value of 9 hours), and c) glutaraldehyde-lactose ratio and crosslinking time (gelatine concentration hold value of 25%$^{w}/_{v}$).*

### 6.3.4.4 Residual analysis of the Box-Behnken experimental design for optimization of the outer pepsin-responsive intestinally-targeted coating

The suitability of the multiple regression model was assessed based on the residual plots for MDT and $MDT_P$ as depicted in Figure 6.13. The normal probability plots of the residuals of MDT showed a normal distribution of data points on a linear line with 'clustering' along the line. $MDT_P$ also showed data points falling uniformly on a linear line with the absence of 'clustering'. The residual plots of the standardized residuals versus the fitted values for both MDT and $MDT_P$ exhibited a random distribution of data points with no discernible pattern. Furthermore, since the points fluctuated around zero, the assumptions of zero-means and constant variance of the regression model held true. Finally, the plot of the residuals versus the order of data also showed a random distribution of data points with rapid changes in the signs (+/-) between consecutive residuals.

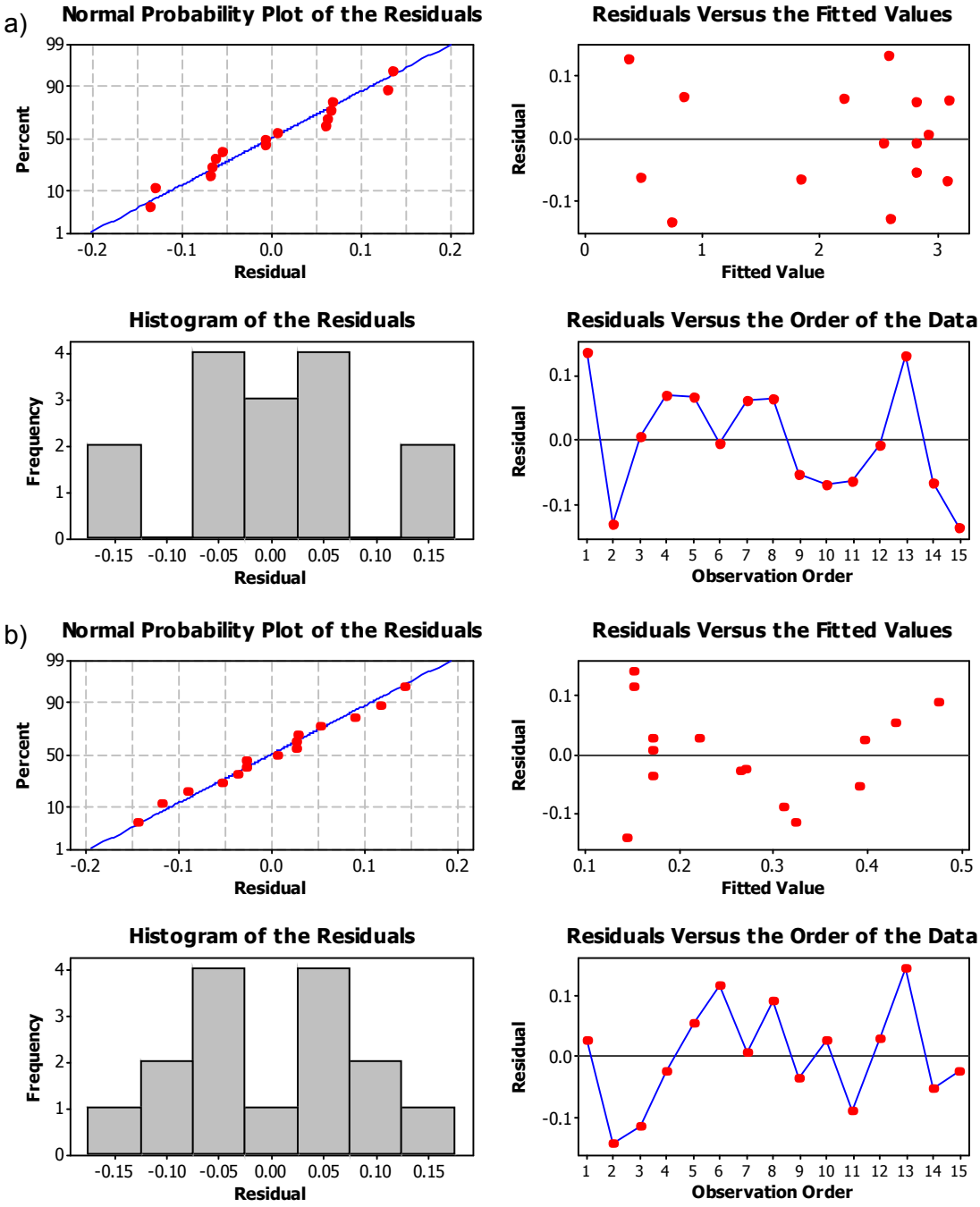



***Figure 6.13:*** *Residual plots of a) mean dissolution time in the absence of pepsin and b) mean dissolution time in the presence of pepsin.*

A full ANOVA analysis of the measured formulation responses revealed that none of the factors of $MDT_P$ exhibited any significant influence with $p \geq 0.05$. However numerous factors of MDT showed statistical significance with p-values ≤0.05. These are depicted in Table 6.5.

***Table 6.5:*** *Estimated p-values for mean dissolution time in the absence (MDT) and presence of pepsin ($MDT_P$)*

| Term | p-value | |
|---|---|---|
| | **MDT** | **$MDT_P$** |
| **[Gelatine]** | 0.000 | 0.567 |
| **[GA:lactose ratio]** | 0.965 | 0.523 |
| **Crosslinking time** | 0.013 | 0.333 |
| **[Gelatine]*[Gelatine]** | 0.000 | 0.681 |
| **[GA:lactose ratio]*[GA:lactose ratio]** | 0.032 | 0.626 |
| **Crosslinking time*crosslinking time** | 0.032 | 0.172 |
| **[Gelatine]*[GA:lactose ratio]** | 0.021 | 0.235 |
| **[Gelatine]*Crosslinking time** | 0.424 | 0.329 |
| **[GA:lactose ratio]*Crosslinking time** | 0.004 | 0.268 |

The complete regression equations generated for mean dissolution time in the absence of pepsin (MDT) and presence of pepsin ($MDT_P$) are depicted in Equations 6.5 and 6.6 respectively.

$$\begin{aligned}MDT = {} & -1.06288 + 0.0773553[\text{Gelatine}] + 23.3017[\text{GA : lactose ratio}] + 0.337375[\text{Crosslinking time}] \\ & - 0.00386296[\text{Gelatine} * \text{Gelatine}] - 56.3748[\text{GA : lactose ratio} * \text{GA : lactose ratio}] \\ & - 0.00612126[\text{Crosslinking time} * \text{Crosslinking time}] + 0.253747[\text{Gelatine} * \text{GA : lactose ratio}] \\ & - 6.94367E-04[\text{Gelatine} * \text{Crosslinking time}] - 0.949119[\text{GA : lactose ratio} * \text{Crosslinking time}]\end{aligned}$$

Equation 6.5

$$\begin{aligned}MDT_P = {} & 1.59332 - 0.0201592[\text{Gelatine}] - 8.68395[\text{GA : lactose ratio}] - 0.0709428[\text{Crosslinking time}] \\ & + 0.000139504[\text{Gelatine} * \text{Gelatine}] + 9.57342[\text{GA : lactose ratio} * \text{GA : lactose ratio}] \\ & + 0.00319061[\text{Crosslinking time} * \text{Crosslinking time}] + 0.0995970[\text{Gelatine} * \text{GA : lactose ratio}] \\ & - 8.31791E-04[\text{Gelatine} * \text{Crosslinking time}] - 0.229599[\text{GA : lactose ratio} * \text{Crosslinking time}]\end{aligned}$$

Equation 6.6

#### 6.3.4.5 Constrained optimization of the outer pepsin-responsive intestinally-targeted coating

The optimization process and thus determination of the optimum level for each formulation variable was performed employing Minitab® V15 statistical software. Ultimately the optimization approach resulted in a formulation with a desirability of 1.00 and which included a gelatine concentration of 14.379%$^{w}/_{v}$, a GA:lactose ratio of 0.1810 and a crosslinking time of 6.060 hours (Figure 6.14).

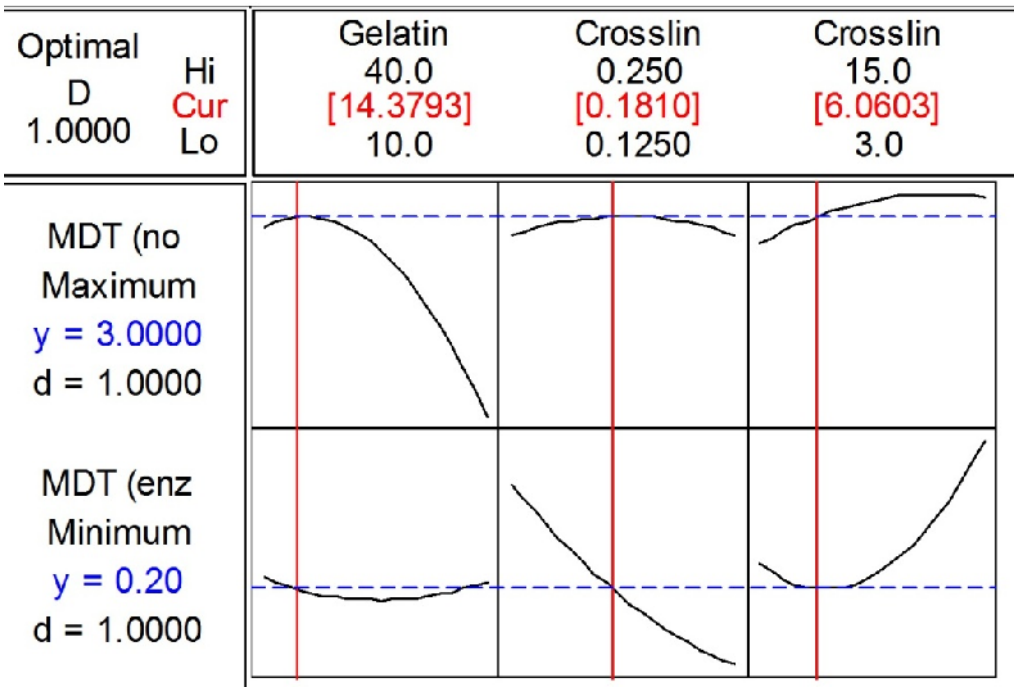



***Figure 6.14:*** *Optimization plots displaying factor levels and desirability values for the optimized formulation.*

The optimization approach provided an optimized formulation with a predicted MDT of 3.00 and a statistical desirability of 100% however data on the experimental formulation resulted in an MDT of 2.84 with a deviation of 0.114 from the predicted value. In comparison, the experimental $MDT_P$ value deviated from the predicted $MDT_P$ value by only 0.059 (Table 6.6).

***Table 6.6:*** *Comparison of the predicted vs. experimental values of the response parameters*

| Response parameter | Predicted | [1]Desirability (%) | Experimental | [2]Deviation (%) |
|---|---|---|---|---|
| **Mean Dissolution Time (MDT) (no pepsin)** | 3.00 | 100 | 2.8382 | 0.114 |
| **Mean Dissolution Time ($MDT_P$) (with pepsin)** | 0.20 | 100 | 0.2836 | 0.059 |

[1] *Statistical desirability values of the predicted optimized formulation*
[2] *Deviation of the experimental response values from the predicted*

The close correlation of the experimental and predicted MDT and $MDT_P$ values suggest the reliability of the optimization process. Moreover, the optimization process proved efficient in predicting the *in vitro* drug release behaviour of the outer polymeric coating in response to pepsin which ultimately insinuated toward the intestinal targeting ability of the formulation.

The optimized formulation contained an average of 6.731±0.861mg of loperamide HCl. Subsequent to coating and crosslinking of the gelatine coatings the tablets experienced an average of 2.92±0.049% increase in the overall dimensions and an average weight increase of 7.93±0.033%.

The *in vitro* release profile depicted in Figure 6.15 illustrates the pepsin-responsiveness of the optimized formulation. It is evident that within the 90 minutes in SGF void of pepsin complete loperamide HCl release was achieved whereas in this same time only 0.182 fractional drug release was achieved in the presence of pepsin. Essentially complete drug release was then achieved within the 4 hours in SIF cementing the intestinal targeting ability of the optimized outer coating.

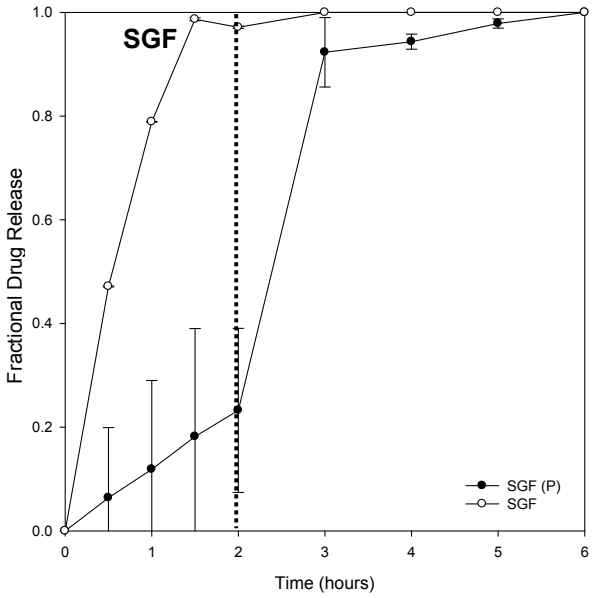



***Figure 6.15:*** *Fractional drug release profile of loperamide HCl from the optimized outer coating in the presence and absence of pepsin in simulated gastric fluid.*

*In vitro* drug release studies conducted on the conventional commercially available loperamide HCl formulation, Imodium®, resulted in complete disintegration and thus complete release of loperamide HCl within the first 5 minutes of dissolution studies in SGF (N=3), in the presence and absence of pepsin.

### 6.3.5 The effect of pepsin on the swelling and erosion of the outer pepsin-responsive coating

The influence of pepsin on the outer crosslinked gelatine coatings was assessed gravimetrically and the results depicted in Figure 6.16. Figure 6.16a illustrates the substantially greater percentage weight gain of the formulations in SGF without pepsin for the entire 6 hour duration, signifying the greater degree of swelling in the absence of pepsin. Nevertheless, despite the reduced weight increase in the presence of pepsin, the formulations follow an almost identical pattern of weight increase to those not exposed to pepsin (Figure 6.16b).

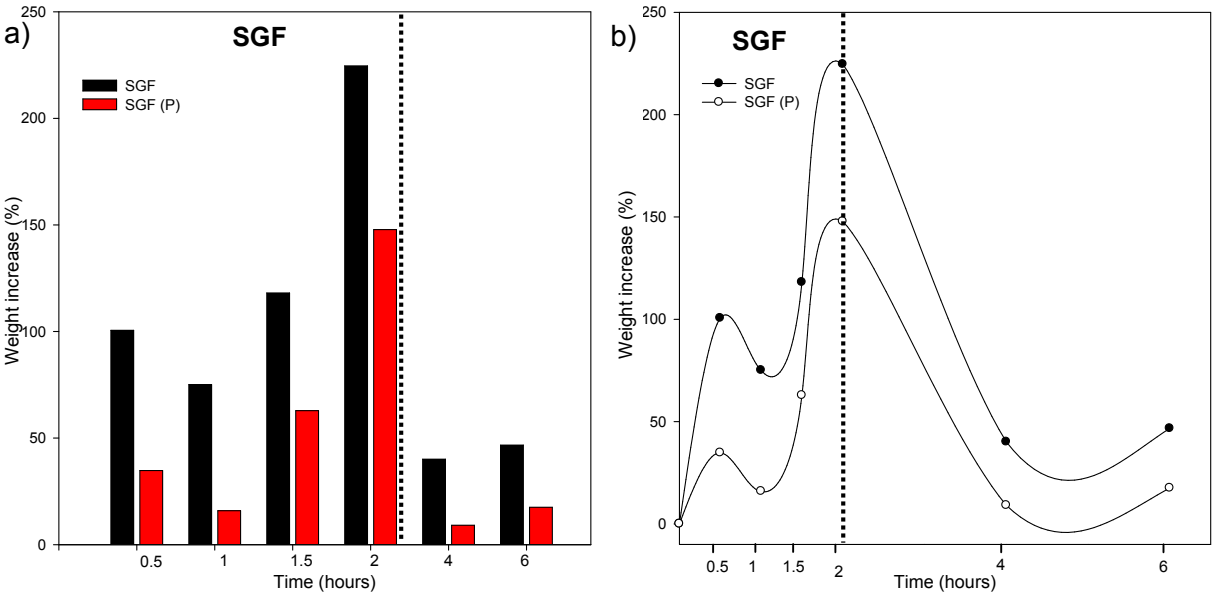



***Figure 6.16:*** *a) Histogram of the weight increase (%) of the optimized outer coating in the presence and absence of pepsin, b) illustration of the similar pattern of weight increase (%) in the presence and absence of pepsin.*

Subsequent to the weight increase study, the hydrated samples were dried and also gravimetrically evaluated to assess the loss in weight of the formulations as a consequence of its exposure to pepsin for the various time periods. Figure 6.17 illustrates the difference in the weight of the optimized formulation in the presence and absence of pepsin and distinctly shows the greater loss in weight of the formulations exposed to pepsin in SGF.

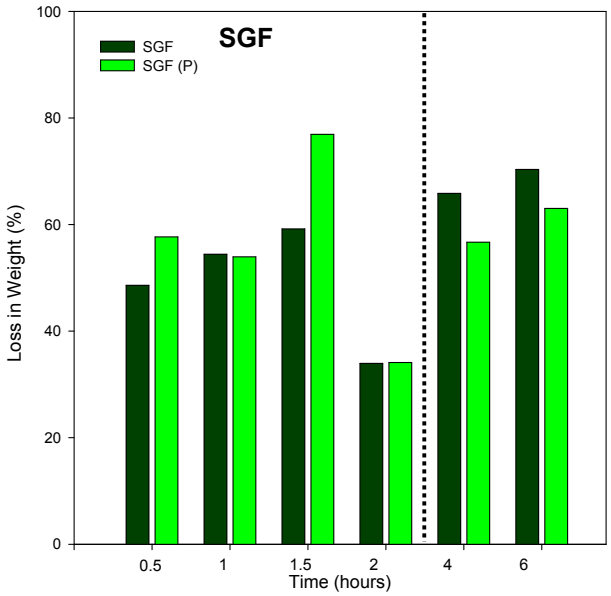



***Figure 6.17:*** *Histogram of the difference in the loss in weight of the optimized coatings due the presence of pepsin in simulated gastric fluid.*

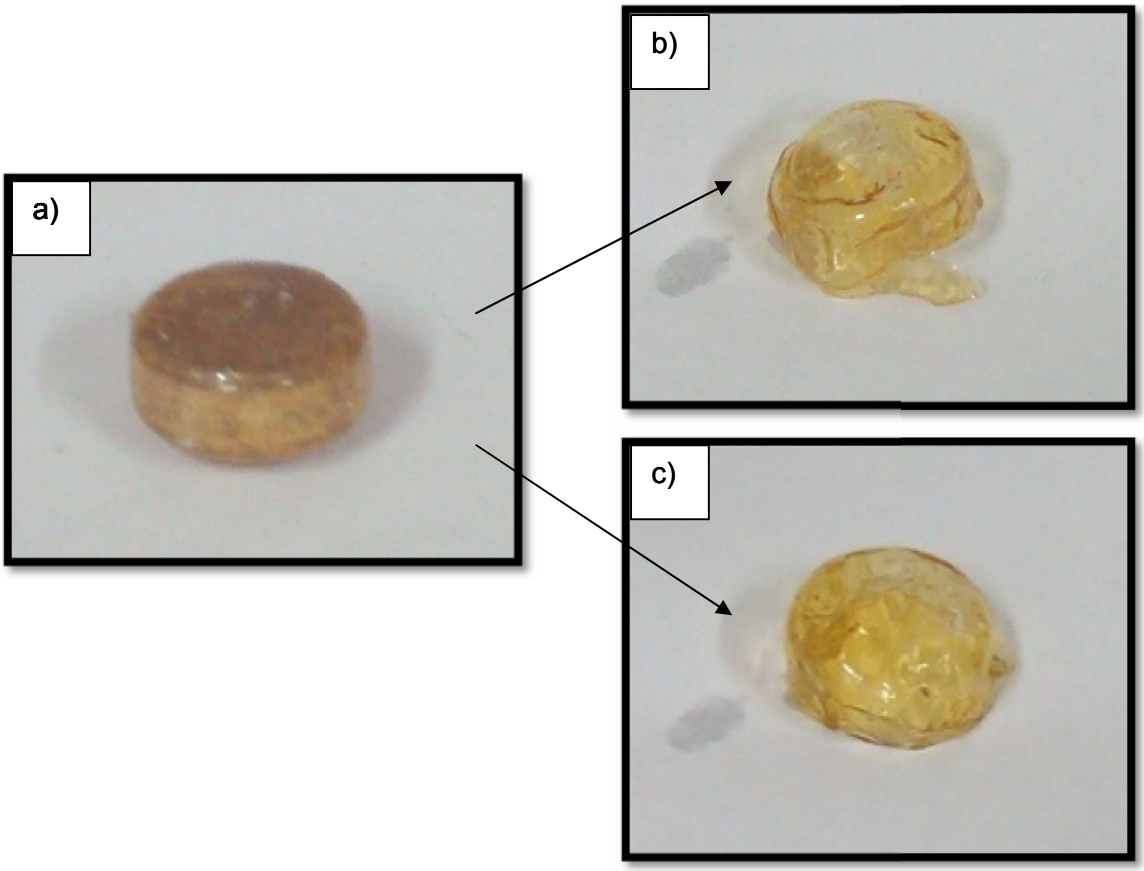



***Figure 6.18:*** *Digital images of the stimuli-responsive oral tablet system with a) the outer pepsin-responsive coating, b) the hydrated coating after 2 hours in simulated gastric fluid with pepsin and c) the hydrated coating after 2 hours in simulated gastric fluid without pepsin.*

### 6.3.6 Explication of the reaction mechanism involved in crosslinking gelatine with glutaraldehyde

Essentially, gelatine contains extended left-handed proline helix conformations which are incorporated with 300 to 4000 amino acids. However the most prominent of these amino acid residues include glycine which is arranged to every third residue in its structure, proline and 4-hydroxyproline. The other constituting amino acids are depicted in Table 6.7.

***Table 6.7:*** *Amino acid composition of pigskin and bovine hide gelatine (Talebian et al., 2007; Farris et al., 2010)*

| Amino acid | Pigskin gelatine (mol %) | Bovine hide (mol %) | Amino acid | Pigskin gelatine (mol %) | Bovine hide (mol %) |
|---|---|---|---|---|---|
| Alanine | 11.05 | 10.88 | Leucine | 2.35 | 3.00 |
| Arginine | 4.96 | 9.90 | Lysine | 2.65 | 3.46 |
| Asparagine | 0.60 | - | Methionine | 0.32 | 0.42 |
| Aspartic acid | 4.4 | 7.46 | Phenylalanine | 1.38 | 1.99 |
| Glutamic acid | 7.10 | 11.28 | ***Proline*** | ***13.10*** | ***12.52*** |
| ***Glycine*** | ***32.20*** | ***32.63*** | Serine | 3.40 | 3.62 |
| Histidine | 0.45 | 0.77 | Threonine | 1.80 | 2.11 |
| ***Hydroxyproline*** | ***9.80*** | - | Tyrosine | 0.35 | 0.40 |
| Hydroxylysine | 0.75 | - | Valine | 1.90 | 2.18 |
| Isoleucine | 1.02 | 1.44 | | | |

The strength of gelatine is attributed to its triple helix conformation such that the greater the triple-helix content, the higher the strength of the film and as a consequence the lower the swelling property of gelatine in water (Oakenfull and Scott, 2003; Bigi *et al.*, 2004). However, the gelling/swelling properties of gelatine can be altered by introducing chemical crosslinks between particular amino acid residues (Pal *et al.*, 2007).

The fundamental evidence of crosslinking of gelatine with GA is essentially observable from the obvious improvement in the mechanical properties, water resistance, and proteolytic resistance of the coating, however evidence may also be provided based on the FTIR spectra obtained of the crosslinked coatings in relation to the components thereof. Generally, the crosslinking chemistry of the aldehyde groups of GA with the free non-protonated ε-amino groups (-$NH_2$) of lysine or hydroxylysine occurs through a nucleophilic addition type reaction. In addition, it has been established that neutral to slightly alkaline pH values are more favourable to crosslinking gelatine since pH directly influences the charge density and charge distribution of gelatine thus affecting the degree of protonation of –$NH_2$ groups and the presence of negative charges on the carboxylic groups (Farris *et al.*, 2010).

FTIR spectra of gelatine revealed several characteristic bands such as the very broad band at 2600-3400$cm^{-1}$ attributable to H-bonded OH stretching of the –COOH groups present on the protein molecule. Furthermore, the broad band at 3284$cm^{-1}$ of gelatine presented with a 'shoulder' at the region of 3500$cm^{-1}$. This 'shoulder-like' peak corresponds to the non-bonded –OH stretching band of gelatine. However, this peak disappears in the spectrum of the crosslinked gelatine coating and thus provides evidence of the involvement of the –OH groups with GA during crosslinking (Figure 6.19).

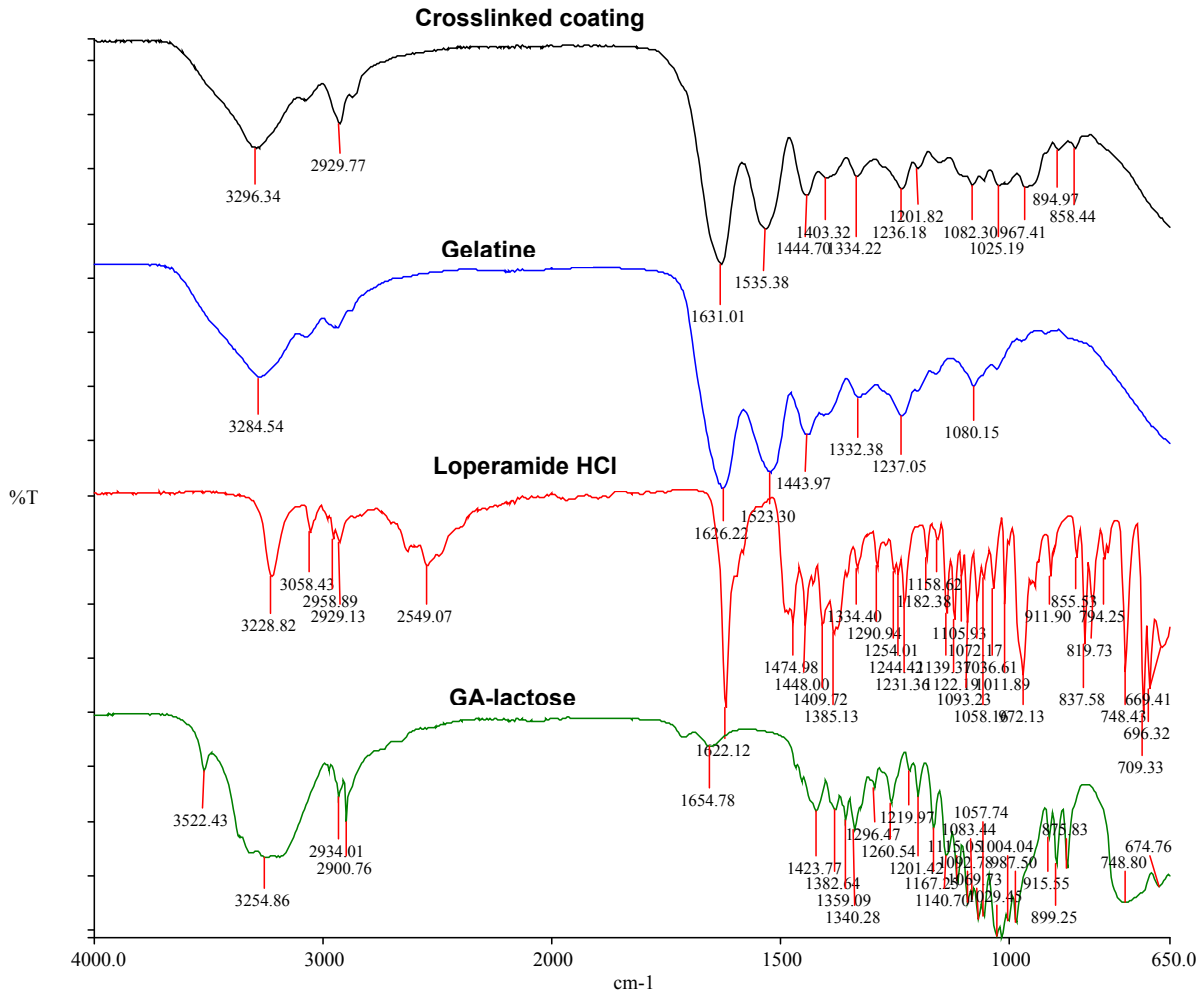



***Figure 6.19:*** *FTIR spectra of the components of the outer pepsin-responsive coating, as well as the spectrum of the crosslinked coating.*

In the gelatine molecule the peak at 1626cm$^{-1}$ is indicative of an amide I band, mainly associated with the C=O stretching vibration, however since this peak shifts to 1631cm$^{-1}$ in the FTIR spectrum of the crosslinked gelatine coating it suggests that the carbonyl groups of the amino acids are also likely involved in the crosslinking reaction. Moreover, the peak at 1523cm$^{-1}$ of gelatine due to an amide II band originating from N-H bending vibration and the C-N stretching vibration shifts to 1535cm$^{-1}$ in the spectrum of the crosslinked coating. This shifting of peaks essentially provides evidence that the amide groups may be involved in the crosslinking reaction to form Schiff bases. Based on the evident shifts of these prominent peaks it can be deduced that the crosslinking reaction between GA and gelatine occurs at an acidic pH value where GA interacts with other functional groups on the gelatine molecule along with the few unprotonated amino groups that still exist at low pH's. The crosslinking chemistry can thus be explained by the reaction mechanism proposed by Farris and colleagues in 2010 (Figure 6.20).

Essentially, the α-carbon of GA forms a highly reactive carbocation resulting in the nucleophilic attack of the –OH groups of hydroxyproline and hydroxylysine of gelatine. A

hemiacetal is thus formed and $H^+$ is regenerated. Due to the homogenous charge distribution along the hemiacetal oxygen bridge a stable system is obtained.

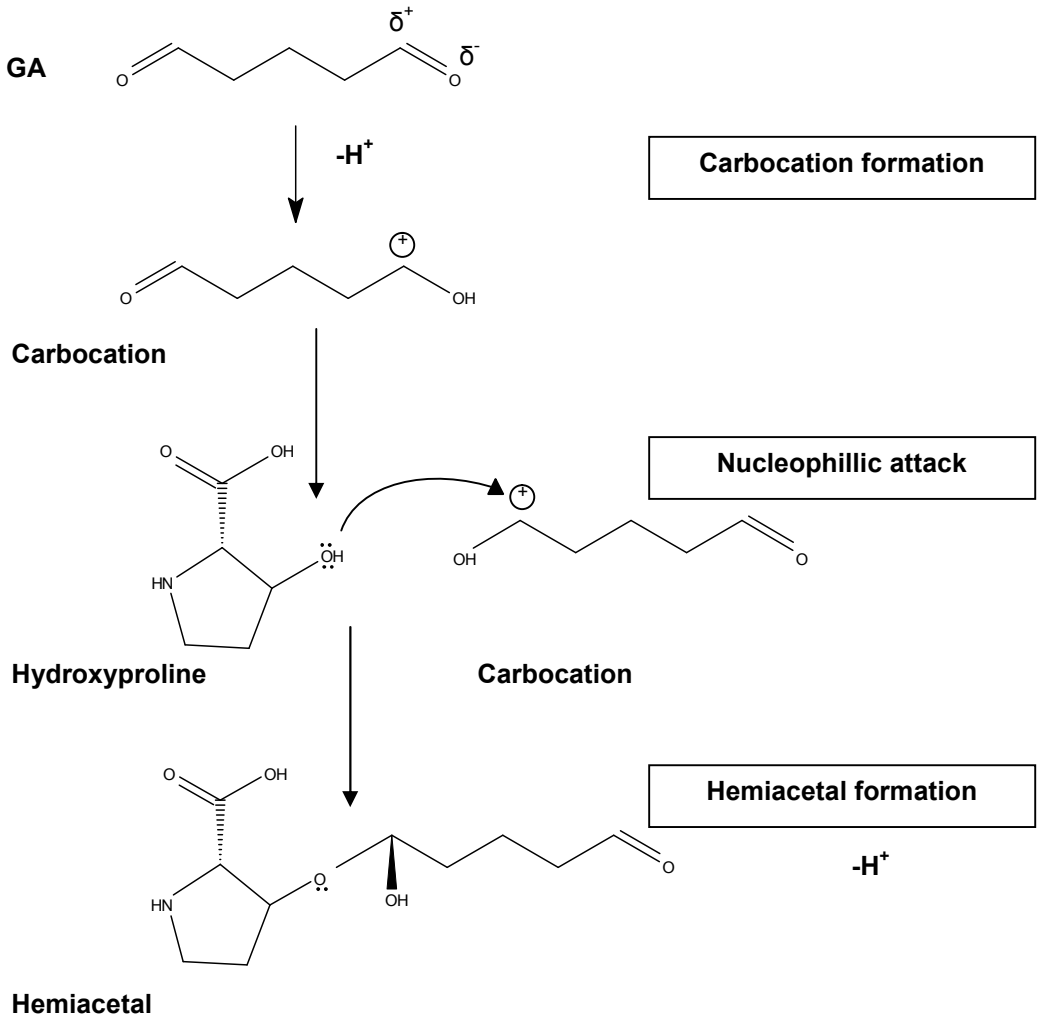



***Figure 6.20:*** *Proposed crosslinking reaction mechanism between gelatine and glutaraldehyde (Farris et al., 2010).*

In addition, nucleophilic addition of the free unprotonated amino groups to the C=O groups of GA occurs to form a tetrahedral unstable intermediate, carbinolamine. Thereafter, the –OH groups become protonated and a water molecule is lost. Consequentially, a conjugated Schiff base is formed (Farris *et al.*, 2010).

### 6.3.7 The effect of pepsin on the structural variations and polymeric backbone of the loperamide HCl-loaded crosslinked gelatine coatings

A significant peculiarity of pepsin lies in its ability to accomplish extensive proteolytic degradation without significantly increasing the presence of titratable groups. It has been suggested that this may be due to the disintegration of the protein molecule into smaller fragments which become united in the original molecule. The fundamental basis of pepsin action on gelatine occurs due to the hydrolysis of peptide linkages between the α-amino and

α-carboxyl groups of amino acids. However, the inequivalent liberation of –COOH and –$NH_2$ molecules leads to the impression that linkages may occur in the protein molecule in which –COOH is united to some other group other than primary -$NH_2$ groups (Cannan and Muntwyler, 1930). This theory may be supported by the FTIR spectra attained of the crosslinked gelatine coatings after exposure to simulated media with and without pepsin for 0.5 hours and 2 hours (Figure 6.20).

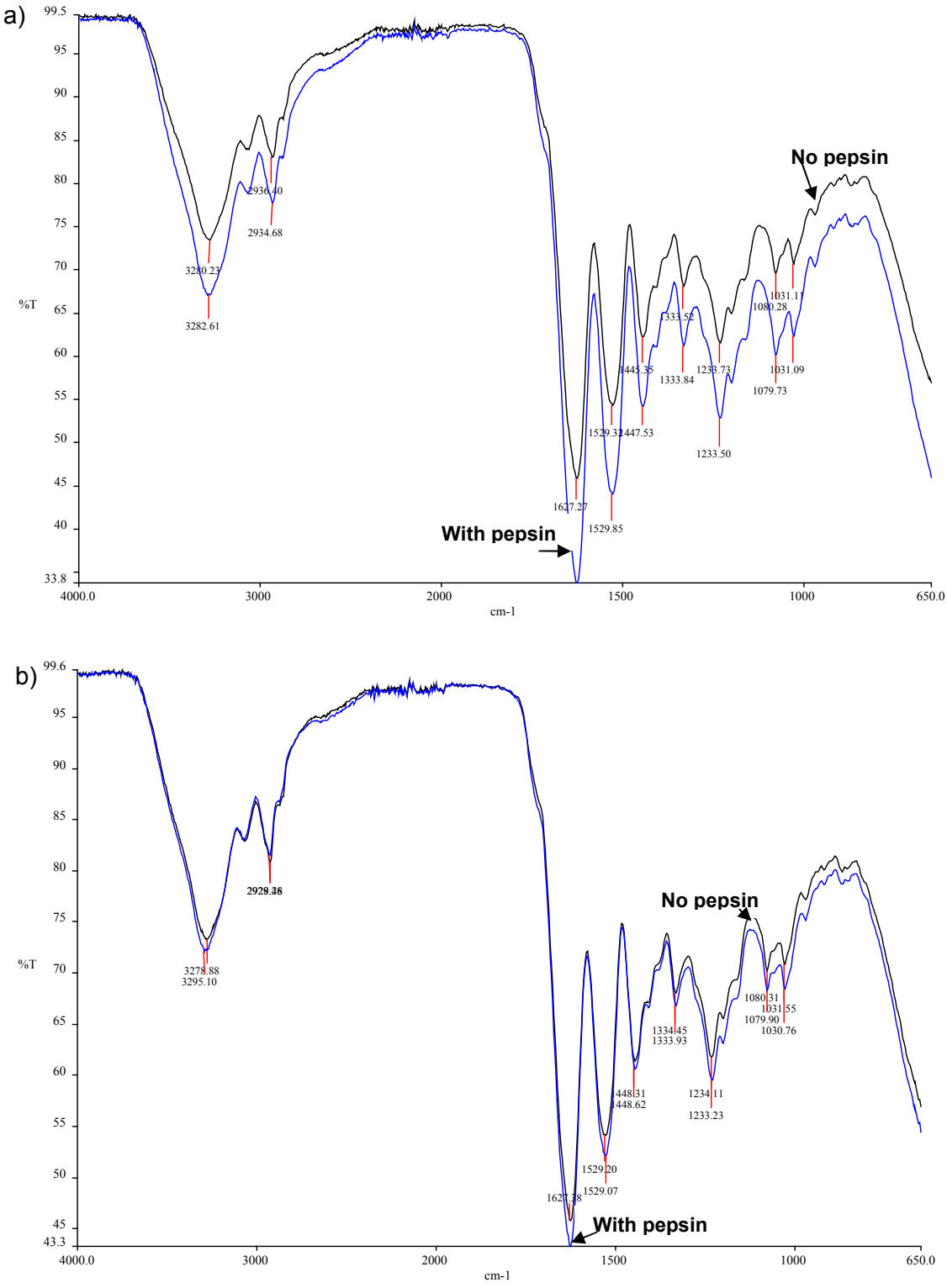



***Figure 6.21:*** *FTIR spectra of crosslinked gelatine coatings after exposure to simulated gastric fluid with and without pepsin for a) 0.5 hours, and b) 2 hours.*

Since an excess of –COOH is liberated during proteolytic degradation of pepsin, the FTIR spectra suggest that at 0.5 hours (in the absence of pepsin) the reduction in the intensity of the peak at ~3280cm$^{-1}$ is attributable to the loss of –COOH in SGF due to the dissolution of the uncrosslinked gelatine (Figure 6.21a). Similarly, there is a reduction in the intensity of the peak at 1627cm$^{-1}$ due to loss of –$NH_2$ groups in solution. In contrast, the peaks at 3282cm$^{-1}$ and 1627cm$^{-1}$ from formulations exposed to pepsin, showed a relatively greater intensity suggesting that both the –COOH and –$NH_2$ groups remain on the crosslinked molecule probably due to the formation of a complex on the existing molecule subsequent to proteolytic cleavage of the relevant groups.

After exposure of the crosslinked formulations to SGF with and without pepsin for 2 hours, the relative difference in the intensity of the 2 spectra are much less pronounced. This may be due to the subsequent dissolution of the newly formed complexes. In addition, the peaks between 1445-1031cm$^{-1}$ of the spectra attained from the formulations exposed to SGF for 0.5 hours and 2 hours are indicative of loperamide HCl presence in the coating (Figure 6.21b). These FTIR spectra provide the reason for the minimization of loperamide HCl in the presence of pepsin, where only 0.233 fractional release was observed compared to complete loperamide HCl release in the absence of pepsin after 2 hours. In addition the reduced ability of the crosslinked coatings to absorb SGF containing pepsin and the reduced erosion of the formulations exposed to pepsin is also validated.

### 6.4 Concluding Remarks

Through the construction and experimental investigations performed in accordance with the Box-Behnken experimental design, an optimized outer pepsin-responsive intestinally-targeted coating was formulated. The pepsin-responsive ability of the formulation was evident from the minimal loperamide HCl release in SGF containing pepsin compared to when pepsin was absent. Furthermore, on entry to simulated small intestinal conditions complete drug release was achieved. The drug release characteristics of the formulation could also be related to its ability to absorb SGF and its erosion at each time point. Ultimately, the pepsin-responsiveness and the intestinal-targeting ability of the formulation were as a result of the unique crosslinking method employed as well as the distinct action of pepsin on these crosslinked formulations.

# CHAPTER 7

# *IN VIVO* EVALUATION OF THE STIMULI-RESPONSIVE ORAL TABLET SYSTEM IN THE PIG MODEL

## 7.1 Introduction

In the evolving and critical field of human medical research in particular, drug delivery design, the search for an ideal experimental animal model which is analogous to humans in as many aspects as possible, proves to be daunting. Nevertheless, even prior to the 1960's it had been noted that "pigs are just not pigs, but almost humans" (Douglas, 1972). Pigs have extensively been used to study the rate of passage of food through the gastrointestinal tract (GIT) due to the anatomical comparability of the GIT as well as the similar total body weight ranges to humans (Castle and Castle, 1956; Castle and Castle, 1957; Kidder *et al.*, 1961; Ochia, 1973; Pond and Houpt, 1978). Furthermore, due to the many biological similarities, data obtained from the pig model is sufficient for extrapolation to man (Bustad and McClellan, 1968; Oberle and Das, 1994).

### 7.1.1 The stomach

The anatomical and functional diversities of the GIT of different species exist as a direct influence of their dietary preferences (Steves and Humes, 1995; Cunningham, 1997). The stomach of the pig, like humans, is differentiated into 4 regions with each region responsible for specific physiological functions. Essentially, only the proper gastric region (fundus) and pyloric region secretes pepsinogen, intrinsic factor, hydrochloric acid and gastrin. The top 2 regions namely, the nonglandular stratified squamous region and the cardiac region, are relatively larger in man than in pigs and are solely responsible for mucus and bicarbonate production (Martinez *et al.*, 2002).

Normally food is emptied in a bimodal manner from the stomach of pigs with 30-40% of ingested material entering the duodenum within 15 minutes. Essentially, food passes through the stomach and small intestine much more rapidly than through the large intestine (Auffrey and Martinent, 1967; Pond and Houpt, 1978). Gastric emptying is typically incomplete so food may constantly be present in the stomach leading to the false perception regarding the fasted state of the pig. The migrating myoelectric complex (MMC) responsible for the gastric emptying of indigestible solids occurs with a periodicity of 2 hours in man in the fasted state compared to 75-80 minutes in pigs (Szurszewski, 1969; Code and Marlett, 1975, Feldman *et al.*, 1984; Kumar *et al.*, 1986; Gupta and Robinson, 1988).

### 7.1.2 The small intestine

Pigs, as omnivores, possess a well-developed small intestine and a more complex lower intestine to compensate for their diverse diet. The differences in the transit time through the small intestine of different species may be related to the disproportionate relationship between the body length and intestinal length. However, pigs in particular have a body length to intestinal length ratio of 1:14, and an approximate small intestinal length of 16-21m (Argenzio, 1984). Furthermore, due to the low pH of the stomach, jejunum and upper ileum there are a limited number of microorganisms that can exist in these regions resulting in the minor role played by intestinal microflora on the metabolism of most drugs (Martinez *et al.*, 2002).

### 7.1.3 The large intestine/colon

In the GIT of monogastric species the colon houses the largest population of microorganisms and thus is a major site of production and absorption of volatile fatty acids in sheep, pigs, rabbits, rats, dogs and humans (Kararli, 1995). Furthermore, due to the particularly similar diets of man and pig the population of colonic flora in these species are greatly similar (Yamada *et al.*, 1995; Gardner *et al.*, 1996). The structural and anatomical differences of the large intestine of monogastric mammalian species in particular pigs vs. humans are illustrated in Figure 7.1.

Even though the GIT of the pig has numerous anatomic differences from humans, the physiology however remains similar and thus has extensively been used as a gastrointestinal model. The specific functional characteristics of pigs GIT that relate them directly to humans include the ion transport, motility, and splenic blood flow characteristics. These physiological characteristics of the GIT-like in humans-is probably due to their omnivorous diet (Brown and Terris, 1996; Reeds and Odle, 1996; Tumbleson and Schook, 1996).

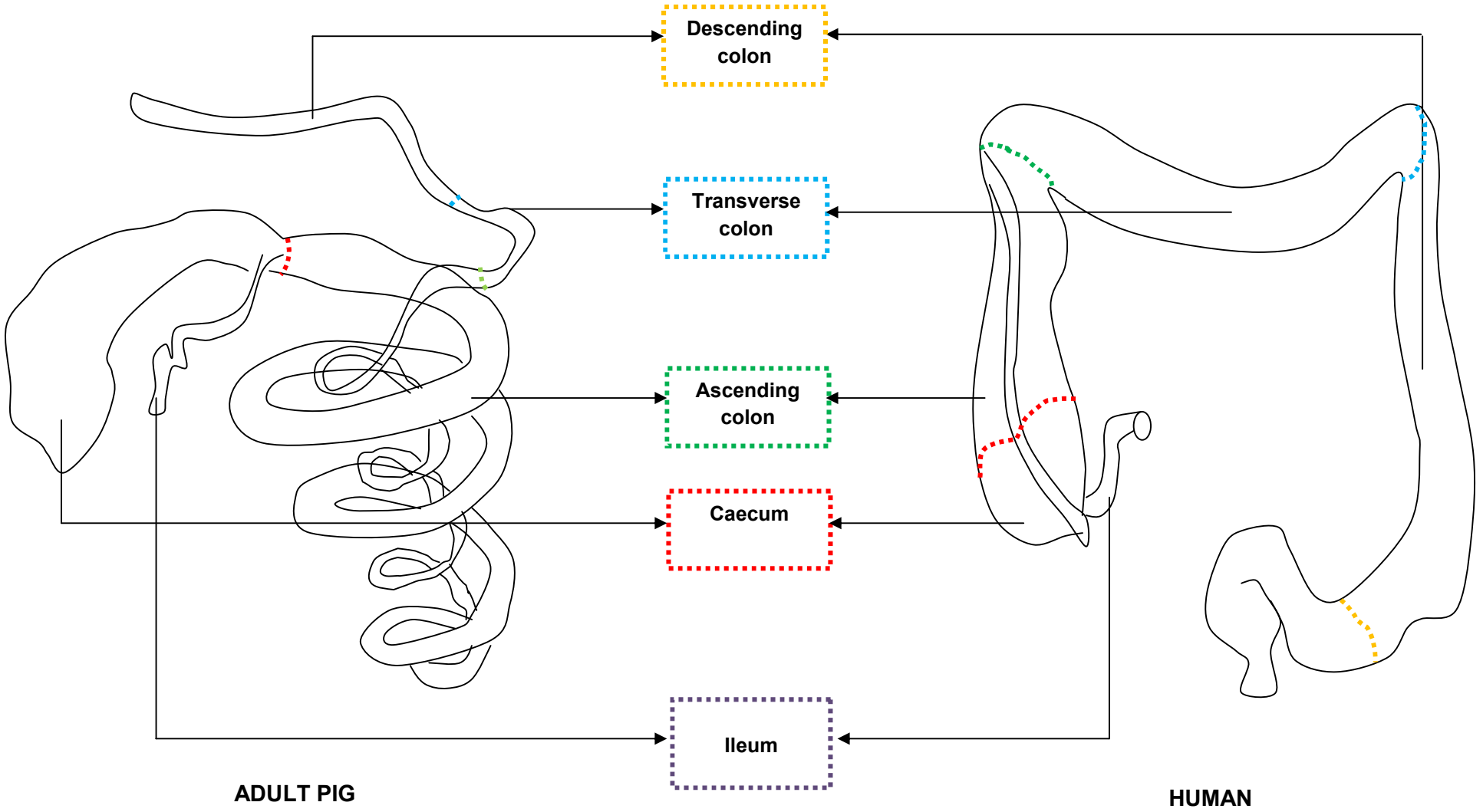



***Figure 7.1:*** *Structural/anatomical differences of the human vs. the pig colon.*

The fundamental purpose of this chapter was to elucidate the plasma-concentration profiles of 5-ASA and loperamide HCl from the stimuli-responsive oral tablet (SROT) system thus allowing the quantitative and qualitative comparison of the SROT with the commercially available conventional systems of Asacol® and Imodium®. However, a prerequisite to the achievement of this aim necessitated the establishment of a suitable and effective method of blood sampling from the pig that avoided excessive stress and pain for the animal. In addition, effective chromatographic methods of analysis for the relevant drugs had to be developed.

## 7.2 Materials and Methods

### 7.2.1 Materials

Chemicals employed for plasma sample preparation for 5-ASA and loperamide HCl determination by UPLC analysis included ortho-phosphoric acid ($H_3O_4P$) (85-90%) purchased from Fluka (Sigma-Aldrich Chemie GmbH, Steinheim, Switzerland), perchloric acid (69-72%) from Saarchem (Merck Laboratory Supplies (Pty) Ltd., Midrand, Gauteng, South Africa), propionic anhydride (99+%) obtained from Sigma-Aldrich (St. Louis, MO, USA), ethyl acetate ($CH_3OOC_2H_5$) (min. 98.0%) purchased from Saarchem (Merck Chemicals (Pty) Ltd., Wadeville, Gauteng, South Africa) and tert-Butyl-Methyl-Ether (MTBE) purchased from Fluka Chemika (Fluka Chemie, Buchs, AG, Swtzerland). The buffer salts employed for mobile phase and sample preparation included di-sodium hydrogen phosphate anhydrous ($Na_2HPO_4$), potassium dihydrogen orthophosphate ($KH_2PO_4$), and sodium carbonate anhydrous ($Na_2CO_3$) purchased from Rochelle Chemicals (Johannesburg, Gauteng, South Africa), Merck Chemicals (Pty) Ltd. (Wadeville, Gauteng, South Africa) and Associated Chemical Enterprises (South-Dale) respectively. Acetonitrile 200 (ROMIL-SpS™ Super Purity Solvent ($CH_3CN$), Assay>99.9%) was purchased from Romil Pure Chemistry (Waterbeach, Cambridge, England) and double de-ionized water was obtained from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, USA). 4-ASA, barium sulphate.

### 7.2.2 Experimental subjects and conditions

Healthy female adult white pigs weighing approximately 35-45kg were employed in the study. The pigs were maintained on a commercially available pig feed diet for the duration of the study and were allowed two medium sized meals twice a day until 12 hours prior to dosing. During the 24 hour study period pigs were fed smaller meals throughout the day, with the occasional ‘treat’ of raisins or vegetable peels at each sampling point. Water was allowed *ad libitum*. Pigs were cared for by the personnel at the Central Animal Service (CAS) at the University of Witwatersrand and were housed in small adjacent individual pens with metal ‘cage-like’ divisions and concrete floors with a bed of hay. Each pen was cleaned out twice a

day and the bed of hay regularly replaced. The pens allowed for free movement and 'normal' activity of the pig thus maintaining normal gastrointestinal motility. The housing area was kept lit during the day and dark at night so as to not affect the circadium rhythms of the pigs.

### 7.2.3 Determination of an effective method and route of blood sampling

#### 7.2.3.1 Pilot study of the viability of blood sampling via the marginal ear vein

Prior to dosing with the SROT and conventional commercially available systems (Asacol® and Imodium®) it was necessary to establish a simple, yet effective method of performing blood sampling from the conscious pig. Thus, as a pilot study and in accordance with Animal Ethics Clearance no. 2007/56/04 (see appendix), a catheter was inserted into the marginal ear vein of the pig after anaesthesia was induced by a cocktail of midazolam (0.3mg/kg I.M) and buprenorphine (0.05mg/kg I.M) with anaesthesia being maintained with 2% isoflurane/100% oxygen. The process of catheter insertion and the blood sampling procedure is visually explicated in the sequential digital images shown in Figure 7.2.

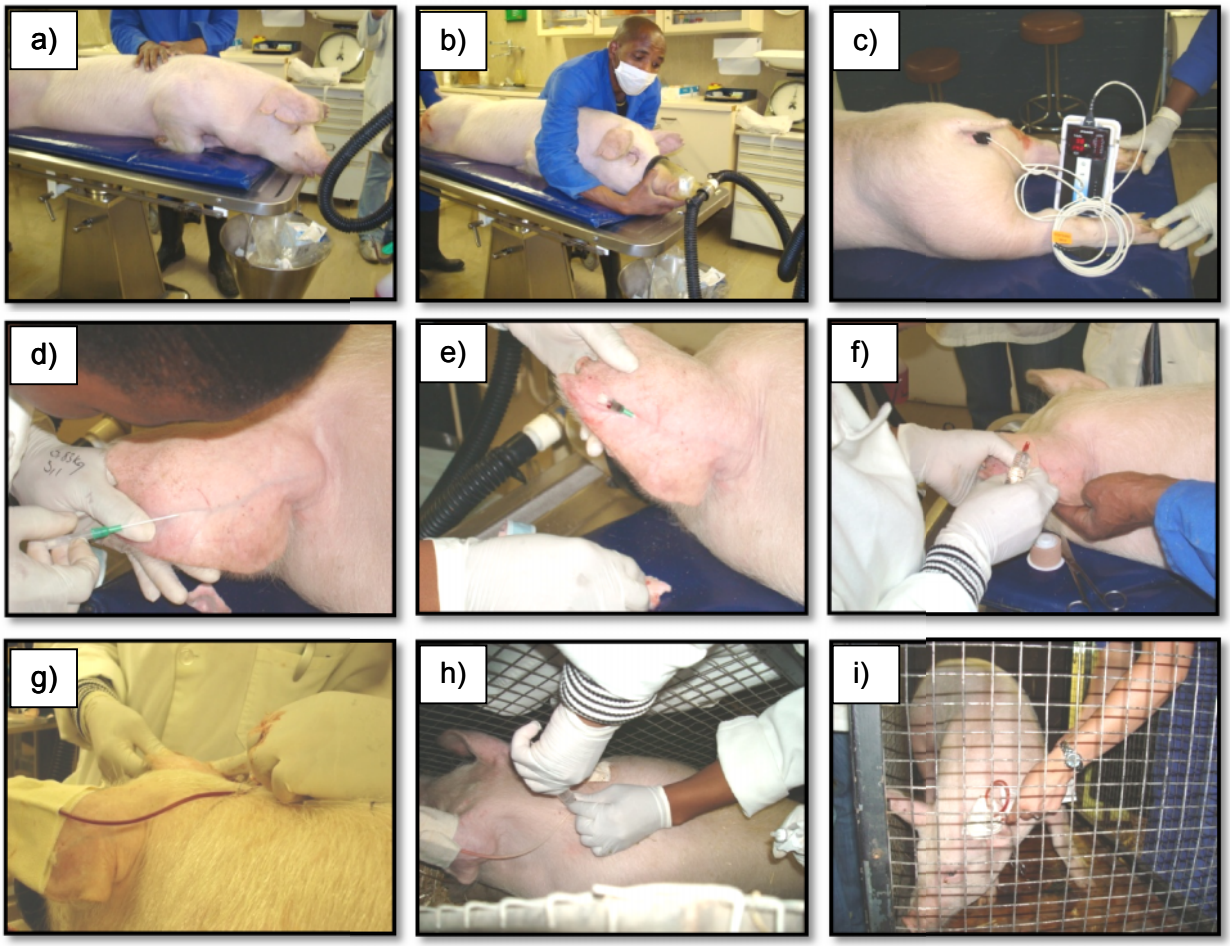



***Figure 7.2:*** *Sequential digital images of the process of catheter insertion in an anaesthetized pig and blood sampling via the marginal ear vein catheter from the conscious pig.*

Briefly, after the administration of the anaesthetic cocktail, the pig was maintained under anaesthesia and its vitals (blood pressure, oxygen saturation etc) constantly monitored. The

catheter was inserted and the tubing sutured from the ear to neck of the pig. The tubing was flushed with heparinized saline (500IU of heparin in 1000mL of 0.9% saline) to prevent clotting within the tubing and to maintain a clear pathway for blood flow. For blood sampling the conscious pig had to be coaxed into a narrow pen that limited excessive movement of the pig. Blood sampling was then attempted/performed from the open top of the cage.

#### 7.2.3.2 Surgical insertion of a chronic jugular catheter

For the surgical placement of the chronic jugular catheters, the pigs were anaesthetized with 11mg/kg ketamine (I.M) and 0.3mg/kg midazolam (I.M). Buprenorphine (0.05mg/kg) and carpofen (4mg/kg) were both administered intramuscularly for analgesia. Pigs were then intubated and anaesthesia maintained with 2% isoflurane in 100% oxygen. Under aseptic conditions, a 7 FR gauge double lumen 35cm catheter (CS-28702) (Arrow Deutschland GmbH, Erding, Germany) impregnated with chlorhexidine gluconate was surgically inserted into the left jugular vein. The jugular vein was exposed by an incision made dorsal to the jugular groove on the left lateral aspect of the neck. Via blunt dissection, the vein was isolated and the catheter inserted 10cm into the lumen of the vein. The lumen of the catheter was then fastened to the wall of the vein using a purse suture technique. The remaining length of the catheter (25cm) was tunnelled subcutaneously, with the use of a trocar, to an exit point on the dorsal aspect of the scapular. The externalized injection ports of the catheter were sutured to the skin of the pig so as to limit excessive movement and kinking. Blood was drawn and the catheter flushed with heparinized saline (1000IU of heparin in 1000mL of 0.9% saline). The pigs were subsequently allowed to recover for approximately 8 days post surgery. During this time, pigs were continually habituated to the process of blood sampling and catheters were flushed with heparinized saline every ±12 hours to prevent clotting and infection due to the catheter.

### 7.2.4 Dosage administration process

Prior to dosage administration, pigs were anaesthetized with a cocktail of midazolam (0.3mg/kg I.M) and buprenorphine (0.05mg/kg I.M) injected directly into the jugular vein catheter. Anaesthesia was then maintained with 2% isoflurane and 100% oxygen. As depicted in Figure 7.3 whilst being held upright, an intragastric tube (with a lumen diameter of 15mm) containing the delivery system in the modified aperture was inserted from the mouth into the stomach, and the delivery system was subsequently flushed out of the tube with 20mL of water. Pigs were then allowed to recover from anaesthesia under observation.

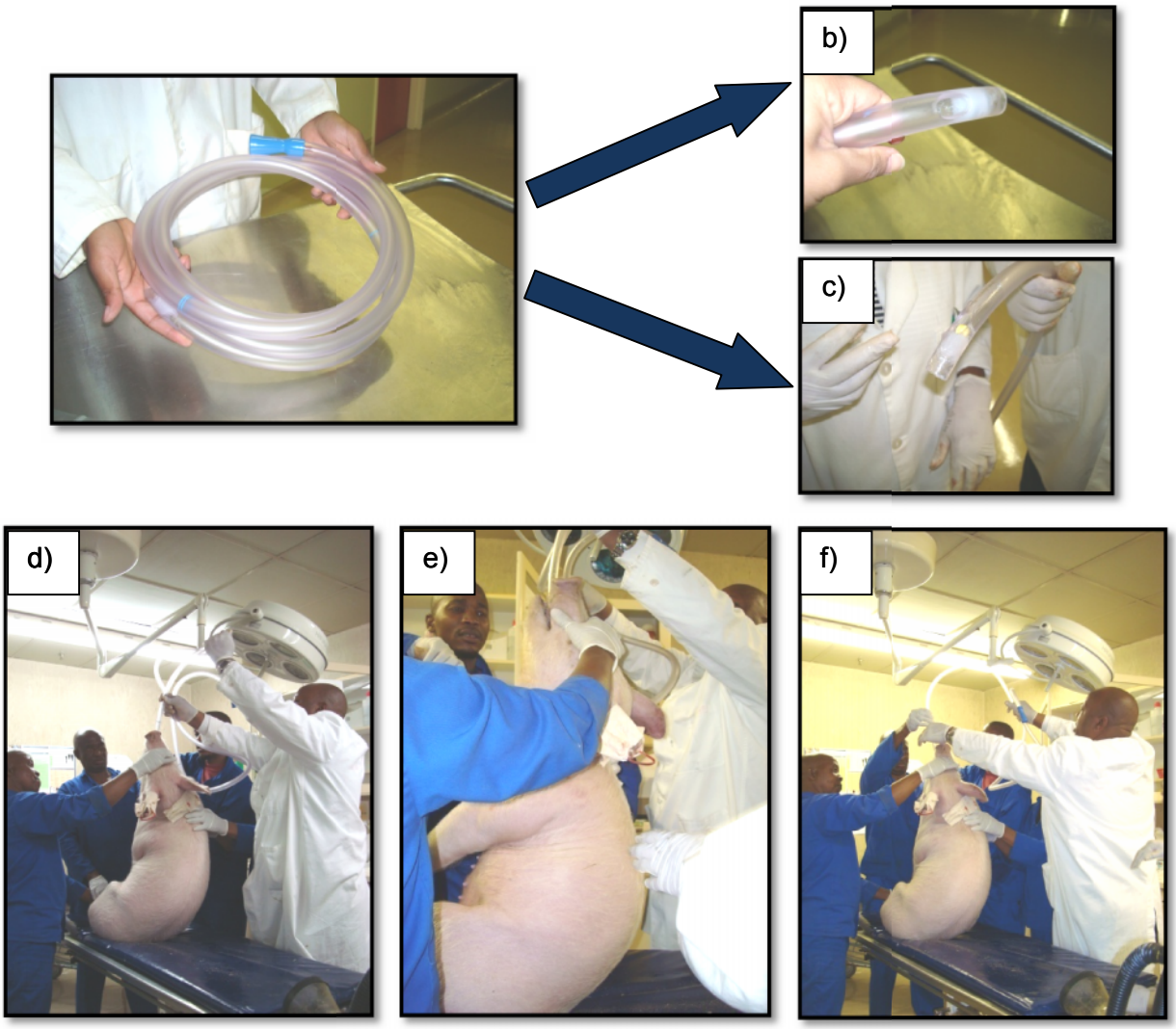



***Figure 7.3:*** *Digital images of a-c) the intragastric tube with the modified aperture for tablet insertion, d-e) the process of insertion of the intragastric tube into the stomach of the pig with subsequent 'flushing out' of the delivery system from the tube.*

### 7.2.5 Determination of the *in vivo* drug release characteristics of 5-ASA from Asacol® and the SROT using UPLC analysis

#### 7.2.5.1 Preparation of solvents used for sample preparation and UPLC analysis

##### 7.2.5.1.1 Priming solvents

The solvents used to prime the UPLC pumps prior to analysis included a weak wash solution of 10%$^{v}/_{v}$ acetonitrile and 90%$^{v}/_{v}$ double de-ionized water, and a strong wash solution of 90%$^{v}/_{v}$ acetonitrile and 10%$^{v}/_{v}$ double de-ionized water. These solutions were also used for 'washing-out' the system after sample analysis.

##### 7.2.5.1.2 Mobile phases

The binary mobile phase employed for sample analysis included acetonitrile and 0.01M $Na_2HPO_4$ buffer at pH 3 in a 17:83 ratio (v/v). The $Na_2HPO_4$ buffer was prepared by dissolving 3.58g of sodium hydrogenphosphate dodecahydrate ($Na_2HPO_4$) in 998mL of

double de-ionized water. Ortho-phosphoric acid (2mL) was added to this solution which resulted in a buffer pH of 3.

#### 7.2.5.1.3 Buffer for sample preparation

A phosphate buffer (pH 7.4) was required for incorporation with the sample prior to analysis. This buffer solution was prepared by dissolving 23.976g of $Na_2HPO_4$ in 1000mL of double de-ionized water. Potassium dihydrogen phosphate ($KH_2PO_4$) (2.27g) was dissolved in 250mL of double de-ionized water. 800mL of the 0.067M $Na_2HPO_4$ solution was then combined with 200mL of the 0.067M $KH_2PO_4$ solution to result in a buffer solution of pH 7.4.

Note: only double de-ionized water (Milli-Q, Millipore, Johannesburg) with a resistivity of 18.2MΩcm$^{-1}$ was used for all UPLC solvents employed for sample analysis. In addition, the mobile phases, weak and strong washes and all solvents used for sample preparation were filtered using a 0.22µm pore size Cameo Acetate membrane filter (Millipore Co., Bedford, Massachusetts).

### 7.2.5.2 Blood sampling and sample preparation

At predetermined time points (0, 2, 4, 6, 8, 10, 12, 16, 20 and 24 hours) after the oral administration of either Asacol® or Imodium® 5mL blood samples were withdrawn via the jugular catheter (Figure 7.4). It must be noted that after the oral administration of the SROT, 10mL blood samples were withdrawn with 5mL set aside for 5-ASA analysis and 5mL for loperamide HCl analysis. The blood samples were collected into heparinized Monovette® syringes (Sarstedt, Germany) and centrifuged immediately at 3000rpm for 15 minutes. The resultant plasma was separated and subsequently stored in 1mL eppendorf tubes at -70°C until analysis.

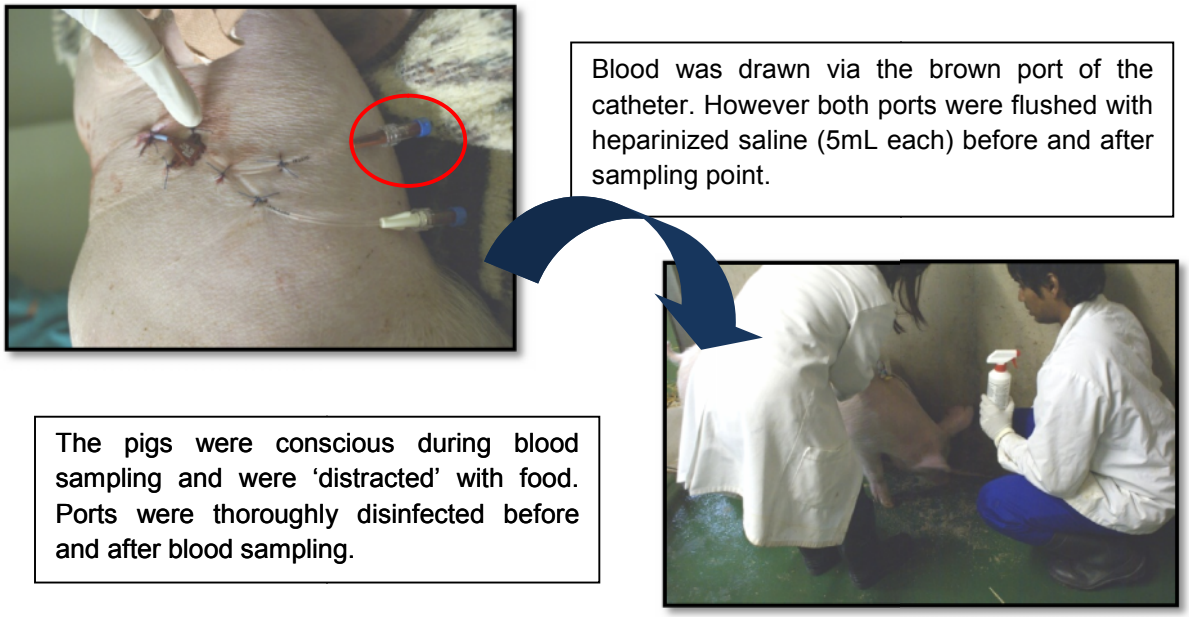



***Figure 7.4:*** *Blood sampling from the conscious pig.*

To each 1mL of defrosted plasma, 20µL of perchloric acid was added to facilitate deproteinization. The samples were subsequently vortexed (Vortex-Genie 2, Scientific Industries Inc., Bohemia, NY, USA) for 1 minute and centrifuged (Desktop Centrifuge TD5A-WS, Shanghai Luxiangyi Centrifuge Intrument Co., Ltd., Shanghai, China) at 2000rpm for 12 minutes. The clear supernatant (500µL) was transferred to a clean test tube to which 500µL of phosphate buffer (pH 7.4) was added. The internal standard employed in the study, 4-ASA, was added to each sample at a constant concentration of 0.208mg/mL (100µL). This solution was vortexed for 30 seconds after which 40µL of propionic anhydride was added to facilitate the derivatization of 5-ASA present in the plasma. The contents were vigorously shaken and allowed to derivatize for 20 minutes at 25°C. Concentrated HCl (50µL) was then added and the sample vortexed for a further 30 seconds. The acyl derivatives of 4-ASA and 5-ASA were then extracted into 3mL of ethyl acetate. After centrifugation (2000rpm for 12 minutes) the tubes were stored at -45°C for 45 minutes until the inorganic layer froze. The organic layer containing the analytes was then decanted into a 10mL glass test tube and the solvent evaporated (under a stream of nitrogen, in a water bath maintained at 45°C). The dry extract was then reconstituted in 1mL of the mobile phase (0.01M $Na_2HPO_4$, pH 3) and transferred into the UPLC vial (Waters® LCMS certified vials with a pre-slit screw top, Waters, Milford, MA, USA) for injection.

**7.2.5.3 Preparation of analytical standards for the calibration series**

Primary stock solutions of 5-ASA (0.254mg/mL) and 4-ASA (0.208mg/mL) were prepared in double de-ionized water maintained at 37°C. The 5-ASA stock solution was serially diluted to obtain solution concentrations ranging from 0.0079375mg/mL to 0.254mg/mL. Drug-free plasma (1mL) was then spiked with 100µL of these serial concentrations of 5-ASA and 100µL of the internal standard (IS) 4-ASA, to result in a final 5-ASA plasma concentration in the range of 0.795-25.452µg/mL. Spiked plasma samples underwent an identical sample preparation process, liquid-liquid extraction procedure and analysis method as described in section 7.2.5.2. The calibration curve was plotted with the peak area ratios of the analyte and IS against the corresponding analyte concentrations (expressed as µg/mL). Linearity equations and correlation coefficients ($R^2$) were obtained by means of the least square method. The limit of detection (LOD) of the derivatized 5-ASA was determined based on the concentration of *N*-propionyl-5-ASA that achieved a peak height of 10 and a signal-to-noise ratio of 3 (Mandrioli *et al.*, 2006, Nobilis *et al.*, 2006).

All solutions were filtered through a 0.22µm pore size Cameo Acetate membrane filter (Millipore Co., Bedford, Massachusetts) prior to incorporation with drug-free plasma.

#### 7.2.5.4 UPLC analysis with tandem photodiode-array (PDA) detection

Ultra Performance Liquid Chromatography (UPLC) analysis was performed on an Acquity® Ultra Performance Liquid Chromatography system (Waters®, Milford, MA, USA) coupled with a PDA detector. Separation of analytes was achieved on the Acquity UPLC® BEH Shield RP18 column (2.1mm x100mm, 1.7µm particle size) maintained at 25°C. The binary mobile phase employed included acetonitrile and 0.01M $Na_2HPO_4$ buffer at pH 3 in a 17:83 ratio (v/v), with an isocratic analysis run time of 15 minutes, an injection volume of 6.00µL, and a flow rate of 0.1mL/min. UV detection was performed at a maximum excitation wavelength of 313nm.

### 7.2.6 Barium X-ray imaging of the SROT through the GIT of the pig

X-ray imaging was undertaken as a means of determining the position of the SROT in the GIT of the pig at which 5-ASA release was initiated. This required the incorporation of a sufficient quantity of $BaSO_4$ into the optimized SROT formulation to ensure detection by the imaging equipment. Tablets were prepared as previously described (Chapter 5, section 5.2.3) except for the incorporation of 600mg of $BaSO_4$ with the rest of the tablet components. However the large weight (600mg+1350mg) and corresponding dimensions of the tablet made it incapable of passing through the intragastric tube. Therefore only 1350mg of the combination of tablet components and the $BaSO_4$ were compressed into tablets and subsequently coated with the enzyme-responsive coating solution as described in Chapter 5, section 5.2.5. Prior to X-ray imaging, pigs were anaesthetized with a mixture of midazolam (0.3mg/kg I.M) and buprenorphine (0.05mg/kg I.M) and were maintained under anaesthesia with 2% isoflurane in 100% oxygen. The vital signs of the pigs were constantly monitored during the X-raying procedure. X-ray imaging was conducted on a 240V FDA compliant mobile X-ray unit (Shimadzu Mobileart Plus MUX-100H, Shimadzu Europa GmbH, Duisburg, Germany), with image capturing on an IP Cassette imaging plate, type-CC in black and white with dimensions of 24x30cm (FCR Fuji IP Cassette), and image development on a Prima System CR-20 (Fujifilm FCR Primax). Pigs were X-rayed at the relevant time-points post-dosing and X-rayed in the dorsoventral, right and left lateral views to enable a more definitive indication of where in the GIT the SROT is at a particular time-point. Subsequently, the pigs were returned to their pens and observed until recovery from the anaesthetic.

### 7.2.7 Determination of the *in vivo* drug release characteristics of loperamide HCl from Imodium® and the SROT using UPLC analysis

#### 7.2.7.1 Sample preparation

Priming of the UPLC pumps, washing-out of the sample column and blood sampling was performed identical to the relevant procedures described in section 7.2.5. A simplistic

schematic of the sample preparation process for extraction of loperamide HCl from plasma prior to analysis is outlined in Figure 7.5.

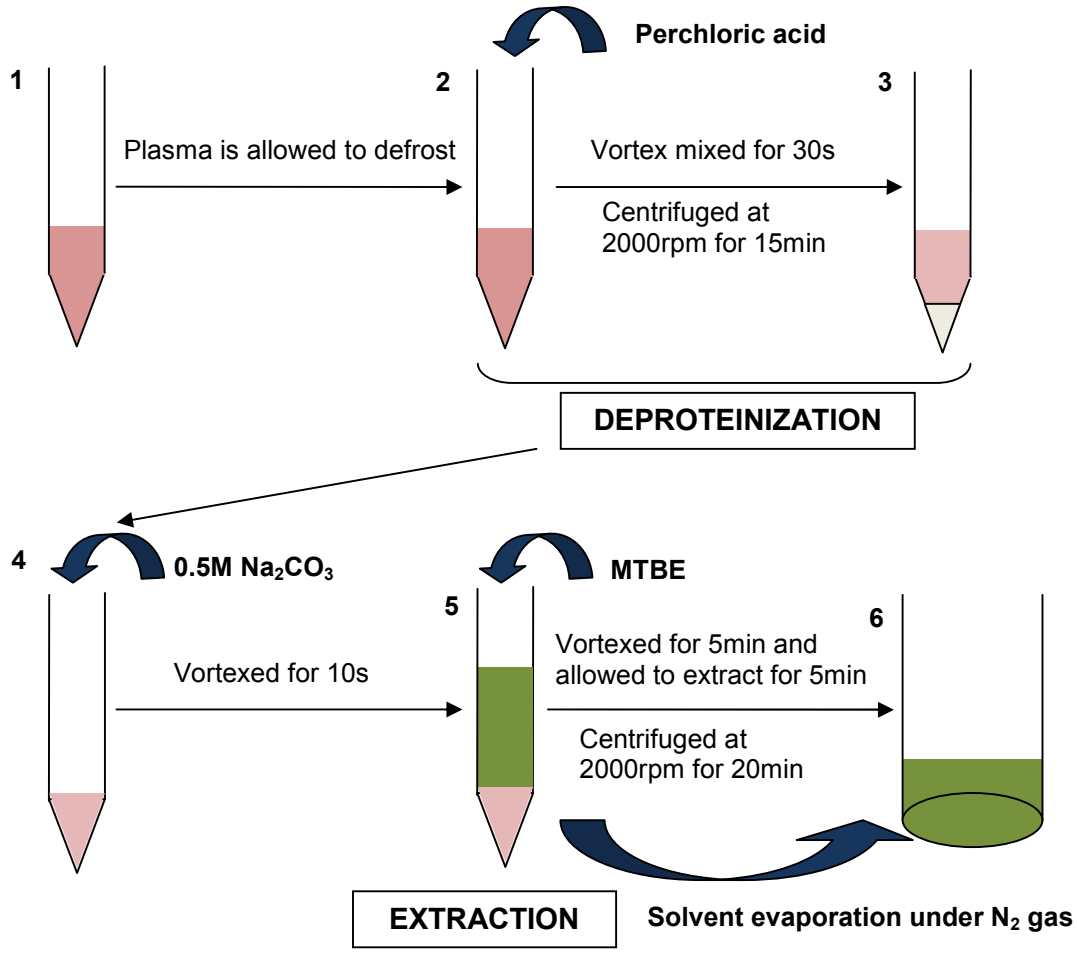



***Figure 7.5:*** *Schematic illustration of the plasma sample preparation process employed prior to UPLC analysis.*

Essentially, this preparatory process required the gradual defrosting of frozen plasma (without dramatic changes in temperature). To 500µL of the defrosted plasma, 20µL of perchloric acid was added and vortex mixed for 30 seconds to facilitate the deproteinization of loperamide HCl from plasma proteins. The murky plasma solution was then centrifuged at 2000rpm for 15 minutes. The clear supernatant (300µL) was then transferred to a clean test tube to which 50µL of a 0.5M aqueous $Na_2CO_3$ solution was added. The test tube was vortexed for 10 seconds after which 3mL of MTBE was added. This was subsequently vortexed for 5 minutes and allowed to extract for a further 5 minutes at 25°C. The test tube was then centrifuged at 2000rpm for 20 minutes. The organic layer was decanted into a clean 10mL glass test tube and the solvent evaporated under a stream of $N_2$ gas, and maintained at 40°C in a water bath. The sample residue was then reconstituted in 2mL of 50%$^{v}/_{v}$ acetonitrile and 50%$^{v}/_{v}$ double de-ionized water. To each 2mL sample, 100µL of the internal standard 4-ASA (0.2mg/mL) was pipetted, agitated until homogenously distributed and subsequently filtered through a 0.22µm Cameo Acetate membrane filter into the UPLC vial for injection.

#### 7.2.7.2 Preparation of analytical standards for the calibration series

The primary stock solution of loperamide HCl (0.1mg/mL) was prepared by dissolving loperamide HCl in 10mL of acetonitrile and subsequently making up the solution to 50mL with double de-ionized water. The stock solution of the internal standard 4-ASA (0.216mg/mL) was prepared in double de-ionized water maintained at 37°C. The loperamide HCl stock solution was serially diluted to obtain solution concentrations ranging from 0.003125mg/mL to 0.1mg/mL. Drug-free plasma (500μL) was then spiked with 100μL of these dilute solutions and 100μL of 4-ASA to result in final loperamide HCl plasma concentrations of 0.377875-3.023μg/mL after consideration of the sample preparatory steps as well as the liquid-liquid extraction procedure described in section 7.2.7.1. Plotting of the calibration curve and LOD determination was then performed as described in section 7.2.5.3.

#### 7.2.7.3 UPLC analysis with PDA detection of loperamide HCl

Separation of analytes was achieved on the Acquity UPLC® BEH Shield RP18 column (2.1mmx100mm, 1.7μm particle size) maintained at 25°C. The binary mobile phase employed included acetonitrile and double de-ionized water in a 50:50 ratio (v/v), with an isocratic analysis run time of 6 minutes, an injection volume of 6.00μL, and a flow rate of 0.22mL/min. UV detection was performed at a maximum excitation wavelength of 200nm for detection of loperamide HCl and 313nm for detection of 4-ASA.

## 7.3 Results and Discussion

### 7.3.1 Assessment of the marginal ear vein vs. the jugular vein as an efficient blood sampling route

Prior to investigating chronic catheterization of a suitable vein in the pig model, a pilot attempt was made to withdraw blood samples at regular periods from the pig via a catheter inserted in the marginal ear vein. This catheter was temporary and in theory would remain viable for a maximum of 24 hours. Despite the efficiency and speed of the catheterization procedure, blood sampling via this route proved challenging in various aspects. Essentially, the major drawback of this method of blood sampling originated from the vulnerability and weakness of the structure of the marginal ear vein which consequently led to the numerous tributary obstacles. The fundamental cause of the failure of this method is due to the collapsing/ballooning of the ear veins which subsequently resulted in a cascade of challenges as is depicted in Figure 7.6.

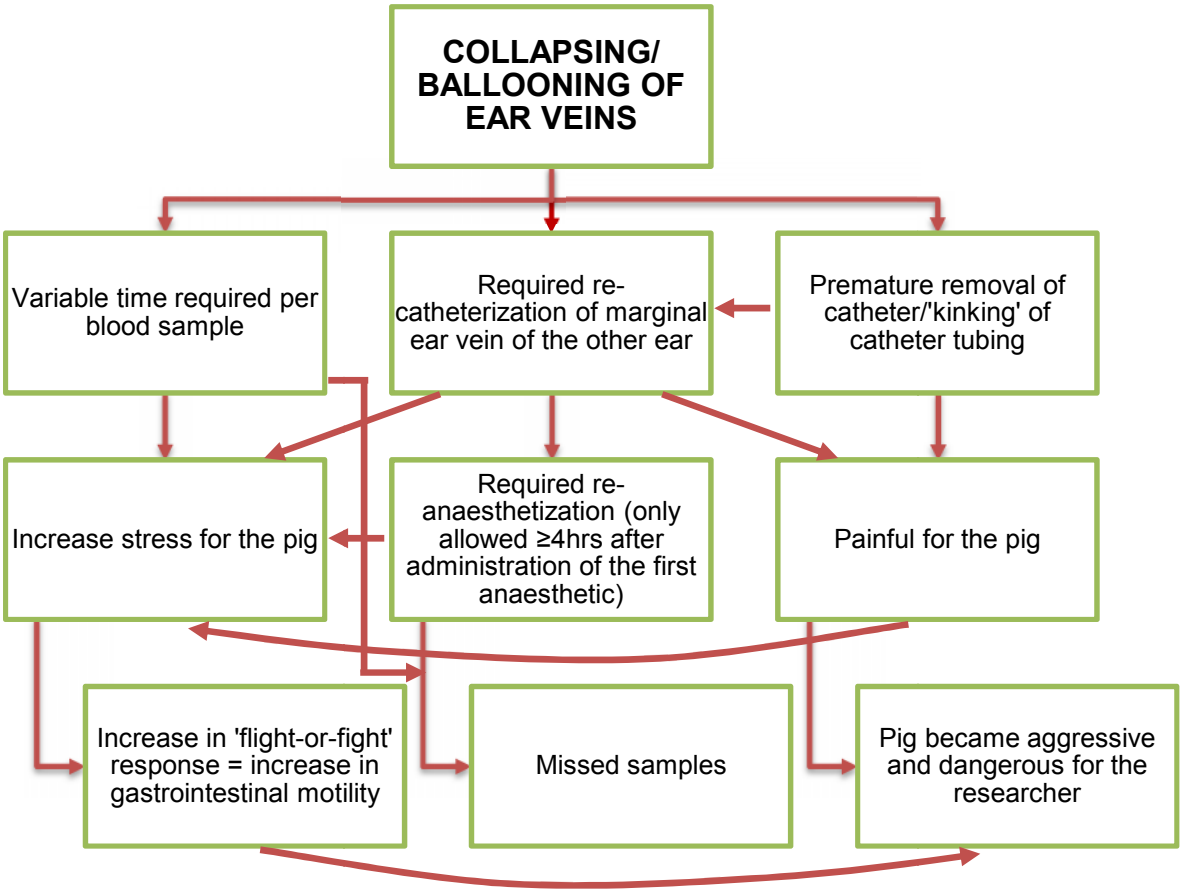



***Figure 7.6:*** *Schematic of the challenges experienced during blood sampling via the marginal ear vein.*

There is an obvious intertwining of the challenges experienced with this method of blood sampling which led to inconsistent and unrepresentative blood samples based on the following reasons:

- The time it takes to coax the pig into the sampling pen and the actual time it takes to withdraw 5mL of blood can range from 5 minutes to 90 minutes if the vein remains viable.
- If the veins collapse or balloon various sampling points are missed especially since the pig may only be anaesthetized a maximum of 3 times per 24 hour period, with at least 4 hours between each dose.
- The time it takes to withdraw each sample also provides variable plasma concentrations of the relevant drug in each bit of the collected blood.
- The stress experienced by the pig considerably alters the physiology and motility of the GIT which directly influences the plasma concentration profiles of drug delivery systems that are intended for release in specific regions of the GIT.

Thus, chronic jugular catheterization was investigated with the aim of establishing a more long-term and reliable means of withdrawing blood samples from the conscious pig. The process of catheter insertion into the jugular vein was explained in section 7.2.3.2, and is visually explicated in Figure 7.7.

Despite the lengthy catheterization process the ultimate benefits of blood sampling via this route was numerous. However several essential process conditions had to be maintained for this route of blood sampling to remain viable for an extended period of time and to assist in the actual blood sampling procedure. These process conditions included the necessary habituation of the pig/pigs at least 7 days prior to surgery and 10 days after surgery (during the post-operative recovery period). This process of habituation allowed the pig to become accustomed to the presence of humans in its pen and also allowed the pig to 'enjoy' human contact. By enabling the close contact between the pig and the researcher it facilitated the withdrawing of blood samples in less than 2 minutes. Thus no stress was placed on the pig and consequently the gastrointestinal motility was not influenced. Another fundamental procedure that had to be regularly and strictly performed involved the flushing of the catheters with heparinized saline at each sampling point and at least 3 times a day during days when no sampling was required. Due to the susceptibility of chronic catheters to infection and the sensitivity of the jugular region this process had to be conducted by thoroughly disinfecting the ports before and after flushing and blood sampling. These stringent procedures allowed the catheters to remain viable for at least 30 days, without any incidences of infection. After the research period the catheters were removed by the registered vet and pigs were returned to stock.

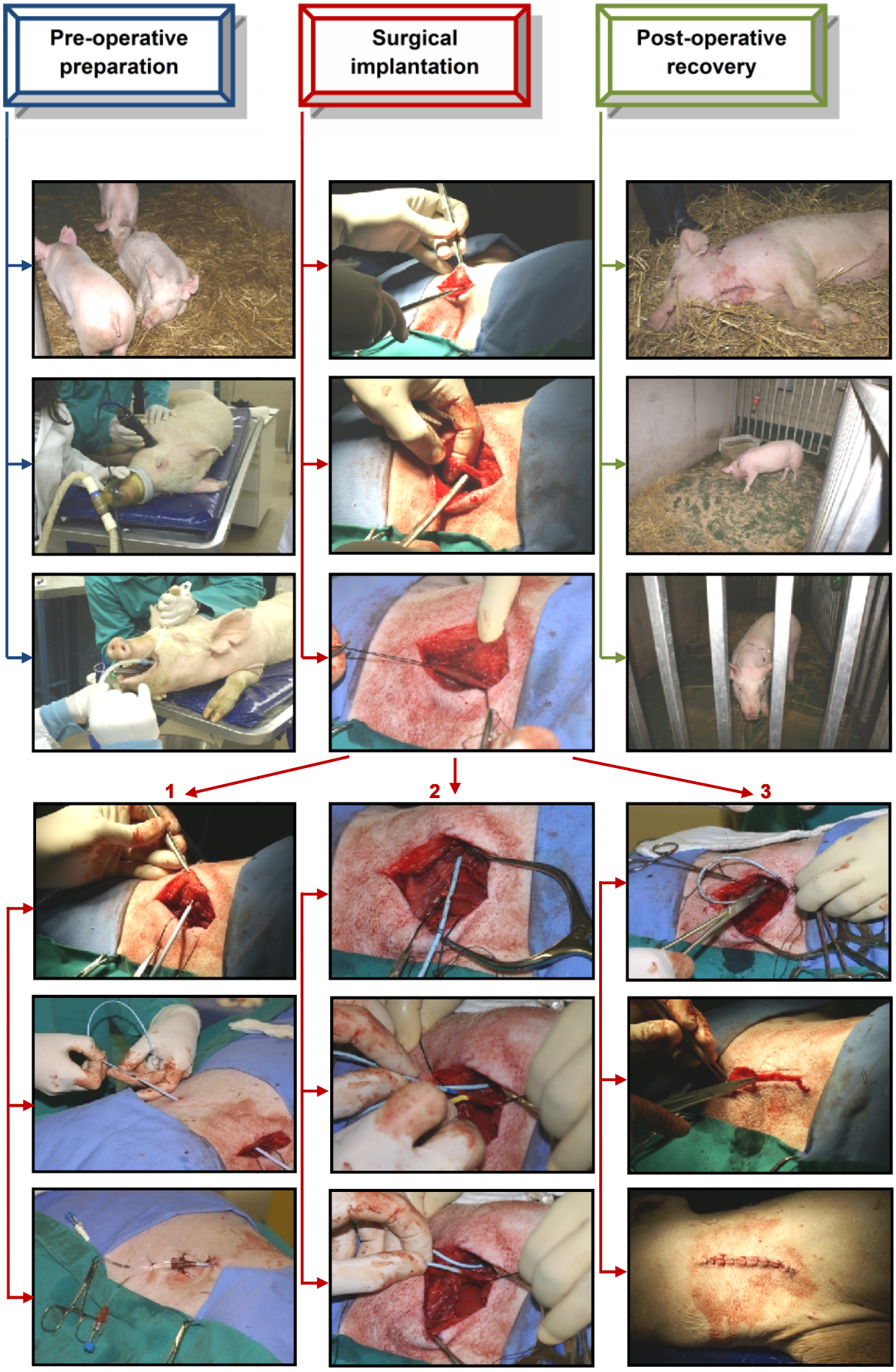



***Figure 7.7:*** *A graphical illustration of the process of jugular vein catheterization from pre-operative preparation to post-operative care.*

### 7.3.2 *In vivo* analysis of plasma samples after administration of Asacol® and the SROT

#### 7.3.2.1 Sample preparatory processes

##### 7.3.2.1.1 Deproteination

Even though 5-ASA is extensively metabolized in the GIT wall and the liver to numerous phase II biotransformation products such as *N*-acetyl-5-ASA, *N*-formyl-5-ASA, *N*-butyryl-5-ASA and *N*-β-D-glucopyranosyl-5-ASA essentially only the active 5-ASA and its principle inactive metabolite (*N*-acetyl-5-ASA) are representative of the plasma concentrations of the parent drug itself (Tjornelund *et al.*, 1989; Tjornelund Hansen, 1991; Tjornelund *et al.*, 1991). However due to the high plasma protein binding ability of 5-ASA (40-50%) and *N*-acetyl-5-ASA (80%), plasma samples required deproteinatization prior to analysis of samples (Klotz, 1985; Parfitt, 1999). Thus concentrated perchloric acid was added to precipitate the plasma proteins thus allowing liberation of the analytes.

##### 7.3.2.1.2 Derivatization

Due to the presence of the primary aromatic amino group (-$NH_3^+$, p$K_a$=6), carboxylic group (-COOH, p$K_a$=3), and phenolic group (-OH, p$K_a$=13.9) in the molecule of 5-ASA, the compound exhibits amphoteric properties, which together with its considerable polarity complicates the extraction and UPLC analysis procedure (Allgayer *et al.*, 1985). Therefore, it was important to derivatize the amino groups in order to suppress its amphoteric properties. The lipophilicity increasing derivatization procedure was thus conducted in the presence of propionic anhydride as it was the only acceptable agent for N-acylation since the other derivatives of the homologous series could be present in the plasma as authentic metabolites of 5-ASA.

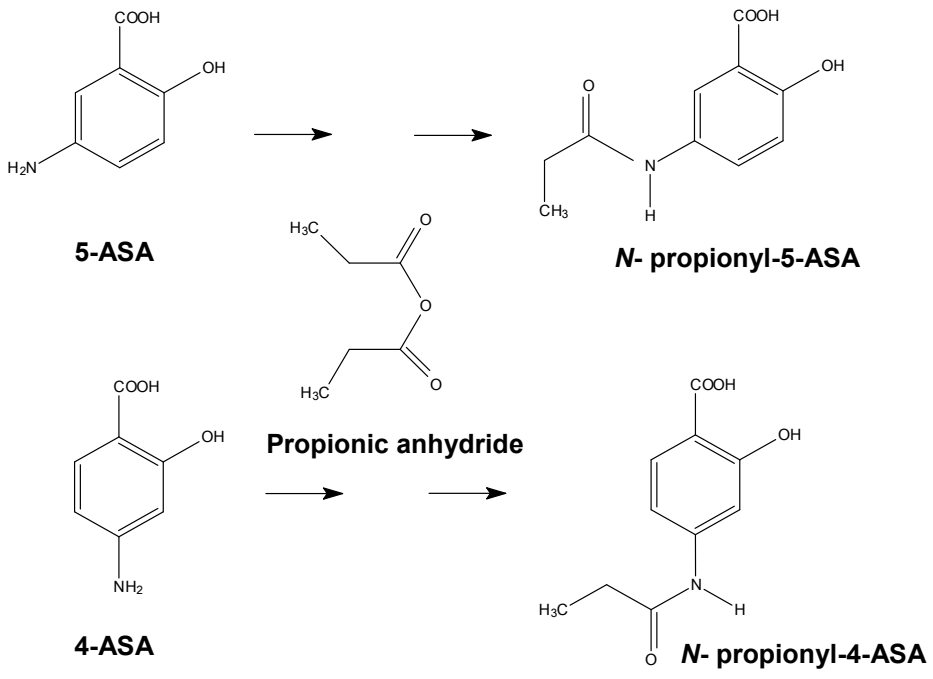



***Figure 7.8:*** *Derivatization of amphoteric 5-aminosalicylic acid and 4-aminosalicylic acid by propionic anhydride (Nobilis et al., 2006).*

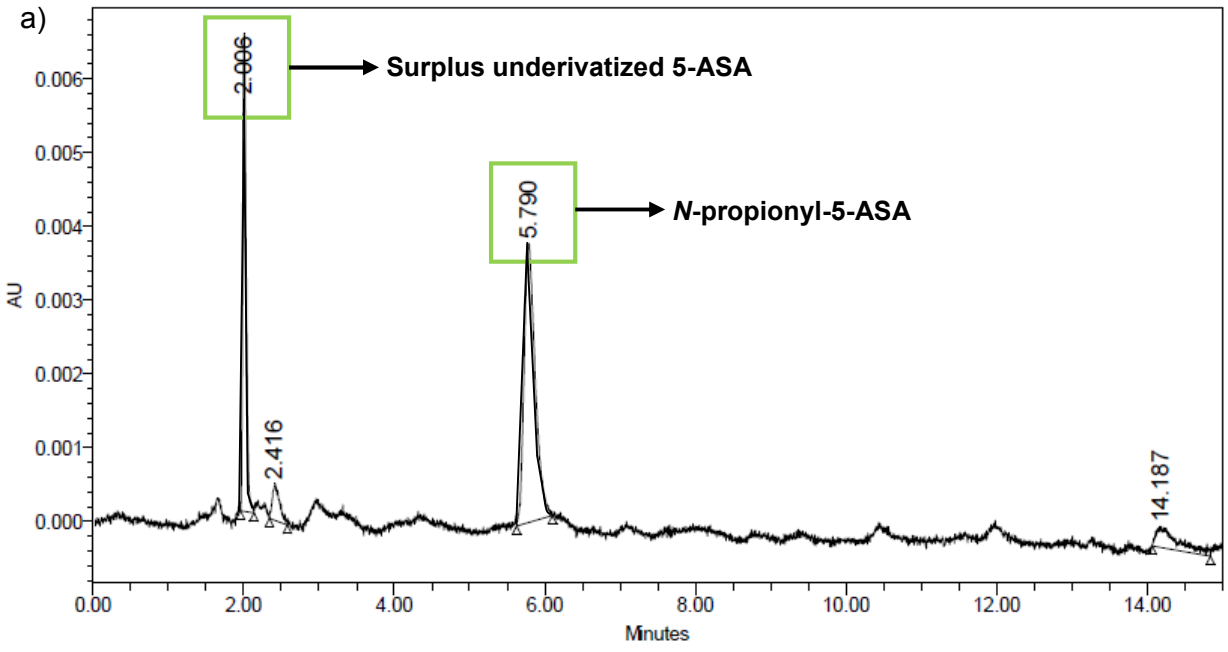
The reactions conditions applied were based on the optimum derivatization obtained in the study conducted by Nobilis and co-workers in 2006. Acylation occurs due to the nucleophilic nitrogen of the primary amino groups in 5-ASA and 4-ASA reacting readily with the deficient acyl anhydride (propionic anhydride) at ambient temperature as depicted in Figure 7.8.

#### 7.3.2.1.3 Extraction

From preliminary method development it was established that due to the extensive sample preparation process, the most appropriate solid phase extraction (SPE) procedure could not be determined despite the numerous methods evaluated. Therefore liquid-liquid extraction in ethyl acetate was performed which was based on a previously established and validated chromatographic method for 5-ASA analysis in plasma (Nobilis *et al.*, 2006). This was basically conducted to remove plasma constituent interferences and to isolate the drug from the blood sample. Furthermore, to optimize the extractability of the drug and internal standard, concentrated HCl was added to the sample prior to extraction.

### 7.3.2.2 Chromatograms of the derivatized drug and internal standard

Subsequent to the application of the sample preparatory process to blank (drug-free) plasma samples spiked either with a dilute aqueous solution of 5-ASA (Figure 7.9a) or 4-ASA (Figure 7.9b) it was established that the deproteinization, derivatization and extraction procedures were effective for eluting the derivatives of both 5-ASA and 4-ASA as the chromatograms revealed peaks of a high resolution and achieved level chromatographic baselines.



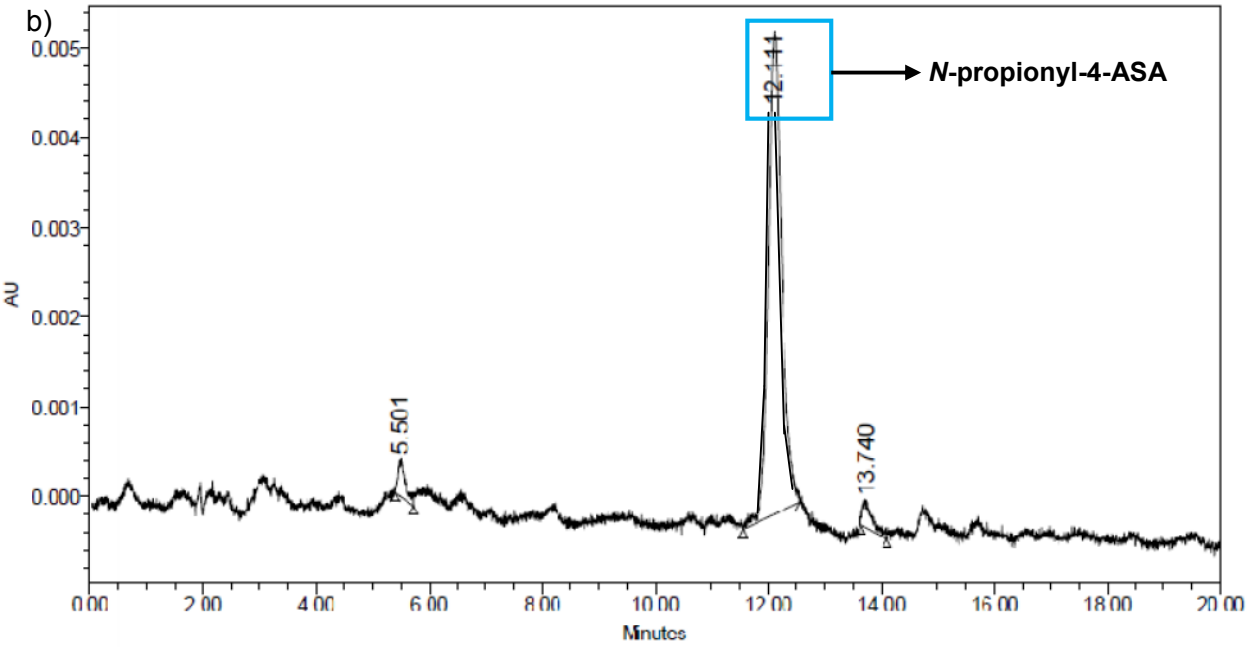



***Figure 7.9:*** *Typical chromatograms of the propionyl derivatives of a) plasma spiked with 5-ASA with peaks for N-propionyl-5-ASA (RT=5.790 minutes) and the excess underivatized 5-ASA (RT=2.006 minutes), b) plasma spiked with 4-ASA with the peak for N-propionyl-4-ASA (RT=12.111 minutes).*

The principle metabolite, *N*-acetyl-5-ASA, was also assessed by UPLC analysis and the chromatogram revealed a peak at a retention time of 3.470 minutes. As indicated in Figure 7.10, this chromatogram also showed a peak at RT=2.014 minutes indicative of the non-acetylated 5-ASA in the sample.

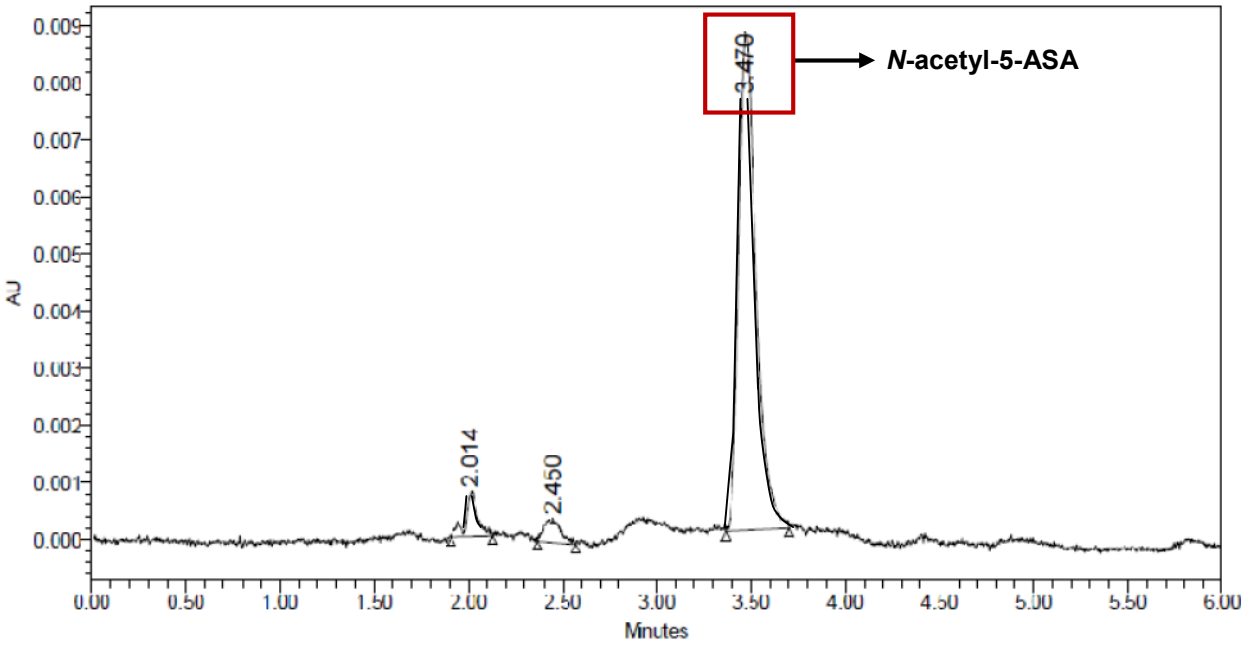



***Figure 7.10:*** *Chromatogram of a blank plasma sample spiked with a dilute aqueous solution of N-acetyl-5-ASA.*

*N*-acetyl-5-ASA was synthesized in the laboratory by dissolving 5-ASA (1g, 0.0065mol) in acetic anhydride and stirring the reaction mixture for 3 hours at 25°C. The reaction mixture was allowed to stand for 24 hours after which the crude crystalline product was filtered off and washed with double de-ionized water and subsequently dried over $P_2O_5$ (Nobilis *et al.*, 2006). Since the purity of the sample was not established it was expected that non-acetylated 5-ASA would remain present in the crystalline product and would be evidenced by UPLC analysis.

Nevertheless, analysis of plasma samples from pigs dosed with both the SROT and Asacol® showed no peaks at the region of 3-4 minutes over the 24 hour period demonstrating the absence of a significant quantity of the metabolite in the plasma. Furthermore, the difference in the plasma concentrations of *N*-propionyl-5-ASA from the SROT vs. Asacol® can be observed from the difference in the area under the curves (AUC) of the drug from the 2 formulations, when the AUC of *N*-propionyl-4-ASA (IS) remains relatively consistent (Figure 7.11a and b).

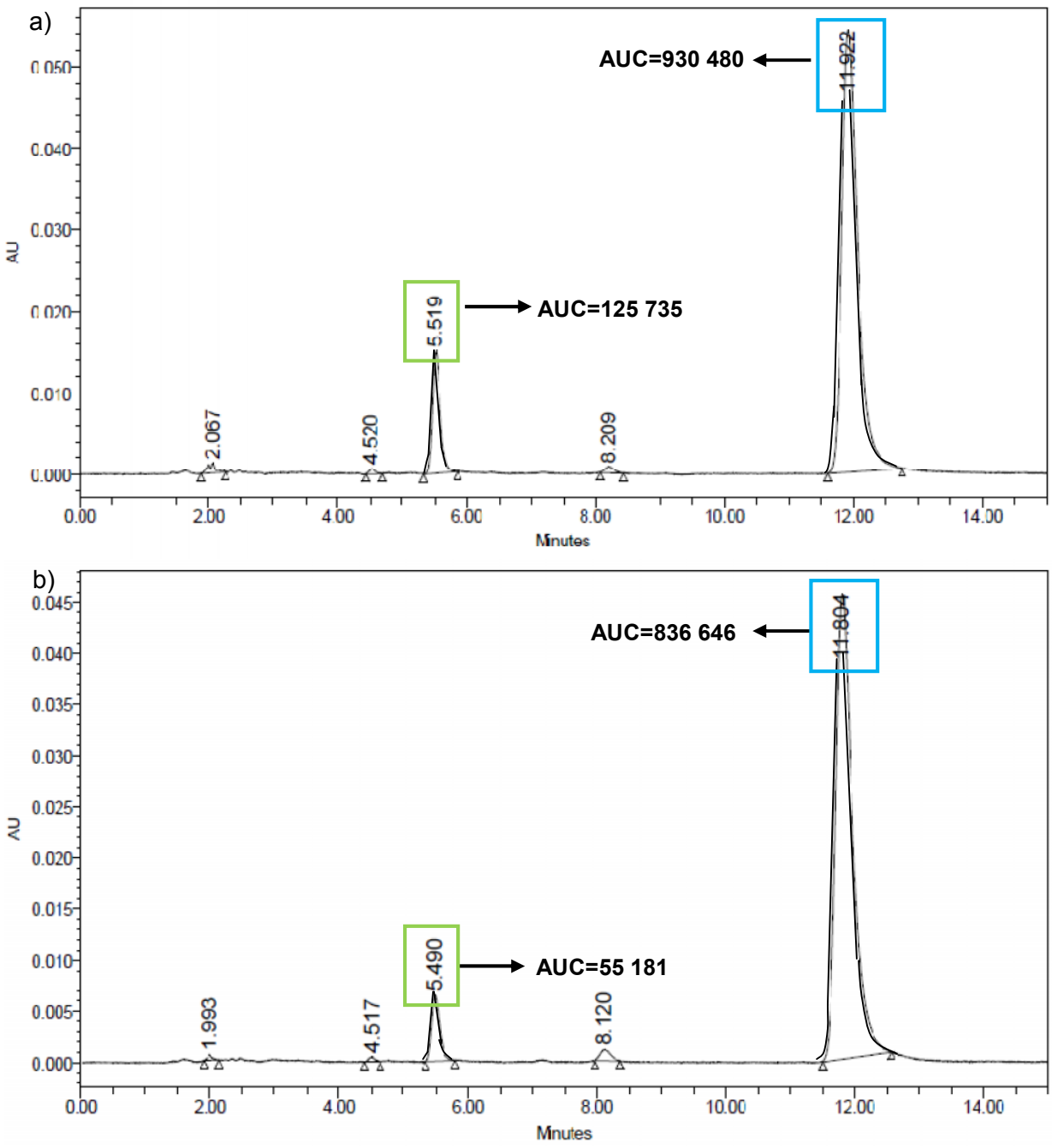



***Figure 7.11:*** *Chromatograms of 5-ASA released from a) Asacol®, and b) the SROT 24 hours after administration.*

### 7.3.2.3 Calibration curve of the derivatized 5-ASA in plasma

The calibration curve of *N*-propionyl-5-ASA in plasma performed by UPLC analysis is depicted in Figure 7.12. Samples were analyzed at a maximum excitation wavelength of 313nm for both the drug and internal standard. The samples at concentration ranges of 0-25.452µg/mL showed good linearity with a correlation coefficient ($R^2$) of 0.999. Furthermore, the limit of detection (LOD) for *N*-propionyl-5-ASA was determined to be 0.0083µg/mL.

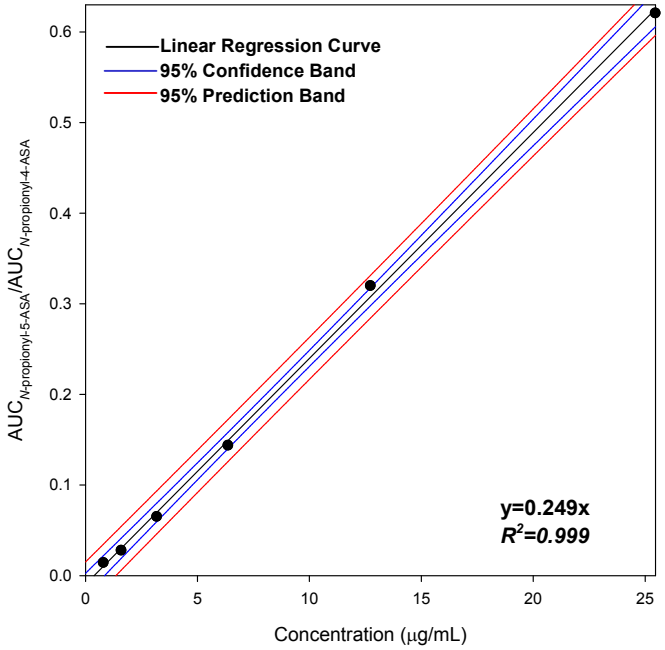



***Figure 7.12:*** *Calibration curve of the derivatives of the drug (5-aminosalicylic acid) and internal standard (4-aminosalicylic acid) in plasma.*

### 7.3.2.4 *In vivo* drug release of 5-ASA from the SROT and Asacol®

The release of 5-ASA from both formulations was assessed over a period of 24 hours (Figure 7.13). Various distinctive release characteristics of the two formulations were identified based on the resultant plasma concentrations at each time point. One such of these characteristics is the significantly lower plasma concentration of 5-ASA from the SROT compared to that of Asacol® over the entire 24 hour period. The SROT identified a $C_{max}$ of only 4.556±1.661µg/mL whilst that of Asacol® was 12.813±3.440µg/mL. The substantially lower concentration of 5-ASA in plasma forms fundamental evidence of the minimal release of 5-ASA in the upper GIT, as well as of the sustained release of 5-ASA in the colon. The minimal 5-ASA absorption will ultimately result in reduced side-effects and improved efficacy due to the minimized systemic absorption. In contrast, Asacol® presented with a high variation in the plasma concentration profiles of 5-ASA in each pig, with several peaks and troughs throughout the 24 hour period. This will obviously result in the occurrence of numerous side-effects, reduced efficacy and patient compliance.

Based on the physiological characteristics of the GIT of the pig it can be estimated that the time of arrival of both formulations in the colon should be approximately 3-4 hours post dosing in the 'fasted' pig. However plasma concentrations of Asacol® show a significant premature release of 5-ASA with $C_{2hrs}$=10.563±4.444µg/mL compared to the SROT with $C_{2hrs}$=0.070±0.121µg/mL, where the delivery system is theoretically still in the proximal small intestine.

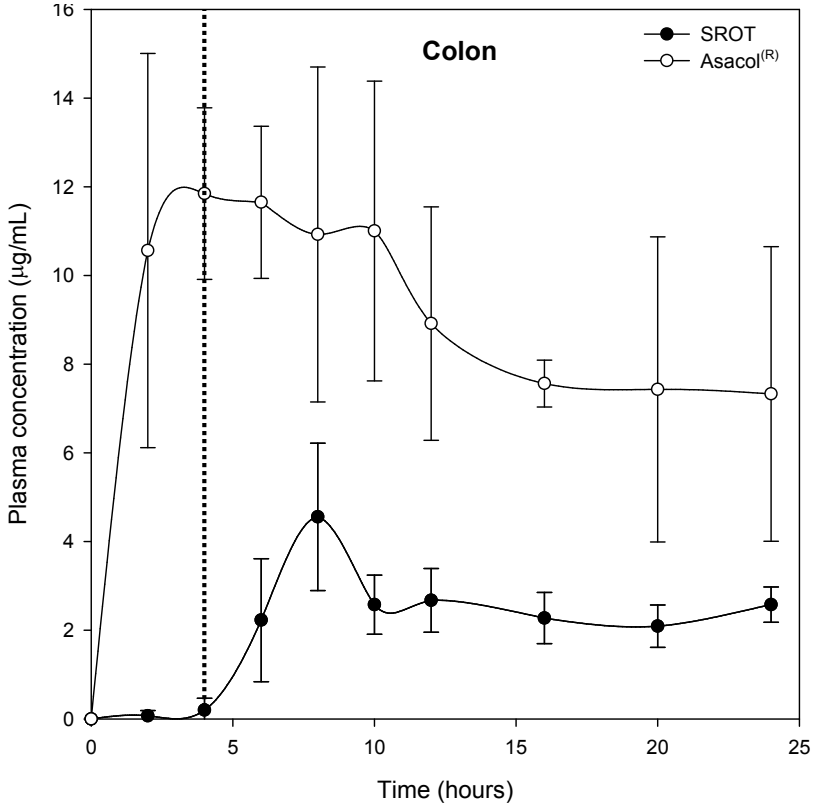



***Figure 7.13:*** *Plasma concentration profiles of the stimuli-responsive oral tablet (the 5-aminosalicylic acid-loaded colon-targeted component) in comparison to the gold-standard marketed comparator product, Asacol®.*

#### 7.3.2.5 Barium x-ray imaging of the SROT in the GIT of the pig

A right lateral view of the abdominal section of the pig, 3 hours post-dosing, the SROT appears to be present in the stomach (Figure 7.14a), however when compared to the left lateral view of the same region the SROT appears to be in the small intestine (Figure 7.14b). Thus to more definitively determine the location of the SROT a dorsoventral image of the abdominal region was acquired. This x-ray image clearly depicts the presence of the SROT in the region succeeding the stomach in particular the proximal small intestine (Figure 7.14c).

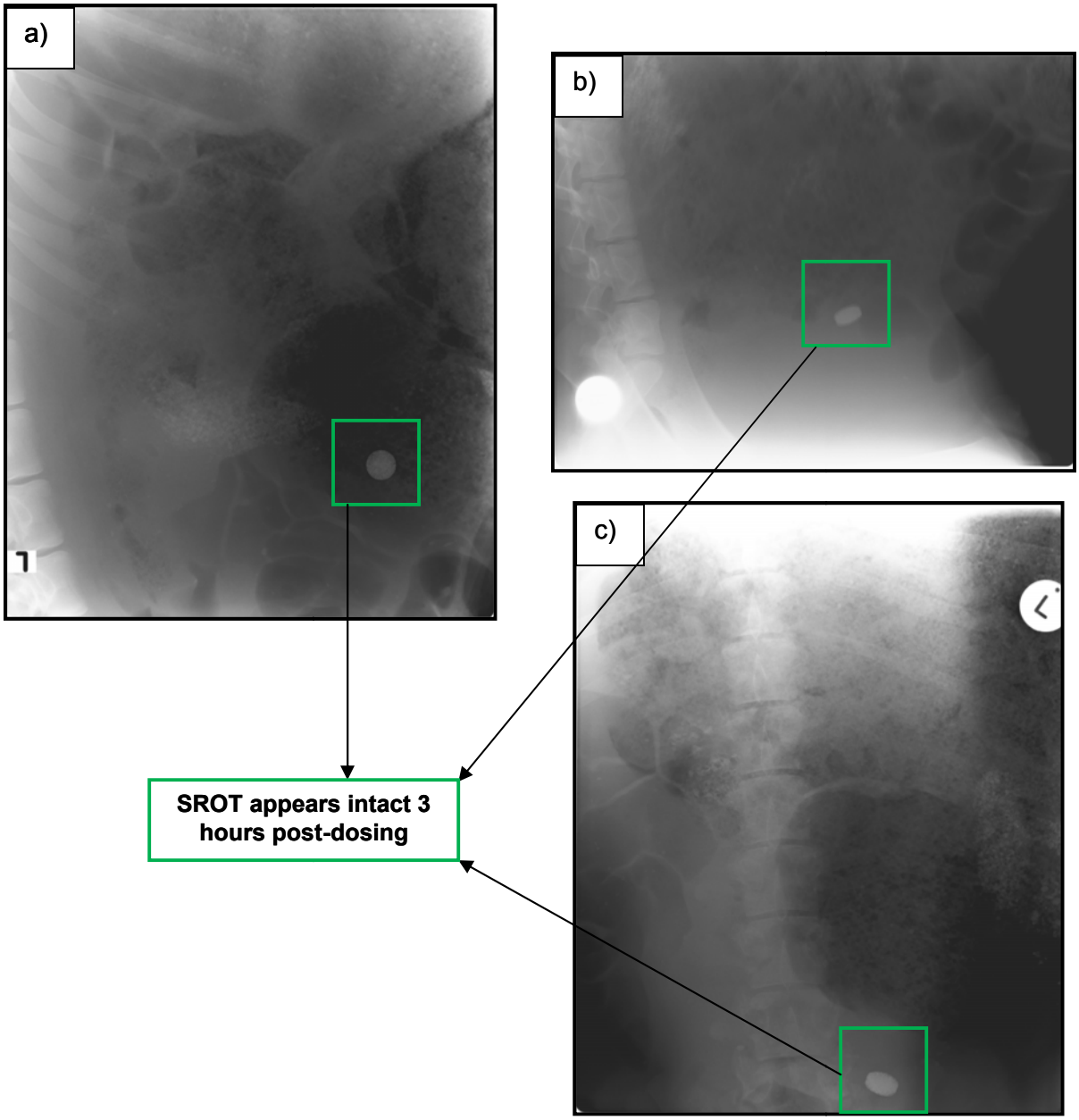



***Figure 7.14:*** *X-ray images of the abdominal region of the pig in the a) right lateral view, b) left lateral view, and c) dorsoventral view 3 hours post-dosing with the stimuli-responsive oral tablet.*

Another dorsoventral view of the pig (Figure 7.15), approximately 30 minutes after the first dorsoventral x-ray was taken showed that the SROT had moved from the small intestine to the caecum or had just entered the proximal colon.

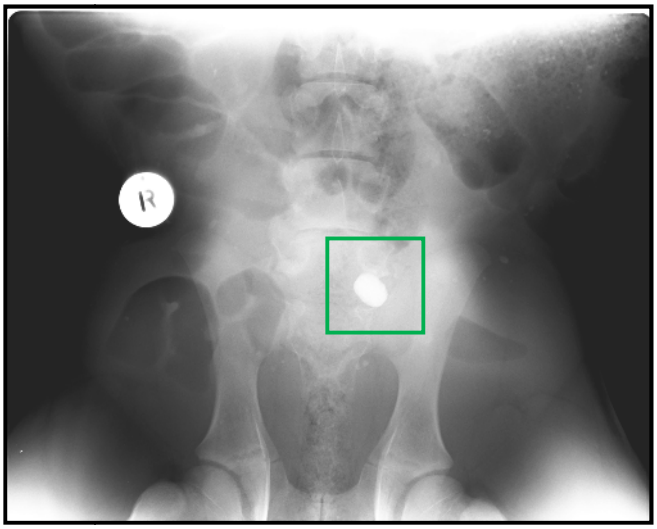


***Figure 7.15:*** *Dorsoventral x-ray image of the pig 3.5 hours post-dosing with the stimuli-responsive oral tablet.*

Figure 7.16a and b obtained 6.5 hours post-dosing showed that the SROT was visible below the ribs of the pig (in the dorsoventral view). It appeared to be in the stomach however the positioning and physiology of the GIT is such that a portion of the ascending and transverse colon is situated behind the stomach, causing the illusion. The tablet also appears 'fuzzy' indicating that erosion of the enzyme-responsive polymer coating had begun which can also be substantiated by the identification of 5-ASA in plasma at this time point (Figure 7.13)

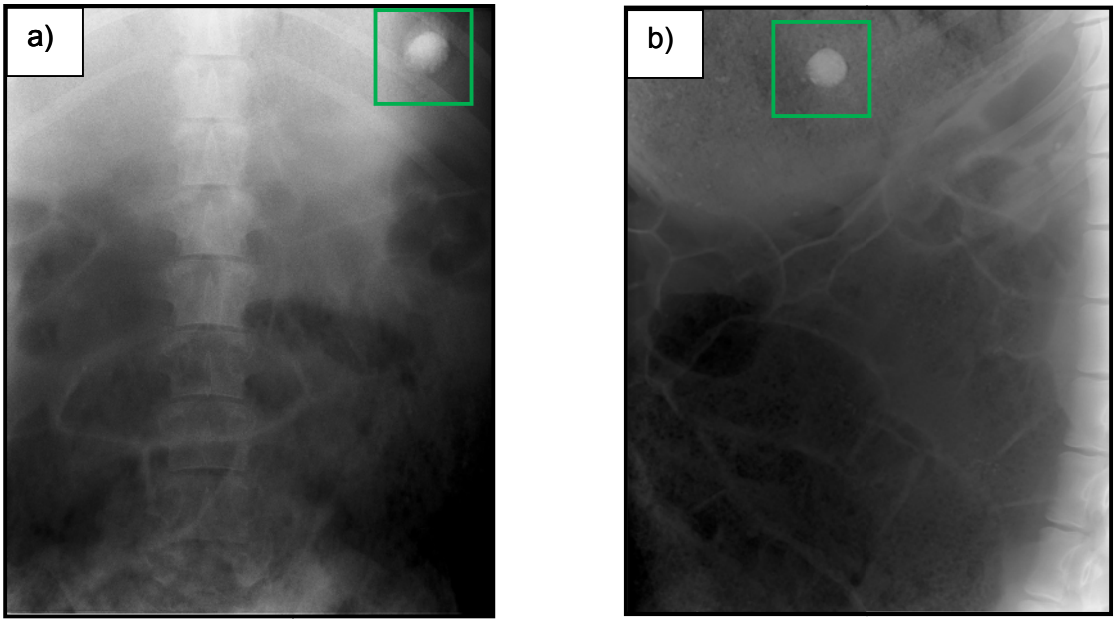



***Figure 7.16:*** *X-ray images of the stimuli-responsive oral tablet in a) the dorsoventral view, and b) the lateral view 6.5 hours post-dosing.*

### 7.3.3 *In vivo* analysis of plasma samples after administration of Imodium® and the SROT

#### 7.3.3.1 Sample preparatory process and method development

There exists an extensive methodology database for the determination of loperamide HCl in biological fluids such as plasma, however the principle drawback of these methods lie in their insufficient sensitivity to the drug for pharmacokinetic studies (Yu *et al.*, 2004). The fundamental reason for the poor sensitivity of typical chromatographic methods is attributed to the low molar absorptivity of the UV chromophores in loperamide and the presence of UV absorbing interference in plasma (He *et al.*, 2000). Thus a supplementary analysis technique has to be implemented in conjunction with the chromatographic technique to improve the method sensitivity for example, HPLC with mass spectrometry (MS), GC-MS, LC-MS, HPLC with electrospray ionization (ESI-MS), HPLC with a radioimmunoassay technique as well as HPLC with tandem mass spectrometry using electrospray ionization and selective reaction monitoring (SRM) (Leung *et al.*, 1988; Leis and Gleispach, 1989; Kamali *et al.*, 1992; De Luca *et al.*, 1993; Knupp *et al.*, 1993; Doser *et al.*, 1995; Hewala, 1995; He *et al.*, 2000).

In contrast, the UPLC allows for analysis of samples with the shortest run time and highest sensitivity without compromising the selectivity of the assay (Dongre *et al.*, 2008). Furthermore, by optimizing UPLC parameters such as pH, flow rate, column type, and buffer concentration the optimum sensitivity, peak shape and selectivity can be achieved (Everley and Croley, 2008). Therefore, based on selected principles of previously established HPLC methods, a unique UPLC method was developed, where perchloric acid was used as a deproteinating agent, $Na_2CO_3$ was used to alkalinize the sample prior to extraction, and MTBE was used as the extraction solvent.

#### 7.3.3.2 Chromatograms of loperamide HCl and the internal standard

The plasma sample preparation process which included the deproteinization and extraction procedure of loperamide HCl resulted in a chromatogram which showed a peak at a retention time (RT) of 2.310 minutes at 200nm (Figure 7.17a). A chromatogram of the internal standard (4-ASA) showed a peak at RT=1.777 minutes for 4-ASA at 313nm (Figure 7.17b).

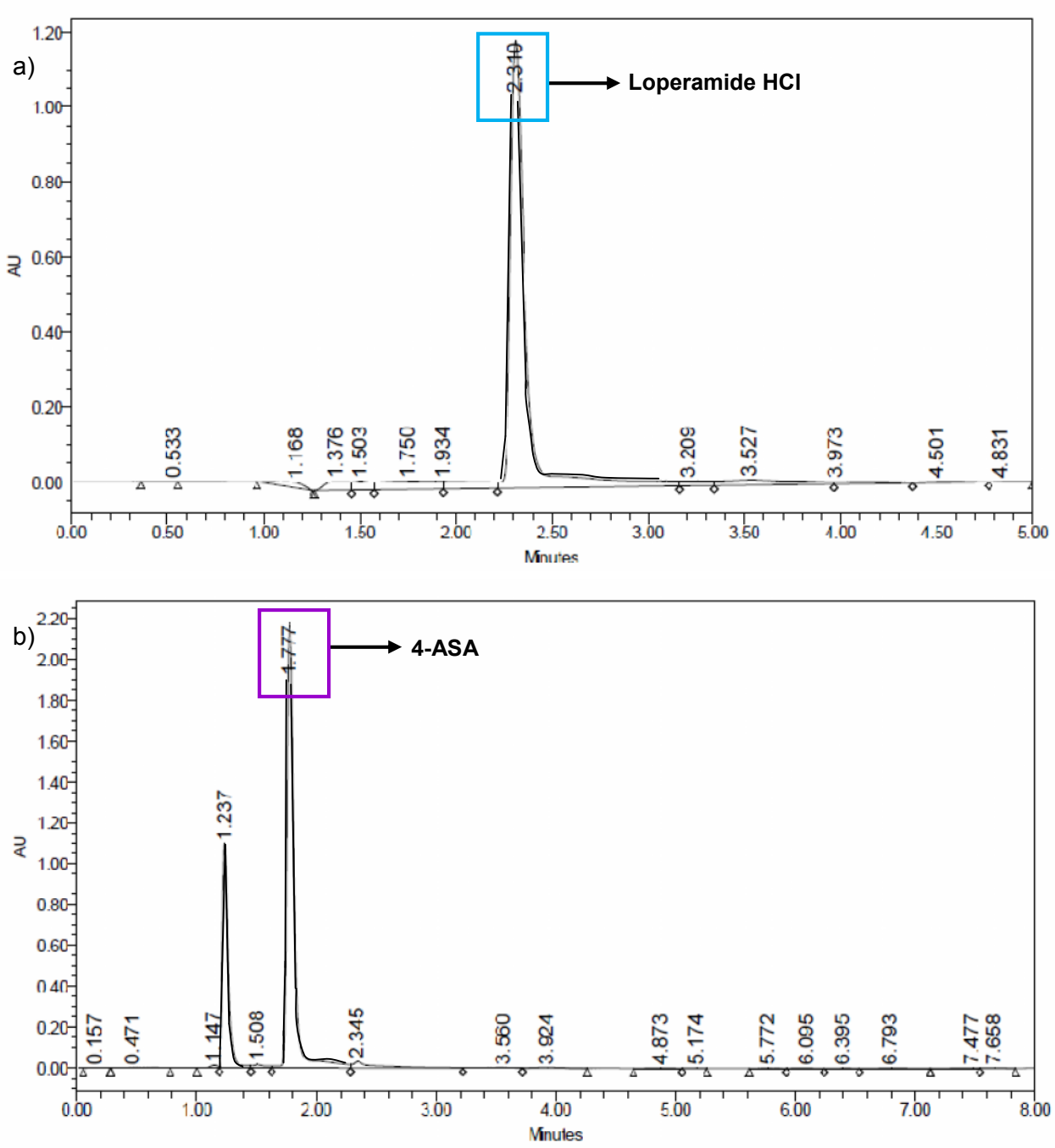



***Figure 7.17:*** *Chromatograms of a) loperamide HCl in plasma at 200nm and b) 4-ASA in plasma at 313nm.*

Due to the ability of the PDA detector to concurrently process chromatograms at 2 different wavelengths, 4-ASA was used as the internal standard even though the maximum excitation wavelength of this molecule was different to that of the drug. Nevertheless 4-ASA also showed an absorbance at 200nm as is evident from the chromatogram of a combination of loperamide HCl and 4-ASA (Figure 7.18). This chromatogram also showed a clear resolution of the separate peaks and a level chromatographic baseline.

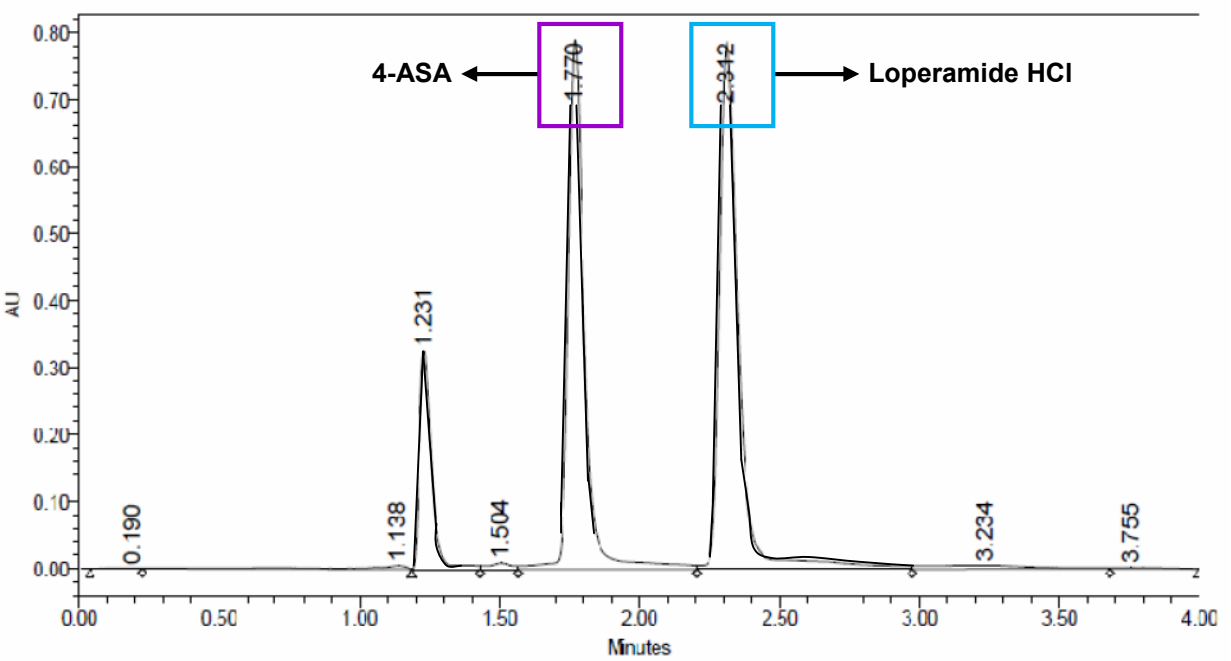



***Figure 7.18:*** *Chromatogram of a spiked plasma sample with a combination of loperamide HCl and 4-ASA analyzed at a wavelength of 200nm.*

### 7.3.3.3 Calibration curve of loperamide HCl

A calibration curve calculated based on the ratio of the AUC of loperamide HCl at 200nm and the AUC of 4-ASA at 313nm showed good linearity with a correlation coefficient ($R^2$) of 0.959 (Figure 7.19). The wavelength of 313nm was preferentially used for 4-ASA as a significantly more resolute peak was obtained comparatively. The sample concentrations analyzed were between 0.189-1.512μg/mL.

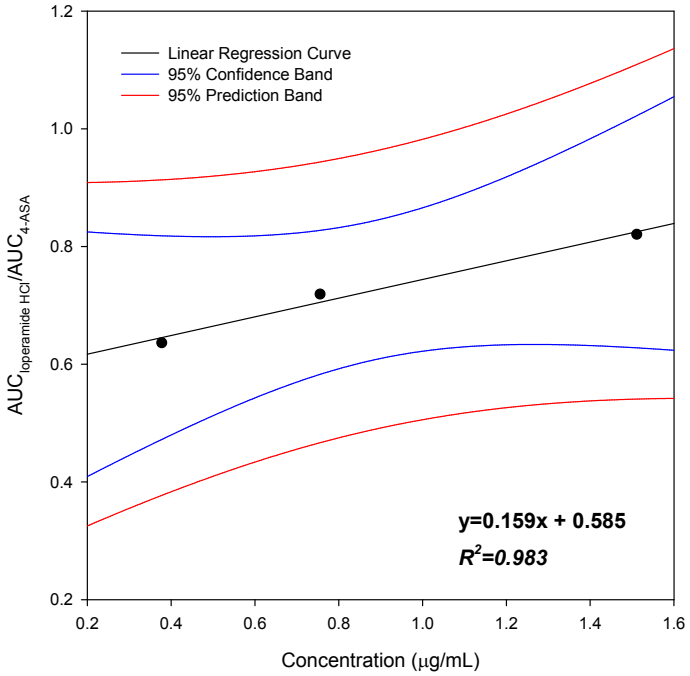



***Figure 7.19:*** *Calibration curve of loperamide HCl in plasma.*

### 7.3.3.4 *In vivo* drug release of loperamide HCl from the SROT and Imodium®

The efficacy of the outer pepsin-responsive intestinally targeted coating can essentially only be recognized based on a quantitative and qualitative comparative analysis of *in vivo* loperamide HCl release from the SROT and Imodium®. Using the x-rays described in section 7.3.2.5 the time of arrival of the SROT into the small intestine can be estimated to be between 1.5-3 hours post-dosing and thus plasma presence of loperamide HCl is essentially required only 90 minutes post-dosing.

The *in vivo* plasma concentration profiles of the SROT and Imodium® shown in Figure 7.20 show that for the first 2 hours post-dosing minimal loperamide HCl is released from the SROT, whereas a concentration of 0.334µg/mL is achieved from Imodium in this same time. Essentially, the results from the plasma samples acquired in the first 2 hours post-dosing provided the fundamental evidence of the efficiency and pepsin-responsiveness of the SROT. The minimization of drug release in the stomach enabled a greater quantity of the contained drug to target the small intestine where its local therapeutic action is seen. Furthermore, the prevention of loperamide HCl release in the stomach minimizes the systemic absorption and consequently the side-effect profile of the drug. A bio-equivalence study conducted in 2006 of 2 different solid-dose formulations of loperamide HCl, showed that a single oral dose of loperamide HCl (4mg) resulted in peak plasma concentrations at approximately 6 hours, and an elimination half-life ($t_{1/2}$) of ±19 hours in humans (McNeil Study Report 15-003, 2006). Thus, similarly a $C_{max}$ of 0.390µg/mL is evidenced 4 hours post-dosing of Imodium® however the SROT achieved a $C_{max}$ of 0.267µg/mL after only 8 hours. The long $t_{1/2}$ of loperamide HCl is responsible for the presence of loperamide HCl in plasma even after the 24 hour study period.

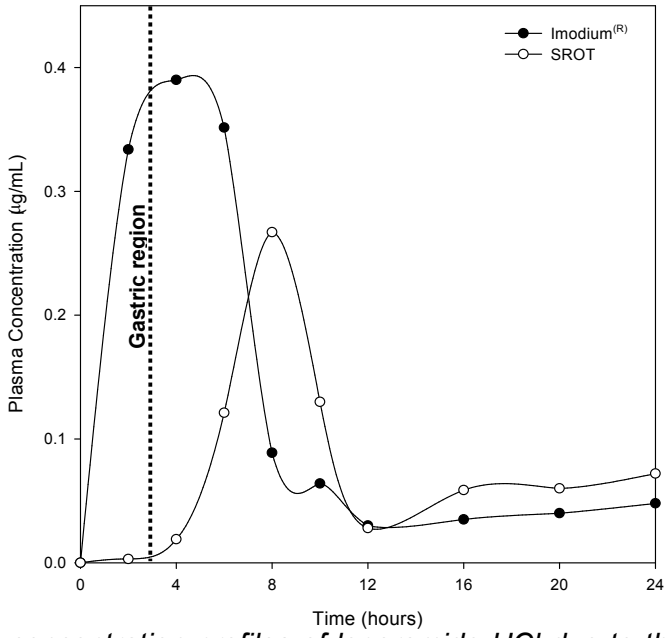



***Figure 7.20:*** *Plasma concentration profiles of loperamide HCl due to the pepsin-responsive coating (SD:0.094-0.386) and Imodium®(SD:0.118-0.470).*

## 7.4 Concluding Remarks

Ultimately, this chapter served to provide evidence of the efficiency of the outer loperamide HCl-loaded coating and 5-ASA-loaded colon-targeted tablet of the SROT to target the small intestine and colon respectively, with the administration of a single delivery system. This simultaneous targeting ability of the 2 different drugs is depicted in Figure 7.21. Apart from the benefits associated with the targeted delivery of the respective drugs, the SROT augmented the therapeutic effect of each drug by providing a more reproducible, consistent and non-fluctuating release behaviour. This ultimately generally results in the reduction of dosage required for therapeutic efficacy, an improvement in pill burden and patient compliance and most importantly an improved quality of life for the patient.

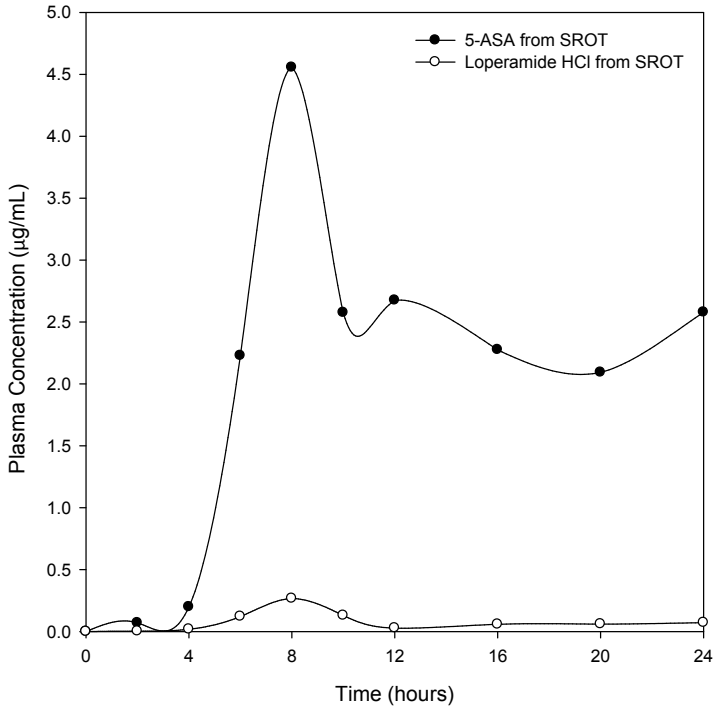



***Figure 7.21:*** *Plasma concentration profiles of loperamide HCl and 5-aminosalicylic acid from a single administration of the stimuli-responsive oral tablet system.*

# CHAPTER 8

# SYNTHESIS OF A NOVEL COMPOSITE POLYACRYLAMIDE-POLYSACCHARIDE HYDROLYZED ELECTROLYTIC MATRIX FOR POTENTIAL APPLICABILITY AS A COLON-TARGETED DRUG DELIVERY SYSTEM

## 8.1 Introduction

As the biomedical and biotechnological sciences make huge strides in advancing drug delivery technology, increased pressure is being placed on the development of novel polymeric materials derived from natural polymers. An effective and simple means of creating these new materials is by a process termed 'blending'. Blends of 'natural-natural' polymers and 'synthetic-synthetic' polymers have been widely investigated. Even though natural polymers may possess superior biocompatibility, their mechanical properties are often unsatisfactory (Yu *et al.*, 2006). On the other hand, synthetic polymers have desirable mechanical properties and only satisfactory biocompatibility. Therefore, blends of natural-synthetic polymers are targeted as a more efficacious means of meeting the needs of modern materials science (Xiao *et al.*, 2001; Coombes *et al.*, 2002; Fares *et al.*, 2003; Zeng *et al.*, 2004; Barnes *et al.*, 2007; Li *et al.*, 2009).

Chemically modified natural polysaccharides have shown diverse potential due to their enhanced hydrophilic properties and their intrinsic polyelectrolyte character (Pedram *et al.*, 2000; Reis *et al.*, 2006; Derkaoui *et al.*, 2008). The latter characteristic provides for the electrostatic interaction between two oppositely charged polyelectrolytes when blended in aqueous solutions to form what is known as polyelectrolyte complexes (PEC's) (Michaels and Miekka, 1961; Lee *et al.*, 1997; Simsek-Ege *et al.*, 2003; Lammertz *et al.*, 2009). These PEC's have been shown to exhibit unique physical and chemical properties due to the considerably stronger electrostatic interactions compared to most other secondary binding interactions (Lee *et al.*, 2003). Oppositely charged polysaccharides are also capable of interacting in the same manner and considering their numerous desirable properties such as their biodegradability, biocompatibility, hydrophilicity and protective properties, increased interest is being placed on them (Barichello *et al.*, 1999; Chellat *et al.*, 2000; Peniche and Argüelles-Monal, 2001; Shchipunov and Postnova, 2009). A variety of polysaccharide-based PEC's have been formulated and evaluated for diverse applications (Macleod *et al.*, 1999a; Qiu *et al.*, 2001; Nichifor *et al.*, 2004; Nagahata *et al.*, 2005; Sarmento *et al.*, 2006; Schoeler *et al.*, 2006; Argin-Soysal *et al.*, 2009; Sæther *et al.*, 2008). Amongst their numerous benefits these complexes are also capable of providing a greater barrier to drug release in the upper

gastrointestinal tract (GIT) than either material alone and thus show increasing promise in colon-targeted drug delivery.

### 8.1.1 The polymers

Polyacrylamide (PAAm) is a water-soluble non-ionic synthetic polymer typically obtained by free radical polymerization of acrylamide in an aqueous medium (Lipp and Kozakiewicz, 1991). It is generally used as a strengthening agent, an erosion-preventing and infiltration-enhancing polymer. It is also commonly applied in gel electrophoresis (Seshi, 1994; Sharma *et al.*, 2005; Byrne and Toscano, 2006; Entry *et al.*, 2008; Yan and Forster, 2009). Transformation of the essentially neutral PAAm molecule into a strong anionic molecule is generally achieved as a consequence of hydrolysis which results in a partially hydrolyzed PAAm (HPAAm) molecule due to the partial conversion of its amide groups to carboxylate groups (Volk and Friedrich, 1980). Therefore when in aqueous solution this polymer presents with a polyelectrolyte character. The fundamental process of HPAAm synthesis is based on a so-called simultaneous polymerization-hydrolysis process whereby the hydrolysis reaction of PAAm is conducted concurrently with the polymerization of the acrylamide (Zeynali and Rabbii, 2002). The most prevalent means of HPAAm formation is by alkaline hydrolysis of PAAm using hydrolyzing agents such as alkali metal carbonates, alkali metal hydroxides, alkali metal sulphates or alkali metal chlorides (Kurenkov *et al.*, 2001).

Chitosan (CHT) is a partially de-acetylated derivative of chitin which possesses unique physicochemical and biological properties such as an enhanced biocompatibility and antimicrobial capability. However apart from these factors, its cationic nature and high charge density in solution along with its mucoadhesive properties has made it one of the most popular natural polymers for use in oral controlled drug delivery technology (Sokker *et al.*, 2009). In addition, the ability of CHT to form non-covalent complexes with other polyelectrolytes (bio-polyelectrolytes, modified natural polyanions or synthetic polyanions) adds to its attractiveness for use in controlled drug delivery (Krayukhina *et al.*, 2008).

Pectin, a naturally occurring anionic polysaccharide has also been greatly explored for its potential in drug delivery technology, especially for targeted colonic delivery (Ashford *et al.*, 1994; Wakerly *et al.*, 1996a, b; Macleod *et al.*, 1999b; Ahrabi *et al.*, 2000; Liu *et al.*, 2003a; Wei *et al.*, 2006; Maestrelli *et al.*, 2008). However, the high hydrophilicity of pectin warrants the need to identify methods of effectively reducing the premature solubility whilst maintaining its polymeric backbone. By polyelectrolyte complexation of this phyto-polysaccharide with CHT, the intrinsic beneficial properties of pectin can be maintained whilst allowing a reduction in the hydrophilicity thus facilitating its use in many controlled release drug delivery systems (Macleod *et al.*, 1999a; Bernabé *et al.*, 2005; Bigucci *et al.*, 2008). In

addition, the susceptibility of polysaccharide polymers to colonic enzymatic degradation may be enhanced by reducing the premature solvation of the relevant polymer in the upper GIT, thus facilitating a more targeted and site-specific delivery of pharmaceutical agents in the distal small intestine and colon.

Accordingly, this chapter focused on the formulation of a lyophilized polyelectrolyte matrix constituting combinations of natural polymers such as pectin and CHT and a partially hydrolyzed synthetic polymer, hydrolyzed polyacrylamide (HPAAm), derived from polyacrylamide. This formulation approach was aimed at the facilitation of a reduction in the premature solvation of the polysaccharide polymer, either CHT or pectin or a combination thereof, in turn reducing upper gastrointestinal release of the model highly water-soluble drug, diphenhydramine HCl (DPH), and providing an intact matrix for entry into the colon.

## 8.2 Materials and Methods

### 8.2.1 Materials

The natural polysaccharide polymers employed included chitosan (CHT) (food grade powder) (Wellable Group Marine Biological & Chemical Co., Ltd., Shishi City, Fujian, China) and pectin (Classic Cu 701) (DE: 34-38%) (Herbstreith & Fox KG, Neuenbürg, Baden-Württemberg, Germany) of molecular weights ($M_w$) 160.9g/mol and 70000g/mol respectively. Polyacrylamide (PAAm) ($M_w$=5×$10^6$-6×$10^6$g/mol), the synthetic non-ionic polymer, was purchased from Fluka Biochemika (St. Louis, MO, USA) and glacial acetic acid was acquired from Rochelle Chemicals (Johannesburg, Gauteng, South Africa). Sodium persulphate (SPS) (SigmaUltra, minimum 98%) as the hydrolyzing agent was purchased from Sigma-Aldrich (St. Louis, MO, USA) and N,N,N',N'-tetramethylethylenediamine (TEMED) (absolute ≥99%, GC) as the accelerant was purchased from Fluka (Buchs, AG, Switzerland). Diphenhydramine HCl (DPH) with a water solubility of 100mg/mL at 25°C was employed as the model drug and was purchased from Sigma-Aldrich-Chemie GmbH (Steinheim, Luxembourg, Germany). All materials employed were of analytical grade and used without further purification.

### 8.2.2 Synthesis of the composite polyelectrolyte matrices

Three formulations consisting of various combinations of the polysaccharide (CHT and/or pectin) and PAAm were synthesized (Table 1). Briefly, a 10%$^w/_v$ aqueous solution of the relevant polysaccharide was blended with a 5%$^w/_v$ solution of PAAm in a 2:1 ($^v/_v$) ratio. All solutions were agitated until a visibly homogenous solution was obtained. DPH was then dissolved into the homogenous blend and was further agitated to facilitate a uniform distribution of the drug. Hydrolization of PAAm was permitted with the addition of SPS, in

conjunction with TEMED as an accelerant in a 4:1 ratio. The reaction was allowed to proceed under constant agitation at 25°C for 3 hours. Matrices consisting of pectin, CHT and HPAAm were prepared in an identical manner as described above and in the same concentrations. However all three polymers were used in equal volumes i.e. in a 1:1:1 ratio. After 3 hours of constant agitation, 1mL aliquots of each polyelectrolyte blend was pipetted into pre-lubricated cylindrical moulds (13mm×5mm) and were subsequently frozen at -70°C for 24 hours prior to lyophilization (FreeZone® 2.5, Labconco®, Kansas City, Missouri, USA) at 25mtorr for 48 hours. Lyophilization of the polyelectrolyte DPH-loaded blends was performed with the aim of removing excess solvent involved in the hydrolysis and complexation process, thus ensuring a more stable and robust formulation of defined dimensions.

***Table 8.1:*** *Composition of each lyophilized polyelectrolyte matrix formulation*

| Formulation | Polymer Type | Concentration ($\%^{w}/_{v}$) | Volume Ratio ($^{v}/_{v}$) | [1]SPS: [2]TEMED ($^{w}/_{v}$) |
|---|---|---|---|---|
| **1** | CHT:HPAAm | 10:5 | 2:1 | 4:1 |
| **2** | pectin:HPAAm | 10:5 | 2:1 | 4:1 |
| **3** | CHT:pectin:HPAAm | 10:10:5 | 1:1:1 | 4:1 |

[1]*Sodium persulphate (hydrolyzing agent)*
[2]*N,N,N',N'-Tetramethylethylenediamine (accelerant)*

### 8.2.3 Elucidation of the successful synthesis of the polyelectrolyte matrices

Fourier Transmission Infrared Spectroscopy (FTIR) was performed on all native polymers involved in matrix formulation as well as on the lyophilized matrices as a means of validating the successful synthesis of the polyelectrolyte complex between HPAAm and the relevant polysaccharides. The method of FTIR analysis is described in Chapter 5, section 5.2.10.

### 8.2.4 Chemometric molecular mechanics for polyelectrolyte modelling

All modeling and computations, including energy minimizations in Molecular Mechanics, were performed using HyperChem™ 8.0.8 Molecular Modeling Software (Hypercube Inc., Gainesville, FL, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK). The decamer of acrylamide (polyacrylamide) was drawn using ChemBio3D Ultra in its syndiotactic stereochemistry as a 3D model, whereas the structures of CHT (10 glucosamine saccharide units) and pectin (10 galactopyranosyl uronic acid units) were built from standard bond lengths and angles using the polysaccharide Builder Module on HyperChem 8.0.8. The models were initially energy-minimized using MM+ Force Field and the resulting structures were once again energy-minimized using the Amber 3 (Assisted Model Building and Energy Refinements) Force Field. The conformer having the lowest energy was used to create the polymer-polymer complex. A complex of one polymer molecule with another was assembled by parallel disposition of the molecules and an identical procedure of energy-minimization was repeated to generate the final models: CHT-HPAAm, pectin-HPAAm and pectin-CHT-

HPAAm. Full geometrical optimization was performed in vacuum employing the Polak–Ribiere Conjugate Gradient method until a Root Mean Square (RSM) gradient of 0.001kcal/mol was reached. Force Field options in the AMBER (with all hydrogen atoms explicitly included) and MM+ (extended to incorporate non-bonded limits, restraints, and periodic boundary conditions) methods were set as defaults. For Molecular Mechanics computations, the Force Fields were utilized with a distance-dependent dielectric constant scaled by a factor of 1. The 1-4 scale factors were electrostatic 0.5 and Van der Waals 0.5. Furthermore, various energies and hydrogen bond lengths involved in the molecular interactions between CHT-HPAAm, pectin-HPAAm and CHT-pectin-HPAAm were computed.

### 8.2.5 Determination of the influence of the polymer blends on the physicomechanical properties of the polyelectrolyte matrices

The physicomechanical properties of the matrices were evaluated in terms of their Matrix Resilience (MR), Matrix Hardness (MH) and Deformation Energy (DE). A calibrated Texture Analyzer (TA.XT*plus*, Stable Microsystems, Surrey, UK) fitted with a cylindrical steel probe (50mm diameter; for MR) and a flat-tipped steel probe (2mm diameter; for MH and DE) was employed. The parameters employed for the analysis is outlined in Table 8.2. All studies (N=3) were conducted at room temperature (25°C).

***Table 8.2:*** *Textural profiling parameter settings employed for physicochemical characterization of the polyelectrolyte matrices*

| **Parameters** | **[1]MR (%)** | **[2]MH (N/mm$^2$)** | **[3]DE (J)** |
|---|---|---|---|
| Pre-test speed | 1mm/sec | 1mm/sec | 1mm/sec |
| Test speed | 0.5mm/sec | 0.5mm/sec | 0.5mm/sec |
| Post-test speed | 10mm/sec | 10mm/sec | 10mm/sec |
| Trigger type | Auto | Auto | Auto |
| Trigger force | 0.05N | 0.05N | 0.05N |
| Load cell | 5kg | 5kg | 5kg |
| Compression strain | Variable | N/A | N/A |
| Target mode | Strain (40%) | Distance | Distance |

[1]*Matrix Resilience*
[2]*Matrix Hardness*
[3]*Deformation Energy*

MR (%) was calculated by the percentage of the ratio between the area under the curve (AUC) of the peak to baseline (after the force is removed) ($AUC_{2-3}$) and the baseline to peak (before the force is removed) ($AUC_{1-2}$) from a Force-Time profile (Figure 8.1a). MH (N/mm$^2$) and DE (J) were both determined based on Force-Distance profiles, in particular, MH was elucidated from the gradient between the initial force and the maximum force attained, and DE from the AUC (Figure 8.1b).

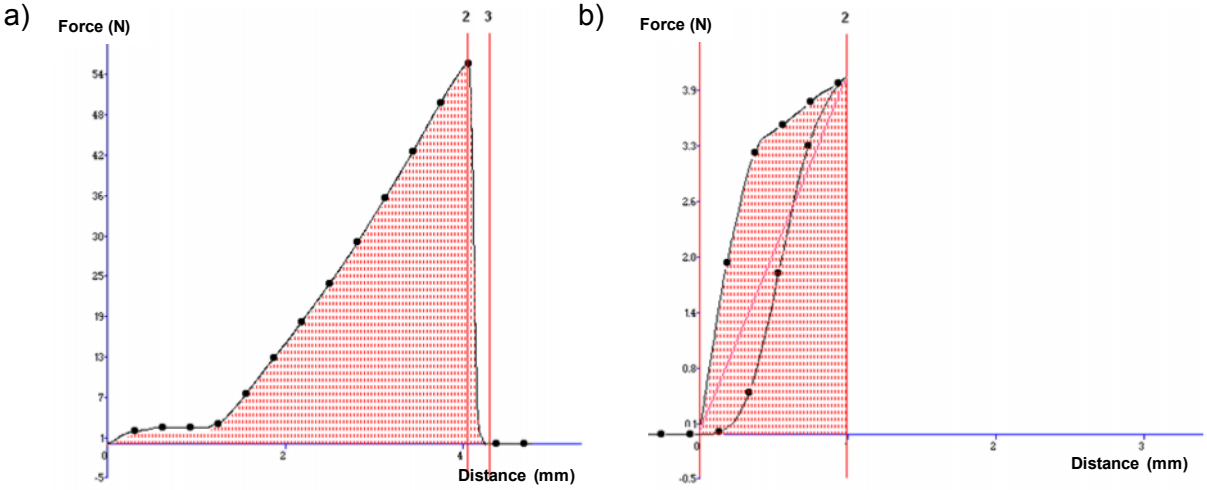



***Figure 8.1:*** *Typical Force-Time and Force-Distance profiles of the polyelectrolyte matrices for determining a) Matrix Resilience, and b) Matrix Hardness and Deformation Energy.*

### 8.2.6 Elucidation of the influence of the polymer blends on the porositometric characteristics of the polyelectrolyte matrices

Surface area and porosity analysis was performed on all polyelectrolyte matrices using a Porositometric Analyzer (Micromeritics ASAP 2020, Norcross, GA, USA). Samples were initially degassed to aid the removal of surface moisture and contaminants prior to analysis. Sample preparation briefly involved the weighing of each matrix (13mm×5mm) with subsequent division of the matrices into quadrants capable of passing through the neck of the sample tube (I.D=9.53mm). A glass filler-rod was then inserted into the sample tube to reduce the total free space volume within the tube facilitating a reduction in the time required for complete degassing to occur. Samples were completely degassed after a period of between 7-9 hours, the process of which encompassed an evacuation and heating phase. The respective parameter settings are shown in Table 8.3.

***Table 8.3:*** *Evacuation and heating phase parameters employed for porositometric analysis*

| Parameter | Rate/Target |
|---|---|
| **Evacuation phase** | |
| Temperature ramp rate | 10°C/min |
| Target temperature | 40°C |
| Evacuation rate | 50.0mmHg/s |
| Unrestricted evacuation from | 30mmHg |
| Vacuum set point | 500µmHg |
| Evacuation time | 60 min |
| **Heating phase** | |
| Temperature ramp rate | 10°C/min |
| Hold temperature | 30°C |
| Hold time | 900 min |

Subsequent to complete degassing, the sample tube was transferred to the analysis port where data such as surface area, pore volume and pore size was obtained in accordance

with Barrett, Joyner and Halenda (BJH) and Brunauer-Emmett-Teller BET computations. The most widely used standard procedure for the determination of surface area of porous materials is based on the BET gas adsorption method which occurs in a two-stage process (Sing *et al.*, 1985). Firstly, the BET equation was used in the linear form to determine the monolayer capacity based on Equation 8.1.

$$\frac{p}{n^a(p^0 - p)} = \frac{1}{n^a_m \times C} + \frac{(C-1)p}{n^a_m \times Cp^0} \qquad \text{Equation 8.1}$$

*Where, $n^a$ refers to the quantity of $N_2$ adsorbed at the relative pressure $P/P_0$, $n^a_m$ was the monolayer capacity and C was exponentially related to the enthalpy of adsorption in the first adsorbed layer.*

The surface area was then determined from the monolayer capacity using Equations 8.2 and 8.3 in the determination of the total and specific surface areas. This, however, required data regarding the molecular cross-sectional area $a^m$ occupied by the adsorbate in the complete monolayer.

$$A_s(BET) = n^a_m \times L \times a_m \qquad \text{Equation 8.2}$$

$$a_s(BET) = A_s(BET)/m \qquad \text{Equation 8.3}$$

*Where $A_s(BET)$ and $a_s(BET)$ are the total and specific surface areas, respectively, of the adsorbent (of mass m) and L is the Avogadro constant.*

### 8.2.7 Surface morphological analysis of the polyelectrolyte matrices in relation to the isothermic porositometry data

The surface morphology of the polyelectrolyte matrices were characterized by Scanning Electron Microscopy (SEM) (JSM-840 Scanning Electron Microscope, JEOL 840, Tokyo, Japan), at an accelerating voltage of 19kV. This was performed to assess the surface characteristics of the matrices in relation to the nature and blend of polymers employed. Matrix samples were mounted on aluminum stubbs and sputter-coated with a thin layer of gold-palladium. Samples were then stored in a desiccator until analysis to avoid absorption of moisture onto the lyophilized matrices. Photomicrographs were captured at magnifications of x1400 and x2300.

### 8.2.8 Construction of calibration curves of DPH

The construction of the calibration curves for DPH in simulated gastric fluid (SGF) (pH 1.2; 37°C) and simulated intestinal fluid (SIF) (pH 6.8; 37°C) was performed as described in Chapter 3, section 3.2.6.

### 8.2.9 Determination of the drug entrapment efficiency within the polyelectrolyte matrix

With the aim of ascertaining the quantity of DPH entrapped within each matrix, individual matrices (N=3) were finely triturated and subsequently dissolved in 100mL of SGF (pH 1.2; 37°C). After complete dissolution, the solutions were filtered through a hydrophilic 0.45µm Millipore membrane filter (Millipore® Millex-HV) (Millipore Corporation, Billerica, MA, USA) and analyzed spectroscopically (Lambda 25, UV/VIS Spectrometer, PerkinElmer®, Waltham, MA, USA) at a wavelength of 254nm. The absorbances attained were then fitted to relevant calibration curves ($R^2$=0.996) for the calculation of DPH content per matrix.

### 8.2.10 *In vitro* drug release characteristics of the polyelectrolyte matrices as a function of the polymer blends employed

*In vitro* drug release studies were performed in a USP Bio-Dis® Apparatus (Caleva RRT 8) (Caleva Ltd, Sturminster Newton, Dorset, England) based on identical parameters and sample withdrawal processes as described in Chapter 3, section 3.4.7, except the pH differential employed included test media of: SGF (pH 1.2; 37°C) ($R^2$=0.996) for 2 hours and SIF) (pH 6.8; 37°C) for the subsequent 4 hours to complete the 6-hour study period. Samples were removed at predetermined time-points and analyzed by UV spectroscopy (Lambda 25, UV/VIS Spectrometer, PerkinElmer®, Waltham, MA, USA) at a wavelength maximum of 254nm (N=3). The fractional drug release achieved at each time point was then quantified based on the calibration curves constructed in the relevant simulated fluids.

## 8.3 Results and Discussion

### 8.3.1 Synthesis validation of the polyelectrolyte matrices

Band shifts, changes in the peak intensity and peak broadening revealed by FTIR spectra of the various polymer blends in relation to the native polymers provided evidence that these modifications were specifically derived from chemical interactions and/or electrostatic interactions due to synthesis of the polyelectrolyte matrices. From the FTIR spectra of CHT the presence of amine groups was substantiated by a broad band from 3360-3340$cm^{-1}$ signifying $NH_2$ stretching. The peak at 2870$cm^{-1}$ and 1587$cm^{-1}$ was assigned to the C-H stretching and $NH_2$ deformation respectively. The native PAAm was dominated by primary amide groups (-$CONH_2$) that essentially resulted in spectra indicating $NH_2$ stretching (3400-3150$cm^{-1}$) and C=O stretching (1680-1660$cm^{-1}$). Without the application of hydrolyzing

agents, PAAm remained resistant to hydrolysis in aqueous solutions maintained at room temperature (Veitser and Mints, 1984). However with the inclusion of SPS to the polymeric blend partial alkaline hydrolysis of PAAm occurred. This resulted in the partial conversion of the amide groups to randomly distributed polar carboxyl groups carrying negative charges that rendered PAAm as a strong anionic polyelectrolyte. Furthermore, due to the employment of acetic acid as a solvent, the $-NH_2$ groups on the CHT molecule were converted to $-NH_3^+$ groups, resulting in the cationic nature of the polymer solution. Thus a combination of these polymers results in an electrostatic attraction between the relevant oppositely charged polyions to form a polyelectrolyte complex (PEC). The proposed reaction mechanism is illustrated in Figure 8.2.

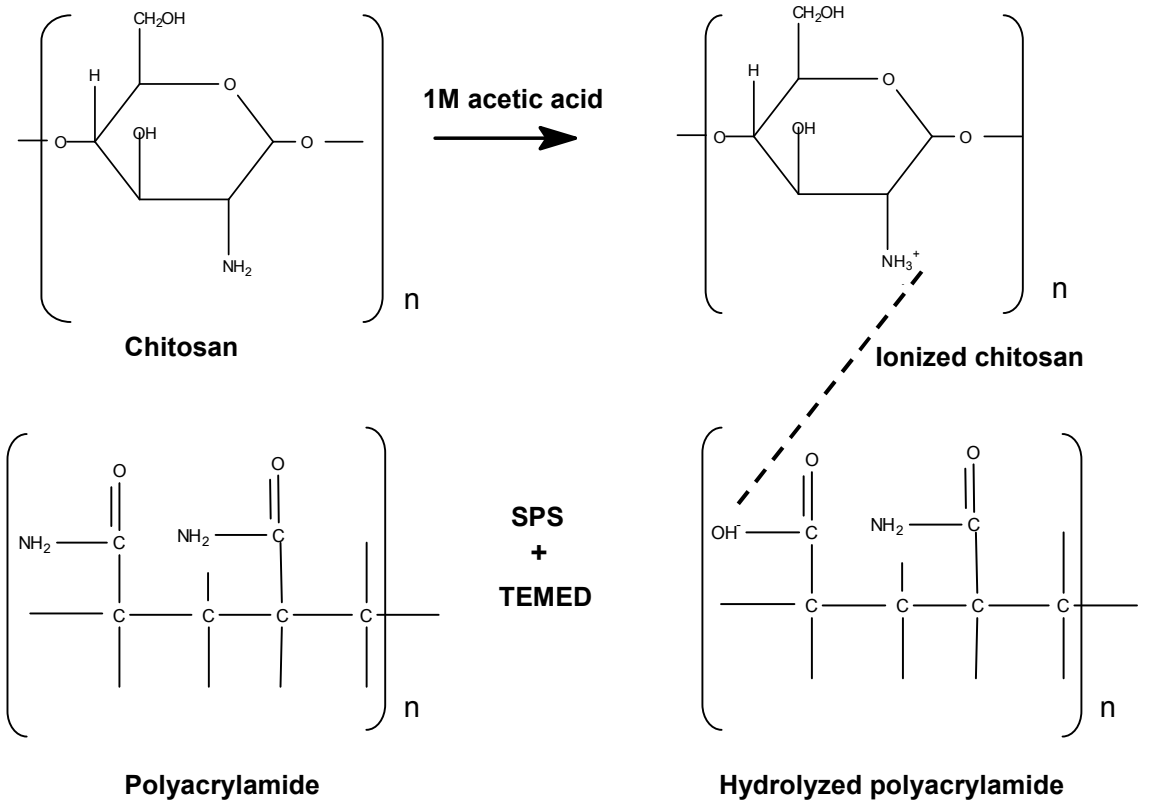



***Figure 8.2:*** *Proposed reaction mechanism between chitosan and polyacrylamide subsequent to hydrolysis with a) depicting the ionization of chitosan in a dilute acetic acid solution, and b) depicting the hydrolysis of polyacrylamide due to sodium persulphate.*

The corresponding FTIR spectra of these matrices revealed unique distinctive peaks between 1425-1390$cm^{-1}$ indicating the presence of $-COO^-$ symmetrical stretching and a distinctive peak at 1535$cm^{-1}$ signifying $-NH_3^+$ deformation. These peaks essentially form the necessary validation for the formation of a PEC between CHT and HPAAm. However there was still evidence of the presence of remaining amide groups from the non-hydrolyzed amide groups of HPAAm.

According to Liu and co-workers (2003b), typically at low ionization, polyacids function as hydrogen donors and can thus form intermolecular hydrogen bonds with the non-ionic PAAm, which is a proton acceptor, to form an intermolecular complex. In addition, the intermolecular interaction between a polyacid and PAAm is also dependent on the degree of ionization of the polyacid as well as the structural compatibility between the two polymers. Since PAAm was only partially hydrolyzed the numerous remaining $–NH_2$ groups present on the polymer backbone provided the basis for the electrostatic interaction with the $–COO^-$ groups of pectin in this study. The FTIR spectrum of these matrices revealed a predominant broad band between $2500cm^{-1}$ and $3500cm^{-1}$ indicative of H-bonded OH stretching due to the free carboxylic groups of the HPAAm. However, the characteristic feature of these matrices was the disappearance of the peak at $1590cm^{-1}$ which signified $NH_2$ deformation of the primary amine groups. Reactions between anionic and cationic macromolecules in aqueous solutions lead to the formation of inter-polymeric complexes and since pectin and CHT are electrostatically complementary macromolecules, it is theorized that an electrostatic interaction between the positively charged amino groups at C-2 of the CHT pyranose ring and negatively charged carboxyl groups at C-5 of the pectin pyranose ring may have led to the formation of a PEC (Rashidova *et al.*, 2004; Tripathi *et al.*, 2010).

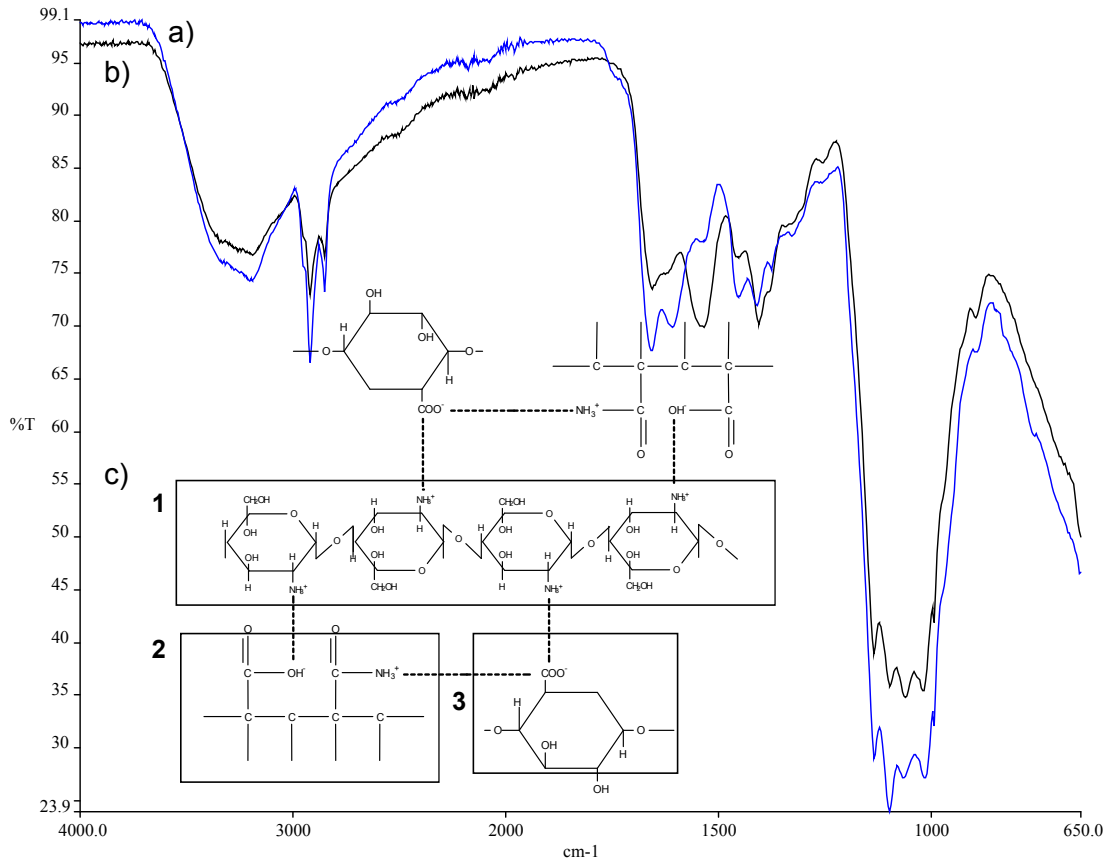



***Figure 8.3:*** *FTIR spectra of a) CHT-HPAAm matrices, and b) CHT-pectin-HPAAm with inset c) possible chemical structure of the electrostatic interaction between CHT, pectin and HPAAm with 1) indicative of the chitosan polymeric chain, 2) partially hydrolyzed polyacrylamide, and 3) pectin.*

Based on FTIR spectra of CHT-pectin-HPAAm matrices relative to the spectra of CHT-HPAAm matrices it was found that the spectra were identical except for the disappearance of the peak at 1535cm$^{-1}$ in the CHT-pectin-HPAAm matrices. As described previously, this peak was indicative of $NH_3^+$ deformation. Thus the existence of this peak in CHT-HPAAm matrices indicated a surplus of $NH_3^+$ sites due to the partial hydrolization of PAAm and thus an insufficient number of $COO^-$ sites available for 'total' electrostatic interaction. However, the inclusion of pectin as part of the blend provided additional $COO^-$ anionic sites that facilitated the electrostatic interaction between the $NH_3^+$ ions of CHT and the $COO^-$ ions of pectin. In addition to these electrostatic interactions, a further interaction was observed between the remaining $–NH_2$ groups of HPAAm and the $–COO^-$ groups of pectin (Figure 8.3).

### 8.3.2 Mechanistic elucidation of polyelectrolyte formation employing chemometric molecular mechanics modelling

In order to further support the synthesis validation and the FTIR results obtained, Molecular Mechanics simulations was undertaken in the form of energy minimizations to demonstrate electro- and structure-selective binding of saccharidic moieties, CHT and pectin, to partially hydrolyzed PAAm. Molecular Mechanics described the energy of the molecules in terms of a simplified function that accounted for distortion from ideal bond distances and angles, as well as for non-bonded Van der Waals and Coulombic interactions. The molecular tectonics of PECs in this study was found to be affected by various types of attractive interactions such as Van der Waals contacts, H-bonds and electrostatic interactions. Table 8.4 and Figure 8.4 show the results of Molecular Mechanics computations performed in vacuum. The final conformation models of molecular networks were generated by the Molecular Mechanics computations for formable complex structures in relation to the cooperative ion-pair binding between the carboxyl (-COO) and the protonated amine ($-NH_3^+$) groups.

The strong binding affinity in CHT-HPAAm was due to the electrostatic interaction caused by the $–NH_3^+$ and –COO ions that proceed in accordance with the short-range Van der Waal attractions and secondary interactions such as H-bonding. In contrast, -COO ions of pectin interacted with the $–NH_3^+$ of HPAAm. Although the pectin-HPAAm complex was electrostatically more stable than CHT-HPAAm, the $E_{elec\ (CHT\text{-}HPAAm\ -\ CHT)}$ and $E_{elec\ (pectin\text{-}HPAAm\ -\ pectin)}$ were computed to be 5kcal/mol and 1kcal/mol, respectively indicating that the electrostatic interactions in pectin-HPAAm complexes were weaker than the interactions in the CHT-HPAAm complexes. Hence, HPAAm was playing a dual role as an anionic electrolyte in the presence of CHT and a cationic polysaccharide in the vicinity of pectin. Interestingly, the Van der Waals energy was more stabilized in the case of pectin-HPAAm (decreased by 58kcal/mol) than in CHT-HPAAm (44kcal/mol) which demonstrated the

significance of a structural backbone fit between the host and guest molecule. In addition, pectin-HPAAm was more stabilized by H-bonds as shown in Table 8.4 and Figure 8.4a-c.

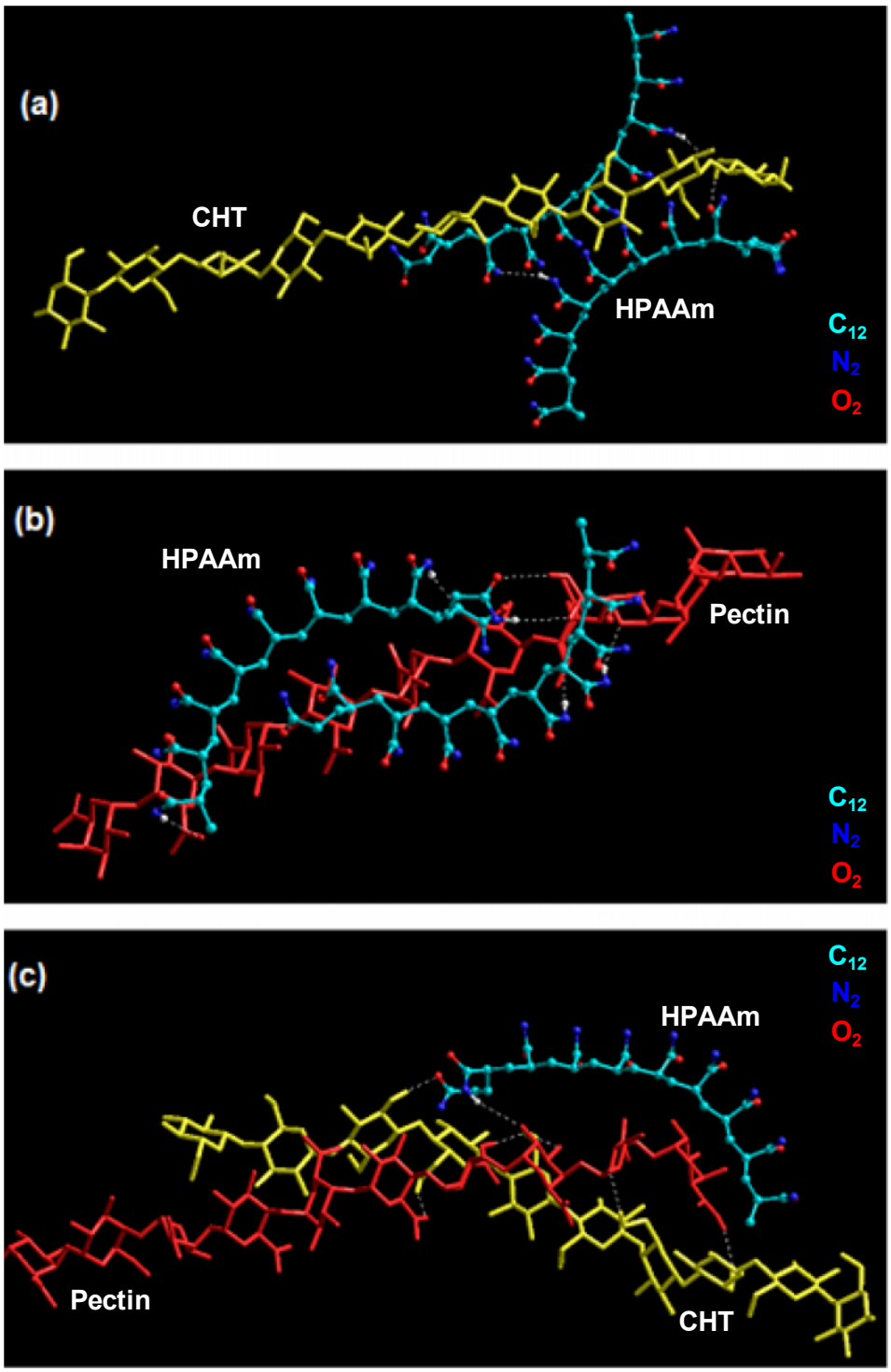



***Figure 8.4:*** *Energy minimized geometrical preferences of the polyelectrolyte complexes derived from molecular mechanics calculations: a) CHT-HPAAm, b) pectin-HPAAm and c) CHT-pectin-HPAAm complex. The HPAAm color codes for elements $C_{12}$, $N_2$ and $O_2$ are also shown.*

Finally, these complex interactions were also explored through the CHT-pectin-HPAAm complex and observed the formation of a PEC represented by a novel tri-polymeric ionic-

quadrilateral (TPIQ) consisting of $–NH_3^+$ ions of CHT, $-COO^-$ ions of pectin and $–NH_3^+$ and $-COO^-$ ions of HPAAm as shown in the FTIR spectra of Figure 8.3. Figure 8.4c clearly demonstrates the polyelectrolyte selection of the pectin-CHT, pectin-HPAAm and CHT-HPAAm polymers. Figure 8.4c also depicts the energy minimized structures of CHT-pectin-HPAAm where due to the flexibility of the polymer chain, the relevant segments of polymers orientated their configuration to form a remarkable structure fit between the $–NH_3^+$ and –COO ions, the electrostatic, Van der Waals and H-bond interactions being pseudo-optimized.

Comparison of the complexes revealed that the CHT-pectin-HPAAm complex was superiorly stable in total energy, Van der Waals, H-bond and electrostatic interactions by 92, 17, 0.88 and 136kcal/mol respectively in comparison to CHT-HPAAm and 45, 20, 1.38 and 70kcal/mol respectively in comparison to pectin-HPAAm. The difference in the electrostatic interactions of the TPIQ and the biopolymer complexes of approximately 136 and 70kcal/mol suggested that the complex was stabilized mainly by Coulombic attractions (Table 8.4) and supported by the Van der Waals forces with a difference of 17 and 20kcal/mol, respectively. Although their H-bond lengths ranged from 2.1579-3.1772A˚ (Figure 8.5) and may be considered as H-bonds of medium strength, it is likely that these interactions may be significant for the stability of the TPIQ. Therefore, the highly stable structure of TPIQ was due to the formation of a pectin-CHT PEC in the vicinity of HPAAm which again demonstrated the significance of a structural backbone fit due to the superior fit between the location of the positive charges and the position of the negatively charged groups.

***Table 8.4:*** *Computed energy parameters (kcal/mol) of the polyelectrolyte complexes formed between chitosan, hydrolyzed polyacrylamide and pectin*

| Structure | Energy (kcal/mol) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Total** | **Bond** | **Angle** | **Dihedral** | **VDW** | **H bond** | **Elec** |
| **CHT** | 35.556 | 3.1201 | 18.035 | 25.7749 | 13.3231 | 0 | -24.6973 |
| **Pectin** | -7.5603 | 5.0315 | 24.4993 | 28.571 | 29.0303 | 0 | -94.6923 |
| **HPAAm** | 10.3568 | 1.4247 | 5.46554 | 8.5739 | -5.0725 | -0.0349 | 0 |
| **CHT-HPAAm** | 13.1292 | 5.8508 | 27.9893 | 42.3994 | -31.8998 | -0.576 | -29.7597 |
| **Pectin-HPAAm** | -34.2502 | 7.5611 | 36.3226 | 46.28 | -28.8202 | -0.0748 | -95.5188 |
| **CHT-pectin-HPAAm** | -79.0868 | 10.868 | 57.0293 | 68.2813 | -48.8639 | -1.4509 | -165.825 |

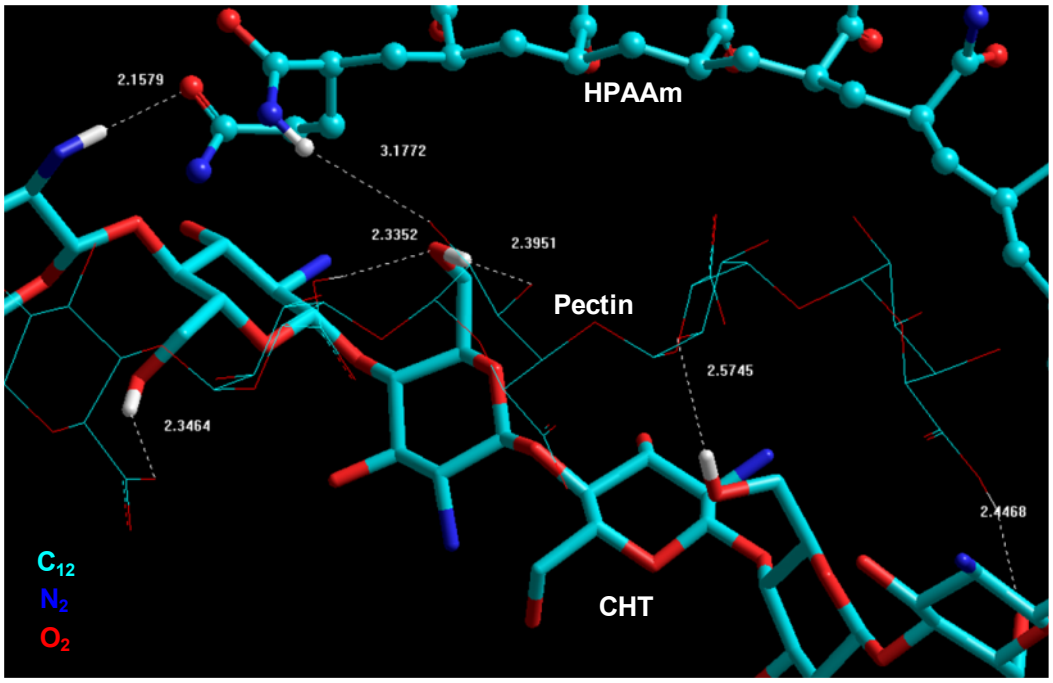



***Figure 8.5:*** *H-bond lengths (Å) involved in the pectin-CHT-HPAAm complex as computed via AMBER Force Field. Color codes for the elements $C_{12}$, $N_2$ and $O_2$.*

### 8.3.3 Physicomechanical analysis of the polyelectrolyte matrices

Matrix resilience (MR) was employed as a measure of the cohesiveness of the polyelectrolyte matrices and referred to the ability of the matrices to recover to their original dimensions after a compressive stress was applied by the textural probe (Pillay and Fassihi, 1999b). It was determined that at a consistent strain of 40%, the CHT-HPAAm matrices proved to be the most resilient at 6.1±0.03%, compared to pectin-HPAAm matrices with a resilience of 5.3±0.02% (Figure 8.6). According to Platé (1976), on forming a complex the reacting chains of components of a PEC lose their flexibility and intermolecular mobility. Considering that CHT-pectin-HPAAm matrices had additional electrostatic interactions between CHT-pectin and pectin-HPAAm, along with the interaction between CHT and HPAAm, it was thus warranted that these matrices would have the least resilient nature.

The energy required to overcome the adhesive and cohesive forces within the matrices is regarded as the Deformation Energy. As expected the energy dissipated in the causation of this effect was markedly lower for CHT-pectin-HPAAm matrices compared to the other variants. Furthermore, the Matrix Hardness of CHT-pectin-HPAAm matrices was lower than pectin-HPAAm matrices and CHT-HPAAm matrices (Figure 8.6).

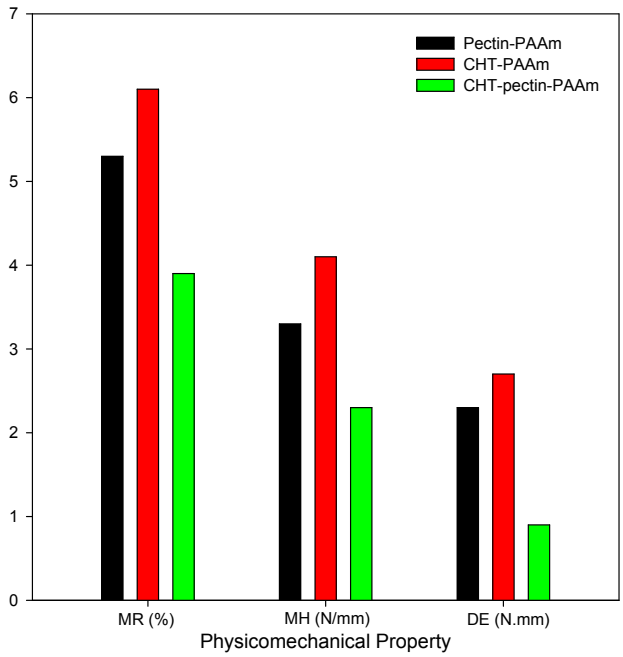



***Figure 8.6:*** *Physicomechanical properties of the polyelectrolyte matrices produced from different polymer blends.*

### 8.3.4 Influence of the polymer blend on the porositometric properties of the polyelectrolyte matrices

The technique of physical gas adsorption was used for assessing the pore characteristics of the various polyelectrolyte matrices as a direct measure of its porous properties and structure (Groen *et al.*, 2003). When applied over a wide range of relative pressures ($P/P_0$), $N_2$ adsorption isotherms provided critical data on the pore size distributions in terms of their micro-, meso-, and macropore ranges (±0.5-200nm). The BJH model of determining pore volume and pore size distribution (PSD) in the mesopore and macropore range was based on the Kelvin equation and corrected for multi-layer adsorption (Barret *et al.*, 1951). Several assumptions were made with the BJH pore size distribution computation, namely pores were rigid and of a well-defined shape, the distribution was confined to the mesopore range and filling/emptying of pores did not depend on the location. It was generally considered that matrices that were purely mesoporous and comprised of non-intersecting mesopores of cylindrical geometry and similar size exhibited Type IV isotherms with a Type H1 hysteresis loop (Groen *et al.*, 2003). However, due to the random distribution of pores and an interconnected pore system of the matrices, the hysteresis loop was of Type H2 or H3. Figure 8.7 provides an illustration of typical isotherms and hysteresis models employed.

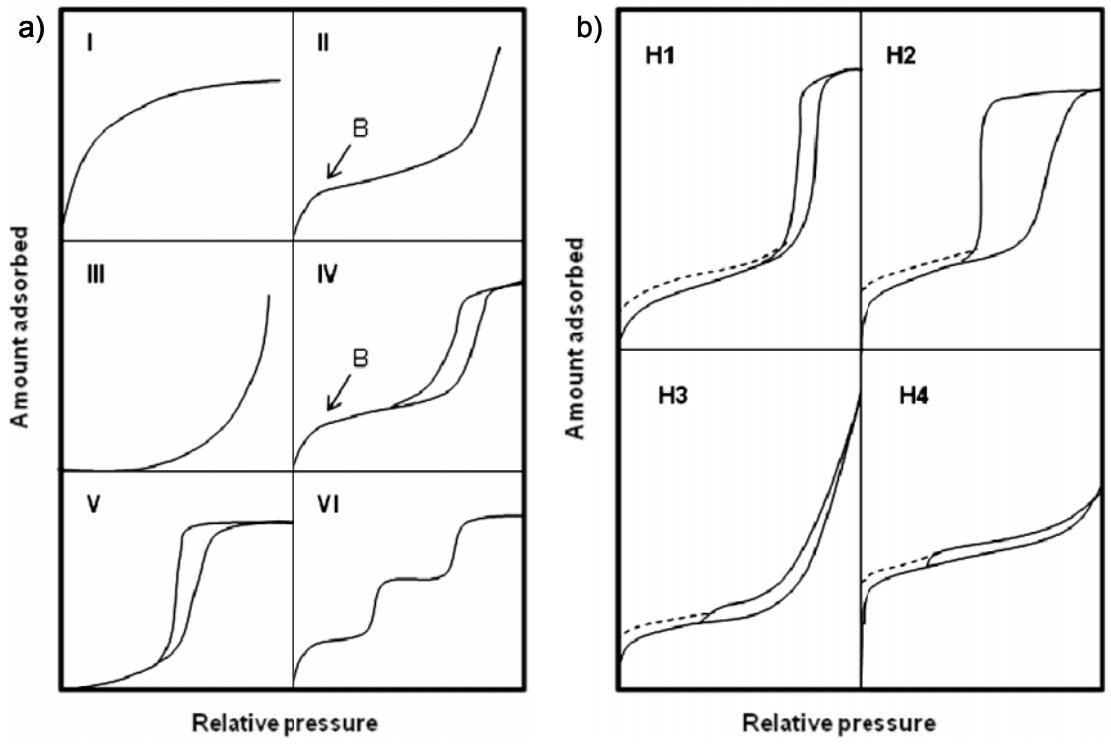



***Figure 8.7:*** *Schematics depicting a) types of isotherms and b) types of hysteresis loops according to the IUPAC classification system (adapted from Sing et al., 1985).*

The isotherm of CHT-pectin-HPAAm matrices (Figure 8.8) was typical of a Type IV isotherm accompanied by a H3 hysteresis. The characteristic hysteresis loop was associated with capillary condensation that took place in mesopores. This was supported by data obtained where the pore sizes of the matrices were found to be 22.46nm according to the desorption curve of the isotherm. Furthermore, according to the isotherm a forced closure of the hysteresis occurred at $P/P_0>0.45$ which was due to a sudden drop in the volume of $N_2$ adsorbed along the desorption curve. This phenomenon was referred to as the Tensile Strength Effect (TSE). The isotherm increased rapidly near $P/P_0=1$ that was indicative of the presence of macropores with a significant vertical rise signifying the large diameters of the macropores.

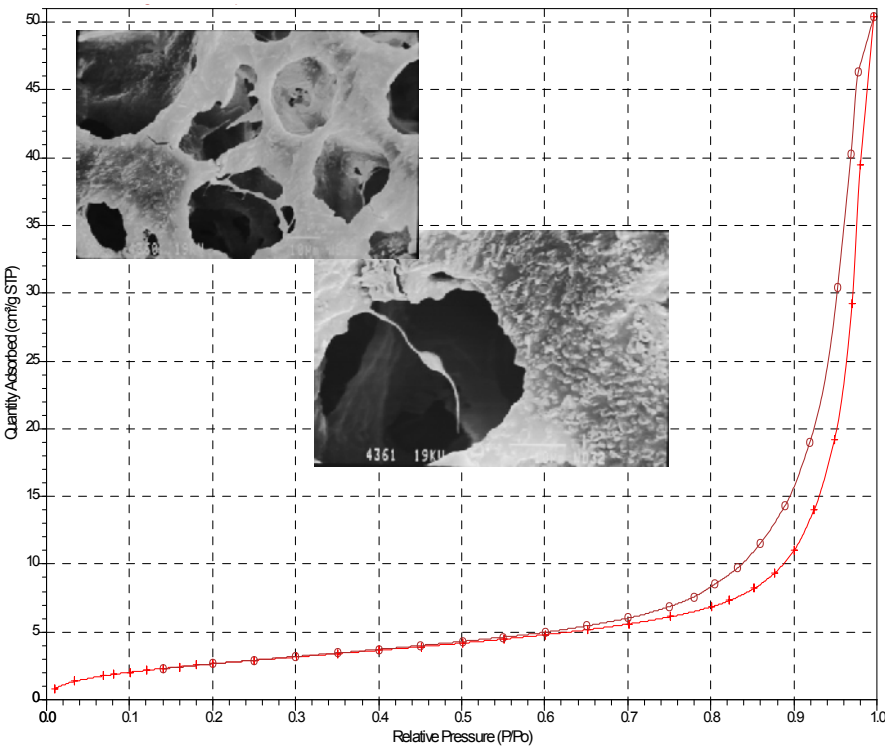



***Figure 8.8:*** *A linear isothermic plot of CHT-pectin-HPAAm matrices with insets of SEM micrograph images at magnifications of x1400 and x2300.*

In contrast, pectin-HPAAm matrices exhibited an isotherm not characteristic of any isothermic types according to the IUPAC classification system. However, certain regions of the isotherm were similar to that of a Type IV isotherm accompanied with a H4 hysteresis loop (Figure 8.9). According to the data obtained pectin-HPAAm matrices had a pore size of 5.15nm based on the desorption isotherm. This was within the mesopore range and explained the presence of the H4 hysteresis of the isotherm. Furthermore, the presence of a low pressure hysteresis ($P/P_0$<0.45) suggested that micropores were also present in the matrices. However, Groen and co-workers (2003) described that a high degree of mesoporosity may lead to a higher mesopore surface area, and this can in turn significantly affect the low-pressure region (micropore range) of the isotherm. This explanation is validated by the elevated surface area of the matrices despite the smaller pore sizes compared to the CHT-pectin-HPAAm and CHT-HPAAm matrices (Table 8.5).

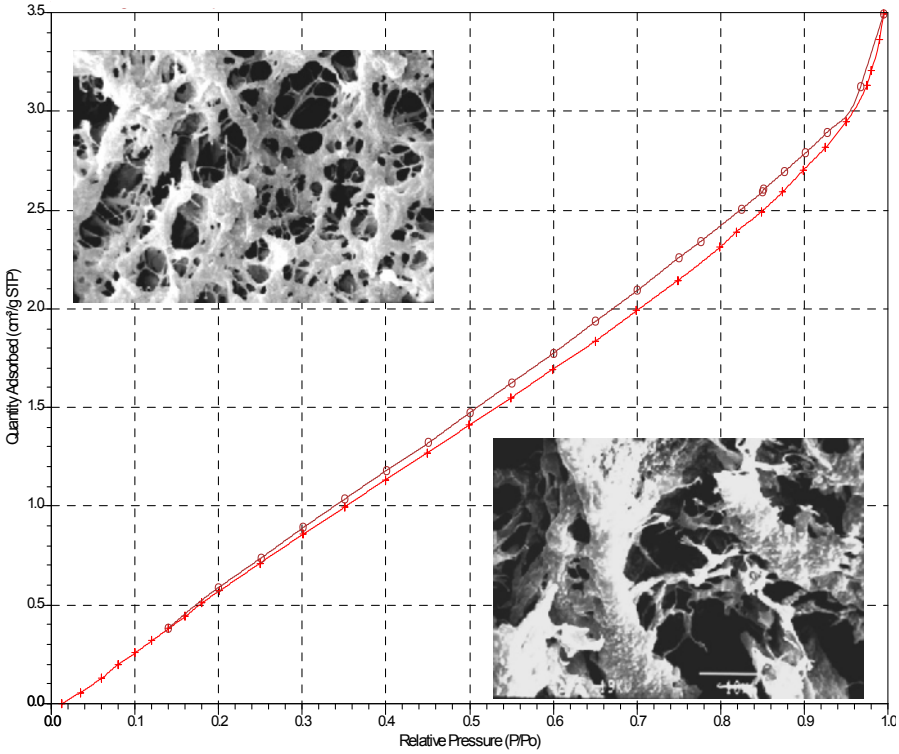



***Figure 8.9:*** *A linear isothermic plot of pectin-HPAAm matrices with insets of SEM micrograph images at magnifications of x1400 and x2300.*

CHT-HPAAm matrices exhibited a similar isotherm to that of CHT-pectin-HPAAm matrices, with similar pore sizes (Table 8.5). However, it deviated from the CHT-pectin-HPAAm matrices with its reduced surface area due to the reduced number of pores present in the matrix.

***Table 8.5:*** *Surface area and porosity characteristics of the various polyelectrolyte matrices*

| | Surface Area($m^2$/g) | Pore Volume ($cm^3$/g) | | Pore Size (nm) | |
|---|---|---|---|---|---|
| | BET | [1]A | [2]B | [1]A | [2]B |
| **CHT-HPAAm** | 2.70 | 0.021 | 0.021 | 26.069 | 21.647 |
| **Pectin-HPAAm** | 22.67 | 0.005 | 0.005 | 5.425 | 5.152 |
| **CHT-pectin-HPAAm** | 10.50 | 0.078 | 0.078 | 25.379 | 22.462 |

[1]*BJH adsorption branch*
[2]*BJH desorption branch*

SEM micrographs of all matrices showed highly porous surface characteristics. Surface area and porosity studies suggested that the porosity of the matrices followed a random distribution and an interconnected pore system. This was evidenced from SEM images at various magnifications for each matrix formulation (Figure 8.8 and 8.9). According to a Type II and Type IV isotherm, the point marked 'B' on Figure 8.7a refers to the point at which monolayer adsorption was complete and was visible at the beginning of the almost linear mid-section of the isotherm. This point was clearly visible as a 'sharp knee' in the isotherm when $C \approx 100$ (Equation 8.1). However, when $C < 20$ this point was not present as a single

point on the isotherm (Sing *et al.*, 1985). CHT-pectin-HPAAm matrices exhibited a Type IV isotherm with no discernable point 'B' that correlated with its *C* value of 28.19. CHT-HPAAm matrices also had no discernible point 'B' with a *C* value of 12.16. This difference in the enthalpy of adsorption resulted in the difference in the surface areas of these matrices.

### 8.3.5 Effect of the polymer blend on the drug release performance of the polyelectrolyte matrices

It has been reported that pore diameter may have a significant effect on the rate of drug release and can also affect the migration of the drug from the matrix into the release media (Jelvehgari *et al.*, 2006). However, such a relationship between pore diameters and release rate is not always apparent. In addition to pore diameter, the number of pores may also be an important factor in controlling the release rate of drug from matrices.

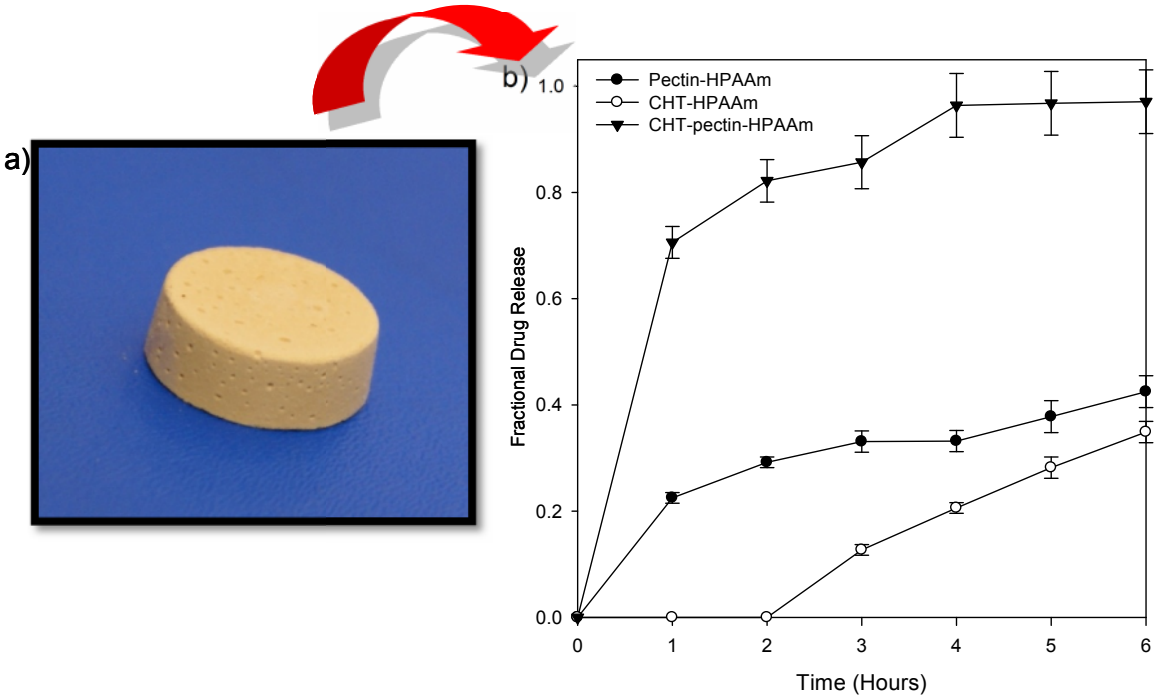



***Figure 8.10:*** *a) A digital image of the lyophilized polyelectrolyte matrix, and b) the drug release profiles obtained for the polyelectrolyte matrices in conditions simulating the gastrointestinal tract.*

Drug release data indicated a marked difference in the release profiles of the various polyelectrolyte matrices, particularly in the first 2 hours in simulated gastric conditions. In this time, no drug was released from the CHT-HPAAm matrices, whereas 28.2% and 82.2% of DPH was released from the pectin-HPAAm and CHT-pectin-HPAAm matrices respectively. After 4 hours of dissolution in simulated intestinal conditions complete drug release was achieved from the CHT-pectin-HPAAm matrices in contrast to only 35.0% release from CHT-HPAAm matrices (Figure 8.10). The pH differential employed which simulated conditions of ]the gastric and small intestinal environments, showed that CHT-HPAAm matrices may hold applicability in the targeted delivery of pharmaceutical agents to the small intestine and colon

as no drug was released in the gastric region. This modulated release profile would prove to be beneficial for the localized treatment of chronic intestinal pathologies such as ulcerative colitis where distal small intestinal and colonic inflammation is prevalent. In contrast, since pectin-HPAAm matrices exhibited controlled release of DPH in SGF and SIF with <40% released, it may still allow for an almost 60% delivery of drug in the colonic region and thus may be beneficial for the treatment of Crohn's disease where the entire GIT is inflamed. The drug release data complements results obtained from the surface area and porosity studies. The surface area ($m^2/g$) of CHT-HPAAm matrices was significantly lower than that of the other matrices (2.7 vs. 22.7 and 10.5$m^2/g$, respectively). The lower surface area sustained the absorption of dissolution media into the matrix thus reducing the swelling rate and subsequently the rate of DPH diffusion out of the matrix. On the contrary, despite the lower surface area of CHT-pectin-HPAAm matrices compared to pectin-HPAAm matrices, the rate of drug release was still higher. This finding may be supported by the higher pore volume and pore sizes as well as the presence of macropores in the CHT-pectin-HPAAm matrices compared to pectin-HPAAm matrices.

### 8.4 Concluding Remarks

After consideration of the numerous benefits of employing modified polysaccharides in controlled drug delivery applications, the synthesis of these novel polyelectrolyte matrices that remain resistant to conditions of the upper GIT prove to be immensely promising.The interaction between partially hydrolyzed PAAm molecules to both anions (pectin) and cations (CHT) formed three polymer complexes which were accommodated by electrostatic, van der waals and H-bonding interactions. The polyelectrolyte matrices demonstrated controlled release of the highly water-soluble model drug, DPH. Drug release behaviour was pivoted to the porositometric properties of the various matrices. It was found that by modifying the concentrations of the polymers employed (CHT, pectin and PAAm) and the combinations thereof, the applicability of these matrices may be accentuated for effective targeted and controlled oral drug delivery applications more specifically, for application in colon-targeted drug delivery.

# CHAPTER 9

# CONCLUSIONS, RECOMMENDATIONS, CHALLENGES AND POTENTIAL PROSPECTS OF THE STIMULI-RESPONSIVE ORAL TABLET SYSTEM

## 9.1 Conclusions

Ulcerative colitis (UC), notorious for its unpredictable attacks of inflammation of the large intestine, is estimated to affect as many as 1.4 million people in USA and 2.2 million people in Europe with 15000-30000 new cases being diagnosed annually worldwide (Loftus, 2004), however despite the prevalence of UC in South Africa there is an absence of definitive statistics regarding the incidence rates. The chronic inflammatory process is limited to various regions of the colonic mucosa and is postulated to occur due to a dysregulated mucosal response in the intestinal wall, facilitated by defects in the protective barrier function of the intestinal epithelium and mucosal immune system (Klotz, 2005).

Generally, UC is characterised by periods of remission and relapsing exacerbations with symptoms including weight loss, diarrhoea accompanied with blood, and abdominal pain (Podolsky, 2002a). Essentially 50% of patients remain asymptomatic, 30% experience mild symptoms and 20% suffer from moderate to severe symptoms (Hendriksen *et al.*, 1985). Furthermore the cumulative probability of remaining 'attack-free' decreases with an increase in years (Allan *et al.*, 1997). Since a definitive aetiology of UC remains obscure, currently the only curative option relies on performing a procto-colectomy (Farrell and Peppercorn, 2002; Podolsky, 2002b; Klotz, 2005). However more appropriately the therapeutic goal in active UC is to achieve and maintain a state of remission for which the aminosalicylates, in particular 5-ASA, have been established as the best treatment option. Consequently, these aminosalicylates should be ideally targeted directly to the affected area namely, the colon. By acting at the luminal site, effective drug concentrations can be achieved in the colonic mucosa and systemic availability limited, thus reducing the potential for drug-related adverse effects. Furthermore, due to the chronicity of colonic inflammation, a consequent symptom of diarrhoea is almost always present and therefore necessitates concurrent therapy with an anti-diarrhoeal agent such as loperamide HCl.

Therefore, this research undertook to develop a novel stimuli-responsive oral tablet (SROT) system that served to provide both symptomatic and chronic treatment for UC with a single tablet administration. Essentially, the novelty of the system is based on its ability to target the release of 2 different drugs to specific regions of the gastrointestinal tract (GIT) in response to a stimulus specific to the relevant region. The stimulus serves as a trigger that either

inhibits or initiates drug release, for example pepsin inhibits loperamide HCl release in the stomach, whereas colonic enzymes initiate and accelerate 5-ASA release in the colon.

Extensive *in vitro* studies were conducted in lieu of the complexity of the proposed system. These studies established an effective SROT as a direct result of the exhaustive preliminary investigations undertaken to identify suitable polymers, polymeric combinations, crosslinking agents, and release modifying agents that imparted not only a robust formulation but also one that is enzyme-specific responsive. *In vitro* studies on the 2 components of the SROT namely, the outer loperamide HCl shell and the 5-ASA loaded inner tablet, were conducted separately since drug release from either component was mutually independent.

The optimization of the 5-ASA loaded colon-targeted tablet component was conducted employing a Box-Behnken experimental design that identified 15 formulations which were subjected to *in vitro* drug release studies to assess the enzyme-responsiveness and colon-targeting ability of the tablets. Ultimately, the optimized tablet not only exhibited significant enzyme-responsiveness when subjected to colonic enzymes but also prevented premature drug release in the upper GIT, cementing its colon-targeting ability. The robustness of the optimized formulation was assessed based on the matrix hardness and friability of the tablets. The influence of colonic enzymes on the tablet was not only established based on the comparative 5-ASA release in the presence and absence of colonic enzymes, but also on the relevant degree of simulated fluid uptake and the corresponding erosion of the tablet, which was also influenced by the structural and polymeric vibrational changes of the tablet due to the enzymes.

The outer loperamide HCl loaded coating of the SROT was formulated to specifically target drug release to the small intestine and thus required a mechanism that inhibited/minimized the release of the drug in the stomach. In order to achieve this minimization in drug release, preliminary *in vitro* studies served to identify suitable polymers, polymeric configurations and methods of crosslinking that would provide a formulation that was capable of responding to the enzyme pepsin in such a way that drug release was inhibited in its presence. Optimization of the coating was conducted from a constructed Box-Behnken experimental design where the pepsin-responsiveness of the coating was established based on the *in vitro* drug release in the presence and absence of the enzyme. The small intestinal targeting ability of the formulation was then determined based on drug release studies conducted in simulated intestinal fluid.

*In vitro* studies not only established a successful SROT but also resulted in a formulation design approach that was easily reproducible, requiring minimal preparatory processes and

employing biocompatible, biodegradable and readily available polymeric materials. Thus based on the promise of the SROT *in vitro*, *in vivo* evaluation of the system followed in the pig model. The colon-targeted tablet component exhibited minimal 5-ASA release in the upper GIT, followed by a sustained plasma concentration of 5-ASA throughout the rest of the 24 hour period. In contrast the commercially available 5-ASA-loaded formulation, Asacol®, presented with greatly fluctuating plasma concentrations and ineffective targeting of the colon. The success of the outer coating was evident from the inhibition of drug release in the first 2 hours post-dosing (indicative of the stomach), with peak plasma concentrations achieved 8 hours post-dosing. The gold-standard formulation, Imodium®, initiated drug release immediately post-dosing resulting in peak plasma concentrations in only 4 hours. Essentially, the SROT proved to be superior to the commercially available counterparts with the added benefit of delivering both drugs with a single tablet administration.

Furthermore, preliminary *in vitro* investigations were undertaken to develop an alternative polymeric matrix comprised of polysaccharide polymers and of a partially hydrolyzed polyacrylamide that were degradable by colonic enzymes. Physicomechanical analysis of the lyophilized polyelectrolyte matrices were performed to establish the matrix resilience, matrix hardness and deformation energy, as well as the surface area and porosity of the matrices comprised of varying polymeric combinations. *In vitro* drug release studies revealed the differing release characteristics of the different polyelectrolyte matrices as a function of the polymers employed. Ultimately, the promise of these matrices for sustaining and controlling the release of a highly water-soluble drug implies the flexibility of the matrices for colon-targeted drug delivery applications as well. In addition, the formulation exploits the attractive properties of polysaccharide polymers whilst concurrently minimizing the hindering characteristics thereof.

### 9.2 Recommendations

It has been said that as the first decade of the 21st century closes we stand at the threshold of unprecedented advances in knowledge of the exact pathogenesis of UC. Furthermore, the simultaneous progress in biotechnology is fostering the development of newer agents that strategically target pivotal processes in the disease pathogenesis (Sands, 2000). However until such time the potential of the existing therapeutic options for UC should be maximized.

Therefore, owing to the immense promise of the SROT as a treatment option, it is recommended that further elaborative studies be performed to determine the exact location in the human GIT at which the relevant drugs are released. It is recommended that this be undertaken by employing gamma-scintigraphic technology which would safely and

definitively establish the targeting efficacy of the SROT. Such data would allow a correlation of the *in vivo* plasma concentrations of 5-ASA and loperamide HCl with the relevant locations. Furthermore, identical gamma-scintigraphic studies should also be performed on the conventional systems so that the SROT may be effectively compared to a 'bench-mark' formulation/s.

It is further recommended that *in vivo* pilot studies be performed in human subjects ascribed to the success of *in vivo* studies conducted in the pig model. Such data would thus permit a correlation between drug plasma concentrations and the location of the delivery system in the GIT of humans.

In addition, it is recommended that the composite polyacrylamide-polysaccharide hydrolyzed electrolytic matrices be further evaluated with the incorporation of 5-ASA and consequently evaluated for the drug release characteristics in conditions simulating the entire GIT namely, the stomach, small intestine and colon with the presence and absence of colonic enzymes.

### 9.3 Challenges experienced with regard to the study

Despite the success of the SROT and the potential future benefits of the formulation, the process of formulation development was marred with several challenges.

Despite the potential of an easily scaled-up manufacturing process for the tablet, the manual dip-coating process that had been employed for the production of the outer shell may possess certain process limitations, principally due to its time-consuming manufacturing process. However, this can be simply overcome by employing a similar manufacturing process to that commonly used to produce gelatine capsules.

The most prominent challenge experienced in the completion of the study was experienced during the *in vivo* study performed in the pig model. Numerous specific challenges were encountered for example determination of an effective method of dosing the pig with the delivery system which ensured that the SROT was not chewed, determination of an effective method of blood sampling, and ensuring that the pigs remained free from infection due to the chronic catheters. Nevertheless, the principle challenge is attributed to the time-consuming, strenuous and at times dangerous experimental conditions experienced. Even though these challenges were ultimately overcome, it is suggested that an alternative method of determining the *in vivo* effectiveness of delivery systems be established, by developing a more sophisticated *in vitro* gastrointestinal dissolution system which as close as possible mimics the human physiological gastrointestinal functions such as fluid content, viscosity,

enzyme populations, elimination processes, absorption processes and distribution processes etc. This would negate the need for performing *in vivo* drug release studies in the animal model.

### 9.4 Potential prospects of the stimuli-responsive oral tablet system

While the SROT has been developed for the simultaneous symptomatic and chronic treatment of UC, with the incorporation of loperamide HCl and 5-ASA, the delivery system itself (excluding the drugs) is not exclusive to this disease. By substituting loperamide HCl and 5-ASA with other pharmaceuticals or nutriceuticals or even a combination of both this system can be employed for numerous other disease conditions.

Even though the SROT was developed for the incorporation of 2 different drugs for targeting 2 different sites in the human GIT, this technology can be easily modified for numerous drug delivery purposes. The enzyme-responsive colon-targeted tablet component can be manufactured without the outer pepsin-responsive coating, thereby allowing the incorporation of numerous other pharmaceutical agents for specific colon-targeting. This tablet can thus incorporate anti-cancer agents, laxatives, corticosteroids for localized action, and non-steroidal anti-inflammatories (NSAIDs) (to prevent gastric and duodenal ulcers) amongst others. Similarly, the outer pepsin-responsive coating can be employed exclusively. It holds potential benefit for drugs with specific absorption windows in the small intestine, for probiotics, vitamins, proteins, anti-tuberculosis drugs, several anti-retroviral drugs etc.

Ultimately, the optimum benefit of the SROT will be experienced if the system is employed in its entirety. Thus the SROT holds immense potential in the nutriceutical market especially since there is a markedly increased awareness by populations of the benefits of supplemental vitamin and mineral administration in daily life. By incorporating vitamins into the SROT, not only will the vitamins be protected from the harsh gastric environment but the two phases of vitamin release, particularly in the small intestine and colon, will allow for a sustained release of the incorporated vitamins throughout the 24 period, thus maximizing its therapeutic benefit.

Ultimately, Sands (2000) most eloquently explained the benefit of novel drug delivery systems by stating that:

*“the refinements in drug formulation have provided the ability to target distinct sites of delivery, enhancing the safety and efficacy of older agents.*” – Sands, 2000

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# APPENDICES

# APPENDIX A

## Abstracts of papers published/submitted from this dissertation

IOP PUBLISHING BIOMEDICAL MATERIALS
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TOPICAL REVIEW

# Stimuli-responsive polymers and their applications in drug delivery

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**Abstract**
Interest in stimuli-responsive polymers is steadily gaining increasing momentum especially in the fields of controlled and self-regulated drug delivery. Delivery systems based on these polymers are developed to closely resemble the normal physiological process of the diseased state ensuring optimum drug release according to the physiological need. Also termed 'environmental-sensitive' or 'smart', these polymers experience rapid changes in their microstructure from a hydrophilic to a hydrophobic state triggered by small changes in the environment. The changes are reversible; therefore, the polymer is capable of returning to its initial state as soon as the trigger is removed. Stimuli may occur internally (e.g. a change in pH in certain organs or diseased states, a change in temperature or the presence of specific enzymes or antigens). External stimuli include magnetic or electric fields, light, ultrasound, etc. This review will delve into the various internally and externally stimuli-responsive polymers and the drug delivery systems that exploit them.

## APPENDIX A2

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*Research Article*

# A Composite Polyelectrolytic Matrix for Controlled Oral Drug Delivery

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***Abstract.*** The purpose of this study was to formulate drug-loaded polyelectrolyte matrices constituting blends of pectin, chitosan (CHT) and hydrolyzed polyacrylamide (HPAAm) for controlling the premature solvation of the polymers and modulating drug release. The model drug employed was the highly water-soluble antihistamine, diphenhydramine HCl (DPH). Polyelectrolyte complex formation was validated by infrared spectroscopy. Matrices were characterized by textural profiling, porositometry and SEM. Drug release studies were performed under simulated gastrointestinal conditions using USP apparatus 3. FTIR spectra revealed distinctive peaks indicating the presence of $-COO^-$ symmetrical stretching (1,425–1,390 $cm^{-1}$) and $-NH_3^+$ deformation (1,535 $cm^{-1}$) with evidence of electrostatic interaction between the cationic CHT and anionic HPAAm corroborated by molecular mechanics simulations of the complexes. Pectin–HPAAm matrices showed electrostatic attraction due to residual $-NH_2$ and $-COO^-$ groups of HPAAm and pectin, respectively. Textural profiling demonstrated that CHT-HPAAm matrices were most resilient at 6.1% and pectin–CHT–HPAAm matrices were the least (3.9%). Matrix hardness and deformation energy followed similar behavior. Pectin–CHT–HPAAm and CHT–HPAAm matrices produced type IV isotherms with H3 hysteresis and mesopores (22.46 nm) while pectin–HPAAm matrices were atypical with hysteresis at a low $P/P_0$ and pore sizes of 5.15 nm and a large surface area. At $t_{2\ h}$, no DPH was released from CHT–HPAAm matrices, whereas 28.2% and 82.2% was released from pectin–HPAAm and pectin–CHT–HPAAm matrices, respectively. At $t_{4\ h}$, complete DPH release was achieved from pectin–CHT–HPAAm matrices in contrast to only 35% from CHT–HPAAm matrices. This revealed the release-modulating capability of each matrix signifying their applicability in controlled oral drug delivery applications.

**KEY WORDS:** composite polyelectrolytes; controlled oral drug delivery; hydrolyzed polyacylamide; matrix characterization; polysaccharides.

## APPENDIX A3

Submitted

**Formulation design, in vitro and in vivo evaluation of a novel stimuli-responsive colon-targeted oral tablet (SROT)**

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**Abstract**
The purpose of this study was to formulate a stimuli-responsive colon-targeted oral tablet (SROT) that was capable of preventing release of the therapeutic agent mesalamine (5-ASA) in the upper gastrointestinal tract (stomach and small intestine) thus ensuring the delivery of a maximal quantity of 5-ASA to the colon. The formulation comprised of 5-ASA-loaded crosslinked chitosan (CHT) granules dispersed within a matrix of pectin, barium chloride ($BaCl_2$), carboxymethylcellulose (CMC) and xanthan gum (XG). The combination of matrix components and granules were compressed into shallow concave tablets and coated with a combination coating solution of pectin and ethylcellulose. Formulation batches were prepared according to the Box-Behnken experimental design and each formulation was evaluated for the in vitro drug release characteristics at various residence times and pH gradients. The mean dissolution time (MDT) in the presence and absence of the colonic enzymes was employed in the determination of the optimized SROT. In vitro drug release studies revealed a distinct responsiveness of the SROT to colonic enzymes with a fractional release of 0.402 in the presence of enzymes vs. 0.152 in the absence of enzymes, after 24 hours. The commercially available comparator product showed irreproducible release profiles over the 24 hour period (SD: 0-0.550) compared to the SROT (SD: 0-0.037). FTIR spectra of the coating showed a disappearance of peaks from 1589-1512$cm^{-1}$ after exposure to colonic enzymes. In addition, with increasing exposure time to colonic enzymes there was a disappearance of peaks between 1646-1132$cm^{-1}$ of the tablet indicating polymeric cleavage by colonic enzymes and release of 5-ASA from the tablet. Plasma concentration profiles of the SROT in the pig model produced a $C_{max}$ of only 3.77±1.375µg/mL compared to 10.604±2.846µg/mL from the comparator product. Overall, the SROT proved beneficial over the commercially available comparator product due to the mechanism of drug release and responsiveness to colonic enzymes.

**Keywords:** Colon-targeting, enzyme-responsive, pH-independent, polysaccharides, crosslinking, stimuli-responsive, ethylcellulose-pectin aqueous dispersion, chitosan, β-glucosidase, pectinase, plasma concentrations

# APPENDIX B

## Abstracts of conference proceedings

## APPENDIX B1

### Effect of the degree of esterification and amidation on drug release from crosslinked zinc pectinate matrices

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**Purpose**

The purpose of this study was to compare the effects of the degree of esterification and amidation on drug release from crosslinked zinc pectinate matrices intended for site-specific drug release in the gastrointestinal tract.

**Methods**

Preparation of crosslinked zinc-pectinate tablets: Tablets were prepared by a direct compression approach. Zinc sulphate ($ZnSO_4$) crystals were ground in a pestle and mortar and combined with magnesium stearate ($1\%^{w}/_{w}$), model drug diphenhydramine HCl, and various grades of apple (Pectin A) and citrus (Pectin B and C) pectins. The degree of amidation and esterification varied. Permutations were blended for 20 minutes until homogeneity and subsequently compressed into tablets (13mm×5mm). Tablets serving as a control were also prepared devoid of any crosslinking agent. In vitro drug release studies: Release studies were conducted in a rotating paddle apparatus in simulated gastric fluid (SGF) (pH 1.2; 37ºC) and simulated intestinal fluid (SIF) (pH 6.8; 37ºC). Samples (5mL) were withdrawn at pre-determined intervals over 24 hours and analyzed by UV spectrophotometry.

**Results**

Results in SGF indicated that pectin A crosslinked with $ZnSO_4$ showed retardation of drug release compared to the non-crosslinked pectin A (43.7% vs. 56.16% after 6 hours). A burst effect was noted in the first 3 hours in both crosslinked and non-crosslinked formulations, however was not significant for the crosslinked formulations (14.95% vs. 4.41% drug release in 30 minutes). Citrus pectins B and C crosslinked with $ZnSO_4$ displayed greater drug release compared to the non-crosslinked formulations. UV spectrophotometry analysis in SIF showed similar results as conducted in SGF i.e. crosslinked pectin A retarded drug release. Pectin B also showed greater drug release (17.6% in 30 minutes) when crosslinked compared to 2.62% drug release from non-crosslinked formulations. In SIF Pectin C was the only citrus pectin to control drug release over a period of 5 hours. A low methoxy pectin with a degree of esterification between 38-44% proved to be the most efficient in retarding drug release in both SIF and SGF. However, in SIF citrus pectins such as Pectin C also proved to control drug release over a similar period.

## The influence of the choice of solvent in wet granulation as a means to retard drug release in the stomach

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### Purpose

The purpose of the study was to determine the drug release characteristics of drug-entrapped granules prepared by wet granulation using different solvents and to determine the most appropriate solvent for the preparation of granules intended for site-specific colonic delivery.

### Methods

Preparation of drug-entrapped granules: Permutations of constant concentrations of a model drug, Diphenhydramine HCl, Sodium Alginate, Chitosan and Zinc sulphate ($ZnSO_4$) were thoroughly blended for 20 minutes until homogenous. Three different solvents were investigated and samples were prepared in duplicate. A Eudragit® S100 latex was prepared and investigated as a solvent in the first set of granules (Set A). The second and third sets were prepared using de-ionized water as a solvent. All three sets of granules were prepared by granulating through a 2mm mesh sieve and allowed to dry at 40ºC for 12 hours. After drying, the second set of granules (Set B) were sprayed with the Eudragit® S100 latex and the third set (Set C) of granules were sprayed with a 10% ethylcellulose solution. The first set of granules were also sprayed with the Eudragit® S100 latex after drying. Each set of granules was incorporated into a matrix of Pectin, Barium chloride, Poly(ethylene oxide) and magnesium stearate. These were then compressed into tablets (13mmx5mm) with a compression force of 8N.

In vitro drug release studies: Release studies were conducted in a rotating paddle apparatus in simulated gastric fluid (SGF) (pH 1.2; 37ºC). Samples (5mL) were withdrawn at pre-determined intervals over 6 hours and analyzed by UV spectrophotometry.

### Results and Discussion

Since the average gastric transit time is approximately 2 hours, the first two hours after drug administration remains the most important when analyzing drug release specifically in the gastric region especially when trying to achieve minimal drug release in this region. After 2 hours of dissolution in SGF, Set B had a drug release of 5.58%. This was substantially lower than the drug release achieved by Set A and C (8.85% and 17.12% respectively). After 5 hours of dissolution, Set C had the highest percentage of drug release (40%) vs. 25.53% and 18% from Set A and B respectively. When attempting to limit drug release in the stomach and target drug release specifically to the distal part of the gastrointestinal tract, de-ionized water as a solvent is most efficient as it facilitates crosslinking of the granules better than the latex solution. Also, dried granules sprayed with Eudragit® S100 were more effective at limiting drug release than the same granules sprayed with the hydrophobic ethylcellulose solution.

**A grafted co-polymerized poriferous matrix for oral drug delivery**

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## Introduction

The purpose of the study was to formulate a lyophilized graft co-polymerized sponge-like drug delivery system using various combinations of natural and synthetic polymers.

## Methods

Three combinations of the sponge-like delivery systems were prepared. Briefly, aqueous stock solutions of 10%$^{w}/_{v}$ pectin (PEC), 5%$^{w}/_{v}$ polyacrylamide (PAAm), and 10%$^{w}/_{v}$ chitosan (CHT) were prepared in 1M acetic acid. The three combinations included PEC-PAAm in a 2:1 ratio, CHT-PAAm in a ratio of 2:1 and CHT-PEC-PAAm in a ratio of 1:1:1. Diphenhydramine (DPH) was incorporated as a model drug in all formulations. The respective combinations were combined until homogenous and underwent redox-initiation by the addition of sodium persulphate (SPS) and N,N,N',N'-tetramethylethylenediamine (TEMED) in a ratio of 2:1. 1mL aliquots of the composite solutions were placed into pre-oiled cylindrical moulds and were frozen at -70°C for 24 hours prior to lyophilization for 48 hours at 25mtorr. Drug release studies were performed at 37±0.5ºC using USP25 Apparatus 3 (Bio-Diss II Release Rate Tester, Vankel Industries) (220mL per vessel). Each formulation was subjected to a continuous run under a pre-determined pH differential of pH 1.2 for 2 hours, pH 6.8 for 4 hours and pH 7.4 for 6 hours. A standard oscillating rate of 10dpm was employed throughout the study. Samples (5mL) were withdrawn at pre-determined intervals over 12 hours and analyzed by UV spectrophotometry at 254nm. Sponges were completely dissolved in 100mL of simulated gastric fluid (SGF) (pH 1.2; 37ºC) and analyzed via UV spectrophotometry to determine DPH content. SEM was used to elucidate the morphology, size and distribution of pores within the sponge-like delivery system and to relate this to the DPH release dynamics.

## Results

The DEE values of the three combinations differed significantly. PEC-PAAm sponges had a DEE value of 93% vs. 38% for CHT-PAAm and 79% for PEC-CHT-PAAm. In order to accurately simulate the passage of the delivery system through the GIT, pH differentials simulating GIT conditions were employed. At $t_{2hours}$ in SGF, PEC-PAAm released 55.2±2.07% of DPH compared to 49±1.15% and 40.9±7.6% for the CHT-PAAm and PEC-CHT-PAAm sponges respectively. The higher DPH release from PEC-PAAm may be attributed to the greater DPH entrapment within the formulation. A x1400 magnification of the CHT-PAAm sponges revealed an uneven distribution of pores of varying morphologies compared to the more uniform and consistent pores of smaller sizes for the PEC-CHT-PAAm sponges at the same magnification. DPH release from the CHT-PAAm sponges was therefore substantially higher at specific time points (for instance 94.28±1.73% and 85.59±0.60% at $t_{6\ hours}$. Using the time-point approach where $t_x$% represented the time at which 100% DPH was released, results showed that PEC-PAAm and CHT-PAAm sponges had a $t_{100}$% at 8 hours. However, PEC-CHT-PAAm sponges had a $t_{100}$% after only 12 hours. This may be a result of the more consistent and even pore distribution of the PEC-CHT-PAAm sponges.

## Conclusions

The combination of PEC-CHT-PAAm proved to be promising for sustained drug delivery. With careful manipulation of the polymeric ratios further modulation in drug release can be achieved.

## APPENDIX B4

### Comparison of crosslinked and non-crosslinked quaternised chitosan matrices for effectual gastric accentuation of drug release

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**Introduction**

This study investigated the viability of non-crosslinked and crosslinked chitosan formulations prepared by two different approaches and to determine the most effective and reproducible method of preparation to ensure gastric targeted drug delivery.

**Methods**

Solutions of chitosan (CHT) (2%-5%$^{w}/_{v}$) in 1M acetic acid were prepared and incorporated with diphenhydramine (DPH) as a model drug. 1mL aliquots were placed in pre-lubricated moulds, and were frozen at -70°C for 24 hours and subsequently lyophilized. Lyophilized samples were crosslinked with $ZnSO_4$ solutions (5% and 30%$^{w}/_{v}$) for 1 hour and underwent further lyophilization. Non-crosslinked matrices were retained for comparative purposes. DPH-loaded and placebo CHT solutions were prepared as above. 1mL aliquots were placed into pre-lubricated moulds and solvent evaporation was allowed under a fume hood. Dried films were crosslinked with the $ZnSO_4$ solutions for 1 hour and allowed to dry. Non-crosslinked films were also produced. Drug release studies were conducted in simulated gastric fluid (SGF) (pH 1.2; 37ºC), employing a rotating paddle apparatus. Samples (5mL) were withdrawn at 30 minute intervals for a period of 3 hours and analyzed by UV spectrophotometry.

**Results**

Prior to DPH release studies on crosslinked and non-crosslinked formulations it was established that the films were inconsistent. On the contrary, CHT lyophilized formulations were consistent and were found to be reproducible. Release studies showed that the non-crosslinked matrices disintegrated and resulted in complete DPH release in the first 30 minutes. The 30%$^{w}/_{v}$ $ZnSO_4$ crosslinked matrices showed higher DPH release in 30 minutes compared to the 5%$^{w}/_{v}$ $ZnSO_4$ crosslinked matrices (65% vs. 49% DPH release). However, after 2.5 hours complete DPH release was obtained at lower crosslinker concentrations whereas higher concentrations only influenced disintegrated after 3 hours. The 8%$^{w}/_{v}$ CHT matrices crosslinked at the two crosslinker concentrations both achieved complete DPH release after 3 hours. The 30%$^{w}/_{v}$ crosslinked matrices however achieved a burst of 58% DPH release compared to 27% from the 5%$^{w}/_{v}$ crosslinked matrices in the first 30 minutes. The 15%$^{w}/_{v}$ CHT matrices crosslinked with either 5%$^{w}/_{v}$ or 30%$^{w}/_{v}$ $ZnSO_4$ had an almost identical release profile throughout the 3 hours.

**Conclusions**

The solvent evaporation method was ineffective as a means of preparing consistent CHT films. The non-crosslinked matrices were ineffective for allowing modulated DPH release in the gastric region over 3 hours. The most effective concentration of CHT was a 15%$^{w}/_{v}$ matrix with a $ZnSO_4$ concentration of either 5%$^{w}/_{v}$ or 30% $^{w}/_{v}$. Further studies are underway to ascertain the potential of the matrices as a pepsin-responsive gastric targeted drug delivery system.

## APPENDIX B5

### Formulation and evaluation of a novel buoyant prolonged release drug delivery system

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**Purpose:** To formulate a buoyant polymeric system and to assess the potential of sustained drug release by conducting in vitro release studies in simulated gastric fluid (SGF) and to assess the physicomechanical properties in relation to the drug release dynamics.

**Method:** Two variants of a buoyant polymeric system encompassed the following polymers: chitosan (CHT), sodium alginate (ALG) and polyacrylamide (PAAm). Diphenhydramine HCl (DPH) (solubility of 1g/mL of water at 25.5°C) was incorporated as a model drug. The variations viz. CHT-ALG-PAAm and ALG-PAAm underwent redox-initiation in a ratio of 4:1 (g/mL) of redox-intiatior. The solutions were agitated at room temperature until homogenous and underwent lyophilization and subsequent drug release studies in SGF (pH 1.2; ±37°C). Samples were analyzed with UV spectrophotometry. Physicomechanical analysis was conducted by textural analysis (TA XT plus, Stablemicro Systems, England) to evaluate formulation resilience and energy absorbed (EA) in the dry ($t_0$ hours) and hydrated states ($t_5$ hours).

**Results:** Both variants demonstrated sustained release of DPH. Inclusion of chitosan (CHT-ALG-PAAm) (N=3) resulted in considerably higher drug release with 91±6.08% at $t_5$ hours, compared to formulations without chitosan (ALG-PAAm) (N=3) (52±2.61%). However, a 'burst' effect was observed with ALG-PAAm at $t_1$ hours. At $t_0$ hours, ALG-PAAm had an EA value of 11.21±1.45N.mm compared to 14.72±1.75N.mm of CHT-ALG-PAAm. This provided an indication of pore size. A lower EA indicated smaller pore sizes and a greater swelling ability which allowed an elevation in drug diffusion out of the polymeric system. At $t_5$ hours, CHT-ALG-PAAm had an EA value of 1.5±0.11N.mm and ALG-PAAm had a value of 1.77±0.04N.mm. Both formulations remained buoyant for the duration of the study. A matrix resilience of 56±2.04% at $t_0$ hours was achieved by both formulations. At $t_5$ hours ALG-PAAm had an average resilience of 18±0.57% compared to CHT-ALG-PAAm which had a resilience of only 10±1.52%.

**Conclusion:** Both formulations demonstrated potential as a prolonged release buoyant drug delivery system with the appropriate modification of the polymeric constituents.

## An Active Pre-Programmed Gastro-Stimulus Drug Delivery System

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**Purpose:** To formulate a buoyant stimuli-responsive drug delivery system and to assess the drug release dynamics in relation to the physicomechanical properties.

**Methods:** Two variants of a diphenhydramine-loaded polymeric delivery system comprising chitosan (CHT), alginate (ALG) and polyacrylamide (PAAm) were prepared. The variations viz. CHT-ALG-PAAm and ALG-PAAm underwent redox-initiation and lyophilization. Drug release studies were performed in SGF (pH 1.2; 37°C). Samples were analyzed at 254nm under UV. Physicomechanical analysis was conducted on a Textural Analyzer where the energy absorbed (EA) in the non-hydrated ($t_0$ hours) and hydrated states ($t_5$ hours) was determined.

**Results:** Both variants demonstrated modulated release of DPH. Inclusion of CHT (N=3) resulted in considerably higher drug release (91±6.08%) when hydrated, compared to formulations without CHT (N=3) (52±2.61%). At $t_0$ hours, ALG-PAAm had an EA value of 11.21±1.45N.mm compared to 14.72±1.75N.mm of CHT-ALG-PAAm. A lower EA indicated smaller pore sizes and greater swelling ability which allowed an elevation in drug diffusion and subsequent increase in drug release. At $t_5$ hours, CHT-ALG-PAAm and ALG-PAAm had an EA value of 1.5±0.11N.mm and 1.77±0.04N.mm, respectively. Both formulations remained buoyant during the study.

**Conclusion:** A distinct correlation between the drug release dynamics and EA of the gastro-stimulus drug delivery systems was established.

## Physicomechanical and Physicochemical Analysis of Novel Chemically Modified Lyophilized Polymeric Matrices

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### Purpose

The purpose of this study was to formulate chemically modified matrices of natural and synthetic polymers with the aim of reducing premature solvation of the polysaccharide polymer and in turn reducing upper gastrointestinal release of drug.

### Methods

Synthesis of modified matrices: Three formulations of various combinations of polysaccharide polymer (CHT and/or pectin) and polyacrylamide (PAAm) were synthesized. The DPH-loaded polymer blends were stirred until homogenous and initiation of radical formation was permitted with the addition of SPS and TEMED. Aliquots (1mL) of each blend were pipetted into pre-lubricated cylindrical moulds, frozen at -70°C for 24 hours and subsequently lyophilized. In vitro release studies: Drug release studies were conducted in the BioDiss apparatus in SGF (pH 1.2; ±37°C) for 2 hours and SIF (pH 6.8; ±37°C) for 4 hours. Samples (5mL) were withdrawn after each hour and subsequently analyzed by UV spectroscopy at a wavelength of 254nm. Drug Entrapment Efficiency (DEE): Matrices were completely dissolved in 100mL of simulated gastric fluid (SGF) (pH 1.2; 37ºC) and analyzed via UV spectrophotometry to determine DPH content. Physicomechanical analysis: Matrix resilience (MR) was determined based on a force-time profile acquired by a textural analyzer. Matrix hardness (MH) and deformation energy (DE) was determined based on a force-distance profile for each formulation. Fourier Transmission Infrared Spectroscopy (FTIR): FTIR was performed on all native polymers involved in matrix formulation as well as on lyophilized matrices as a means of validating the successful synthesis of a modified polymeric matrix.

### Results

CHT-PAAm matrices only began releasing DPH after 2 hours of dissolution studies with less than 40% of DPH release after 6 hours. Pectin-PAAm matrices began releasing DPH immediately; however the rate of drug release was sustained with less than 40% DPH release in the 6 hours. The combination matrix of Pectin-CHT-PAAm showed the least favorable release profile as complete drug release was achieved in only 4 hours. CHT-PAAm and Pectin-PAAm matrices showed a greater resilience (6.1% and 5.3% respectively) than the combination matrix (3.9%). The MH and DE were also greater in matrices of CHT-PAAm and Pectin-PAAm compared to the combination matrix. FTIR spectra validated the formation of chemically modified matrices with distinct bands not present in the native polymers e.g. the formation of a primary aliphatic alcohol represented by the band between 3500-3100cm$^{-1}$ for Pectin-PAAm matrices.

### Conclusions

All matrices remained intact after 6 hours of dissolution studies and displayed varying physicochemical and physicomechanical properties dependent on the polymers employed.

## APPENDIX B8

### Synthesis of chemically modified polymeric matrices for potential drug delivery applications

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**Purpose:** To synthesize chemically modified matrices of natural polysaccharide polymers and synthetic polymers and to assess their applicability in drug delivery.

**Method:** Graft co-polymerized polysaccharide-polyacrylamide (PAAm) matrices were prepared using pectin (PEC), chitosan (CHT) and a combination thereof (PEC/CHT). The three variants underwent a redox reaction by addition of sodium persulphate (SPS) and N,N,N',N'-Tetramethylethylenediamine (TEMED) (4:1 g/mL). Diphenhydramine HCl (solubility of 1g/mL of water at 25.5°C) was incorporated as a model drug. Polymerization proceeded at 25°C for 3 hours. 1mL aliquots were pipetted into moulds and subsequently lyophilized. In vitro release studies were conducted in the BioDis apparatus in SGF (pH 1.2; ±37°C) for 2 hours and SIF (pH 6.8; ±37°C) for 4 hours. The surface area, pore volume and pore sizes of matrices were determined with the Micromeritics ASAP analyzer. FTIR analysis was conducted on native polymers as well as matrices to validate the successful synthesis of a graft co-polymerized matrix. SEM was used to elucidate the morphology and distribution of pores within the matrices.

**Results:** PEC/CHT-g-PAAm matrices had a substantial burst of drug release in the first hour (70.57%) compared to only 22.45% and 0% drug release from PEC-g-PAAm and CHT-g-PAAm matrices respectively. Despite no drug being released from CHT-g-PAAm matrices in the first 2 hours, this formulation proved to be the most effective in achieving an almost zero-order drug release profile. The 'burst' in drug release from PEC/CHT-g-PAAm matrices may be attributed to the substantially greater surface area according to the BJH adsorption and desorption isotherms (12.29$m^2$/g and 13.84$m^2$/g respectively). PEC-g-PAAm matrices have smaller pore sizes and pore volumes compared to CHT-g-PAAm matrices. The larger pore volume of PEC/CHT-g-PAAm matrices (0.077$cm^3$/g) compared to CHT-g-PAAm matrices (0.021$cm^3$/g) is also evident in SEM images. The low surface area of CHT-g-PAAm matrices caused the lower rate of drug release. FTIR spectra validated the formation of chemically modified polymers with distinct bands not present in the native polymers e.g. the formation of a primary aliphatic alcohol indicative at a frequency range of 3500-3100$cm^3$.

**Conclusion:** Chemically modified polymeric matrices demonstrated potential for achieving various release profiles dependent on the polysaccharide polymer employed.

## A Novel Enzyme-Responsive Colon-Targeted Drug Delivery System for the Treatment of Local Colonic Pathologies

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**Purpose:**

To formulate an enzyme-responsive drug delivery system that targets the model drug mesalamine directly to the colonic mucosa.

**Methods:**

Tablets were prepared by dispersing crosslinked chitosan-mesalamine granules within a matrix of polysaccharides (pectin, xanthan gum, and carboxymethylcellulose). $BaCl_2$ was used to crosslink pectin *in situ*. Tablets were coated with a solution of pectin/ ethylcellulose to a 9-10% weight increase. The enzyme-responsiveness was determined based on the drug release in simulated human gastric, intestinal, and colonic fluids (SHGF:pH 1.2; SHIF:pH 6.8; SHCF:pH 5.9) at 2, 4 and 18 hours respectively, with and without commercially available colonic enzymes. The influence of colonic enzymes on the formulation (SHCF absorption ability and erosion) was determined gravimetrically. FTIR was performed to assess the influence of colonic enzymes on the vibrational/structural properties of the formulation while energy refinement force field simulations (ERFFS) were performed to corroborate the experimental findings.

**Results:**

For the first 6 hours in SHGF and SHIF no drug was released. However, after 12 hours in SHCF with colonic enzymes 80.3% of drug was released compared to 30.4% in SHCF without enzymes. The extent of uptake of SHCF-containing enzymes was 101.68% compared to 54.57% without colonic enzymes. Colonic enzymes also resulted in greater erosion of the formulations (45.14% vs. 27.74% after 18 hours in SHCF). FTIR spectra and ERFFS revealed structural variations in the polymeric backbone chains of the tablet and coating indicating cleavage of the polymer backbones by the colonic enzymes.

**Conclusion:**

A novel enzyme-responsive drug delivery system was successfully developed for colon-targeted drug delivery.

## APPENDIX B10

### A Pepsin-Responsive Polymeric Drug Delivery System for the Targeted Delivery of Loperamide HCl to the Small Intestine

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**Purpose:**

To formulate a novel drug delivery system comprised of an inner placebo tablet surrounded by an outer polymeric shell that targets loperamide HCl to the small intestine by being negatively-responsive to pepsin.

**Methods:**

Polymeric shells fabricated by dip-coating placebo lactose tablets into drug-loaded gelatine solutions were crosslinked in a glutaraldehyde-lactose mixture for 6 hours. In vitro drug release studies were conducted in simulated human gastric fluid (SHGF) (pH 1.2) with and without pepsin for 2 hours and simulated human intestinal fluid (SHIF) (pH 6.8) for 4 hours. The influence of pepsin on SHGF uptake ability and erosion of the gelatine shells was determined gravimetrically. FTIR spectroscopy and Molecular Mechanics (MM) simulations were performed to visualize the influence of pepsin on the polymeric backbone of the crosslinked shells.

**Results:**

Only 18.2% drug was released in SHGF with pepsin compared to 100% release in SHGF without pepsin in 90 minutes. Complete drug release was achieved within 4 hours in SHIF indicating the intestinal targeting ability and pepsin-responsiveness of the shell. Formulations unexposed to pepsin experienced a greater degree of swelling. A reduced intensity of the peak at ~3280cm$^{-1}$ in the shells is attributable to the loss of –COOH in SHGF while the peaks at 3282cm$^{-1}$ and 1627cm$^{-1}$ from shells exposed to pepsin, showed a relatively greater intensity suggesting that both the –COOH and –$NH_2$ groups remained intact as corroborated by MM geometrical preferences.

**Conclusion:**

A novel pepsin-responsive polymeric shell for the targeted delivery of loperamide HCl to the small intestine was successfully developed.

# APPENDIX C

## Additional outputs from collaborative research

*Review Article*

# A Review of Multi-Responsive Membranous Systems for Rate-Modulated Drug Delivery

**Rubina P. Shaikh,[1] Viness Pillay,[1,2] Yahya E. Choonara,[1] Lisa C. du Toit,[1] Valence M. K. Ndesendo,[1] Priya Bawa,[1] and Shivaan Cooppan[1]**

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***Abstract.*** Membrane technology is broadly applied in the medical field. The ability of membranous systems to effectively control the movement of chemical entities is pivotal to their significant potential for use in both drug delivery and surgical/medical applications. An alteration in the physical properties of a polymer in response to a change in environmental conditions is a behavior that can be utilized to prepare 'smart' drug delivery systems. Stimuli-responsive or 'smart' polymers are polymers that upon exposure to small changes in the environment undergo rapid changes in their microstructure. A stimulus, such as a change in pH or temperature, thus serves as a trigger for the release of drug from membranous drug delivery systems that are formulated from stimuli-responsive polymers. This article has sought to review the use of stimuli-responsive polymers that have found application in membranous drug delivery systems. Polymers responsive to pH and temperature have been extensively addressed in this review since they are considered the most important stimuli that may be exploited for use in drug delivery, and biomedical applications such as in tissue engineering. In addition, dual-responsive and glucose-responsive membranes have been also addressed as membranes responsive to diverse stimuli.

**KEY WORDS:** Dual-responsive membranes; Glucose-responsive membranes; Membranous drug delivery systems; "On–off" gating mechanisms; pH; Stimuli-responsive polymers; Temperature.

## Levodopa Delivery Systems: Advancements in Delivery of the Gold Standard

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**Abstract**
Background:
Despite the fact that Parkinson's disease (PD) was discovered almost 200 years ago, its treatment and management remains an immense challenge since progressive loss of dopaminergic nigral neurons, motor complications experienced by the patients as the disease progresses and drawbacks of pharmacotherapeutic management still persists. Various therapeutic agents have been employed in the management of PD, including levodopa (L-dopa), selegiline, amantadine, bromocriptine, entacapone, pramipexole dihydrochloride and more recently istradefylline and rasagiline. Of all agents, L-dopa though the oldest, remains the most effective. L-dopa is easier to administer, better tolerated, less expensive and is required by almost all PD patients. However, L-dopa's efficacy in advanced PD is significantly reduced due to metabolism, subsequent low bioavailability and irregular fluctuations in its plasma levels. To date, significant strides have been made to improve the delivery of L-dopa in order to enhance its bioavailability and reduce plasma fluctuations as well as motor complications experienced by patients purportedly due to pulsatile stimulation of the striatal dopamine receptors.
Objective:
The ultimate aim was to critically assess the attempts made thus far directed at improving L-dopa absorption, bioavailability and maintenance of constant plasma concentrations, including the drug delivery technologies implicated.
Methods:
Drug delivery systems that have been instituted for the delivery of L-dopa include immediate release formulations, liquid formulations, dispersible tablets, controlled release formulations, dual release formulations, microspheres, infusion and transdermal delivery, among others. In this review, the L-dopa loaded drug delivery systems developed over the past three decades were elaborated on.
Conclusion:
This review highlights the fact that neuropharmaceutics is at a precipice, which is expected to spur investigators to take that leap to enable the generation of innovative delivery systems for the effective management of PD.

**Keywords:** Bioavailability, Conventional dosage forms, Drug delivery systems, Levodopa, Microspheres, Motor complications, Neuropharmaceutics, Nanotechnology, Parkinson's disease, Pulmonary delivery, Transdermal delivery.

# A review on oral drug delivery systems comprising altered geometric configurations for controlled drug delivery

Kovanya Moodley, Viness Pillay*, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo, Shivaan Cooppan and Priya Bawa
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**Abstract**
Recent pharmaceutical research has focused on controlled drug delivery as an advantage over conventional methods. Adequate controlled plasma drug levels, reduced side effects as well as improved patient compliance are some of the benefits that these systems may offer. Controlled delivery systems that can provide zero-order drug delivery have the potential for maximizing efficacy while minimizing dose frequency and toxicity. Thus, zero order drug release is ideal in a large area of drug delivery which has therefore led into the development of various technologies with such drug release patterns. Systems such as multilayered tablets and other geometrically altered devices have been created to perform this function. One of the principles of multilayered tablets involves creating a constant surface area for release. Polymeric materials play an important role in the functioning of these systems. Technologies so far developed include among others, Geomatrix® multilayered tablets which utilizes specific polymers that may act as barriers to control drug release; Procise® which has a core with an aperture that can be modified to achieve various types of drug release, core-in-cup tablets where the core matrix is coated on one surface while the circumference forms a cup around it, donut-shaped devices which have a hole in the middle, Dome Matrix® and “release modules assemblage” which can offer alternating drug release patterns. This review discusses the novel altered geometric system technologies that have been developed to provide controlled drug release, also focusing on polymers that have been employed in such developments.

**Keywords:**
Controlled drug delivery, geometrically altered devices, multilayered tablets, Polymeric materials, “Release modules assemblage”.

# APPENDIX D

## Animal ethics clearance

## APPENDIX D1

AESC 3

**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**

**STRICTLY CONFIDENTIAL**

**ANIMAL ETHICS SCREENING COMMITTEE (AESC)**

**CLEARANCE CERTIFICATE NO. 2007/56/04**

**APPLICANT:** Professor V Pillay

**SCHOOL:** Pharmacy and Pharmacology
**DEPARTMENT:**
**LOCATION:** Medical School

**PROJECT TITLE:** **An in vivo assessment of novel biocompatible polymeric drug delivery system in pigs**

**Number and Species**

30 male/female pigs

**Conditions:**

i. The applicant must negotiate with CAS on the logistics and times
ii. Should blood sampling be done by the applicant or co-workers, they must prove that they are capable of performing this.

Approval was given for to the use of animals for the project described above at an AESC meeting held on 20070626. This approval remains valid until 20090626

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Signed: _____ Date: 12/07/07
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 18/07/07
(Registered Veterinarian)

cc: Supervisor: #
Director: CAS

Works 2000/Iain0015/AESCCert.wps

**APPENDIX D2**

**CENTRAL ANIMAL SERVICE**

**Tel: 011 717-1312**

**Fax: 011 643-4318**

**DEAR ANIMAL USERS**

It is with great satisfaction that we can relay to users, the positive outcome of their respective in vivo research.

Central Animal Service acquired a batch of 30 pigs for in vivo research with the Department of Pharmacy and Pharmacology, for disease screening.

An ongoing animal surveillance program pre and post studies continued on a daily basis to assess the health and well being of the animals. We are confident to say that no unforeseen or fatal circumstance due to researchers or the research material itself had occurred. All pigs maintained an acceptable health status during and post studies.

Kind regards

Dr Leith Meyer (Director of the Central Animal Services) and the CAS team

06th September 2010.

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