

# **DESIGN OF A GASTROFLOATABLE AND GASTROADHESIVE DRUG DELIVERY SYSTEM FOR NARROW ABSORPTION WINDOW AND LOW BIOAVAILABLE DRUGS**

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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

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I, Caragh Synnøve Murphy, 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.

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This ..... day of ..... 2011

## RESEARCH OUTPUTS AND PATENTS

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### A. Publications

1. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Gastroretentive Drug Delivery System: Current Developments in Novel System Design and Evaluation. *Current Drug Delivery*, 6(5): 451-460. (Abstract in Appendix A1).
2. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo, Nthato Chirwa and Pradeep Kumar. Optimization of a Dual Mechanism Gastrofloatable and Gastroadhesive Delivery System for Narrow Absorption Window Drugs. Submitted. (Abstract in Appendix A2).
3. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence Ndesendo, Nthato Chirwa and Pradeep Kumar. Formulation and Development of Gastroretentive Microparticle-Loaded Gastrospheres for the Delivery of Narrow Absorption Window Drugs. Submitted. (Abstract in Appendix A3).

### B. Conference Proceedings

1. Caragh S. Murphy, Viness Pillay and Yahya E. Choonara. Effect of poly(lactic-co-glycolic acid) on drug release, adhesion and buoyancy of a gastroretentive gastrosphere. Abstract submitted at 2<sup>nd</sup> annual BioMATASA symposium, 26 November 2007, Johannesburg, South Africa. (Abstract in Appendix B1)
2. Caragh S. Murphy, Viness Pillay and Yahya E. Choonara. Assessment of the buoyancy and retention of polyacrylic acid blended gastrospheres. Poster presented at Academy of Pharmaceutical Sciences conference, 4-7 September 2007, Langebaan, Western Cape, South Africa. (Abstract in Appendix B2)
3. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit. Development of Low Density Gastrospheres for Enhanced Bioavailability in Iliac-specific Drug Absorption. Poster presented at Faculty of Health Sciences Research Day and Faculty Postgrad Expo, 31 March 2008, University of the Witwatersrand, South Africa. (Abstract in Appendix B3).

4. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit. Design and Development of Retentive Gastrospheres for the Delivery of Narrow Absorption Window and Low Bioavailable Drugs. Poster presented at 35<sup>th</sup> Annual Meeting and Exposition of the Controlled Release Society, 12-16 July 2008, New York, USA. (Abstract in Appendix B4).
5. Caragh S. Murphy, Viness Pillay, Yahay E. Choonara, Lisa C. du Toit. Gastrospheric multi-units for improving the bioavailability of Narrow Absorption Window bioactives through matrix pore mediation. Poster presented at Academy of Pharmaceutical Sciences of South Africa conference, 22- 26 September 2008, Rustenberg, South Africa. (Abstract in Appendix B5).
6. Caragh S. Murphy, Emilia Panayi, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit. Gastroretentive micro-beads for the localized treatment of gastric disorders. Poster presented at Academy of Pharmaceutical Sciences of South Africa conference, 22-26 September 2008, Rustenberg, South Africa. (Abstract in Appendix B6).
7. Caragh S. Murphy, Lisa C. du Toit, Yahya E. Choonara and Viness Pillay. Optimisation of gastroretentive drug delivery system by way of Box-Behnken statistical design. Oral presentation at Faculty of Health Sciences Research Day, 14 May 2009, Johannesburg, South Africa. (Abstract in Appendix B7).
8. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit. Formulation and Development of a Gastroretentive Drug Delivery System for the Delivery of Narrow Absorption Window Drugs. Oral presentation at Academy of Pharmaceutical Sciences of South Africa conference, 23 – 26 September 2009, Potchefstroom, South Africa. (Abstract in Appendix B8).
9. Caragh S. Murphy, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit. Design and Development of Chitosan-Polymethacrylate Microparticles for Rate-Modulated Drug Delivery. Poster presented at AAPS Annual meeting and exposition, 8 – 12 November 2009, California, USA. (Abstract in Appendix B9).

## **C. Patents**

A gastroretentive pharmaceutical dosage form.

Inventors: S. Moonisami; V. Pillay; Y.E. Choonara; C. Murphy.

Patent Number: PCT/IB2009/005828 (Published WO 2009/153632); SA Patent Application 2009/03854

Brief Description: The invention relates to an orally administrable, gastroretentive dosage form which contains at least one pharmaceutically active ingredient and at least one polymeric adjuvant. The adjuvant serves to retain the dosage form in a selected region of the gastrointestinal tract for sufficient time, by floating, for the active ingredient to be released and absorbed. Ideally, the dosage form will contain two or more active ingredients which are delivered to different regions of the gastrointestinal tract

Current Status: SA application pending; Awaiting ISR for PCT.

## **D. Additional Outputs for Collaborative Research**

1. Clare Dott, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo and Caragh S. Murphy. Pharmaceutical Applications of Electro-Spinning. Submitted. (Abstract in Appendix C1).
2. Aameena Wadee, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo and Caragh S. Murphy. Recent Advances in the Design of Drug-loaded Polymeric Implants for the Treatment of Solid Tumours. Submitted. (Abstract in Appendix C2).
3. Yahya E. Choonara, Viness Pillay, Valence M.K. Ndesendo, Lisa C. Du Toit, Riaz A. Khan, and Caragh S. Murphy (2009). The simultaneous in vitro characterization of poly(lactic co-glycolic acid) and poly(glucuronide)-rich nanoparticles employing various sol-gel synthetic wet chemical processing strategies. *Biomedical Materials* (BMM), submitted. (Abstract in Appendix C3).

## SUMMARY

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It has always been a major challenge to ensure that drugs possessing a narrow absorption window are delivered in such a way as to ensure that plasma concentrations are within the therapeutic window while maintaining good patient compliance. This is due to the inherent low bioavailability caused by the narrow site of absorption, which is usually located in the upper parts of the small intestines. The logical solution to this problem is to increase the duration in which the drug is held within the stomach. Many mechanisms have been investigated in order to achieve this, some more successful than others.

This study sought to develop two novel dual mechanism gastroretentive drug delivery systems which will deliver the model drugs, metformin and ciprofloxacin, in a zero order fashion over a period of 12 hours. In so doing, the drug plasma concentrations will be maintained within the therapeutic window, thus preventing side-effects commonly associated with the peaks and troughs in plasma concentrations. The mechanisms of buoyancy and gastroadhesion were employed in order to achieve gastroretention.

Polymers were selected due to their individual characteristics and include poly-lactic-co-glycolic acid (PLGA), polyacrylic acid (PAA), alginate and pectin and the model drug employed was metformin hydrochloride. Fifteen formulations were obtained using the design of experiments Box-Behnken approach, all of which displayed excellent buoyancy. The yield was found to be above 80% in all cases, although due to the high water solubility of metformin, drug entrapment efficacy was only between 18 and 54%. Mean dissolution time (MDT<sub>12</sub>) and gastroadhesive strength were used as the formulation responses in order to optimize the formulation. Furthermore, the molecular mechanics force field simulations were performed to corroborate the experimental findings. Drug release profiles revealed three different release kinetics, namely burst; first-order and zero-order release. Varying gastroadhesive results were obtained, and were highly sensitive to changes in polymer concentrations. Physicochemical characterisation of the gastrospheres was conducted on the optimized formulation, including Fourier Transform Infrared spectroscopy (FTIR), surface area and porosity analysis and Scanning Electron Microscopy (SEM). The spatial disposition and energetic profile of the sterically constrained and geometrically optimized multi-polymeric complex of alginate, pectin, PAA and PLGA corroborated the experimental results in terms of *in vitro* drug release and gastroadhesive strength of the fabricated gastrospheres.

A novel microparticle formulation was developed which was loaded within a pre-developed gastrosphere design which is both gastrofloatable and gastroadhesive. A combination of chitosan and polymethacrylate were used in order to form the ionically crosslinked microparticles. A face-centered central composite design was constructed for this study which resulted in 14 statistically derived formulations. In order to identify the optimal polymeric concentrations, microparticle drug entrapment, microparticle yield and mean dissolution time (MDT<sub>12</sub>) were utilized as formulation constraints. Physicochemical and morphological characterisation was conducted on the optimized formulation, including Fourier Transition Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM) characterisation. Regression analysis and constraint optimization was utilized in order to obtain the optimal formulation. Surface response analysis was utilised in order to determine the interactions between chitosan and Eudragit®. Microparticle yield was relatively high, ranging from 77 to 92%, while drug entrapment ranged between 50 to 73%. Drug release profiles displayed an initial burst release followed by zero order release over a 12 hour period. To mechanistically elucidate the complexation and crosslinking mechanism of Eudragit® and sodium tripolyphosphate with respect to chitosan, we employed computer-aided modelling of the three-dimensional structure of the active residues of the guest molecules with the respective substrate, to predict the possible orientation of residues most likely affecting the drug delivery system's performance.

The *in vivo* release of ciprofloxacin and metformin was investigated through UPLC analysis of plasma samples obtained from white pigs following the administration of the drug delivery systems. The release of each system was compared to the release of a product currently on the market containing the same drug. Drug release from both delivery systems was superior to that of products currently available in South Africa, displaying a more controlled rate of release.

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---

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To my family at Med-Leigh Pharmacy, the lessons you have taught me about life are invaluable.

## DEDICATION

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This work is dedicated to every person who has been a part of my life.  
Without your influence I wouldn't be the person I am now.

## **ANIMAL ETHICS DECLARATION**

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I hereby confirm that the following study entitled "DESIGN OF A GASTROFLOATABLE AND GASTROADHESIVE DRUG DELIVERY SYSTEM FOR NARROW ABSORPTION WINDOW AND LOW BIOAVAILABLE DRUGS" has received the approval from the Animal Ethics Screening Committee of the University of the Witwatersrand with ethics clearance number 2007/56/04. (Appendix D).

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Animal ethics clearance certificate

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## CHAPTER 1

### Background, Rationale and Motivation for this Study

---

#### 1.1. INTRODUCTION

The time taken for a drug delivery system to pass through the stomach remains fairly constant at three hours, while the transit time through the small and large intestines may extend to over twenty hours (Davis, 2005). The gastric time will determine the duration that drug remains in contact with its specific site of absorption (Davis, 2005). The efficacy of drug therapy may therefore be enhanced by prolonging transit time of a drug through the gastrointestinal tract (Tang et al., 2007).

Drugs which have a poor bioavailability or a narrow absorption window are ideal candidates for incorporation into gastroretentive prolonged release drug delivery systems (Streubel et al., 2006). Examples of such drugs include acyclovir (23%), captopril (65%), riboflavin (15%), metformin (50%) levodopa (30%), nitrofurantoin (40%) and ciprofloxacin (69%) (El-Gibaly, 2002; Davis, 2005; Fukuda et al., 2006; Ahmed and Ayres, 2007).

Prolonging the release of drugs within the gastrointestinal tract by way of increasing gastric residence time offers numerous advantages over conventional oral immediate-release drug delivery systems (Chavanpatil et al., 2006; Streubel et al., 2006; Tang et al., 2007). Advantages that are more specific to prolonged release, due to the enhanced gastric residence time, are that the drug is released into the stomach and intestines over a longer period of time, allowing an increase in time available for drugs with low bioavailability or narrow absorption windows to be absorbed without flooding their specific site of absorptions.

There are numerous factors which affect gastric emptying and as a result may influence the gastric retention time of an oral drug delivery system. The size and shape of the system affects its transit through the pyloric sphincter, while the density will determine whether the system would float on the gastric contents or sink to the base of the stomach. These factors are important to consider when designing a gastroretentive drug delivery system. Biological factors also play an important role in the functioning of the gastrointestinal tract and include the age and gender of the patient, the presence of disease, as well as the patients level of physical activity, body mass index and posture. Further factors that influence gastric emptying include the ingestion of food

and particular drugs which may have an impact on gastrointestinal motility (Fukuda et al., 2006; Streubel et al., 2006).

In addition to systemic treatment, this multi-unit drug delivery system may also be applied to the local treatment of conditions affecting the stomach itself, including nausea, vomiting and gastric ulcers. Referring specifically to domperidone, a potent peripheral dopamine antagonist, is commonly indicated for the treatment of nausea, vomiting and dyspepsia. It may be a suitable drug model to be incorporated within a gastroretentive drug delivery device, offering an increase in its duration of action.

Various approaches have been investigated in order to retain a drug delivery system in the stomach for an extended period of time. These approaches include swelling and expanding systems, floating systems and the use of a bioadhesive system (El-Gibaly, 2002). Of late, bioadhesive polymers have received much attention as absorption enhancers, allowing the drug delivery system to be anchored at a specific site, ensuring maximum absorption of the drug (Tur and Ch'ng, 1998).

The major drawback of a gastroadhesive drug delivery system is that of mucosal shedding, where mucosal cells of the stomach are regularly sloughed off and replaced in order to retain the integrity of the mucosal lining, which may reduce the adherence of the polymeric system. Furthermore, there is the possibility of the delivery system to adhere to other mucosal linings such as that of the oesophagus (Streubel et al., 2006). These failings may be overcome by the incorporation of the drug within multiple units.

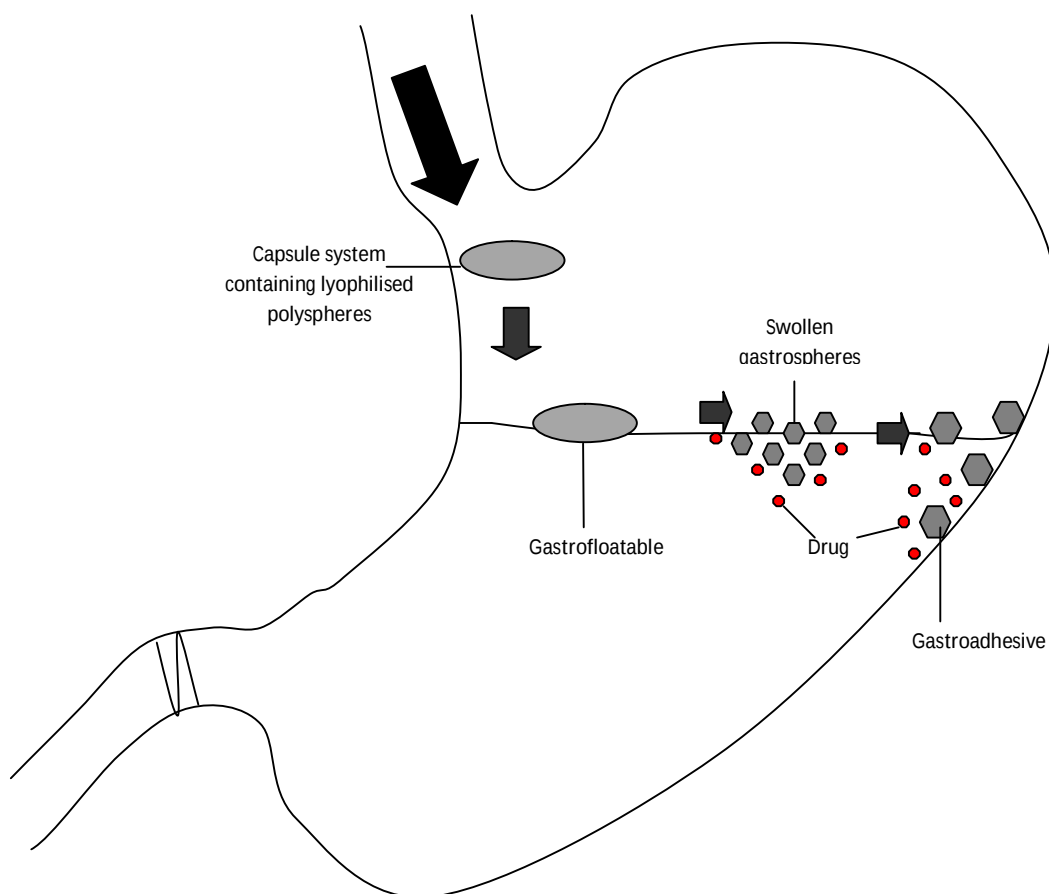
A gastric retentive prolonged-release drug delivery system can be developed either as a single or multiple-unit formulation. The major disadvantage of a single-unit formulation is their 'all-or-nothing' emptying process, which could lead to gastric irritation and a high variability in bioavailability (El-Gibaly, 2002). However, multi-unit drug delivery systems offer more reproducible gastric residence times, reduced absorption variability between subjects and offers a superior dispersion pattern through the gastrointestinal tract, resulting in a reduced risk of damage to the local mucosa (Goole et al., 2007). Mucoadhesive drug delivery systems have not yet reached their full potential to deliver drugs within the gastro-intestinal tract due to the failure to achieve sufficiently prolonged controlled release of drugs (Bernkop-Schnürch, 2005).

In this study, the proposed drug delivery system for increasing gastric retention time will be designed and formulated in such a manner as to overcome the above mentioned limitations. Therefore, in order to further increase the gastric residence time of the drug delivery system, it may be advantageous to make use of more than one gastroretentive mechanism when formulating the delivery system. In this study, a multi-unit approach will be taken that will possess both gastrofloatable and gastroadhesive properties. In so doing, the drug delivery system would initially be buoyant on the surface of gastric contents, and as the gastric contents move into the intestine, the multi-units would adhere to the mucosal surface of the stomach, extending the length of time in which the drug delivery system is retained within the stomach. Further, by utilising a multi-unit approach has the added benefit that it will overcome the disadvantages caused by sloughing of the gastric mucosa.

## **1.2. RATIONALE AND MOTIVATION**

Through the utilisation of a double gastrofloatable and gastroadhesive approach incorporated into a multi-unit drug delivery system, numerous limitations with conventional gastrofloatable and gastroadhesive drug delivery systems may be overcome. Due to the buoyancy of the drug delivery system there may be a desirable portion that would remain suspended within the gastric contents and later have the ability to adhere to the mucosal lining. This will provide a solution to overcome the difficulties brought about due to mucosal shedding. The use of the multi-unit system will also ensure that there is no risk of all-or-none drug release.

In order to deliver the multi-units, polymeric spheres will be incorporated into a capsule. On contact with gastric fluid, the capsule, which is in itself buoyant, will float on the surface of the stomach contents. After the capsule has become hydrated and dissolves, the polymeric spheres will be released. The polymeric spheres will hydrate and swell, enabling them to remain buoyant. Buoyancy of the spheres would also be improved as a result of lyophilization. Lyophilization of spheres results in a highly porous structure, which ultimately results in a reduced density, allowing it to remain buoyant within the stomach contents. The spheres will be able to adhere to the mucosal lining of the stomach due to the adhesive characteristics of the polymers.



**Figure 1.1:** Schematic of the proposed mechanism of drug release from the gastrofloatable and gastroadhesive drug delivery system.

### 1.3. AIM AND OBJECTIVES OF THIS STUDY

The aim of the present study is to develop an oral drug delivery system which will enhance the bioavailability of drugs which are normally associated with a narrow absorption window. Commercially available dosage forms are synonymous with repeated high doses of the drug in order to obtain the required therapeutic plasma concentrations. This, however, results in side effects which may inhibit patient compliance. Drug delivery systems which are currently on the market are costly and the method of controlled release may easily be destroyed by uninformed patients i.e. by breaking or crushing of tablets.

To achieve the aforementioned aim, the following objectives are highlighted:

1. To review the diverse novel systems developed for the improved delivery of narrow absorption window and low bioavailability drugs to the patient in order to enhance therapeutic outcomes and improve patient compliance.
2. To identify a single feasible method for the formulation of a gastroretentive gastrosphere and microparticulate system.

3. To experimentally synthesise several variants of the preferred gastrosphere and microparticulate system employing a Box-Behnken Response Surface Design or Face-Centered Central Composite Design to elucidate the effect of independent variables; the upper and lower limits of which were set during preliminary investigations. This will facilitate a mechanistic evaluation of possible correlations between independent variables.
4. To determine the optimum parameters to synthesise an ideal polymeric gastrosphere and multiparticulate system based on statistical optimisation implemented via the Response Surface Methodology.
5. To assess the *in vivo* drug release characteristics of the optimum gastrosphere and multiparticulate system.

#### **1.4. POTENTIAL BENEFITS OF STUDY**

- i. The development of a multi-unit gastrofloatable and gastroadhesive drug delivery system, intended for the delivery of drugs possessing a narrow absorption window and low bioavailability.
- ii. Present research findings related to the developed technology at national and international conferences as well as publication in international scientific peer-reviewed journals.
- iii. Attract interest in the developed technology from pharmaceutical industry.
- iv. The application for a patent on the developed technology.

#### **1.5. OVERVIEW OF THE DISSERTATION**

The dissertation was constructed as follows for attainment of the aforementioned aims:

**Chapter 1** of this study contains an introduction into the topic of gastric retentive drug delivery systems, the rationale for the research and aim and objectives.

**Chapter 2** concisely provides a descriptive review of the current global situation of research into gastroretentive drug delivery systems. This section elaborates on the factors that need to be taken into account and the different methods utilised in order to attain gastric retention and recent innovations are described.

**Chapter 3** of this study describes the development, design and optimisation of the gastrosphere drug delivery system. A candidate formulation, possessing the advantages of simple and effective manufacture, and favourable *in vitro* release behaviour and mucoadhesion, was identified utilising a model-independent approach

for further investigation and optimisation. A Box-Behnken experimental design was employed to synthesise several variants of the candidate formulation, which were characterised in terms of their mucoadhesion and drug release properties. The optimum gastrosphere system, having appropriate mucoadhesion and drug release characteristics, was identified by instituting the principles of Response Surface Methodology.

**Chapter 4** focuses on the development of a novel microparticle formulation loaded within gastrospheres developed in Chapter 3. A Face-Centered Central Composite Design was constructed which resulted in 14 statistically derived formulations. In order to identify the optimal polymeric concentrations, microparticle drug entrapment, microparticle yield and mean dissolution time ( $MDT_{12}$ ) were utilized as formulation constraints. Regression analysis and constraint optimization was utilized in order to obtain the optimal formulation.

**Chapter 5** discusses the *in vivo* animal testing and UPLC analysis of metformin and ciprofloxacin loaded within the gastrosphere and microparticle loaded drug delivery systems respectively and comparisons are made between these two delivery systems and products currently available on the South African market.

**Chapter 6** concludes the dissertation and ties together the significant issues addressed regarding the formulation of a gastroretentive drug delivery system, with recommendations for future investigations.

## **CHAPTER 2**

### **Gastroretentive Drug Delivery Systems: Current Developments in Novel System Design and Evaluation**

---

#### **2.1. INTRODUCTION**

Oral drug delivery has become the mainstay of treatment due to higher patient compliance and reduced patient discomfort. However, there are still many drugs demonstrating poor efficacy and low bioavailability via this route. These drugs include those which a) act locally within the stomach such as amoxicillin (Whitehead et al., 2000), b) are absorbed within the stomach or specific parts of the upper intestines such as furosemide (Sakkinen et al., 2002), c) are unstable in intestinal fluids such as captopril (Seta et al., 1988) and d) are poorly soluble within the alkaline environment of the intestines such as diazepam (Wurster et al., 2003).

The most important factor leading to the low bioavailability of many drugs is due to their narrow absorption window (NAW), most commonly situated in the upper part of the small intestines, namely the duodenum and jejunum. These segments contain extensive absorption properties although, due to the rapid transport past the sites, absorption is limited (Hoffman et al., 2004). It is therefore evident that by extending the time that a drug is in contact with its specific site of absorption, the greater the amount of drug that may be absorbed. This can be achieved through the design of a drug delivery system which is retained within the stomach.

Through the use of a gastroretentive drug delivery system, the delivery system is retained within the stomach by either one or a combination of mechanisms, allowing the drug to go into solution within the stomach, pass through the pyloric sphincter and ultimately be delivered to its specific site of absorption within the small intestines.

#### **2.2. CONSIDERATIONS FOR GASTRORETENTIVE SYSTEM DESIGN**

##### **2.2.1. Absorption windows**

It has been observed that many drugs are only absorbed in specific regions of the gastrointestinal tract. These specificities may be attributed to many factors, including drug solubility due to varying pH's, enzymatic degradation, interaction with endogenous compounds such as bile, and the necessity for active transport mechanisms which are only present in specific regions (Davis, 2005).

Drugs which display a NAW are also found to have low bioavailability when administered by conventional immediate release drug delivery systems. This, however, results in poor drug efficacy and is detrimental to overall drug therapy. Most drugs with a narrow absorption window are absorbed in the proximal part of the small intestines, or duodenum. Drugs that fall into this category include riboflavin, metformin, captopril, acyclovir, ciprofloxacin, levodopa and nitrofurantoin (Davis, 2005; Fukuda et al., 2006; Ahmed and Ayres, 2007).

There are some drugs which are not suitable for use in a gastroretentive system, such as those which have adverse effects on the stomach lining or are absorbed equally throughout the entire gastrointestinal tract (GIT); however there are certain drugs which may benefit from being incorporated into a gastroretentive system. These drugs usually possess one or more of the following characteristics: local action within the stomach, primary absorption within the stomach, poorly soluble or unstable in the alkaline environment of the small and large intestines, narrow absorption window and rapid absorption from the GIT (Gutierrez-Rocca et al., 2003).

### **2.2.2. Gastrointestinal transit**

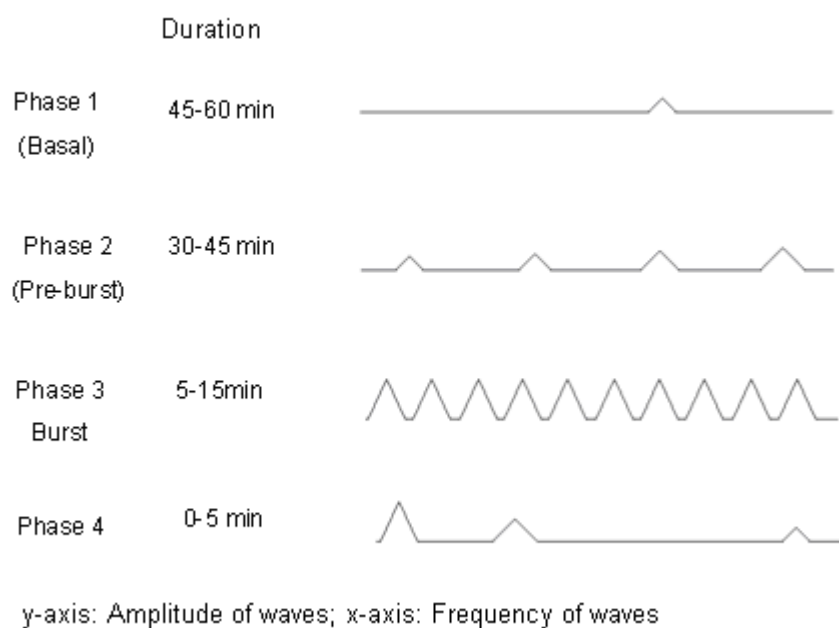
In order to fully comprehend the need for gastroretentive devices and conceptualize their design, it is important to understand the anatomy and physiology of the stomach.

The stomach is made up of three anatomical parts; the fundus, body and antrum. The fundus and body make up the proximal portion, acting as a reservoir for ingested material, while mixing and grinding occurs in the distal region (antrum) (Thibodeau and Patton, 1996). The pylorus is an anatomical sphincter that is situated between the antrum and the duodenum that serves as a sieve as well as a mechanical stricture to the passage of large particles into the small intestines (Thibodeau and Patton, 1996; Klausner et al., 2003).

Gastric contents are removed from the stomach via cyclical gastric motor contractions (Chen et al., 2000). Gastric emptying time therefore has a huge effect on the fate of any oral dosage form. The process of gastric emptying occurs both in the fed and the fasted state; however, there are marked differences between the patterns of motility in both states (Singh and Kim, 2000; Klausner et al., 2003).

The cyclical motor activity that occurs during the fasted state is termed interdigestive migrating myoelectric complex (IMMC). The IMMC follows a cycle lasting between 80

minutes to 2 hours, with the aim of cleaning debris from the stomach and small intestines (Chen et al., 2000; Singh and Kim, 2000; Klausner et al., 2003). The IMMC is divided into four phases. Phase 1 (basal state) is a quiescent period with only rare contractions, lasting 40 to 60 minutes; phase 2 (pre-burst phase) lasts 30 to 45 minutes, showing intermittent peristaltic contractions with increasing frequency and amplitude; phase 3 (burst period) demonstrate intense frequent bursts of contractions 3-5 times per minute, lasting up to 15 minutes. Phase 3 is also commonly known as the 'housekeeper waves' due to their sweeping motion. Phase 4 is the transitional period between phase 3 of one cycle and phase 1 of the next cycle. The IMMC begins in the stomach and migrates through the small intestines. As phase 3 of one cycle reaches the colon, the next phase 3 begins in the stomach (Chen et al., 2000; Klausner et al., 2003).



**Figure 2.1:** The four phases of the interdigestive migrating myoelectric complex (Davis, 2005).

The cyclical motor contractions in the fed state are initiated 5 – 15 minutes after a meal is ingested, lasting for as long as the food persists within the stomach, usually 3-4 hours. The gastric residence time is related to the nutritive and physical properties of the food. These contractions are similar to those observed in phase 2 of the IMMC, and are responsible for mixing and grinding of the stomach contents. The pylorus is

closed, allowing only small particles to enter the duodenum, while retaining large particles within the stomach (Singh and Kim, 2000; Klausner et al., 2003).

Gastric motility may be influenced by a variety of biological factors, including age, gender, posture, body position, body mass index, stress levels and disease states (Singh and Kim, 2000; Klausner et al., 2003), while the rate of gastric emptying of a dosage form will be affected by its shape, size and density as well as the concomitant intake of food and specific drugs (Singh and Kim, 2000).

### **2.2.3. Dosage form factors influencing gastric residence time (GRT)**

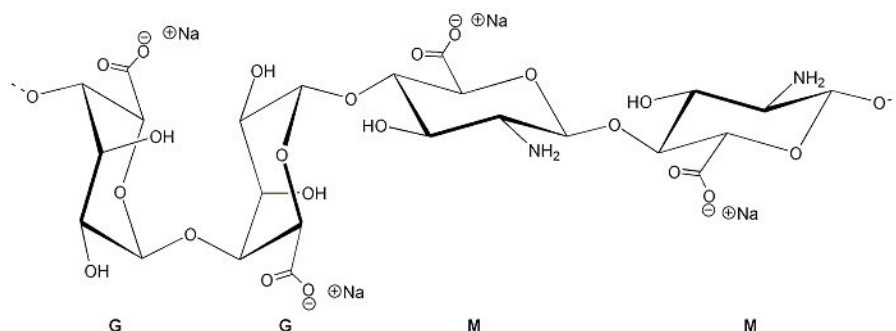
Most of the approaches employed to attain gastroretention are influenced by a number of factors, namely (Garg and Sharma, 2003):

1. Density – GRT is a function of dosage form buoyancy that is dependent on the density
2. Size – dosage form units with a diameter of more than 7.5mm are reported to have an increased GRT compared with those with a diameter of 9.9mm
3. Shape of dosage form – tetrahedron and ring-shaped devices are reported to have better GRT  $\approx$  90% to 100% retention at 24 hours compared with other shapes
4. Single units vs. multiple units - due to the high variability in bioavailability, risk of local irritation and unreliable gastric prolongation, single-unit delivery systems are not generally preferred for controlled drug release (El-Gibaly, 2002). It is due to this that it is preferable to utilize a multiple unit approach when developing a drug delivery system. The advantages of multiple units are most probably due to the ability of the units to pass uniformly through the gastrointestinal tract, and include: a more predictable drug release profile, less chance of localized damage to the mucosal lining, decreased risk of the delivery system failing, units with different release profiles can be included in one delivery system and no significant impairment if a few units fail to perform (Iannuccelli et al., 1998; Rouge et al., 1998; Goole et al., 2007).

## 2.3. COMMON BIOCOMPATIBLE POLYMERS EMPLOYED FOR THE DESIGN OF GASTRORETENTIVE DRUG DELIVERY SYSTEMS

### 2.3.1. Alginate

Alginate, a natural linear anionic co-polymeric polysaccharide that consists of homopolymeric blocks of (1-4)-linked  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) residues linked covalently in various sequences (Figure 2.2) (Jain et al., 2005). The physical properties of gels that are formed render the application of alginates suitable for gastroretentive drug delivery. The gelation is determined by the matrix composition, sequence of polymer blocks and molecular mass of the alginate grade employed in designing the gastroretentive drug delivery system. Alginates have a wide range of pharmaceutical applications including thickening, gel-forming and colloidal stabilizing agents, binders and disintegrants in tableting and in the development of controlled-release gastroretentive drug delivery systems. Crosslinking of alginate can be initiated by polyvalent cations, resulting in the formation of gel-like matrices. Hydration of an alginate matrix leads to the formation of a gelatinous layer that affects drug diffusion by allowing drug to diffuse outward while the inner core remains unhydrated therefore acting as a non-releasing reservoir of drug and polymer.

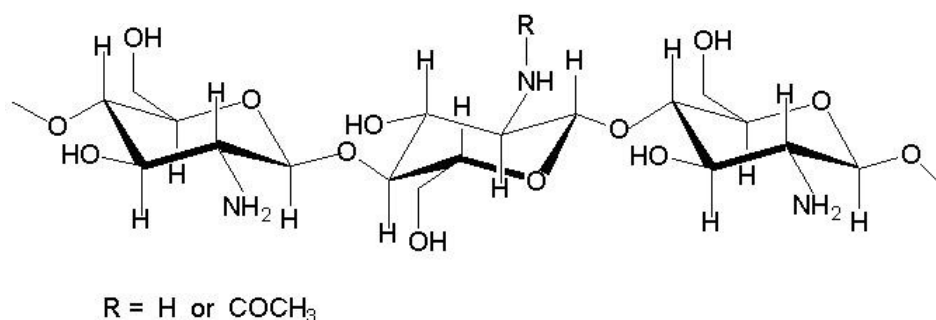


**Figure 2.2:** Chemical structure of alginate depicting the homopolymeric blocks of (1-4)-linked  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) (Lawrie et al., 2007).

### 2.3.2. Chitosan

Chitosan is also a linear natural cationic binary co-polymeric polysaccharide that is obtained from the N-deacetylation of chitin. Chitosan consists of  $\beta$ -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The acetylated and deacetylated units are randomly distributed along the chitosan chain and the degree of acetylation represents the proportion of acetylated units with respect to the total number of units. The physicochemical properties of chitosan may be modified by altering the degree of deacetylation as well as the pH and ionic strength

during formulation. Chitosan is insoluble at neutral and alkaline pH; however it forms water-soluble salts with organic and inorganic acids. In an acidic medium, the amino groups become protonated rendering the molecule positively charged. At neutral pH, most chitosan molecules become non-ionic and therefore precipitate from solution. The biological properties of chitosan can also be modified with the attachment of functional groups to the chitosan polymeric chain. Chitosan has numerous beneficial properties for the design of gastroretentive drug delivery systems such as superior mucoadhesion; gelation; swelling; the ability to form films; mucosal permeation enhancement; tissue growth promotion and the ability to control drug release. The bioadhesive properties of chitosan have been extensively employed to prolong the gastric residence time of gastroretentive drug delivery systems (Bardonnet et al., 2006; Smart, 2005; Thongborisute and Takeuchi, 2008).



**Figure 2.3:** Chemical structure of chitosan illustrating the  $\beta$ -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Lawrie et al., 2007).

### 2.3.3. Hydroxypropylmethylcellulose, polyethylene oxide and polypropylene

The most commonly used polymers in hydrophilic matrix formulations are hydroxypropylmethylcellulose (HPMC) and polyethylene oxide (PEO). This is due to their water solubility, availability in a range of molecular weight/viscosity grades, and their unique swelling/erosion characteristics, which are utilized in order to control drug release (Jamzad and Fassihi, 2006). Polypropylene foam powder is hydrophobic and possesses a highly porous, open-cell structure with pore sizes predominantly in the micro and meso-porous range with a low inherent density (0.905g/cm<sup>3</sup>) (Sher et al., 2007).

### 2.3.4. Ethylcellulose and hydroxypropylcellulose

Ethylcellulose is a hydrophobic polymer used in the design of gastroretentive drug delivery systems to achieve sustained release of both soluble and poorly soluble drugs (Crowley et al., 2004). The physicochemical properties and mechanism of drug release

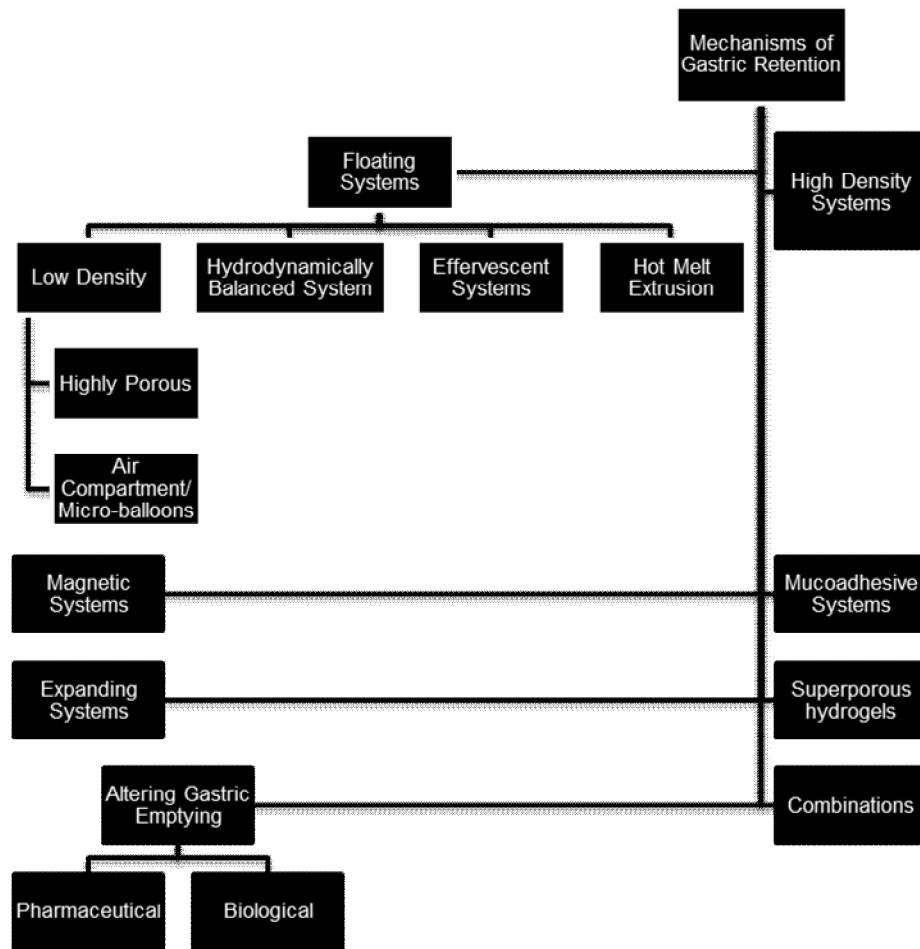
from ethylcellulose-based gastroretentive matrices are usually prepared by direct compression or hot-melt extrusion. Hydroxypropylcellulose (HPC) is widely used for the design of oral drug delivery systems. The specific uses of the various grades for gastroretention are dependent on their structural characteristics, i.e. as the degree of alkyl group substitution that may alter the polymeric interaction with gastric fluids in order to exploit a relevant gastroretentive mechanism. HPC grades that have a medium-high degree of alkyl group substitution are most suited for the preparation of matrix tablets, whereas grades with a lower degree of substitution have superior swelling dynamics.

### **2.3.5. Polymethacrylates**

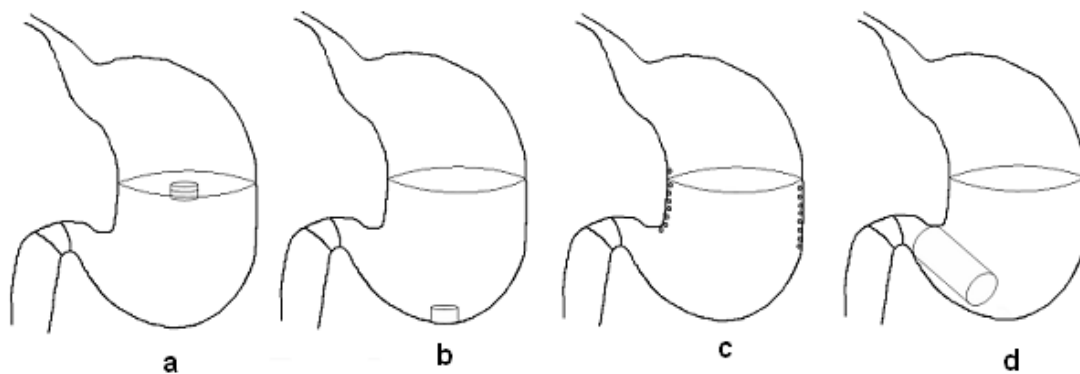
Polymethacrylates (Eudragit®) is commonly used as a retardant in controlled release drug delivery systems. Eudragit® RS functions to retard drug release when melted above its glass transition temperature (Fukuda et al., 2006). The pH of the GIT environment in which gastroretention is intended, results in the polymer acting as a polyelectrolyte, making them suitable for altering the gastric residence time through mucoadhesion, gelation, solubility and density variation. Various Eudragit® grades have been employed for the design of gastroretentive drug delivery systems such as Eudragit® E, RL, RS, and NE that are polycations, while Eudragit® grades L and S are polyanions (Bardonnnet et al., 2006; Jain et al., 2005; Moustafine et al., 2005).

## **2.4. MECHANISTIC ATTEMPTS AT GASTRIC RETENTION**

Various polymeric drug delivery systems have been developed that attempt to exploit the anatomy and physiology of the GIT environment. These include buoyant systems, bioadhesive systems, high density systems, modified shape systems, gastric-emptying delaying devices and co-administration of gastric-emptying delaying drugs (Figures 2.4 and 2.5). Among these, buoyant drug delivery systems have been used most often.



**Figure 2.4:** Classification of gastric retentive systems.



**Figure 2.5:** Schematic depicting mechanisms of retention of: a) Low density buoyant system b) High density system c) Mucoadhesive system d) Swelling/Expanding system.

### **2.4.1. Floating systems**

Buoyant drug delivery systems are by far the most commonly researched mechanisms of achieving gastric retention. In order for a drug delivery system to achieve buoyancy an intimate balance between the weight and volume of the system is required. To obtain immediate buoyancy, the delivery system should have an original density of less than  $1\text{g/cm}^3$ . One of the major advantages of utilizing a buoyant drug delivery system is the ability to achieve an increase in gastric residence time without altering the intrinsic rate of gastric emptying (Stithit et al., 1998; El-Gibaly, 2002). Several factors have been found to influence the buoyancy of a drug delivery system, including mass, density, diameter, nature of excipients, grade of polymers used and the pH of gastric fluids (Singh and Kim, 2000). Numerous novel methods have been developed in order to achieve buoyancy.

#### **2.4.1.1. Low density systems**

##### **2.4.1.1.1. Highly porous system**

The inclusion of low density polymeric carriers in a formulation may result in a matrix with a density of less than  $1\text{g/cm}^3$ , thereby becoming buoyant. There are numerous low density carriers available, including porous silicon dioxide, polypropylene foam, magnesium aluminometa silicate, porous calcium silicate (Jain et al., 2005) and polypropylene foam powder (Streubel et al., 2002; Sher et al., 2007). These porous carriers possess certain characteristics which add to their attractiveness for use in drug delivery system design, including a high surface area, tunable pore sizes with narrow distributions, stable uniform porous structures and well-defined surface properties thus allowing for the absorption of drugs and drug release in a reproducible and predictable manner (Sher et al., 2007).

Streubel et al. (2002) formulated microparticles using a solvent evaporation method. Polypropylene foam powder was dispersed within the organic phase. The resultant microparticles still displayed a high porosity and more than 83% of the particles remained buoyant for at least 8 hours.

Jain et al. (2005) utilized calcium silicate, a highly porous structure for the development of a buoyant system intended for the delivery of repaglinide. Drug was initially adsorbed onto the calcium silicate through ultrasonication. Drug adsorbed calcium silicate was then used to create the highly porous microspheres. This system displayed good buoyancy and excellent drug entrapment.

Sher et al. (2007) combined the principles of floating and pulsatile systems in order to achieve a gastroretentive, chrono-therapeutic drug delivery system. Porous beads were formed by way of melt and solvent evaporation. Both of these methods involved a single step and multiple absorption technique with the inclusion of Accurel MP 1000<sup>®</sup>, a low density polypropylene foam powder.

Besides using a highly porous carrier, pores may also be incorporated within a gastroretentive drug delivery system just before crosslinking occurs. Badve et al. (2007) employed pectin and sodium bicarbonate in order to produce hollow porous floating beads. Sodium bicarbonate is incorporated into the polymeric solution, resulting in the generation of CO<sub>2</sub> gas when it comes into contact with the acid medium of the crosslinking solution. The small pockets of gas become entrapped within the beads during crosslinking, resulting in a highly porous bead.

Another method of obtaining a highly porous matrix is to include lyophilization within the formulation process. Due to the mechanics of the process, sublimation of water occurs, resulting in a stable, yet porous structure. The increased porosity results in a reduction in density of the system thereby facilitating floatation. Whitehead et al. (2000) prepared beads by crosslinking sodium alginate with calcium chloride which were subsequently frozen and lyophilized. *In vivo* studies revealed that the lyophilized beads remained buoyant for extended periods of time, and the concurrent intake of food enhanced buoyancy.

#### **2.4.1.1.2. Air compartment/Microballoons**

In order to ensure immediate buoyancy of a drug delivery system, an air compartment (or buoyancy chamber) can be incorporated within the system. This process, however, is generally complex and complicated.

Iannuccelli et al. (1998) were able to successfully design a delivery system involving a much simpler approach. Units, consisting of a core, separated from a membrane by an air compartment, were prepared by coating a calcium alginate core with a membrane formed from a blend of calcium alginate and polyvinyl alcohol. An air compartment formed between the core and the membrane due to shrinkage of the core during the drying process. *In vitro* tests revealed 100% immediate buoyancy, lasting for more than 24 hours. It was observed during *in vivo* human studies that the delivery system achieved effective buoyancy for extended periods of time, and that buoyancy was improved with the concurrent intake of food (Iannuccelli et al., 1998).

Chitosan, which has played an important role in the design of controlled release drug delivery systems, crosslinks with multivalent counter-ions via ionotropic gelation to form microspheres. Kas (2007) reported that these microspheres displayed a limited strength and poor buoyancy. El-Gibaly (2002), however, was able to develop hollow chitosan microspheres through the interaction with a negatively charged surfactant, sodium dioctyl sulphosuccinate, leading to the formation of chitosan gel sacs that were insoluble at a low pH (Sato et al., 2003).

Sato et al. (2003) developed a multiple-unit intragastric floating system involving hollow microspheres or microballoons, by solvent diffusion. It was observed that the temperature at which the microspheres were prepared had an appreciable effect on the buoyancy, drug release and friability. Further investigations through pharmascintigraphic evaluation demonstrated gastric retention of up to 300 minutes following administration (Sato et al., 2004).

Hollow microspheres are currently considered to be one of the most promising systems intended to maintain buoyancy. This is due to their inherent superior buoyancy in combination with a multi-unit system (Bardonnet et al., 2006).

#### **2.4.1.2. Hydrodynamically balanced systems**

A hydrodynamically balanced system is a single-unit delivery system. Low density, hydrophilic polymers are blended with the drug and usually added to empty gelatin capsules. Once in contact with gastric fluids, the capsule dissolves and exposes the polymeric blend, allowing for hydration and swelling to occur. Due to the low densities of the polymers, the swollen mass floats on the surface of the gastric contents. Various excipients may be incorporated in order to delay the erosion of the system (Bardonnet et al., 2006).

Ali et al. (2007) investigated the release of metformin via this approach. Varying concentrations of hydroxypropylmethyl cellulose (HPMC) and polyethylene (PEO) were investigated, as well as the effect of various release modifiers, including ethylcellulose, cellulose acetate phthalate (CAP) and liquid paraffin. A zero order release was achieved and the drug delivery system remained buoyant for 12 hours. *In vitro* studies showed an increase in drug absorption compared to an immediate release capsule.

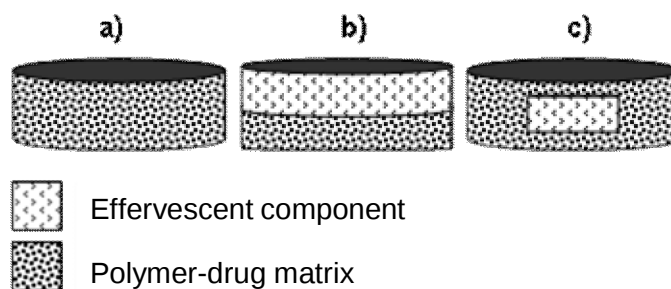
#### **2.4.1.3. Effervescent tablets**

Sodium bicarbonate, citric acid and tartaric acid generate carbon dioxide (CO<sub>2</sub>) gas which may be entrapped within the system matrix once in contact with acidic gastric contents. The release of CO<sub>2</sub> gas therefore results in an upward motion maintaining buoyancy of the system (Singh and Kim, 2000). These CO<sub>2</sub> generating components are incorporated in a tablet matrix in one of two forms, either intimately incorporated within the matrix, or separated within its own layer as depicted in Figure 2.6 (Bardonnnet et al., 2006).

Ichikawa et al. (1991) developed a double layered granular system incorporating a core of bicarbonate and tartaric acid, each formed separately in sub-layers in order to prevent direct contact. The system was fully buoyant within 10 minutes due to the formation of a balloon-like swollen tablet with a density of less than 1g/ cm<sup>3</sup>. This system was observed to be successful over a range of pH values.

Bicarbonate loaded microparticles, coated with an ion-exchange resin containing theophylline, have been formulated by Atayabi et al (1996). The microparticles were then coated with a semi-permeable coating, thereby allowing the permeation of gastric fluid which resulted in an exchange of chloride and bicarbonate ions. The generated CO<sub>2</sub> gas became trapped within the membrane, thereby resulting in buoyancy of the system.

There are, however, a few disadvantages related to effervescent drug delivery systems. A notable disadvantage is that the rate and degree of effervescence are highly pH dependent, and is easily affected by the intake of food and certain disorders such as achloriaemia. Another disadvantage is that the delivery system may be prematurely removed from the stomach due to its lag phase, prior to becoming buoyant (Fukuda et al., 2006).



**Figure 2.6:** Schematic of a) a single layer tablet matrix, b) a bi-layered tablet and c) a bi-layered tablet with a central effervescent core.

#### 2.4.1.4. Hot-melt extrusion

Hot melt extrusion is a method of continuous mixing and design of mouldable materials. It is possible to produce tablets, microspheres, granules, transdermal and transmucosal delivery systems through this process (Mididoddi and Repka, 2007). Polymethacrylate (Eudragit®) polymers are the most commonly used polymers for this approach, due to their thermoplastic properties. When selecting a polymer, it is important to consider the glass transition temperature, melt viscosity and stability under high temperatures. Hot-melt extrusion is associated with numerous advantages. These advantages include fewer steps involved, the absence of solvents, no need for compression and thorough mixing of formulatory components (Bruce et al., 2005; Mididoddi and Repka, 2007).

Fukuda et al.(2006) utilized a single screw extruder in order to produce the extrudates, which, once at room temperature, were then cut into tablets. Sodium bicarbonate was included in the formulation that underwent thermal degradation resulting in the generation of CO<sub>2</sub> gas. The CO<sub>2</sub> became entrapped within the tablet matrix, resulting in a highly porous, buoyant tablet. However, due to the pH dependence associated with the generation of CO<sub>2</sub> gas the delivery system was not suitable for achlorhydric patients, who have a high fasted gastric pH value (pH=7).

#### 2.4.2. High density systems

The density of gastric fluid is roughly 1.004g/cm<sup>3</sup> (Bardonnet et al., 2006). Pellets with a density of between 2.4 and 2.8g/cm<sup>3</sup> have been found to sink to the bottom of the stomach when the patient is in the upright position. The pellets become entrapped within the folds of the mucosa, therefore withstanding the effects caused by peristalsis (Clarke et al., 1993). Rouge et al. (1998) conducted a comparative study with an immediate release system, a high density system and a low density system. The results showed gastric residence times of 0.5, 1 and 2 hours respectively, indicating

that the high density system did not demonstrate any significant extension of gastric residence time. Excipients which are commonly used in order to increase the density of drug delivery systems include: barium sulphate, zinc oxide, iron powder and titanium dioxide (Bardonnnet et al., 2006). Although high density drug delivery systems have not shown remarkable significance for the delivery of drugs to a human model, success has been illustrated with the administration of pellets with a density of  $2.0\text{g/cm}^3$  in the bovine model (Rouge et al., 1998).

#### **2.4.3. Magnetic systems**

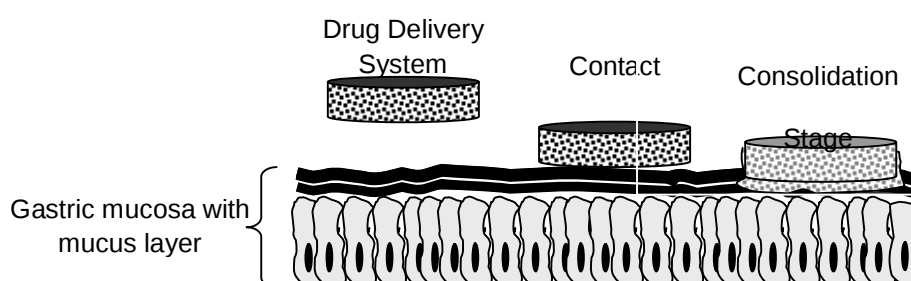
Gröning et al. (2007) designed a novel drug delivery system by incorporating a small magnet within the delivery system, and guiding it with an extracorporal magnet attached to the abdomen. The capsule was effectively delayed within the stomach, therefore extending the gastric residence time and increasing the absorption of the drug at its specific absorption window. It was however found that results differ, depending on whether the patient is in a fed or fasting state. A high patient variability has been observed due to variability in gastric motility and peristaltic waves (Gröning et al., 2007). Clinical investigations were conducted involving three different delivery systems. The first system involved the magnetic depot tablet with the use of the extracorporal magnet, the second system excluded the use of the extracorporal magnet and the third system was an immediate release formulation. A gastric retention time of 12 hours was obtained, and drug plasma concentrations showed an increase in drug absorption associated with the magnetic depot tablet when the extracorporal magnet was used. The most probable system limitation associated with a magnetic system is the reduced patient compliance due to the precision with which the magnet must be placed externally (Bardonnnet et al., 2006).

#### **2.4.4. Mucoadhesive systems**

The basis of mucoadhesive systems is that the delivery system adheres to a site in the gastrointestinal tract, thereby extending the residence time. Research into mucoadhesive drug delivery systems first began in the field of ophthalmics (Bernkop-Schnürch, 2005), but has to date shown the potential to prolong residence times of nasal (Tafaghodi et al., 2004), ocular (Hornof et al., 2003), buccal (Korbonits et al., 2004) and vaginal (Kast et al., 2002) drug delivery systems.

The first generation of mucoadhesive polymers were hydrophilic macromolecules containing numerous hydrogen bonding groups. These include chitosan, carbomers and sodium alginate (Smart, 2005). These polymers become adhesive on exposure to

moisture and adhere non-specifically to bio-surfaces, although adhesion is greater when in contact with a dry, inert surface. The various types of bonds involved in mucoadhesion include hydrogen bonds, ionic bonds, covalent bonds, van der Waals forces and hydrophobic bonds. There are many theories which have been hypothesized as to the mechanism of mucoadhesion. Although a definite mechanism has not been proven to be true for all scenarios, two steps have been identified in the process of adhesion, namely the contact stage and the consolidation stage (Figure 2.7). The contact stage involves the wetting of the mucoadhesive surface, which comes into intimate contact with the mucous membrane. The consolidation stage involves the physicochemical interactions that occur in order to consolidate and strengthen the adhesive joint, resulting in a prolonged adhesion.



**Figure 2.7:** The two stages involved in mucoadhesion (Smart, 2005).

Chavanpatil et al. (2006) developed a delivery system comprising HPMC and psyllium husk for the delivery of ofloxacin. It was observed that both the HPMC and psyllium husk displayed significant bioadhesivity and resulted in enhanced gastroretention.

Säkkinen et al. (2003) investigated the use of microcrystalline chitosan as a mucoadhesive agent. Although all grades were found to be adhesive, further investigation revealed that microcrystalline chitosan with the largest molecular mass displayed the greatest adhesion. *In vivo* studies, however, did not produce evidence that the system provided gastric-retention in humans.

Illum et al. (2001) have formulated a mucoadhesive microsphere intended for the local treatment of *Helicobacter pylori*. The microspheres are formed through the process of spray drying and comprise an inner core containing drug, a rate controlling layer of water insoluble polymer and an outer bioadhesive layer.

Tur and Ch'ng (1998) investigated whether there was a relationship between the bioadhesivity of a polymer and its zeta potential. Different polymers were synthesized

and swelling, bioadhesivity and zeta potential studies were conducted. The results were correlated and it was confirmed that a relationship existed. It is believed that the zeta potential may provide the initial driving force for bioadhesion, followed by interpenetration and the subsequent secondary bonds. The study also indicated the importance of the degree of ionization of the polymer.

Thongborisute and Takeuchi (2008) have developed a method for measuring the mucoadhesive properties of polymers (Figure 2.8). This method involved measuring the change in particle size and zeta potential of mucin particles after the addition of a polymer. A large change in what is termed the RU response signified stronger mucoadhesive properties.

|                    | Polymer              |
|--------------------|----------------------|
| Most mucoadhesive  | Chitosan             |
|                    | Carbopol 971 PNF     |
|                    | Carbopol 974 PNF     |
|                    | Low molecular weight |
|                    | Chitosan             |
|                    | HPMC                 |
|                    | PVP 25               |
| Least mucoadhesive | PVA 205              |

**Figure 2.8:** Rank order of strength of mucoadhesiveness of a selection of polymers (adapted from Thongborisute and Takeuchi, 2008).

Existing molecules are currently being modified in order to increase their mucoadhesive properties. An example of this which is growing in popularity is the thiolation of polymers. This results in the in-situ formation of disulfide bonds, not only between the polymers, but also between the polymers and the mucus membrane, thus resulting in the strengthening of the mucoadhesivity. This has been applied to chitosan (Guggi et al., 2003), alginates, carbomers (Bernkop-Schnürch, 2005), polycarbophil (Kast et al., 2003) and poly(acrylic) acid (Caliceti et al., 2004)

The major limitation to mucoadhesive drug delivery systems is the natural turnover of mucus, which occurs between every 12 to 24 hours within the stomach (Bernkop-Schnürch, 2005; Bardonnnet et al., 2006).

#### **2.4.5. Expanding systems**

Expanding drug delivery systems are retained within the stomach due to their size which is larger than the diameter of the pyloric sphincter, thereby inhibiting its transport into the intestine. When developing an expanding drug delivery system, there are a few criteria which should be met (Klausner et al., 2003; Bardonnnet et al., 2006; Gröning et al., 2007). These criteria include: a) the delivery system should be small enough and convenient to swallow, b) it should expand rapidly to an effective size so as to prevent premature evacuation from the stomach and c) the delivery system should degrade in order to prevent a luminal blockage. It has been suggested that the minimum size required in order to be retained within the stomach is a length of at least 5cm or a diameter of 3cm (Klausner et al., 2003). Expanding gastroretentive drug delivery systems have been utilized by the veterinary sector for numerous years and was first developed by Laby in 1974 for the prevention of bloat in ruminants. Consequent to that, much more extensive research was conducted within the veterinary sector (Brewer and Griffin, 1980; Griffin and Brewer, 1981).

Expansion of the delivery systems is achieved by two mechanisms namely swelling and unfolding. Both these mechanisms result in an increase in size which inhibits the passage of the delivery system through the pyloric sphincter into the intestine. Swelling occurs due to the absorption of water, usually by osmosis, whereas unfolding occurs due to the mechanical shape memory of the pharmaceutical carrier.

Swelling systems demonstrate an additional benefit over other gastroretentive delivery systems, due to the bulk of the system located within the stomach; a “fed” state is maintained, resulting in the suppression of housekeeper waves, offering a prolonged period of gastric retention (Bardonnnet et al., 2006). Mamajek and Moyer (1980) patented a drug reservoir surrounded by an elastic and swellable membrane that is permeable to the drug and body fluids, thereby controlling the rate of release of the drug. Due to the absorption of fluids causing an osmotic pressure within the drug reservoir, the drug was released. Due to the loss of the drug and expanding agent the size eventually decreases, allowing its evacuation.

Urquhart and Theeuwes (1984) developed a system consisting of tiny wax coated tablets, controlling the rate of drug release. The tablets were then dispersed within a polymeric hydrogel. In the stomach, the system swelled up to 50 times its original volume, allowing the drug to release from the tiny tablets. The system left the stomach after hydrolysis and bioerosion had occurred.

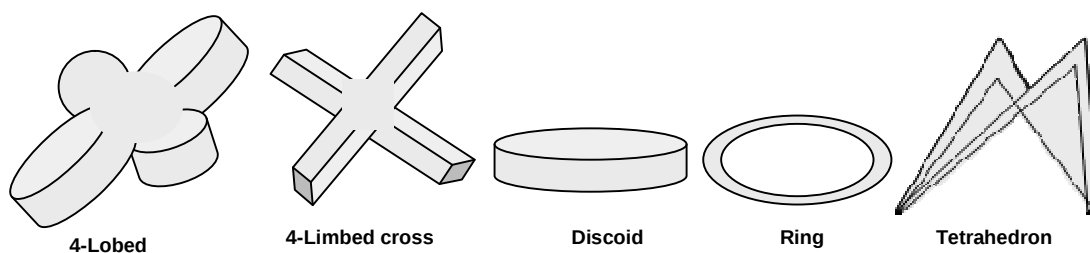
Gröning et al. (2007) developed a swelling gastroretentive delivery system based on the compression of a drug-loaded collagen sponge. The collagen had been initially freeze-dried, resulting in a porous and spongy structure. The collagen was then compressed using both a pneumatic pump and a tablet press. The surface of the collagen was coated with a thin layer of magnesium stearate, allowing the delivery system to remain stable when in contact with saliva. On contact with the gastric fluids, the tablet expanded to almost its original size. Both the rate and extent of expansion is pH dependant. Although the final volume was slightly smaller in an acidic environment, the expansion occurred at a much faster rate. *In vivo* studies demonstrated that the duration of gastric residence was extended and disintegration was observed after 180 minutes.

Edgren et al. (2004) patented a multiple layered delivery system formed from polymers which swell in the presence of gastric fluids. The rigidity of the system is maintained by covering a portion of the matrix with a band of insoluble material, thereby preventing that specific portion from swelling.

Shell and Louie-Helm (1999) developed a method of incorporating drug loaded liposomes and nanoparticles, or enteric coated drug particles into a swellable/erodible polymer, which can be made into a tablet or capsule. On contact with gastric fluids, the capsule or tablet disintegrates, allowing the polymer to swell. Erosion occurs over an extended period of time, releasing the drug in a controlled manner.

Unfolding systems should demonstrate specific properties in order to be effective and non-hazardous to the patient. These properties include: resistance to forces within the stomach; allowance for unhindered passage of food through the stomach while in residence; expansion to a circumference of greater than 5cm and flexible enough not to puncture the gastrointestinal wall (Curatolo et al., 1995; Klausner et al., 2003).

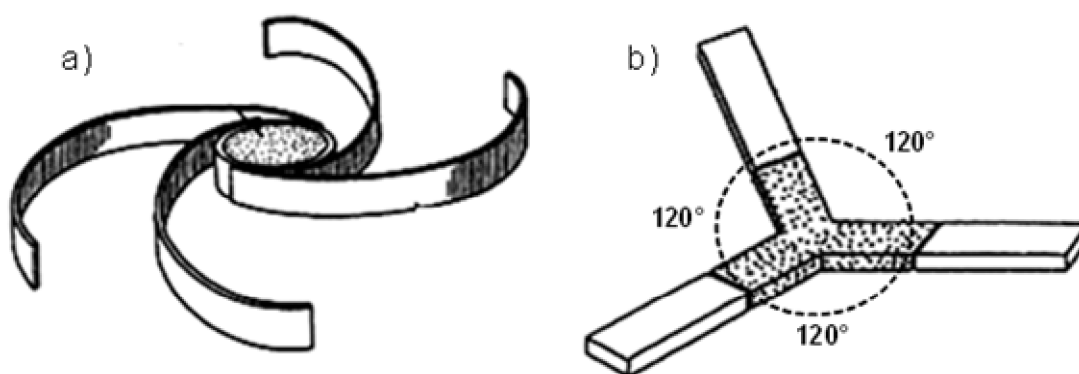
Caldwell et al. (1988) proposed and evaluated different geometric shapes of bioerodible polymers which could be compressed within a tablet or folded into a capsule. These shapes included the tetrahedron, ring or planar membrane (Figure 2.9).



**Figure 2.9:** Different geometric forms of unfoldable systems (Source: *Bardonnnet et al. 2006*).

Krumme (2004) has patented a triangular shaped drug delivery system with an internal drug compartment. When the device comes into contact with gastric fluid, it unfolds and its shape prevents passage through the pylorus. Asmussen et al. (2001) formulated an expanding drug delivery system, comprising multiparticulate drug dispersed within the expandable component, and covered with a polymer, creating a membrane which is permeable to gastric juice and the active compounds. Kagan et al. (2006) developed an Accordion Pill™ which comprises three layers, a central layer providing the frame sandwiched between two envelope membranes, and the drug reservoir in the centre. *In vivo* studies demonstrated that the concurrent intake of a high calorie meal was not necessary for this particular delivery system. This is a huge accomplishment as the retention times of gastroretentive drug delivery systems are detrimentally affected by the fasted state.

Sonobe et al. (1991) developed an unfolding “Y” system demonstrating prolonged shape memory (Figure 2.10) in which the centre of the “Y” was comprised of a polymer which was able to maintain its shape memory for an extended period of time, while the limbs of the “Y” consisted of a drug-loaded erodible material, the rate of erosion of which determines the residence time. A third component provided the link between the centre and the limbs. Curatolo et al. (1995) developed a spiral configuration comprising four short shape memory arms arranged concentrically around a tablet. As seen in Figure 2.10, a shape memory material (a), which assures unfolding, is connected to the erodible material which serves as a drug reservoir (b) and whose rate of degradation controls the gastric retention time. Both of these systems achieved a gastric retention of longer than 24 hours in *in vivo* animal studies.



**Figure 2.10:** Diagrammatic representation of: a) The spiral configuration unfolding system developed by Curatolo et al., 2005, and b) The unfolding 'Y' system developed by Sonobe et al., 1991 (Source: Klausner et al., 2003).

Gastroretentive delivery systems are currently in use as medical devices within Europe for the treatment of overweight patients. This indication is due to the bulk effect within the stomach, resulting in a feeling of satiety and reduced hunger (Gröning et al., 2007).

Despite the interest that expandable systems have attracted, there are still a few drawbacks associated with the unfolding systems which have to be considered. The most important is that this type of system is difficult to manufacture and is not cost-effective. Furthermore, the mechanical shape memory of the polymers is relatively short-lived and due to the prolonged storage of the drug delivery systems, the efficacy of the unfolding process may be compromised (Bardonnnet et al., 2006).

With reference to the swelling systems, commercialization of the technology is considerably simpler, and stands a better chance of entering the clinical setting (Klausner et al., 2003).

#### **2.4.6. Superporous hydrogel**

The superporous hydrogel is a novel expanding system which is currently being researched by Chen et al. (2000). It is due to the unique characteristics associated with these superporous hydrogels that they are classified as a new mechanism of gastric retention. The superporous hydrogel, when dried, contains open pores which form capillary channels. It is due to these open pore channels within the dehydrated hydrogel, through which water is rapidly absorbed which allows swelling to take place within a few minutes, up to a few hundred times its original volume. The most unique aspect of the superporous hydrogel is that the average pore size is usually in the range of a few hundred micrometers. On hydration, water is taken up by capillary

wetting as opposed to diffusion. In order to increase the mechanical strength of the hydrogels to withstand peristaltic pressure, the superporous hydrogel composites were synthesized by adding croscarmellose sodium (Ac-Di-Sol®; FMC Biopolymer). Superporous hydrogels can be divided into two groups, which are differentiated by their swelling ratio and their mechanical stability. A superporous hydrogel or SPH is a soft polymer which swells very quickly, but has poor mechanical stability, whereas a superporous hydrogel composite (SPHC) has a slower swelling rate, but is mechanically stable. The SPHC is therefore utilized as a retentive drug delivery system (Dorkoosh et al; 2004).

Through the incorporation of biodegradable crosslinkers, the superporous hydrogel will degrade in the body, thus preventing obstructions within the gastrointestinal tract. *In vivo* animal studies demonstrate that the superporous hydrogel remained within the stomach for more than 24 hours after feeding. After about 30 hours there was evidence of fragmentation and the delivery system was cleared from the stomach (Chen et al., 2000).

#### **2.4.7. Naturally or pharmaceutically altering gastric emptying**

##### **2.4.7.1. Pharmaceutical**

A rather simple method to improve gastric retention involves the inclusion of either an excipient or pharmaceutical substance which possesses gastric motility retardation characteristics.

Stops et al. (2006) investigated the use of citric acid to prolong the gastric residence time of floating calcium alginate beads. It has been proposed that the intake of citric acid decreases duodenal pH to below 6, thereby halting gastric motility. A negative feedback system is thus activated to restore the pH, allowing gastric emptying to recommence (Hunt and Knox, 1962). *In vivo* studies demonstrated that citric acid effectively delayed gastric emptying in the fasted state.

Osinski et al. (2002) conducted studies on the gastric emptying rate of mice. It was observed that metaclopramide and bethanechol display an increase in gastric emptying, while propantheline, an anticholinergic compound and CRF (corticotrophin releasing factor), a hypothalamic hormone, resulted in increases in gastric emptying time. Marathe et al. (2000) conducted human studies in order to determine the effect of metaclopramide and propantheline on the absorption of metformin. The results obtained suggested that metaclopramide did not affect the absorption of metformin as it resulted in a decrease in gastric emptying time, while pre-treatment with

propantheline actually resulted in an increased absorption due to the prolongation of gastric emptying time.

#### **2.4.7.2. Biological**

Certain dietary components, such as fats, peptides and some amino acids, possess the characteristic of prolonging gastric emptying and intestinal transit. This phenomenon is known as the ileal brake, which is believed to be a feedback process in order to improve digestion of dietary components.

Components from other biological species have been investigated for their ability to delay gastric and intestinal transit.

It is known that tapeworms decrease intestinal transit in hosts. For this reason, Kroening et al. (2003) investigated the effect of compounds excreted by tapeworms and concluded that cGMP is most likely responsible for this delay. It was therefore suggested that the addition of cGMP into pharmaceutical formulations may improve drug absorption.

Groups of researchers have investigated the use of plant lectins and purified fimbriae of *E. Coli* in order to increase bioadhesivity of microspheres. Studies conducted on both of these investigations have revealed supporting data (Illum and Ping, 1998; Montisci et al., 2001; Davis, 2005).

#### **2.4.8. Combinations of gastroretentive mechanisms**

Researchers have recently been investigating the possible advantages of combining more than one mechanism of gastroretention in order to achieve an additional enhancement and prolongation of gastric residence time (Chueh et al., 1995; Chavanpatil et al., 2006).

### **2.5. VISUALIZATION OF GASTRORETENTIVE DRUG DELIVERY SYSTEMS**

Visualization is a vital step in the development of a novel gastroretentive drug delivery system. Many methods have been used in order to clearly see the positioning and characteristics of gastroretentive systems, although the following four techniques may produce superior results:

### **2.5.1. Radio-labeling and $\gamma$ -scintigraphy**

A radioactive isotope which emits  $\gamma$ -rays is incorporated into the formulation (Klausner et al., 2003). The most commonly used radioisotope is technetium (Tc99m), which is prepared through the elution of pertechnetate (TcSO<sub>4</sub>-99m) with a 0.9% sodium chloride solution from a molybdenum-99 generator (Badve et al., 2007). The major advantages of using Tc99m are its short half-life of 6 hours, very low radiation dose and its easy availability in a sterile, pyrogen free and carrier free state (Ali et al., 2007). When the  $\gamma$ -scintigraphy is performed, the location of the delivery system can easily be observed.

### **2.5.2. Radiology**

Through the incorporation of radiopaque threads, such as barium sulphate (BaSO<sub>4</sub>), it is possible to determine the positioning and movement of delivery systems from x-rays taken at different time periods (Chen et al., 2000; Hoffman et al., 2004). Radiology is commonly used in preclinical trials due to its simplicity and cost effectiveness. However, due to health risks from high levels of exposure, its use has become limited and  $\gamma$ -scintigraphy may be preferred (Klausner et al., 2003).

### **2.5.3. Magnetic resonance imaging (MRI)**

MRI's may be performed in order to improve the visualization of delivery systems within the stomach. These scans are normally done in the supine position, and scans are taken in both the axial and coronal planes (Kagan et al., 2006). Sequential images may assist in the determination of gastric retention.

### **2.5.4. Multichannel superconducting quantum interference device (SQUID)**

Newer, non-invasive and radiation free methods, known as biomagnetic techniques have been developed for the evaluation of delivery systems. Multichannel superconducting quantum interference device (SQUID) devices measure the magnetic field of an ingested delivery system which is magnetically marked. Although the SQUID has expensive operating costs, it is designed to detect extremely weak biomagnetic fields, in a magnetically shielded environment (Cora' et al., 2007).

### **2.5.5. Alternate current biosusceptometry (ACB)**

A new promising technique, the alternate current biosusceptometry (ACB), has shown accuracy in the evaluation of physiological properties of the GI tract. Induction coils are used to record the magnetic flux variation obtained by the response of an ingested

magnetic material (ferrite— $\text{MnFe}_2\text{O}_3$ ). Continuous improvements of the ACB has allowed for the gradual increase of sensitivity (Cora' et al., 2007).

## CHAPTER 3

### Development, Design and Optimization of Gastrospheres

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#### 3.1. INTRODUCTION

Metformin hydrochloride is a di-substituted biguanide (N-1,1-dimethylbiguanide) anti-hyperglycaemic agent used in the treatment of Type II non-insulin-dependent diabetes mellitus. It is highly water-soluble and has a relatively low bioavailability of 50% as well as a short, variable biological half-life of 0.9–2.6 hours. Gastrointestinal absorption is completed after 6 hours with peak plasma concentrations reached after 2–3 h (Corti et al., 2008; Porta et al., 2008). Initial doses start between 500mg administered twice a day or 850mg once a day. With a maximum dosage of 3g per day administered in divided doses (South African Medicines Formulary, 2010).

Alginate is a linear copolymer made up of  $\beta$ -D-mannuronic acid and  $\alpha$ -L-guluronic acid in different configurations (Tu et al., 2005; Xu et al., 2007). The carboxyl groups present on the alginate molecule is responsible for its pH-sensitive nature (Xu et al., 2007). Contact with a multivalent cation such as calcium results in instantaneous gelation (Tu et al., 2005). This gelation process can be explained through the egg-box model where the carboxylic acid groups of two adjacent alginate molecules are bound by the multivalent ion (Tu et al., 2005; Al-Kassas et al., 2007). Pectin, a naturally occurring polysaccharide, mainly consists of  $\alpha$ -(1,4)-galacturonic acid. The characteristics of pectin are highly dependent on its level of esterification and gels in the presence of particular multivalent ions such as calcium through the binding of galacturonic acid on adjacent chains (Itoh et al., 2007). This gelation process reduces hydration and results in a more stable molecule at low pH's (Wei et al., 2006).

Polyacrylic acid (PAA) is commonly used in drug delivery systems due to its biocompatibility, mucoadhesivity, unique properties and multifunctional nature (Khutoryanskiy, 2007; Jin et al., 2009). The mucoadhesive properties of PAA is due to hydrogen bonding with mucin in the gastrointestinal system (Jin et al., 2009). At low pH's, such as within the gastric region, carboxylic acid groups on the PAA chain are non-ionised, displaying the strongest mucoadhesion (Khutoryanskiy, 2007). Poly-lactic-co-glycolic acid (PLGA) is widely used in the area of pharmaceutical drug delivery due to its good biocompatibility, toxicity and biodegradation profiles (Mok and Park, 2008; Klose et al., 2010) and is one of very few synthetic polymers approved for human use (Klose et al., 2010).

The purpose of this chapter therefore, was to design and optimize a gastrofloatable and gastroadhesive multi-particulate drug delivery system for the delivery of model narrow absorption window drug, metformin, with the use of a Box-Behnken experimental design. Parameters which were evaluated include the yield, drug entrapment efficiency, *in vitro* drug release in simulated gastric fluid, buoyancy, gastroadhesion, swelling studies, surface area and porosity analysis as well as an investigation of transitions that might have occurred within the polymeric sphere.

## **3.2. MATERIALS AND METHODS**

### **3.2.1. Materials**

The following materials were all of analytical grade and used as received. The polymers used were Sodium alginate (ALG) (Protanal LF 10/60<sup>®</sup>; FMC BioPolymer, Drammen, Norway), pectin (PEC) (classic CU 701<sup>®</sup>; Herbstreith & Fox, Neuenbürg, Germany), polyacrylic acid (PAA) (Carbopol 974P NF<sup>®</sup>; Noveon, Cleveland, Ohio, USA) and poly lactic-co-glycolic acid (PLGA) (Resomer RG 858 S<sup>®</sup>) (Boehringer Ingelheim Pharma, Ingelheim, Germany). 1,1-Dimethylbiguanide hydrochloride (metformin) was purchased from Aldrich (St Louis, MO, USA). Calcium hydroxide was purchased from BDH Chemicals Ltd. (Poole, Dorset, UK) and dichloromethane which was used as a solvent (Merck Chemicals Ltd, Wadeville, Gauteng, South Africa). All other reagents used were of analytical grade and were employed as purchased.

### **3.2.2. Methods**

#### **3.2.2.1. Construction of a randomized Box-Behnken experimental design**

A randomized Box-Behnken statistical experimental design was constructed (Minitab<sup>®</sup> V15, Minitab Inc., PA, USA) in order to model the number of formulations required for optimization as well as to establish the interaction effects of the independent formulation variables on the physicochemical and physicomachanical properties of the gastrospheres. Experimental trials were performed on 15 statistically-derived formulations of various combinations. Alginate, pectin, PAA (1-2%<sup>w/v</sup>) and PLGA (0-2%<sup>w/v</sup>) were selected as the independent formulation variables, with alginate and pectin at a ratio of 1:1, and the Mean Dissolution Time at 12 hours ( $MDT_{12}$ ). The strength of gastroadhesion was selected as the formulation response. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses. Response surface plots were constructed to visually represent the influence of the polymeric concentrations on the metformin release dynamics from the crosslinked gastrospheres.

### 3.2.2.2. Preparation of gastrospheres

Gastrospheres were prepared by crosslinking and subsequent lyophilization using a combination of polymers in accordance with the Box-Behnken experimental design (Table 3.1).

**Table 3.1:** Box-Behnken design template with randomly generated formulations.

| <b>Gastrosphere Formulations</b> | <b>[Alginate] (%)</b> | <b>[Pectin] (%)</b> | <b>[PAA] (%)</b> | <b>[PLGA] (%)</b> |
|----------------------------------|-----------------------|---------------------|------------------|-------------------|
| 1                                | 1.0                   | 1.0                 | 2.0              | 1.0               |
| 2                                | 2.0                   | 2.0                 | 1.5              | 2.0               |
| 3                                | 1.0                   | 1.0                 | 1.5              | 2.0               |
| 4                                | 1.5                   | 1.5                 | 1.5              | 1.0               |
| 5                                | 1.5                   | 1.5                 | 2.0              | 0.0               |
| 6                                | 2.0                   | 2.0                 | 2.0              | 1.0               |
| 7                                | 1.0                   | 1.0                 | 1.5              | 0.0               |
| 8                                | 1.5                   | 1.5                 | 1.5              | 1.0               |
| 9                                | 1.5                   | 1.5                 | 1.0              | 0.0               |
| 10                               | 2.0                   | 2.0                 | 1.0              | 1.0               |
| 11                               | 1.5                   | 1.5                 | 1.0              | 2.0               |
| 12                               | 1.5                   | 1.5                 | 1.5              | 1.0               |
| 13                               | 1.5                   | 1.5                 | 2.0              | 2.0               |
| 14                               | 1.0                   | 1.0                 | 1.0              | 1.0               |
| 15                               | 2.0                   | 2.0                 | 1.5              | 0.0               |

Fifteen polymeric solutions of alginate, pectin, PAA and metformin were dissolved in 100mL water, and PLGA was dissolved in 5mL dichloromethane. Both polymeric solutions were combined and allowed to stir for 1 hour until a homogenous state was achieved. The combined polymeric solution was injected drop wise into a crosslinking solution of 2%<sup>w/v</sup> calcium hydroxide (500mL). Gastrospheres were allowed to cure for 20 minutes, after which they were removed from the crosslinking solution, washed three times with 500mL deionized water and frozen at -72°C for 24 hours. Frozen gastrospheres were lyophilized (Labconco, Missouri, USA) with a 2 hour condensation phase at -60°C and a 24 hour sublimation phase at 25 mmHg.

### 3.2.2.3. Gastrosphere yield and drug entrapment efficiency

The yield of gastrospheres was determined by measuring the dry weight of the formed gastrospheres and comparing it to the weight of the initial dry formulation components. Drug entrapment efficiency (DEE) studies were performed by stirring ground gastrospheres in 100mL phosphate buffer solution (PBS) (pH 7.6, 37°C). Thereafter, metformin content was determined in triplicate using ultraviolet spectroscopy (CE 3021, Cecil Instruments, Cambridge, England) at the wavelength maximum of 241nm. The DEE was calculated utilizing Equation 3.1 (Streubel et al., 2002).

$$DEE = \frac{\text{Actual amount of metformin}}{\text{Theoretical amount of metformin}} \times 100$$

[Equation 3.1]

### 3.2.2.4. *In vitro* analysis of the drug release from the Gastrospheres

Drug release studies were conducted employing the USP 35 apparatus 2 dissolution test approach (Erweka DT 700, Heusenstamm, Germany). A modification to the approach was made by immersing the samples under a ring-mesh assembly (Pillay and Fassihi, 1999) in 900mL simulated gastric fluid (SGF) (pH 1.2, 37°C) at a rotation speed of 50rpm. SGF was prepared according to USP 32, 2008. 2g sodium chloride (NaCl) was dissolved in 100mL deionized water. 7mL of concentrated HCl was then added to the solution to result in a solution pH of 1.2. Samples of 5 mL were removed at predetermined time intervals and filtered through a 0.45µm Millipore Millex filter (Billerica, Massachusetts, USA). Equal volumes of fresh drug-free SGF were replaced in order to maintain sink conditions. Samples were then analyzed with UV spectroscopy at 241nm. All experiments were conducted in triplicate.

The release data was subjected to a model-independent analysis known as the time-point approach. Briefly, the Mean Dissolution Time set at 12 hours ( $MDT_{12}$ ) for each formulation was calculated. The application of the  $MDT_{12}$  approach provided a more precise analysis of the metformin release performance for comparison of several release data sets. Equation 3.2 was employed in this regard (Hopfenberg and Hsu, 1978; Pillay and Fassihi, 1999).

$$MDT = \sum_{i=1}^n t_i \frac{M_i}{M_{\infty}} \quad \text{[Equation 3.2]}$$

Where  $M_i$  is the fraction of dose released in time  $t_i = (t_i + t_{i-1}) / 2$  and  $M_{\infty}$  corresponds to the loading dose.

### 3.2.2.5. Analysis of the buoyancy of gastrospheres

A total number of 50 gastrospheres of each formulation were immersed in 100mL SGF (pH 1.2; 37°C) and then placed in an orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd., Hahistadrut, Holon, Israel) for 12 hours. Each sample was observed at predetermined time intervals while noting the number of spheres that were/were not buoyant. All experiments were conducted in triplicate.

### 3.2.2.6. Determination of the gastroadhesion of spheres

Gastrospheres were immersed in SGF (pH 1.2, 37°C) for predetermined time periods. Adhesion was measured using a texture analyzer (TA.XT.*plus*, StableMicroSystems, Surrey, UK) with a simulated gastric membrane covering both the probe and stage platform. Samples were tested using an applied force of 2N, a trigger force of 0.05N and a contact period of 15 seconds. Adhesion was determined by measuring the force required to separate the gastrosphere from the membrane, termed the detachment force. This detachment force was determined in terms of the work of adhesion which was obtained by calculating the area under the curve (AUC) of the Force-Distance textural profile. All experiments in this study were conducted in triplicate.

### 3.2.2.7. Evaluation of the hydration of gastrospheres

A total number of 50 spheres of each formulation were weighed and immersed in 100mL SGF (pH 1.2; 37°C) and placed in an orbital shaker incubator for 12 hours. The spheres of each formulation were removed at predetermined time intervals, blotted with filter paper to remove excess SGF and weighed. All experiments were conducted in triplicate. The swelling characteristics of the gastrospheres were expressed in terms of water uptake (Chavanpatil et al., 2006) using Equation 3.3.

$$\text{Water uptake} = \frac{\text{Swollen mass} - \text{Dry mass}}{\text{Dry mass}} \times 100 \quad [\text{Equation 3.3}]$$

### 3.2.2.8. Constraint optimization of formulation responses

A model-independent approach (Minitab® V15, Minitab Inc., Pennsylvania USA) was used to optimize the lyophilized gastrospheres. Statistical optimization was therefore employed to ascertain the ideal polymeric combination with the desired physicochemical properties capable of attaining optimum gastroadhesive strength and a predicted  $MDT_{12}$  value which would conform to zero-order kinetics over 12 hours.

### 3.2.2.9. Fourier Transform Infrared analysis

FTIR spectroscopy was performed on the crosslinked gastrospheres and its constituent polymers. Samples were scanned over a wave number range of 4000 to 650 $\text{cm}^{-1}$  using a Perkin Elmer FTIR spectrometer with a MIRTGS detector, (PerkinElmer Spectrum 100, Llantrisant, Wales, UK). The spectrum was at a ratio of 16 sample scans against 16 background scans. Samples were placed on a diamond crystal and processed by universal ATR polarization accessory for the FTIR spectrum series, at a resolution of 4 $\text{cm}^{-1}$ .

### 3.2.2.10. Analysis of surface area and porosity

Surface area and porosity analysis was conducted using the Micromeritics ASAP Analyzer (Micromeritics ASAP 2020, Georgia, USA). Samples were initially subjected to degassing to remove surface moisture and gas particles prior to analysis. Degassing encompassed an evacuation phase and a heating phase. The respective parameters are shown in Table 3.2.

**Table 3.2:** Evacuation and heating phase parameters during degassing.

| Parameter                    | Rate/Target         |
|------------------------------|---------------------|
| <b>Evacuation Phase</b>      |                     |
| Temperature ramp rate        | 10°C/min            |
| Target temperature           | 90°C                |
| Evacuation rate              | 50.0mmHg/s          |
| Unrestricted evacuation from | 30mmHg              |
| Vacuum set point             | 500 $\mu\text{mHg}$ |
| Evacuation time              | 60 min              |
| <b>Heating Phase</b>         |                     |
| Temperature ramp rate        | 10°C/min            |
| Hold temperature             | 120°C               |
| Hold time                    | 900 min             |

Each polymer matrix was weighed and inserted into the sample tube. Subsequently, a glass filler rod was inserted into the sample tube to decrease the total free space within the tube thus allowing a reduction in the time required for complete degassing to occur. The time required for degassing to be completed ranged from 7 to 9 hours. After being completely degassed, the sample tube was removed, covered with a thermal jacket and transferred to the analysis port where it was cooled with liquid nitrogen prior to the analysis.

#### **3.2.2.11. Analysis of the surface morphology of the gastrospheres**

Scanning Electron Microscope (SEM) analysis was carried out using a Phenom™ scanning electron microscope (FEI Company, OR, USA). Samples were made electrically conductive prior to analysis through the process of gold-sputter coating (SPI Module™ Sputter Coater, SPI Supplies, PA, USA).

Samples were attached to an SEM stub using adhesive carbon tape. The stub was inserted into the stub holder thereafter putting the glass chamber and sputter head in place. Argon gas was allowed to flush the system before the leak valve was sealed and the vacuum was turned on. The sputter coater was turned on for 90 seconds when plasma current reached 18mA, after which the system was turned off and the vacuum released.

#### **3.2.2.12. Molecular mechanics simulations**

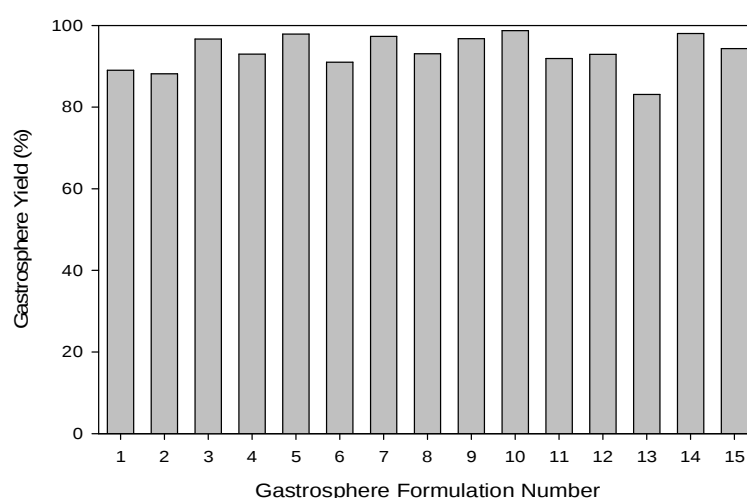
Molecular Mechanics Computations in vacuum, which included the model building of the energy-minimized structures of multi-polymer complexes, were performed using the HyperChem™ 8.0.8 Molecular Modeling System (Hypercube Inc., Gainesville, Florida, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK) on an HP Pavilion dv5 Pentium Dual CPU T3200 workstation. The decamers of PAA and PLGA were generated as 3D models from standard bond lengths and angles employing polymer builder tools using ChemBio3D Ultra in their syndiotactic stereochemistry whereas the structures of alginate and pectin (ten oligosaccharide units each) were generated using sugar builder module on HyperChem 8.0.8. The structure of glycosylated gastric mucopeptide analogue (MUC) was generated on sequence editor module on HyperChem 8.0.8. The glycosylation was carried out at the threonine and serine amino acid residues. The generation of the overall steric energy associated with the energy-minimized structures was initially executed 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 polymer-polymer and polymer-mucin 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: ALG-Pec, ALG-Pec-Ca<sup>2+</sup>, ALG-Pec-PAA, ALG-Pec-PAA-Ca<sup>2+</sup>, ALG-Pec-PAA-PLGA, ALG-Pec-PAA-PLGA-Ca<sup>2+</sup>, MUC-Polymers and MUC-Polymers- Ca<sup>2+</sup>. 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 the following: electrostatic 0.5 and van der Waals 0.5.

### 3.3. RESULTS AND DISCUSSION

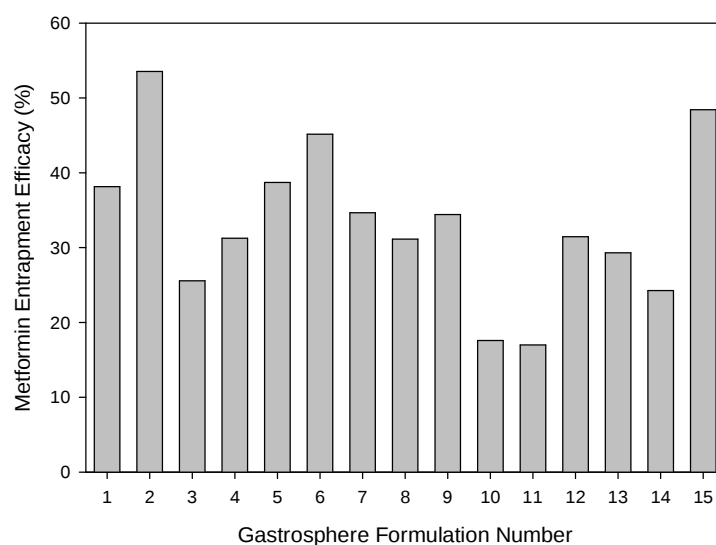
#### 3.3.1. Determination of gastrosphere yield and entrapment efficiency

Gastrosphere yield values obtained from the 15 formulations ranged between 83 and 98%, with an average of 93.5%. Figure 3.1 provides the results obtained for each formulation.



**Figure 3.1:** Gastrosphere yield of various crosslinked gastrospheres formulated as per the statistical experimental design template generated (N=3, SD<9 in all cases).

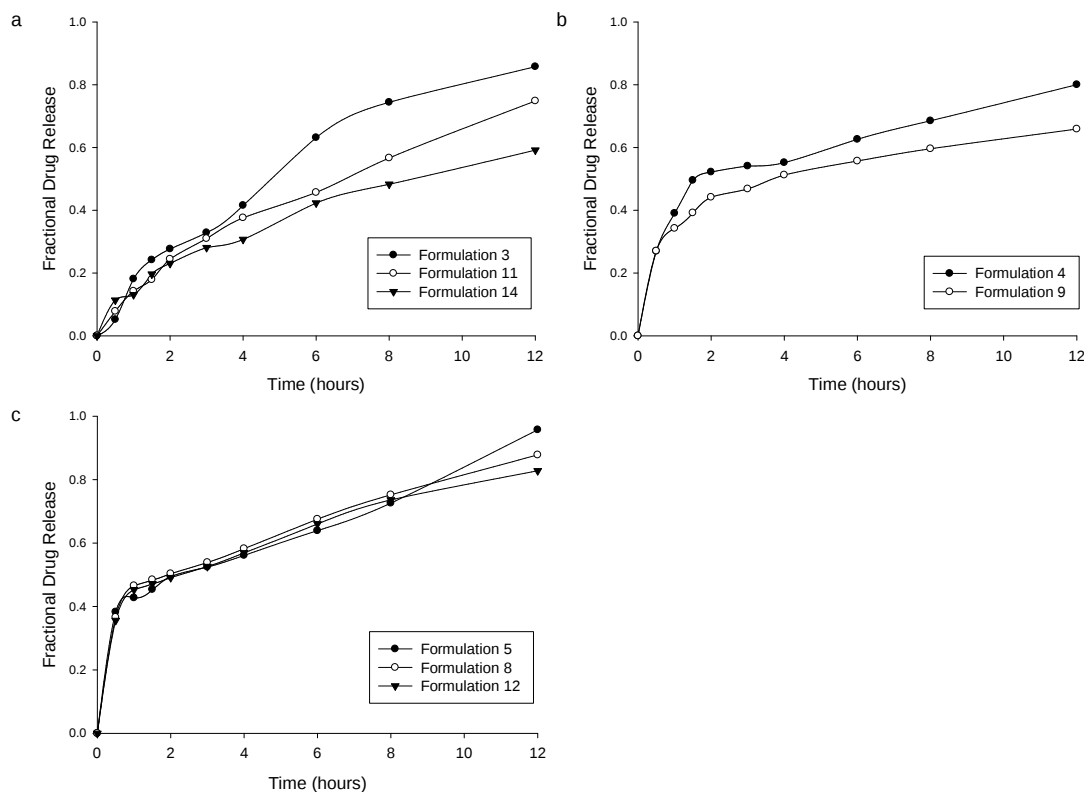
Entrapment efficiency values of metformin within the crosslinked gastrospheres ranged from 18 to 54%. Figure 3.2 provides the drug entrapment efficacy values obtained for each formulation. The rather low entrapment efficiency values are due to drug loss during the crosslinking step, which is exacerbated due to the high water solubility of metformin.



**Figure 3.2:** Drug entrapment efficiency of metformin within various crosslinked gastrospheres formulated as per the statistical experimental design template generated (N=3, SD<13 in all cases).

### 3.3.2. *In vitro* drug release from the Gastrospheres

Due to the intended purpose of this drug delivery system, zero-order drug release is the ideal drug release pattern. Three profiles of drug release were achieved, namely near zero-order release, first order release and burst release.



**Figure 3.3:** Drug release profiles. a) Pseudo zero-order drug release; b) First-order drug release; and c) Burst release of metformin from gastrospheres in SHGF (pH 1.2; 37°C) (N=3, SD≤0.075 in all cases).

Figure 3.3(a) shows that formulations 3, 11 and 13 displayed pseudo zero-order drug release over the 12 hour period, resulting in 85%, 75% and 60% final drug release respectively. In order to achieve a constant plasma metformin concentration over the entire 12 hour period and to avoid peaks and troughs which are commonly associated with side effects, zero order release is ideal.

First-order drug release was observed from formulations 4 and 9, with 80% and 66% final drug release as shown in Figure 3.3(b). Formulations 5, 8 and 12 (Figure 3.3c), displayed an initial burst release, followed by a zero-order release. More than 50% of the final drug concentration was released within the first hour, followed by a gradual zero-order release over the subsequent 11 hours. Although not ideal for our application, this profile may be appropriate for systems which may require a high loading dose and subsequent maintenance of plasma concentration.

In order to evaluate drug release, Mean Dissolution Time after 12 hours ( $MDT_{12}$ ) was employed. The application of the  $MDT_{12}$  approach provides a more precise analysis of the metformin release performance for comparison of several release data sets.

### **3.3.3. Analysis of gastrosphere buoyancy**

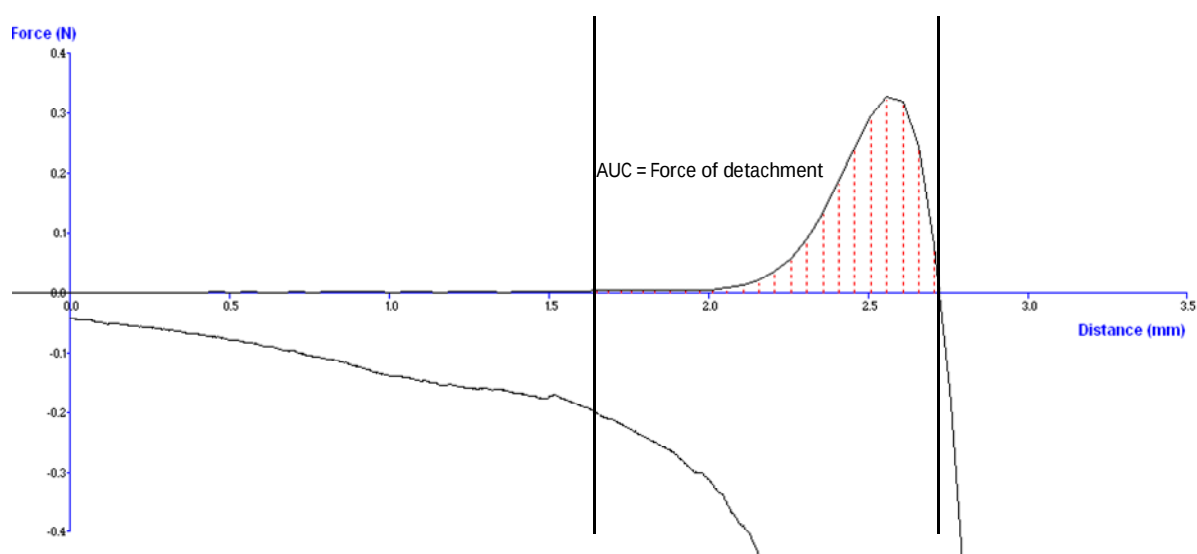
Excellent *in vitro* buoyancy was observed for all 15 formulations. The gastrospheres were immediately buoyant on contact with the SGF. Data obtained revealed that 99.0% of gastrospheres were buoyant after 8 hours. Buoyancy is slightly reduced to 97.5% after the full 12 hour period, although this reduction appears totally insignificant. The buoyancy of this system can be attributed to the low apparent density of the gastrospheres, resulting from the highly porous structure attained from the lyophilization process.

### **3.3.4. Determination of the gastroadhesive strength**

Gastroadhesion was found to be greatly variable between formulations due to the high sensitivity of PAA compared to other polymers. PLGA, due to its hydrophobic nature, inhibited the absorption of water, whereas PAA being hydrophilic resulted into the attraction of water molecules. The results indicated that both high PAA and PLGA concentrations were required for two distinctive purposes; PAA being highly bioadhesive maintained proper gastroadhesivity while PLGA inhibited the water uptake (by virtue of its hydrophobicity) so as to give room for PAA to facilitate adhesion over

the 12 hour period. The concentrations of pectin and alginate also played a significant role in facilitating the adhesion.

It was decided that two factors relating to mucoadhesion had to be taken into account when analysing the results. The area under the curve (AUC) of each formulation was calculated in order to analyse the data (i.e. to obtain the work of adhesion) as illustrated in Figure 3.4. In order to prevent an immediate passage of the gastrospheres from the stomach, the AUC from time 0 to 2 hours ( $T_{0-2}$ ) was calculated as the first factor. The second factor was determined by calculating the AUC from time 2 to 12 hours ( $T_{2-12}$ ), representing the ability to maintain adhesion throughout the full 12 hours. The optimum formulation must possess the greatest of both factors.

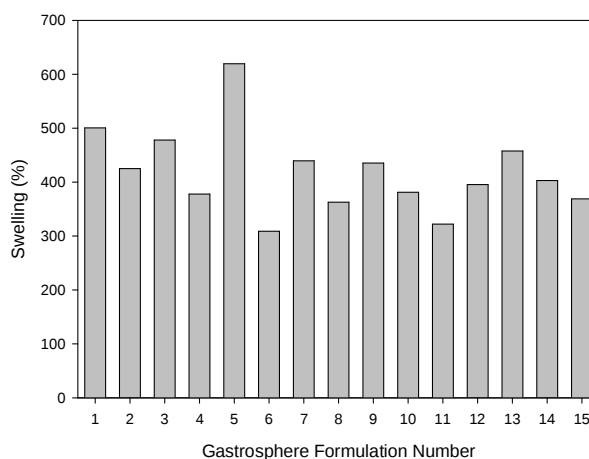


**Figure 3.4:** A typical textural profile illustrating the calculated area under the curve (AUC).

### 3.3.5. Calculation of water uptake and the swelling tendency of the gastrospheres

The degree of swelling for the polymers used has been determined by the water uptake capacity of the gastrospheres. Although the drug delivery system is intended as a twice daily dosage regime, it was found that optimal water uptake occurred at 8 hours, after which, there was a slight reduction in water uptake. Figure 3.5 depicts the effects that each polymer had on the swelling ability of the drug delivery system. It was evident that an increase in PLGA resulted in a reduction of swelling. This was most probably due to the hydrophobic nature of the polymer, reducing the overall attraction of water to the gastrospheres. Pectin and sodium alginate had a similar effect, where the degree of swelling was reduced with increasing concentrations. However, due the

high hydrophilic and bioadhesive nature of PAA, water molecules were strongly attracted to the gastrospheres, thus resulting in an increase in swelling with an increasing concentration.



**Figure 3.5:** Effect of various polymeric concentrations on swelling after 8 hours (N=3, SD<32 in all cases).

### 3.3.6. Response surface analysis

Response surface plots (Figures 3.7, 3.8 and 3.9) were obtained for the measured responses ( $MDT_{12}$ ,  $T_{0-2}$ ,  $T_{2-12}$ ) based on the experimental model, representing the functional relationship between the response and the experimental factors.

#### 3.3.6.1. Analysis of the Box-Behnken response surface design

$MDT_{12}$ ,  $T_{0-2}$  and  $T_{2-12}$  for the experimental formulations were included in the statistical design for the identification of the formulation possessing optimum drug release and gastroadhesion. Residual analysis for  $MDT_{12}$ ,  $T_{0-2}$  and  $T_{2-12}$  (Figures 3.6a, b and c respectively) generally showed random scatter, indicating that no trends were present; however, some grouping was observed for  $T_{0-2}$ . The normal probability plots of the residuals fell on a straight line, thus indicating that the data was normally distributed and there was a non-existence of unidentified variables.

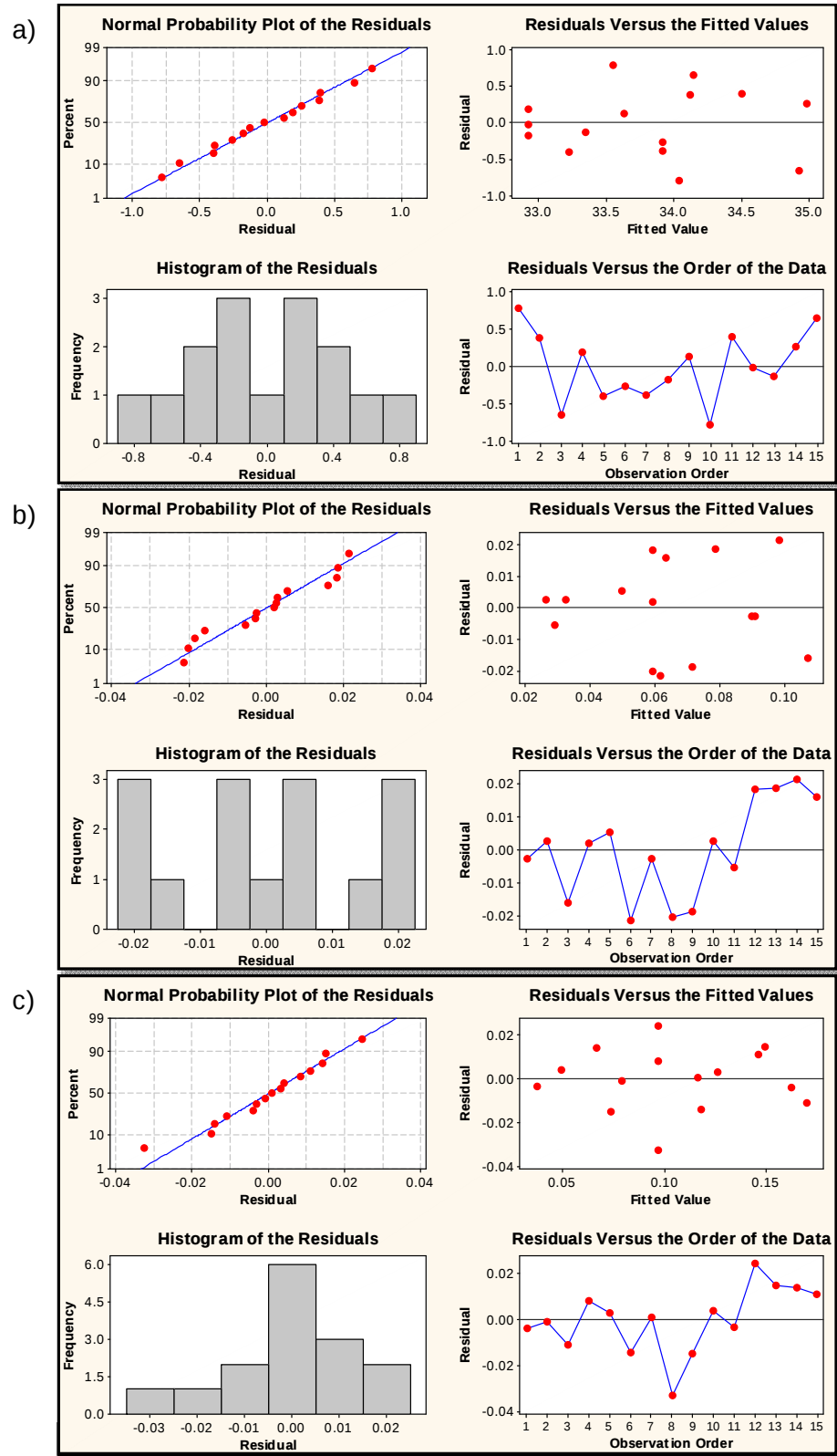


Figure 3.6: Residual Plots for: a)  $MDT_{12}$ ; b)  $T_{0-2}$ ; and c)  $T_{2-12}$ .

The complete regression equations generated for  $MDT_{12}$ ,  $T_{0-2}$  and  $T_{2-12}$  are indicated below:

$$MDT_{12} = 47.1094 - 12.5160[ALG] - 5.94507[PAA] + 0.679866[PLGA] + 3.59370[ALG*ALG] + 1.19402[PAA*PAA] + 0.452163[PLGA*PLGA] + 1.30763[ALG*PAA] - 0.513957[ALG*PLGA] - 0.377558[PAA*PLGA]$$

[Equation 3.4]

$$T_{0-2} = 0.323909 - 0.248121[ALG] - 0.0509750[PAA] - 0.0225549[PLGA] + 0.0524639[ALG*ALG] - 0.0116194[PAA*PAA] + 0.000949306[PLGA*PLGA] + 0.0428333[ALG*PAA] - 0.0241250 [ALG*PLGA] + 0.0355833[PAA*PLGA]$$

[Equation 3.5]

$$T_{2-12} = -0.0499556 - 0.130288[ALG] + 0.267579[PAA] + 0.0134806[PLGA] + 0.0669556[ALG*ALG] - 0.0580778[PAA*PAA] + 0.0142681[PLGA*PLGA] - 0.0273333[ALG*PAA] - 0.0603417[ALG*PLGA] + 0.0301417[PAA*PLGA]$$

[Equation 3.6]

### 3.3.6.2. Response surface analysis for Mean Dissolution Time

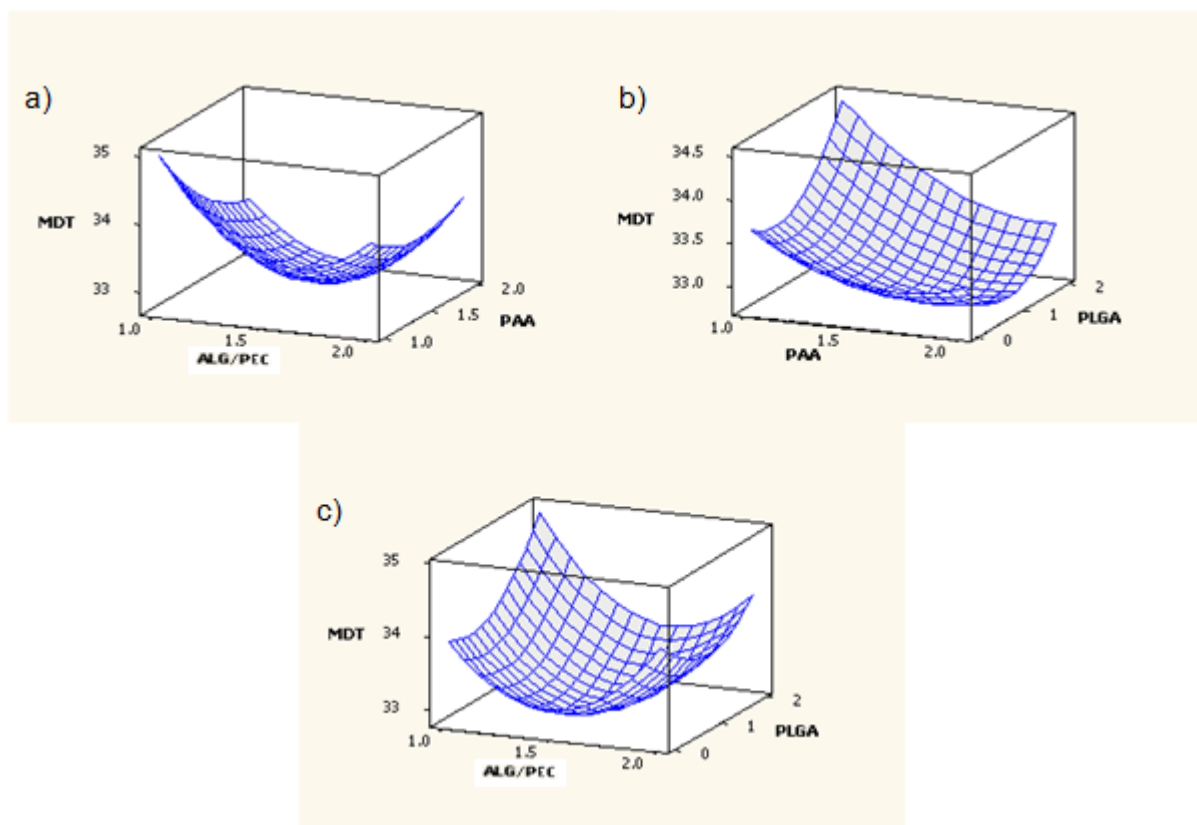
The effect of factors ALG/PEC and PAA at the midpoint of factor PLGA on response  $MDT_{12}$  is shown in Figure 3.7(a). At low levels of factor PAA,  $MDT_{12}$  was high and increasing the factor ALG/PEC from 1 to 1.5% resulted in a reduction of  $MDT_{12}$ , although as the factor was further increased from 1.5 to 2%, an increase in MDT was noted. At high levels of factor ALG/PEC,  $MDT_{12}$  was moderate. An initial increase in factor PAA from 1 to 1.5% resulted in a reduction of  $MDT_{12}$ , although further increasing the level of factor PAA from 1.5 to 2% resulted in the return of  $MDT_{12}$  to its original value.

The effect of factors PAA and PLGA at the midpoint of factor ALG/PEC on response  $MDT_{12}$  is shown in Figure 3.7(b). At low levels of PAA,  $MDT_{12}$  was moderate, increasing with an increase in factor PLGA. At high levels of PAA,  $MDT_{12}$  was low, reducing as PLGA was increased from 0 to 1%, and returning to the original value as PLGA was further increased to 2%.

The effect of factors ALG/PEC and PLGA at the midpoint of factor PAA on response  $MDT_{12}$  is shown in Figure 3.7(c). At low levels of ALG/PEC,  $MDT_{12}$  was moderate; increasing as factor PLGA was increased. At high values of factor ALG/PLGA, an

increase in factor PLGA from 0 to 1% resulted in a reduction of  $MDT_{12}$ , although increasing this factor further to 2% resulted in an increase of MDT, returning to its original value.

The effect of PLGA may be explained due to the fact that it is a lypophilic polymer which does not rapidly degrade or swell, obstructing the release of drug from the gastrospheres, thereby prolonging drug release.



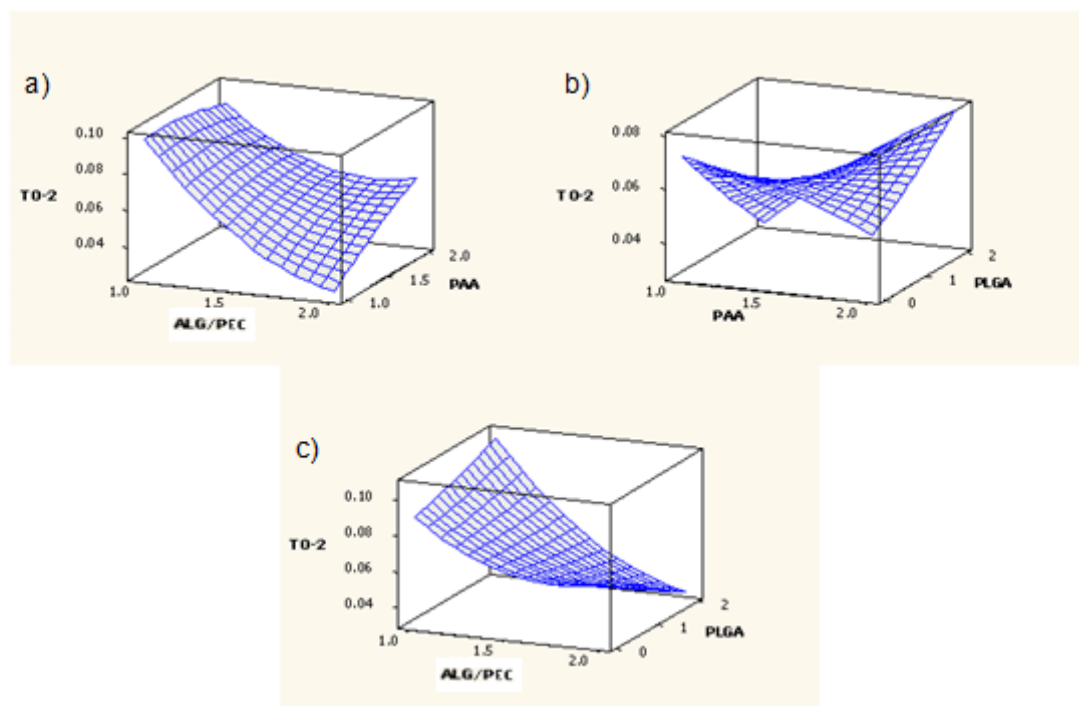
**Figure 3.7:** Response surface plots generated for  $MDT_{12}$ .

### 3.3.6.3. Response surface analysis for mucoadhesion from 0 to 2 hours

The effect of factors ALG/PEC and PAA at the midpoint of factor PLGA on response  $T_{0-2}$  is shown in Figure 3.8(a). At low levels of factor ALG/PEC,  $T_{0-2}$  was high. Increasing the level of factor PAA resulted in a slight reduction of  $T_{0-2}$ . At high levels of ALG/PEC, was  $T_{0-2}$  low, increasing with an increase in PAA.

The effect of factors PAA and PLGA at the midpoint of factor ALG/PEC on response  $T_{0-2}$  is shown in Figure 3.8(b). At low levels of PAA,  $T_{0-2}$  was high; decreasing as the level of factor PLGA was increased. However,  $T_{0-2}$  was low at high levels of PAA, increasing with an increase in the level of PLGA.

The effect of factors ALG/PEC and PLGA at the midpoint of factor PAA on response  $T_{0-2}$  is shown in Figure 3.8(c). At low levels of PLGA and ALG/PEC,  $T_{0-2}$  was high, decreasing with an increase in the level of ALG/PEC. At low levels of ALG/PEC,  $T_{0-2}$  was moderate, increasing as the level of PLGA increased, although at high levels of ALG/PEC,  $T_{0-2}$  decreased as the level of PLGA increased.



**Figure 3.8:** Response surface plots generated for  $T_{0-2}$ .

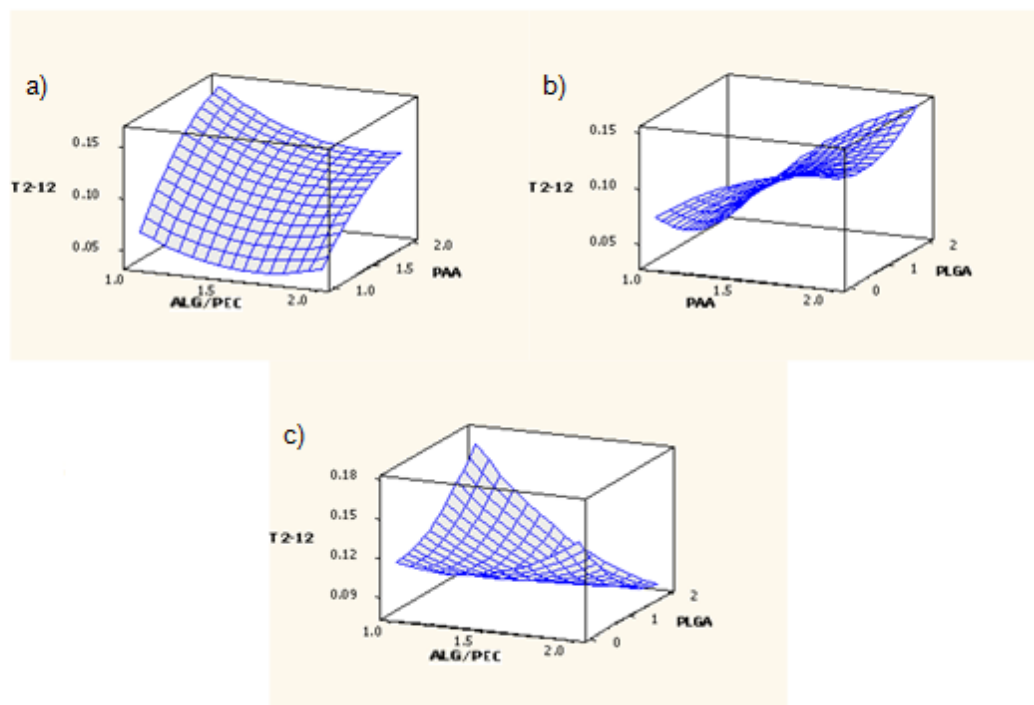
#### 3.3.6.4. Response Surface Analysis for Mucoadhesion from 2 to 12 hours

The effect of factors ALG/PEC and PAA at the midpoint of factor PLGA on response  $T_{2-12}$  is shown in Figure 3.9(a). At low levels of factor ALG/PEC,  $T_{2-12}$  was low, increasing with an increase in factor PAA. At high levels of ALG/PEC,  $T_{2-12}$  was also low and an increase in the levels of PAA had the same effect, although to a lesser degree.

The effect of factors PAA and PLGA at the midpoint of factor ALG/PEC on response  $T_{2-12}$  is shown in Figure 3.9(b). At low levels of factor PAA,  $T_{2-12}$  was low and was further reduced as the level of factor PLGA increased. At high levels of PAA,  $T_{2-12}$  was high, although a reduction in  $T_{2-12}$  was noted as the level of factor PLGA increased from 0 to 1%, followed by a subsequent increase as levels reached 2%.

The effect of factors ALG/PEC and PLGA at the midpoint of factor PAA on response  $T_{2-12}$  is shown in Figure 3.9(c). At low levels of ALG/PEC,  $T_{2-12}$  was moderate,

increasing as the level of PLGA increased. At high levels of ALG/PEC,  $T_{2-12}$  was increased slightly, although a reduction of  $T_{2-12}$  was observed with an increase in the level of factor PLGA.



**Figure 3.9:** Response surface plots generated for  $T_{2-12}$ .

### 3.3.7. Constraint optimization of formulation responses for the crosslinked gastrospheres

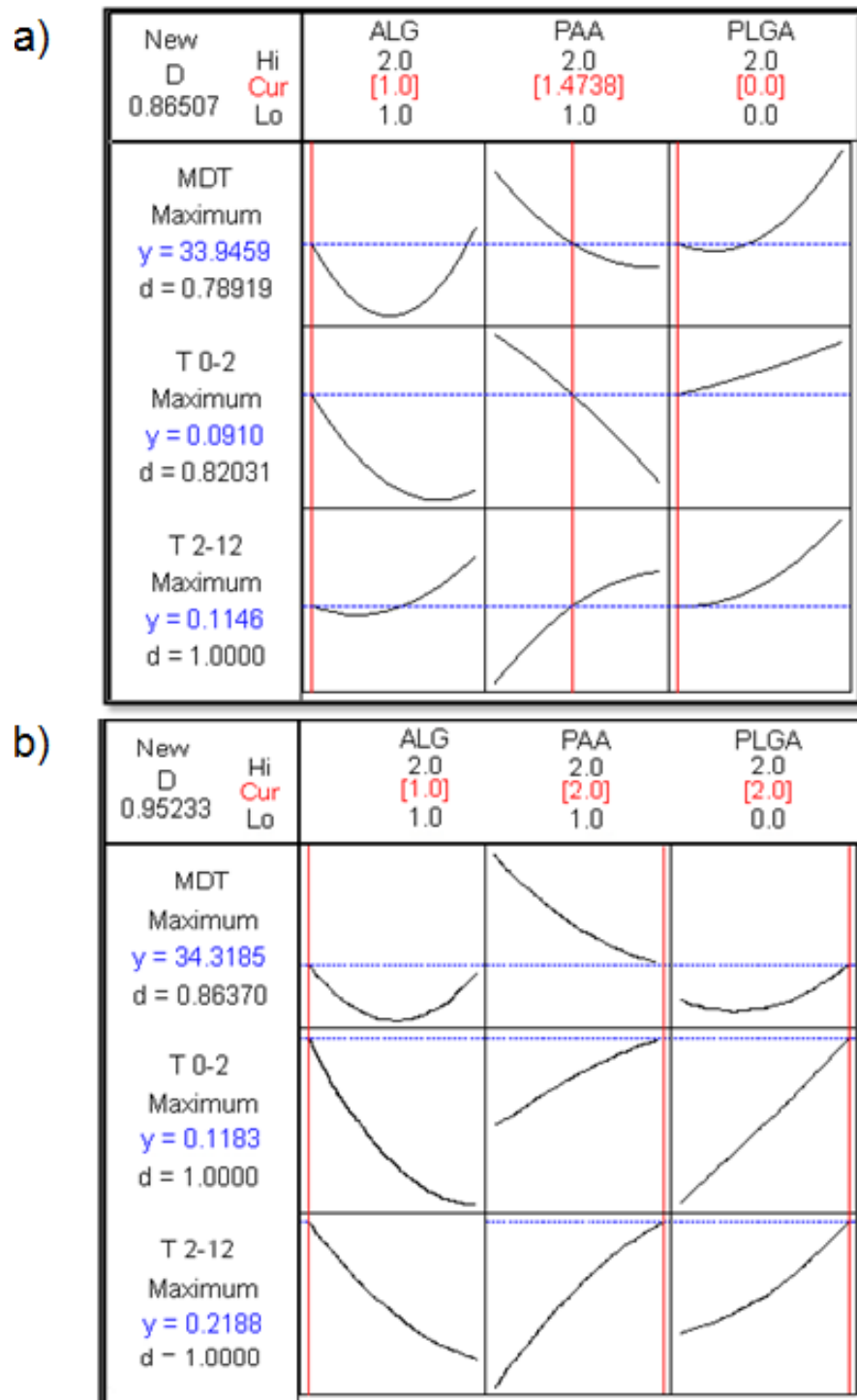
Minitab® V15 (Minitab Inc., California, USA) was used to optimize the formulation responses namely, the  $MDT_{12}$ , strength of adhesion after 2 hours ( $T_{0-2}$ ) and strength of adhesion from 2 to 12 hours ( $T_{2-12}$ ). These responses were selected due to the fundamental role they provide for the qualitative modeling of metformin release from the crosslinked gastrospheres. Optimal responses generated by the inherent optimization function of Minitab® are represented by the desirability plots in Figure 3.10.  $MDT_{12}$  was computed to converge to zero-order kinetics of metformin release from the optimized gastrosphere formulation.  $T_{0-2}$  and  $T_{2-12}$  were computed in a manner that would permit maximum gastroadhesion for both. The constraints that were imposed in order to achieve the desired responses are listed in Table 3.3. According to the predictions of the statistical design, the optimal gastrosphere that would permit a desirable  $MDT_{12}$  value of 34.833 (which is reflective of zero-order kinetics over 12 hours) and the greatest strength of adhesion for both the first 2 hours as well as the subsequent 10 hours would result in two possible formulations (Table 3.4).

**Table 3.3:** Formulation constraints employed for response optimization.

| <b>Variables</b> | <b>Limits</b>                    |
|------------------|----------------------------------|
| Alginate/Pectin  | 1-2% <sup>w</sup> / <sub>v</sub> |
| PAA              | 1-2% <sup>w</sup> / <sub>v</sub> |
| PLGA             | 0-2% <sup>w</sup> / <sub>v</sub> |
| $MDT_{12}$       | 34.883                           |
| $T_{0-2}$        | Maximum                          |
| $T_{2-12}$       | Maximum                          |

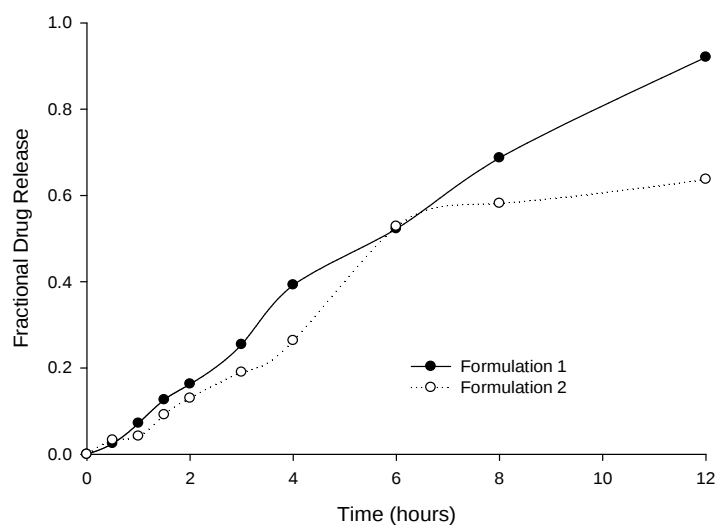
**Table 3.4:** Predicted optimized formulations obtained via the surface response method.

| <b>Gastrosphere Formulations</b> | <b>[Alginate] (%)</b> | <b>[Pectin] (%)</b> | <b>[PAA] (%)</b> | <b>[PLGA] (%)</b> |
|----------------------------------|-----------------------|---------------------|------------------|-------------------|
| 1                                | 1                     | 1                   | 2                | 2                 |
| 2                                | 1                     | 1                   | 1.478            | 0                 |



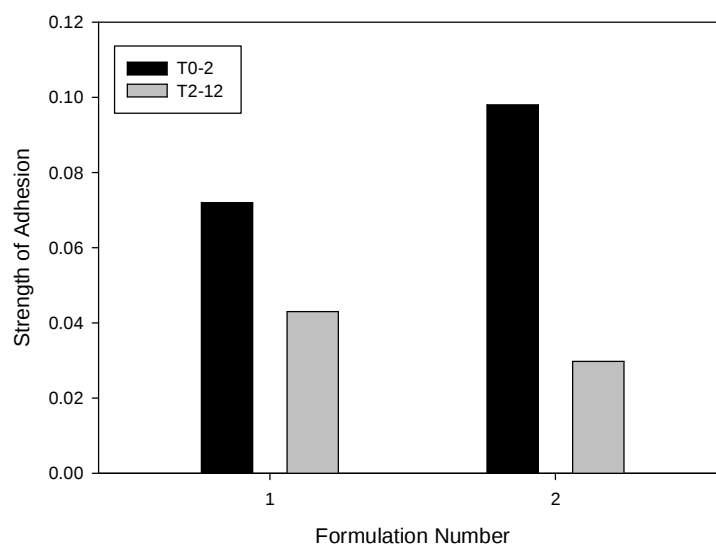
**Figure 3.10:** Optimization plots delineating factor settings and desirability values for optimal formulations: (a) F1; and (b) F2.

Drug release data obtained for the two optimized formulations are shown in Figure 3.11. It can be observed that the first formulation displayed a more preferable profile due to the higher final drug release of 92% as compared to the 63% obtained from the second formulation (Figure 3.11).



**Figure 3.11:** Fractional drug release obtained from the two optimized formulations (N=3, SD<0.12 in all cases).

From Figure 3.12 it is evident that F1 possesses a much greater strength of adhesion after both  $t_{0-2}$  and  $t_{2-12}$  when compared with F2.



**Figure 3.12:** Strength of adhesion of the two optimization formulations (N=3, SD<0.003 in all cases).

### 3.3.7.1. Experimental and predicted response values for the optimized formulations

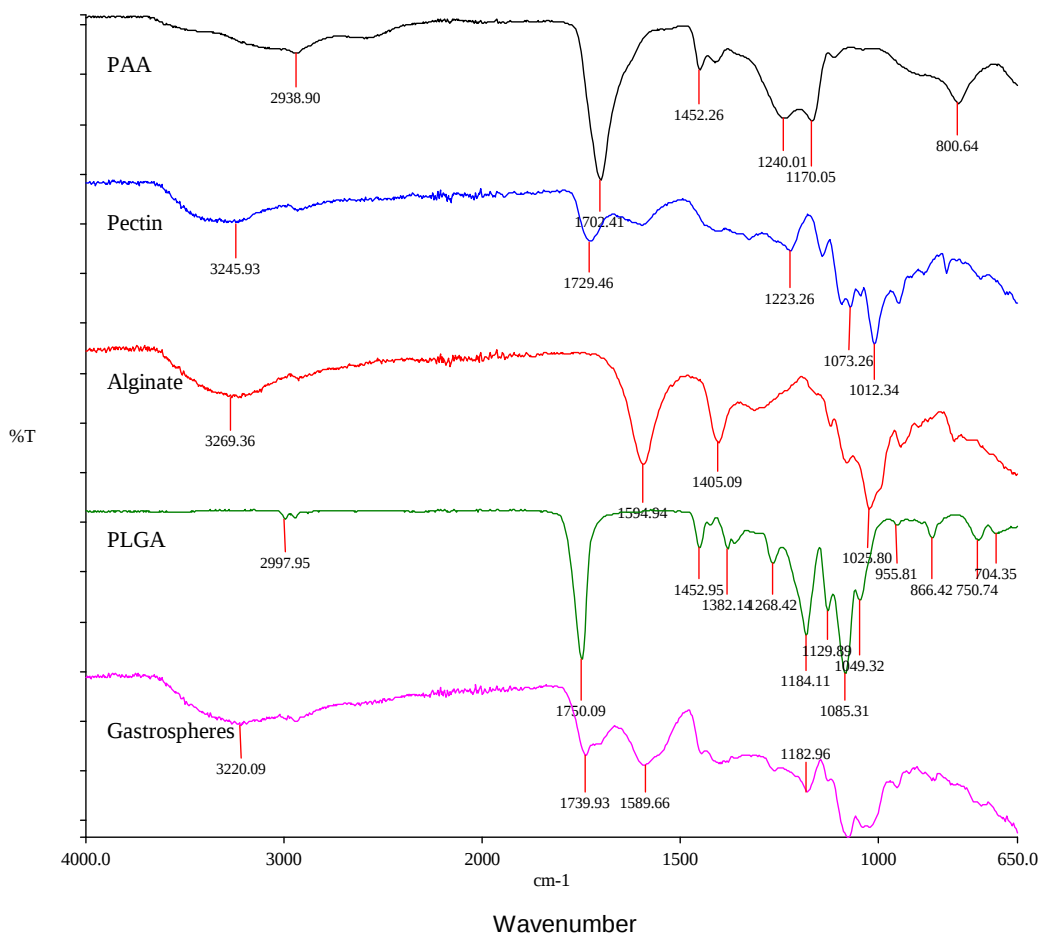
Both ideal formulations were prepared in accordance with the optimal predicted settings. The experimentally derived values for the  $MDT_{12}$ ,  $T_{0-2}$  and  $T_{2-12}$  of formulation F1 were in close agreement with the predicted values (Table 3.5), and is obviously a far superior formulation in comparison to formulation F2.

**Table 3.5:** Experimental and predicted response values for the optimized formulations.

| Measured Response | Formulation | Predicted Response | Actual Response | Desirability |
|-------------------|-------------|--------------------|-----------------|--------------|
| MDT <sub>12</sub> | F1          | 33.9459            | 32.3367         | 0.78919      |
|                   | F2          | 34.3185            | 33.81803        | 0.86370      |
| T <sub>0-2</sub>  | F1          | 0.0910             | 0.0723          | 0.82031      |
|                   | F2          | 0.1183             | 0.0436          | 1.00000      |
| T <sub>2-12</sub> | F1          | 0.1146             | 0.0985          | 1.00000      |
|                   | F2          | 0.2188             | 0.0297          | 1.00000      |

### 3.3.8. Characterisation of the gastrospheres by Fourier-Transform Infrared Spectroscopy

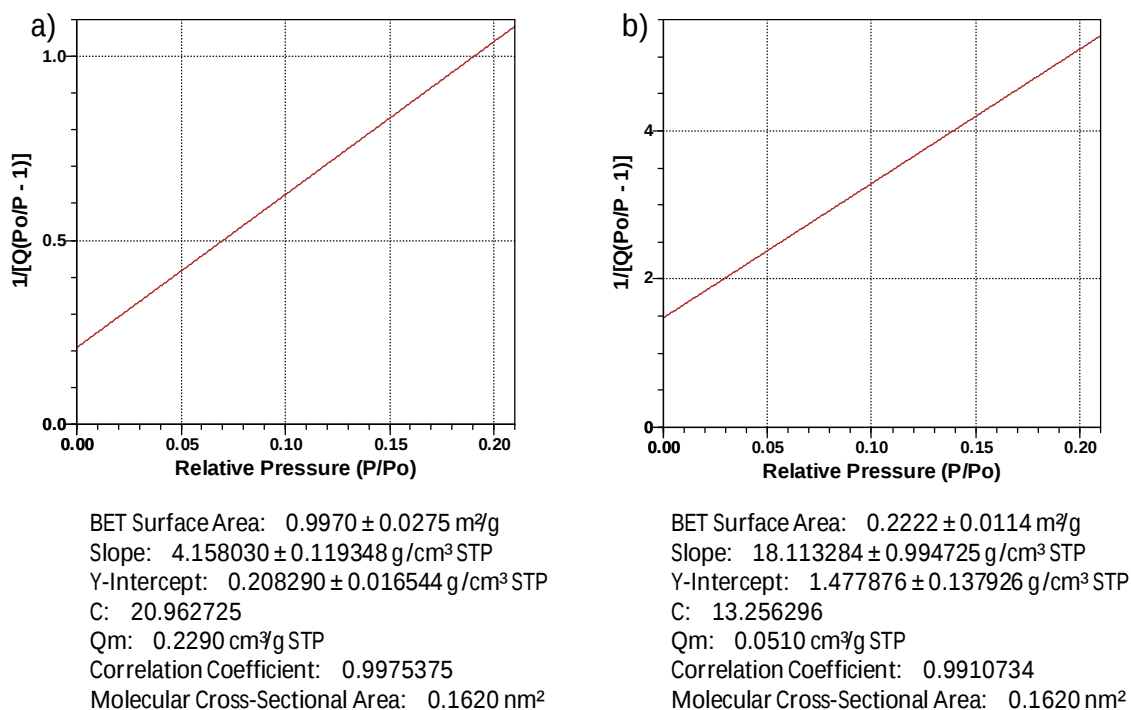
Figure 3.13 shows the FTIR spectra of each component polymer as well as that of the crosslinked gastrosphere. It is evident that most of the strong bonds belonging to PLGA and PAA are still present, although to a lesser degree. These bonds include O-H stretching vibration (2500-3600cm<sup>-1</sup>), ester bonds (1600-1800cm<sup>-1</sup>), C=O stretching vibrations (1590–1750cm<sup>-1</sup>) and C-O-C vibrations (1000-1200cm<sup>-1</sup>). Notably, there was no overlapping tendency that featured between the wave bands of the native polymers and that of gastrospheres (Figure 3.13) indicating that there was no interaction between the drug and the polymers employed in the formulations.



**Figure 3.13:** FTIR spectra of the component polymers and the crosslinked gastrospheres.

### 3.3.9. Analysis of surface area and porosity

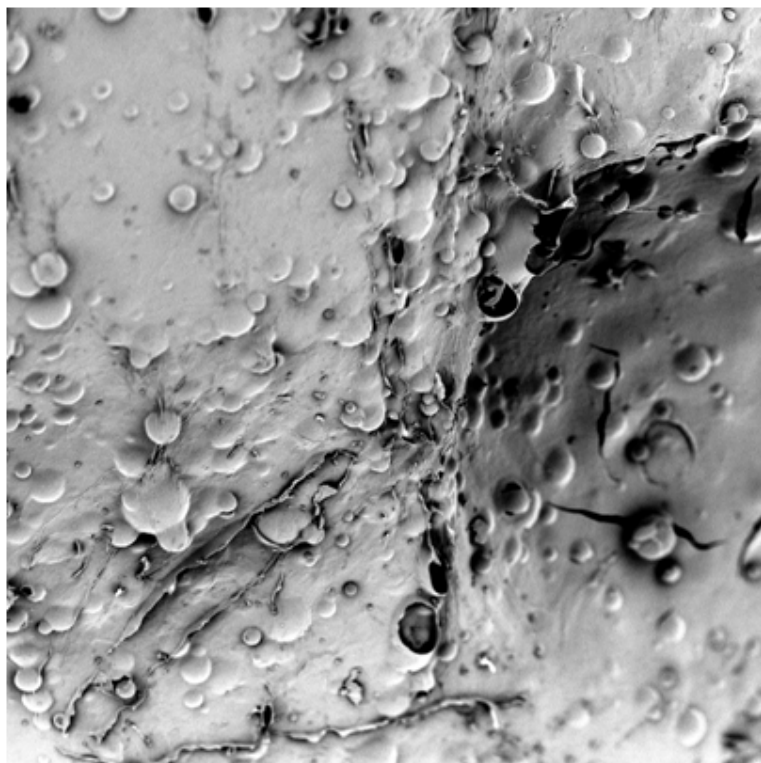
A comparison was made between a sample of gastrospheres which were lyophilized and a sample which was air dried. The results of BET surface analysis are shown in Figure 3.14. It could be visually elucidated that the air dried sample was significantly smaller than the lyophilized sample due to structural collapse during the drying process. This was confirmed by the results obtained from the surface area analysis, which revealed that surface area and pore volume were reduced by 82.3% and 39.3% respectively with the exclusion of the lyophilization process. These results assist in emphasizing the importance of the lyophilization process in the development of a low density gastrospheres, and therefore buoyant, system.



**Figure 3.14:** BET surface analysis. a) lyophilized; and b) air-dried gastrospheres.

### 3.3.10. Surface characterisation of the gastrospheres

Figure 3.15 shows an SEM image of a gastrosphere at a magnification of 440x. It was observed that the entire surface of the gastrosphere was covered with air pockets just below the surface. When these air pockets formed too near the surface, they ruptured, resulting in the formation of pores and channels. The presence of these air pockets visually confirms the presence of pores within the gastrosphere which are responsible for its low density and resultant buoyancy.



**Figure 3.15:** Typical SEM image of the surface of a gastrosphere at a magnification of 440x.

### **3.3.11. Molecular mechanics elucidation of the performance of gastrosphere drug delivery system**

The present communication dealt with the fabrication of a drug delivery system integrating a unique combination of polymers with acidic functionalities such as alginate (mannuronic and guluronic acid residues), pectin (galacturonic acid residues), PAA and PLGA (polyhydroxy acid derivative of lactic and glycolic acid). Among these polymers, all except PLGA are known to exhibit interactions with divalent cations such as  $\text{Ca}^{2+}$  where alginate and pectin display crosslinking with  $\text{Ca}^{2+}$  ions (da Silva et al., 2009) and polyacrylic acid exhibit complexation of calcium ions (Kriwet and Kissel, 1996). It is evident from Table 3.6 that ALG- $\text{Ca}^{2+}$  is energetically stabilized by 37 kcal/mol as compared to ALG because of strong electrostatic interactions along with high torsional energy (Figure 3.16a). However, in case of Pec- $\text{Ca}^{2+}$ , the energy of interaction is about 9 kcal/mol mainly arising due to van der Waals forces. This somewhat hydrophobic stabilization was probably due to the presence of methyl groups (as methyl esters) in pectin structure. Furthermore, the ALG-Pec complex is destabilized by 13 kcal/mol demonstrating the necessity of crosslinking the bipolymeric structure with  $\text{Ca}^{2+}$  ions which was confirmed by the high degree of conformational stability (42 kcal/mol) in ALG-Pec- $\text{Ca}^{2+}$ . In addition, the crosslinked structures was more closely packed displaying the spatial preference of the polymeric chains in

response to the presence of  $\text{Ca}^{2+}$  (Figure 3.16 a, c and d). Both ALG-Pec and ALG-Pec- $\text{Ca}^{2+}$  displayed intra- and inter-polymeric hydrogen bonding, although at different positions, as depicted in Figure 3.16. These results are in line with the earlier reported studies where the polyguluronates (alginate) displayed better strength and the stereospecificity in binding to  $\text{Ca}^{2+}$  through “Egg-box model” as compared to polygalacturonates (pectin) (Braccini and Pe´rez, 2001).

**Table 3.6:** Calculated energy parameters (kcal/mol) of the polymer-polymer and polymer-protein assemblies complexes ALG, Pec, PAA, PLGA and glycosylated MUC.

| Structure                            | Energy (kcal/mol)  |              |                   |                    |                       |                  |                     |                   |
|--------------------------------------|--------------------|--------------|-------------------|--------------------|-----------------------|------------------|---------------------|-------------------|
|                                      | Total <sup>a</sup> | $\Delta E^b$ | Bond <sup>c</sup> | Angle <sup>d</sup> | Dihedral <sup>e</sup> | VDW <sup>f</sup> | H bond <sup>g</sup> | Elec <sup>h</sup> |
| ALG                                  | 74.426             | -            | 5.204             | 32.485             | 34.108                | 30.709           | 0                   | -28.142           |
| ALG Ca <sup>2+</sup>                 | 36.548             | -37.452      | 5.502             | 30.586             | 40.364                | 35.197           | 0                   | -75.102           |
| Pec                                  | -68.280            | -            | 5.231             | 50.779             | 59.971                | 15.331           | -8.022              | -191.564          |
| Pec Ca <sup>2+</sup>                 | -76.894            | -8.614       | 5.194             | 50.977             | 60.072                | 6.704            | -8.024              | -191.817          |
| ALG-Pec                              | 19.509             | 13.363       | 10.923            | 85.783             | 97.369                | 26.523           | -8.034              | -193.054          |
| ALG-Pec Ca <sup>2+</sup>             | -22.862            | -42.371      | 11.215            | 84.019             | 100.076               | 32.13            | -8.699              | -241.603          |
| PAA                                  | 10.258             | -            | 1.517             | 7.178              | 4.244                 | -2.540           | -0.142              | 0                 |
| PAA Ca <sup>2+</sup>                 | 1.597              | -8.661       | 1.504             | 7.202              | 4.151                 | -11.117          | -0.143              | 0                 |
| ALG-Pec-PAA                          | 2.167              | -17.342      | 13.099            | 98.079             | 104.553               | -8.058           | -9.624              | -195.883          |
| ALG-Pec-PAA Ca <sup>2+</sup>         | -48.238            | -50.405      | 13.124            | 96.918             | 104.16                | -11.512          | -11.336             | -239.593          |
| PLGA                                 | 3.941              | -            | 0.423             | 3.764              | 1.789                 | -2.028           | -0.007              | 0                 |
| ALG-Pec-PAA-PLGA                     | -48.608            | -54.346      | 13.786            | 109.649            | 106.446               | -63.481          | -9.367              | -205.64           |
| ALG-Pec-PAA-PLGA Ca <sup>2+</sup>    | -85.372            | -36.764      | 13.326            | 100.403            | 108.598               | -58.105          | -12.569             | -237.027          |
| GlycoMucin                           | -166.812           | -            | 5.474             | 70.351             | 55.173                | -29.066          | -7.096              | -261.649          |
| GlycoMucin-Polymers                  | -315.117           | -99.697      | 20.078            | 187.27             | 189.902               | -114.094         | -23.812             | -574.47           |
| GlycoMucin-Polymers Ca <sup>2+</sup> | -255.515           | -3.331       | 19.762            | 205.956            | 179.337               | -116.819         | -18.079             | -525.673          |

<sup>a</sup> Total steric energy for an optimized structure

<sup>b</sup>  $\Delta E_{interaction} = E(Host:Guest) - E(Host) - E(Guest)$

<sup>c</sup> Bond stretching contributions, reference values were assigned to all of a structure's bond lengths

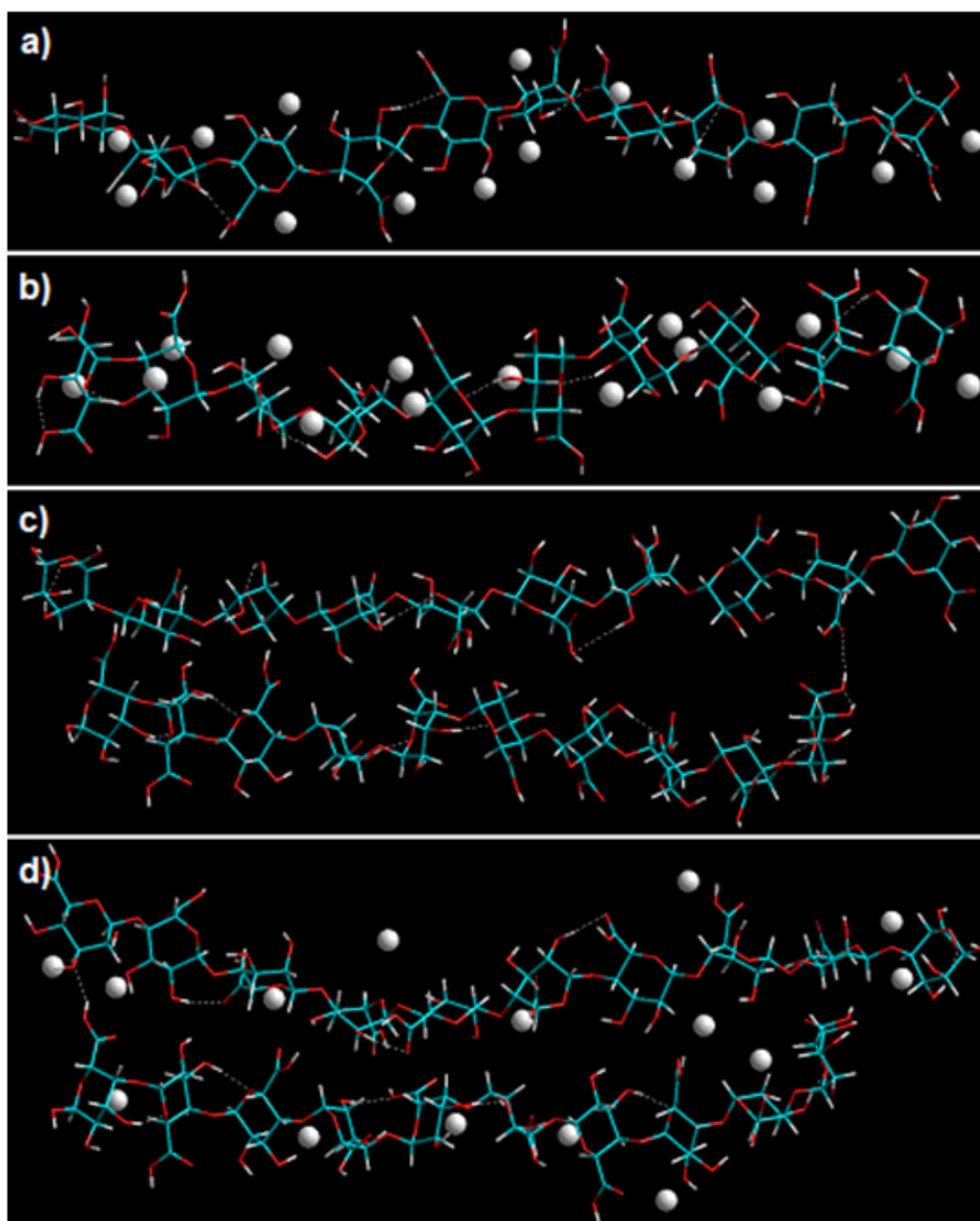
<sup>d</sup> Bond angle contributions, reference values were assigned to all of a structure's bond angles

<sup>e</sup> Torsional contribution arising from deviations from optimum dihedral angles

<sup>f</sup> van der Waals interactions due to non-bonded interatomic distances

<sup>g</sup> Hydrogen-bond energy function

<sup>h</sup> Electrostatic energy



**Figure 3.16:** Energy minimized constrained models of the polymeric assemblies derived from molecular mechanics calculations. (a) Alginate- $\text{Ca}^{2+}$ ; (b) Pectin- $\text{Ca}^{2+}$ ; (c) Alginate-Pectin; and (d) Alginate-Pectin- $\text{Ca}^{2+}$ . Colour codes for elements are: Carbon (cyan); Hydrogen (white-tubes); Oxygen (red); and Nitrogen (blue). Calcium ions are rendered spherically in white.

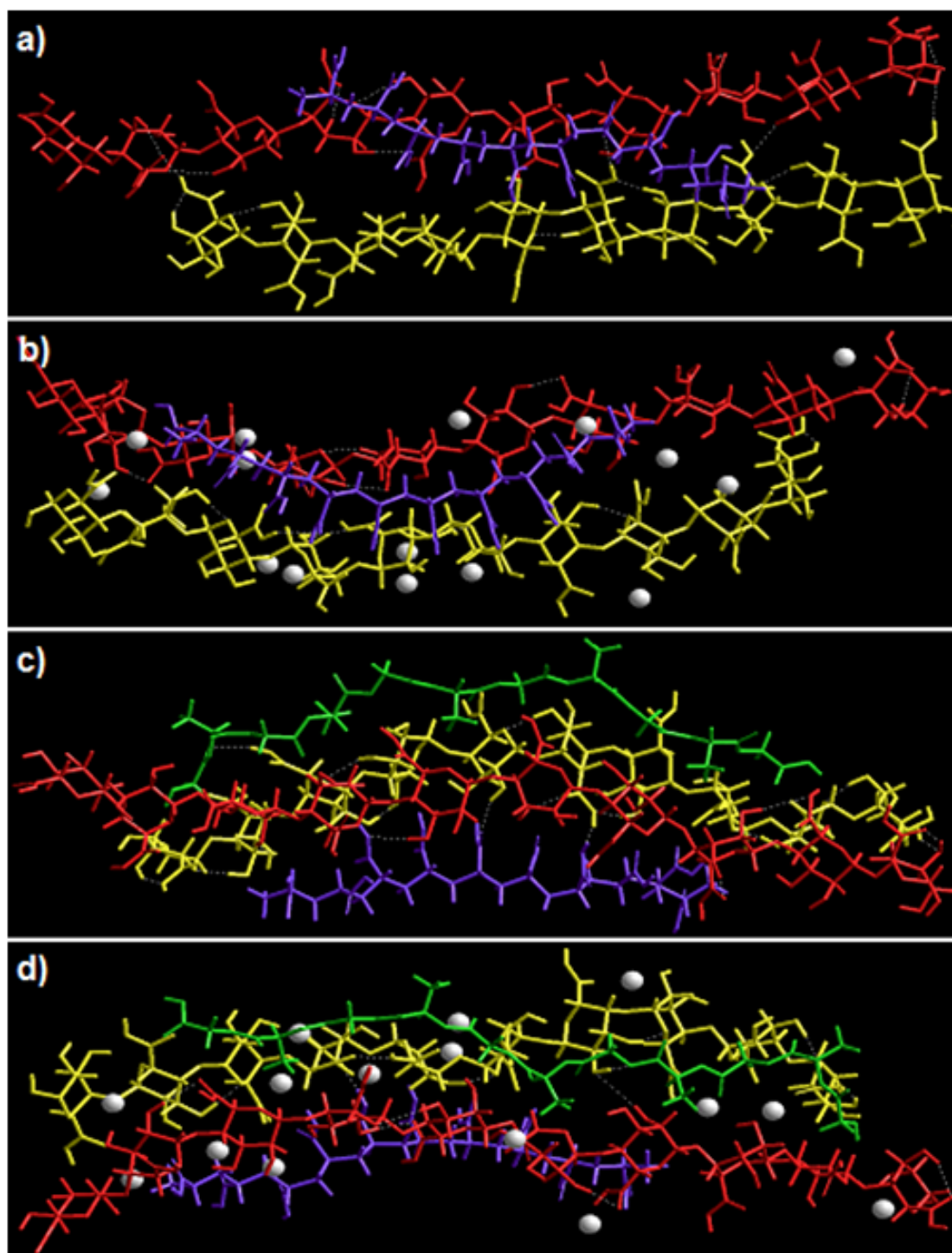
Likewise pectin,  $\text{PAA-Ca}^{2+}$  also displayed a rather small energy of interaction as compared to alginate and was stabilized mainly by the van der Waals forces owing to the presence of methyl groups in the structure of PAA. The addition of PAA to ALG-Pec, further stabilized the polymeric structure by 17 kcal/mol instituted by all the non-

bonding interactions viz., van der Waals forces, H-bonding and electrostatic interactions (Table 3.6). These non-bonding interactions also contributed to the energy minimization of spatially constrained geometrical model of ALG-Pec-PAA- $\text{Ca}^{2+}$  (Figure 3.17a and b). It is noteworthy that the addition of PAA to ALG-Pec increased the interpolymeric hydrogen bonding between alginate and pectin and the addition of  $\text{Ca}^{2+}$  to ALG-Pec-PAA increased the structural integrity of the tripolymer complex as represented in Figure 3.17. The formation of H-bonding between PAA and alginate and PAA and Pectin can also be seen in Figure 3.17 confirming the rationality of incorporating PAA in to the alginate-pectin platform.

As expected, PLGA, a hydrophobic polymer, when added to the tripolymer complex displayed a very high energy of interaction ( $\Delta E = -54.346 \text{ kcal/mol}$ ) attributable to very high van der Waals interactions primarily due to non-bonded interatomic distances. Surprisingly, the total steric energy of this quad-polymer was almost equal to the  $\text{Ca}^{2+}$  crosslinked ALG-Pec-PAA and the  $\Delta E$  of formation of both was also similar. However, the structural integrity in terms of closed packing was still a concern. The incorporation of  $\text{Ca}^{2+}$  to this quad-polymer decreased the bond angle contributions resulting in a more sterically constrained structure which was also responsible for the not so high  $\Delta E$  in case of ALG-Pec-PAA-PLGA  $\text{Ca}^{2+}$ .

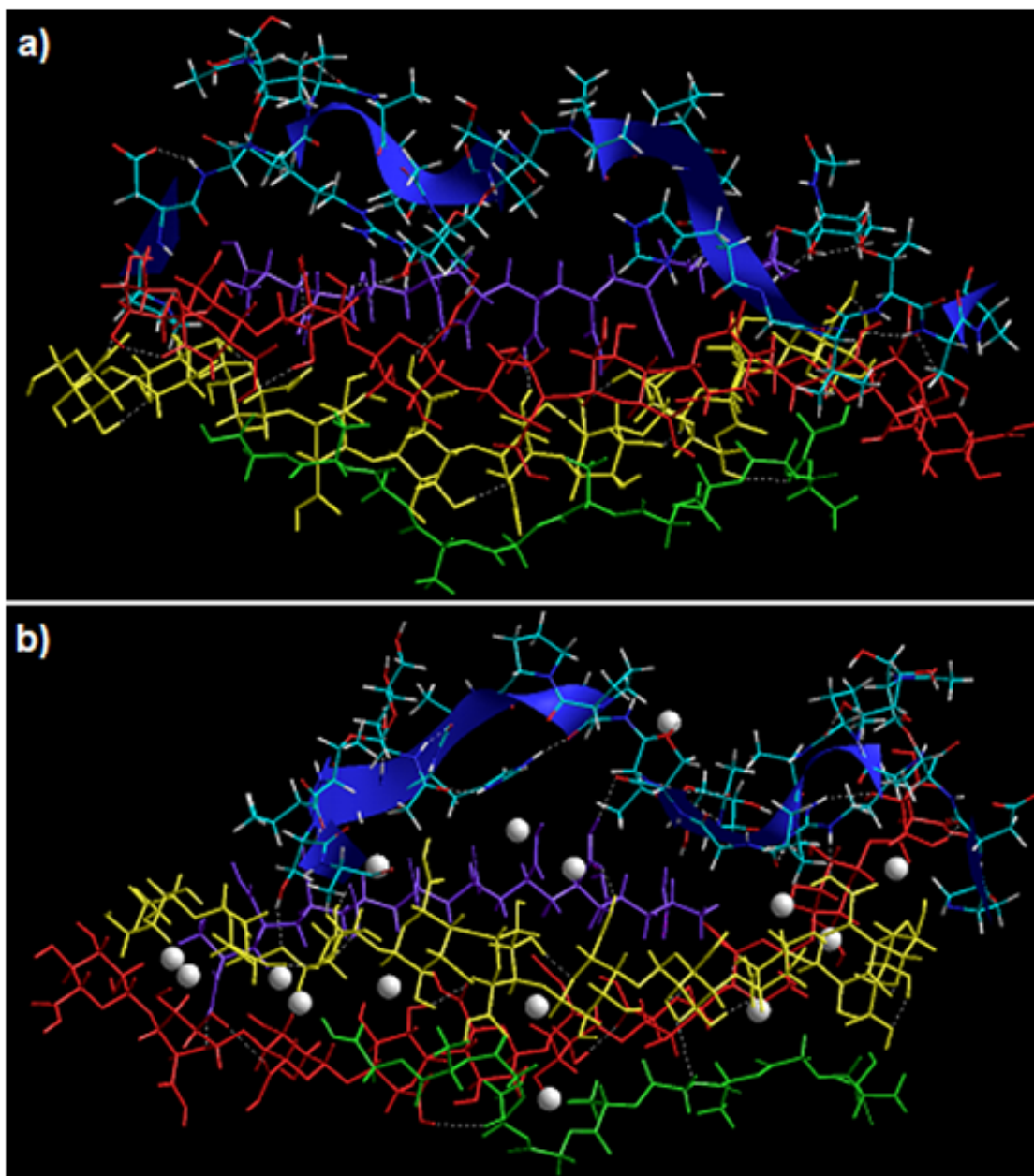
The aforementioned *in silico* results corroborated with the experimental *in vitro* drug release profiles in terms of  $\text{MDT}_{12}$ . The  $\text{MDT}_{12}$  decreased, and hence the rate of drug release increased, with an increase in ALG/PEC and PAA levels from 1 to 1.5% at low and high levels of PAA and ALG/PEC, respectively. This increase in drug release with an increase in ALG/PEC and PAA may be due to the hydrophilic nature and hence increased swelling of the polymers leading to enhanced diffusion of the drug. Furthermore, an increase in polymer level, decrease the  $\text{Ca}^{2+}$  ions available per polymeric fragment resulting in a decrease in crosslinking and hence rigidity of the matrix (Figure 3.17). However, a further increase in polymer levels from 1.5 to 2% increased the  $\text{MDT}_{12}$  and hence decreased the drug release. This may be attributed to the fact that with an increase in level of polymers, the polymer density increases and the space available for swelling decreases thereby decreasing the diffusion of the drug from the polymer matrix (Figure 3.17). Furthermore, the increase in polymer density may have facilitated the  $\text{Ca}^{2+}$  to form all the possible associations with the ordered polyguluronate and polygalacturonate chains to form dimers resulting in a highly crosslinked polymeric framework (Figure 3.17) (Braccini and Pe'rez, 2001). The increase in  $\text{MDT}_{12}$  with an increase in PLGA was obvious due to the fact that PLGA is

hydrophobic and even energetically produced an effect similar to  $\text{Ca}^{2+}$  as described in the previous paragraph.



**Figure 3.17:** Energy minimized geometrical preferences of the multi-polymeric polyelectrolyte complexes derived from molecular mechanics calculations. (a) Alginate-PAA-Pectin; (b) Alginate-PAA-Pectin- $\text{Ca}^{2+}$ ; (c) Alginate-PAA-Pectin-PLGA; and (d) Alginate-PAA-Pectin-PLGA- $\text{Ca}^{2+}$ . Colour codes for polymer chains are: Alginate (red); PAA (violet); Pectin (yellow); and PLGA (green). Calcium ions are rendered spherically in white.

The bioadhesive or mucoadhesive potential of the multiparticulate delivery system was elucidated as being a measure of specific chemical interactions between the polymeric matrix (ALG-Pec-PAA-PLGA) or the  $\text{Ca}^{2+}$ -crosslinked polymeric matrix (ALG-Pec-PAA-PLGA  $\text{Ca}^{2+}$ ) and the glycosylated gastric mucopeptide analogue (MUC) after geometrical optimization using energy minimizations. The stress transduction for energy minimization was found to be a collective phenomenon including interactions in the form of van der Waals forces, H-bonding and electrostatic interactions contributing to the binding energy (Table 3.6) while requiring a large fraction of the surface to establish connectivity between chemically transformed regions. The binding energy of the polymer matrix with MUC was quite high reaching up to 100 kcal/mol confirming the significant interaction between the two (Figure 3.18; Table 3.6). However, the minimized energy increased significantly after introducing the  $\text{Ca}^{2+}$  ions in the MUC-polymer system leading to a comparatively destabilized conformation. Additionally, the H-bonds formed between the polymer matrix and the MUC were lessened in case of  $\text{Ca}^{2+}$ -crosslinked system. A deeper inspection of the MUC-polymer shows that the specificity of this complex arises due to hydrophobic interactions of the methyl groups of the mucopeptide residues with oxygen atoms of the polymers. Furthermore, the binding was more pronounced with PAA and the polysaccharide chains, preferably the alginate, as depicted in Figure 3.18. The inherent mechanism involved in the reduction and stabilization of  $\text{Ca}^{2+}$ -crosslinked matrix may be attributed to the formation of a strained network structure due to calcium crosslinking thereby limiting the complete interaction as observed with the non-crosslinked structure (Figure 3.18).



**Figure 3.18:** The chemical and geometrical binding interactions involving polymers and the glycosylated gastric mucopeptide analogue. (a) GlycoMucin-Polymers; and (b) GlycoMucin-Polymers- $\text{Ca}^{2+}$ . Polymers are depicted in tube rendering and MUC is depicted in stick rendering. Colour codes for elements are: Carbon (cyan); Nitrogen (blue); Oxygen (red); and Hydrogen (white-stick). Colour codes for polymer chains are: Alginate (red); PAA (violet); Pectin (yellow); and PLGA (green). Calcium ions are rendered spherically in white.

The experimental mucoadhesion studies can also be correlated to these *in silico* findings. Like  $\text{MDT}_{12}$  studies, mucoadhesion was also characterized by a "region of maximum" whereby the gastro-adhesion was dependent on the swelling extent of the polymeric matrix. As described earlier in this chapter, an optimum swelling was required for an effective bioadhesion. The 3-D plot depicted an initial increase in mucoadhesion with increase in the amount of polymers up to the intermediate levels

and decreased thereafter. Maximum mucoadhesion, therefore, was seen at the intermediate level of the polymer ratio. It may be because of the fact that the hydrogels swell readily (with higher amount of PAA), when they come in contact with hydrated mucous membrane and hydrogels become progressively elastic due to uncoiling of polymer chains and subsequent increased mobility of the polymer chains resulting in a large adhesive surface for maximum contact with mucosa and flexibility to the polymer chains for interpenetration with mucosa (Kumar and Bhatia, 2010). Increasing the alginate, pectin and PAA amount may provide more adhesive sites and polymer chains for interpenetration with mucosa, resulting consequently in the augmentation of mucoadhesive strength. On the other hand, a further increase in the amount of these hydrophilic polymers may render the network structure too loose to hold the tethered mucous chains thereby decreasing the mucoadhesion (Kumar and Bhatia, 2010). PLGA appeared to play its role here in sustaining the matrix integrity by controlling the swelling of the matrix and hence the mucoadhesion.

### 3.4. CONCLUDING REMARKS

A randomized Box-Behnken statistical experimental design was utilised in order to develop and optimize a novel approach for the formulation of gastrospheres intended for the delivery of narrow absorption window drugs. A range of formulations varying in release characteristics and gastroadhesion, were obtained. Response surface design was employed in order to identify the relationships between the responses ( $MDT_{12}$ ,  $T_{0-2}$  and  $T_{2-12}$ ) and the experimental factors (ALG/PEC, PAA and PLGA). Optimization of experimental factors resulted in the generation of an optimal formulation possessing maximal gastroadhesion over the entire 12 hour period as well as an  $MDT_{12}$  of 32.33, which is capable of displaying a zero-order rate of drug release. The molecular mechanics (MM) simulations ascertained that the *in silico* results corroborated well with the experimentally obtained *in vitro* drug release profiles. Furthermore, the bioadhesive or mucoadhesive potential of the multiparticulate delivery system was elucidated via MM simulations as being a measure of specific chemical interactions between the polymeric matrix (ALG-Pec-PAA-PLGA) or the  $Ca^{2+}$ -crosslinked polymeric matrix (ALG-Pec-PAA-PLGA  $Ca^{2+}$ ) and the glycosylated gastric mucopeptide analogue after geometrical optimization using energy minimizations. Thus, stress transduction for energy minimization was found to be a collective phenomenon including interactions in the form of van der Waals forces, H-bonding and electrostatic interactions contributing to the binding energy. The results obtained give much promise that the developed drug delivery system may find a good application in the delivery of narrow absorption window drugs.

## CHAPTER 4

### Development, Design and Optimization of Microparticle-Loaded Gastrospheres

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#### 4.1. INTRODUCTION

Ciprofloxacin is a broad spectrum, second generation fluoroquinolone antibiotic, active against both gram-positive and gram-negative bacteria, as well as other micro-organisms (Zhanel et al., 2002; Imre et al., 2003; Ge, et al., 2009). Ciprofloxacin hydrochloride is characterised by a short elimination half-life of 4 hours, high water solubility, as well as having a narrow absorption window, mainly being absorbed in the duodenum (Tadros, 2009).

Chitosan is a natural cationic polysaccharide that is obtained from the deacetylation of chitin which is similar in structure to cellulose (Agnihotri et al., 2004; Ko et al., 2002). The presence of primary amine groups gives chitosan special properties which have resulted in its extensive use in drug delivery systems (Agnihotri et al., 2004; Dodane and Vilivalam, 1998; Felt et al., 1998; Kas, 1997). In order to control the rate at which drug is released from a drug delivery system, chitosan can be crosslinked using chemical crosslinking agents such as glutaraldehyde (Jameela and Jayakrishnan, 1995; Blanco et al., 2000), NaOH (Chandy and Sharma, 1996; Lim et al., 1997; Vasudev et al., 1997), ethylene glycol diglycidyl ether (Mi et al., 1999) or using an ionic crosslinker such as tripolyphosphate (TPP) (Shiraishi et al., 1993; Calvo et al., 1997). However, ionic crosslinking is preferable due to the absence of undesirable effects such as irritation and toxicity associated with chemical crosslinkers (Lim et al., 1997; Shu and Zhu, 2001). TPP is a nontoxic multivalent anion which forms a gel through the interaction with the positively charged amino groups of chitosan (Aral and Akbuğa, 1998; Shu and Zhu, 2001). Chitosan has many advantages for use in drug delivery systems. These include biocompatibility and biodegradation, the ability to control the rate of drug release, presence of free amine groups that are available for crosslinking, its cationic in nature (which allows for ionic crosslinking with multivalent anions), the antacid and antiulcer properties (which reduces drug irritation on the stomach) and its mucoadhesive nature (Agnihotri et al., 2004; Berscht et al., 1994).

Eudragit® is the trade name for a range of copolymers derived from esters of methacrylic and acrylic acid. Water permeability and pH-dependent solubility is dependant of the type and frequency of ester substituent's present (Lin et al., 1994).

Due to the broad spectrum of physicochemical properties inherent to the range of Eudragit® polymers, they have been used extensively in the production of pharmaceutical formulations. Their use varies from film coating and enteric coating to the control of the rate of drug release and taste masking (Lin et al., 1990, 1991, 1994). Eudragit® polymers are categorised according to their ionic nature, pH dependence and solubility (Table 4.1).

**Table 4.1:** Properties of Eudragit® polymers

|                  | <b>Ionic Nature</b> | <b>pH dependence</b>    | <b>Solubility</b>                                     | <b>Permeability &amp; Swelling</b> |
|------------------|---------------------|-------------------------|-------------------------------------------------------|------------------------------------|
| Eudragit® S 100  | Anionic             | Dissolves at pH 7.0     | Methanol, ethanol, isopropyl alcohol, acetone,        | -                                  |
| Eudragit® L 100  | Anionic             | Dissolves at pH 6.0     |                                                       |                                    |
| Eudragit® E 100  | Cationic            | Dissolves at pH 1.2-6.0 | As above as well as methylene chloride, ethyl acetate | -                                  |
| Eudragit® RS 100 | Cationic            | Independent             |                                                       | Low                                |
| Eudragit® RL 100 | Cationic            | Independent             |                                                       | High                               |

The purpose of this chapter was to develop and carry out an *in vitro* evaluation on microparticles, formulated through ionic crosslinking of chitosan and Eudragit® RL 100 30D that were incorporated into an ionic crosslinked gastrosphere (developed in Chapter 3), for the controlled release of ciprofloxacin over a period of 12 hours in a zero-order fashion. Chemometric modelling was employed to corroborate the experimental findings.

## **4.2. MATERIALS AND METHODS**

### **4.2.1. Materials**

The following materials are all of analytical grade and were obtained from commercial suppliers and used without further purification. Chitosan (medium molecular weight), ciprofloxacin and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany), alginate (Protanal LF 10/60, FMC BioPolymer, Drammen, Norway), pectin (Classic CU 701, Herbstreith and Fox, Neuenbürg, Germany), polyacrylic acid (Carbopol 974P NF, Noveon, Ohio, USA), poly(lactic-co-glycolic) acid (Resomer RG 858 S) (Boehringer Ingelheim, Ingelheim, Germany) and calcium hydroxide (BDH Chemicals Ltd., Poole, England). Eudragit® RL 100 30D was received from Röhm Pharma Polymers (Darmstadt, Germany). All other reagents used were of analytical grade and were employed as purchased.

#### **4.2.2. Methods**

##### **4.2.2.1. Construction of a Face-Centered Central Composite Design**

A central composite design was constructed in order to provide information regarding the direct additive effects of the study variables on the physicochemical and physicomechanical properties, as well as the pair-wise interaction effects. Experimental trials were performed on 14 statistically derived formulations of various combinations of polymers. Chitosan (0.5 – 1.5%<sup>w/v</sup>) and Eudragit® RL100 30D (0.5-2%<sup>w/v</sup>) were selected as the independent formulation variables. Mean Dissolution Time (MDT<sub>12</sub>) over 12 hours drug entrapment efficiency and microparticle yield were selected as the formulation responses. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses. Response surface plots were constructed to visually represent the influence of the polymeric concentrations on the ciprofloxacin release dynamics from the microparticle-loaded gastrospheres.

##### **4.2.2.2. Preparation of microparticles**

A homogenous solution of chitosan, ciprofloxacin and Eudragit® RL 100 30D was prepared and aerosolized into a vessel containing 6%<sup>w/v</sup> TPP under constant rotation. Chitosan was dissolved in 1%<sup>v/v</sup> acetic acid and Eudragit® was dispersed in distilled water. Aerosolization was achieved by spraying the polymeric solution through the nozzle of a fluid bed drier (Mini Lab Coater, Umang Pharmatech, Maharashtra, India) at a constant rate of 5mL/min. The nozzle was kept at a height of 20cm above the collection vessel and the air pressure was maintained at 0.1MPa. The microparticles were allowed to cure for 30 minutes, after which they were collected via centrifugation, washed, frozen at -72°C and lyophilized at -60°C and 25mmtor.

##### **4.2.2.3. Loading of microparticles into gastrospheres**

An aqueous solution consisting of 1%<sup>w/v</sup> alginate, 1%<sup>w/v</sup> pectin and 2%<sup>w/v</sup> PAA was prepared, and PLGA (2%<sup>w/v</sup>) was dissolved in dichloromethane. Both polymeric solutions were combined and allowed to stir for 1 hour until a homogenous state was achieved. The lyophilized microparticles were uniformly dispersed within this polymeric emulsion and added drop-wise into a 2%<sup>w/v</sup> calcium hydroxide crosslinking solution. The gastrospheres were allowed to cure for 30 minutes, after which they were filtered, washed and frozen at -72°C for 24 hours. The frozen gastrospheres were subsequently lyophilized at -60°C for a further 24 hours.

#### **4.2.2.4. Yield and drug entrapment of microparticles**

The dry weight of the formed microparticles and gastrospheres was measured and compared to the weight of the initial dry formulation components. Drug entrapment efficiency of the microparticles was determined by dissolving 50mg microparticles in 100mL simulated gastric fluid (SGF) (pH 1.2, 37°C). Ciprofloxacin content was measured in triplicate using UV-spectroscopy at 280nm. The entrapment efficiency (DEE) was calculated using equation 3.1 (Streubel et al., 2002).

#### **4.2.2.5. *In vitro* drug release studies**

Drug release studies were conducted on microparticles as well as microparticle loaded gastrospheres employing the USP 32 apparatus 2 dissolution test (Erweka DT 700, Heusenstamm, Germany). A modification to the approach was made by immersing the samples under a ring mesh assembly (Pillay and Fassihi, 1999) in 900mL simulated gastric fluid (SGF) (pH 1.2, 37°C) at a rotation speed of 50rpm. Microparticles were weighed and placed in a tea bag which was closed with thread. The tea bag was subsequently placed below the ring mesh assembly. Samples of 5mL were removed at predetermined time intervals and filtered through a 0.45µm Millipore Millex filter. Equal volumes of fresh SGF were replaced in order to maintain sink conditions. Samples were then analysed with UV spectroscopy (CE 3021, Cecil Instruments, Cambridge, England) at 280nm. All experiments were conducted in triplicate. The release data was subjected to a model-independent analysis known as the time-point approach. Briefly, the Mean Dissolution Time set at 12 hours ( $MDT_{12}$ ) for each formulation was calculated. The application of the  $MDT_{12}$  approach provided a more precise analysis of the ciprofloxacin release performance for comparison of several release data sets. Equation 3.2 was employed in this regard (Hopfenberg and Hsu, 1978; Pillay et al., 1999).

#### **4.2.2.6. Constraint optimization of formulation responses**

A model-independent approach (Minitab® V15, Minitab Inc., PA USA) was used to optimize the microparticle loaded gastrospheres. The independent formulation variables were the concentrations of chitosan and Eudragit® employed in the formulation of the microparticles. Statistical optimization was therefore employed to ascertain the ideal polymeric combination with the desired physicochemical properties capable of attaining a maximum drug entrapment efficiency, microparticle yield and  $MDT_{12}$  value of 34.833 which conforms to zero-order kinetics over 12 hours.

#### **4.2.2.7. Fourier Transform Infrared characterisation**

FTIR spectroscopy was performed on the microparticles and the polymers in their native form as well as the microparticles and gastrospheres individually and in combination. Samples were analysed with a Spectrum 2000 FTIR spectrometer with a MIRTGS detector (PerkinElmer Spectrum 100, Beaconsfield, UK). The spectrum was a ratio spectrum of 16 sample scans against 16 background scans with a resolution of  $4\text{cm}^{-1}$ . Samples were analysed at wave numbers ranging from  $4000\text{--}400\text{ cm}^{-1}$ .

#### **4.2.2.8. Characterisation of surface morphology**

SEM analysis was carried out using a Phenom™ scanning electron microscope (FEI Company, OR, USA). Samples were made electrically conductive prior to analysis through the process of gold-sputter coating (SPI Module™ Sputter Coater, SPI Supplies, PA, USA).

Samples were attached to an SEM stub using adhesive carbon tape. The stub was inserted into the stub holder thereafter putting the glass chamber and sputter head in place. Argon gas was allowed to flush the system before the leak valve was sealed and the vacuum was turned on. The sputter coater was turned on for 90 seconds when plasma current reached 18mA, after which the system was turned off and the vacuum released.

#### **4.2.2.9. Atomistic molecular structural mechanics simulations**

Molecular Mechanics Computations in vacuum, which included the model building of the energy-minimized structures of polymer-polymer and polymer-crosslinker complexes, were performed using the HyperChem™ 8.0.8 Molecular Modelling System (Hypercube Inc., Gainesville, Florida, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK) (Kumar et al., 2011). The structures of Eudragit® (E100-five monomer units) and TPP were generated as a 3D model from standard bond lengths and angles employing ChemBio3D Ultra whereas the structure of chitosan (CHT-ten glucosamine oligosaccharide units) was generated using sugar builder 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 polymer-crosslinker 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-EUD and CHT-TPP. 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.

### 4.3. RESULTS AND DISCUSSION

#### 4.3.1. Preparation of microparticles

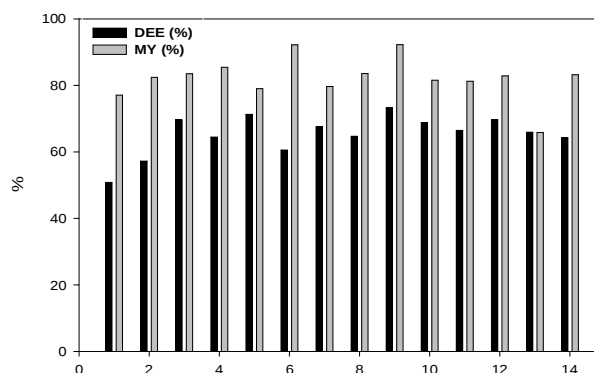
Table 4.2 shows the Face-Centered Central Composite Design template generated and lists the independent formulation variables considered.

**Table 4.2:** Face-Centered Central Composite Design template with statistically generated formulations

|    | StdOrder | RunOrder | PtType | Blocks | [CHT] | [EUD] |
|----|----------|----------|--------|--------|-------|-------|
| 1  | 4        | 1        | 1      | 1      | 1.5   | 2.00  |
| 2  | 3        | 2        | 1      | 1      | 0.5   | 2.00  |
| 3  | 7        | 3        | 0      | 1      | 1.0   | 1.25  |
| 4  | 5        | 4        | 0      | 1      | 1.0   | 1.25  |
| 5  | 1        | 5        | 1      | 1      | 0.5   | 0.50  |
| 6  | 2        | 6        | 1      | 1      | 1.5   | 0.50  |
| 7  | 6        | 7        | 0      | 1      | 1.0   | 1.25  |
| 8  | 11       | 8        | -1     | 2      | 1.0   | 2.00  |
| 9  | 9        | 9        | -1     | 2      | 1.5   | 1.25  |
| 10 | 12       | 10       | 0      | 2      | 1.0   | 1.25  |
| 11 | 14       | 11       | 0      | 2      | 1.0   | 1.25  |
| 12 | 12       | 12       | 0      | 2      | 1.0   | 1.25  |
| 13 | 8        | 13       | -1     | 2      | 0.5   | 1.25  |
| 14 | 10       | 14       | -1     | 2      | 1.0   | 0.50  |

#### 4.3.2. Determination of the yield and drug entrapment efficiency

Yield of the microparticles was relatively high, ranging from 77 to 92%, with one outlying result at 65%, whereas drug entrapment efficiency was within a range of 50% to 73% (Figure 4.1). The polymer which appears to have the largest impact on DEE is Eudragit®. DEE is in the lower range in the formulations where Eudragit® is either at its highest (2%<sup>w/v</sup>) or lowest (0.5%<sup>w/v</sup>) concentrations, whereas DEE is at its maximum when Eudragit® is midway at 1.25%<sup>w/v</sup>. Chitosan appears to be responsible for microparticle yield where yield is highest when chitosan concentrations are highest (1.5%<sup>w/v</sup>) and yield is lowest with chitosan in low concentrations (0.5%<sup>w/v</sup>).

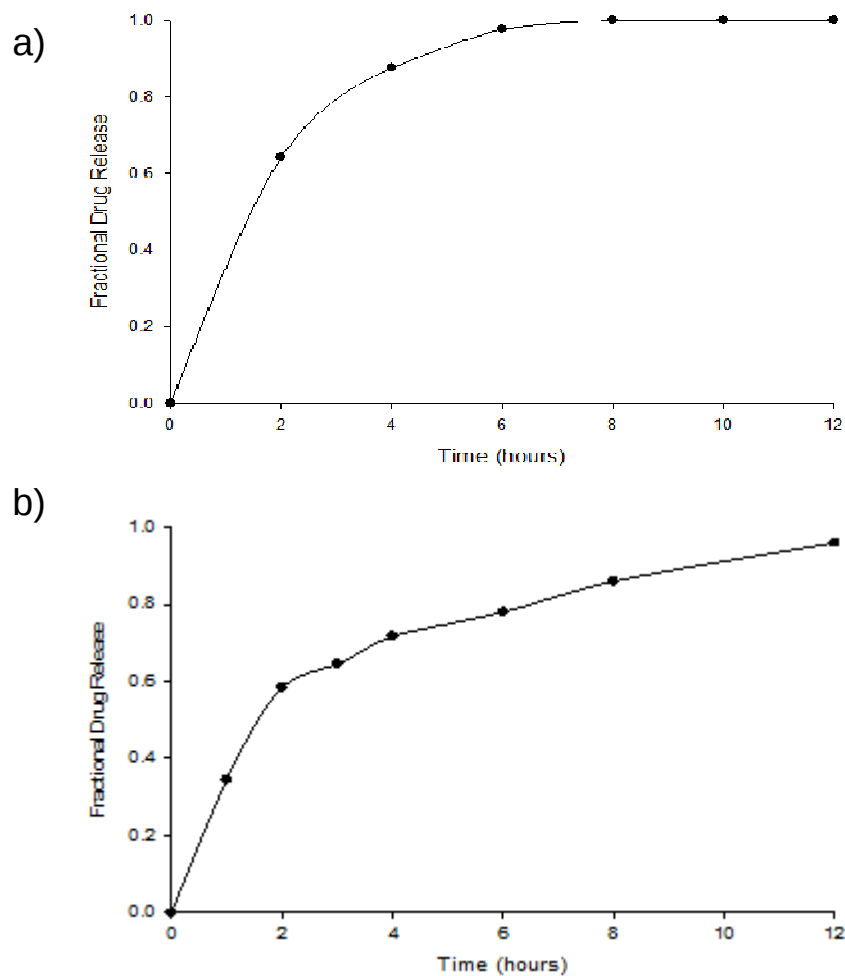


**Figure 4.1:** Graph illustrating results of DEE and microparticle yield (N=3, SD<7.4).

#### 4.3.3. *In vitro* drug release analysis

Drug release profiles obtained from the microparticle formulation 5 is depicted in Figure 4.2a. It was observed that drug release followed a first-order release profile, although almost 90% of drug was released within the first 4 hours.

Drug release from the microparticle loaded gastrospheres resulted in an initial burst release followed by zero order release as shown in Figure 4.2b for the drug release profile of formulation 5. It is evident that the incorporation of the microparticles within the gastrospheres resulted in drug release of a more controlled manner and over an extended period of time. Table 4.3 gives the  $MDT_{12}$  values obtained from all 14 formulations which were employed as a factor for constraint optimization. Eudragit® appears to impact *in vitro* drug release more significantly than chitosan.  $MDT_{12}$  is greatest when Eudragit® is at a moderate concentration of 1.25% $w/v$ , and decreases when Eudragit® concentrations are at their highest (2% $w/v$ ) or lowest (0.5% $w/v$ ). In most cases,  $MDT_{12}$  increased as chitosan concentrations increased.



**Figure 4.2:** Drug release of ciprofloxacin in SGF (pH 1.2) from a) microparticles for formulation 5 (N=3, SD<0.14 ); and b) microparticle loaded gastrospheres for formulation 5 (N=3, SD<0.032) over 12 hours.

**Table 4.3:** MDT<sub>12</sub> values obtained from *in vitro* drug release studies of ciprofloxacin from microparticle loaded gastrospheres (N=3, SD<0.74)

| F# | MDT <sub>12</sub> |
|----|-------------------|
| 1  | 29.78             |
| 2  | 29.79             |
| 3  | 32.24             |
| 4  | 32.07             |
| 5  | 30.82             |
| 6  | 29.48             |
| 7  | 32.4              |
| 8  | 29.33             |
| 9  | 34.91             |
| 10 | 31.87             |
| 11 | 32.10             |
| 12 | 32.00             |
| 13 | 32.47             |
| 14 | 25.36             |

#### **4.3.4. Analysis of the Central Composite Design**

MDT<sub>12</sub>, DEE and yield for the synthesized formulations were included in the statistical design for the identification of the optimal formulation. Residual plots for MDT<sub>12</sub>, DEE and yield are illustrated in Figure 4.3. There was generally a good scattering of points, although slight clustering was observed for MDT<sub>12</sub> (Figure 4.3(a)). The normal probability plots of the residuals for DEE and yield (Figure 4.3(b and c)) show a uniform distribution along the straight line, indicating that the data was normally distributed without outside influences. The histogram of the residuals for yield (Figure 4.3(c)) is slightly shifted to the right, showing that the frequency of residuals was not evenly distributed.

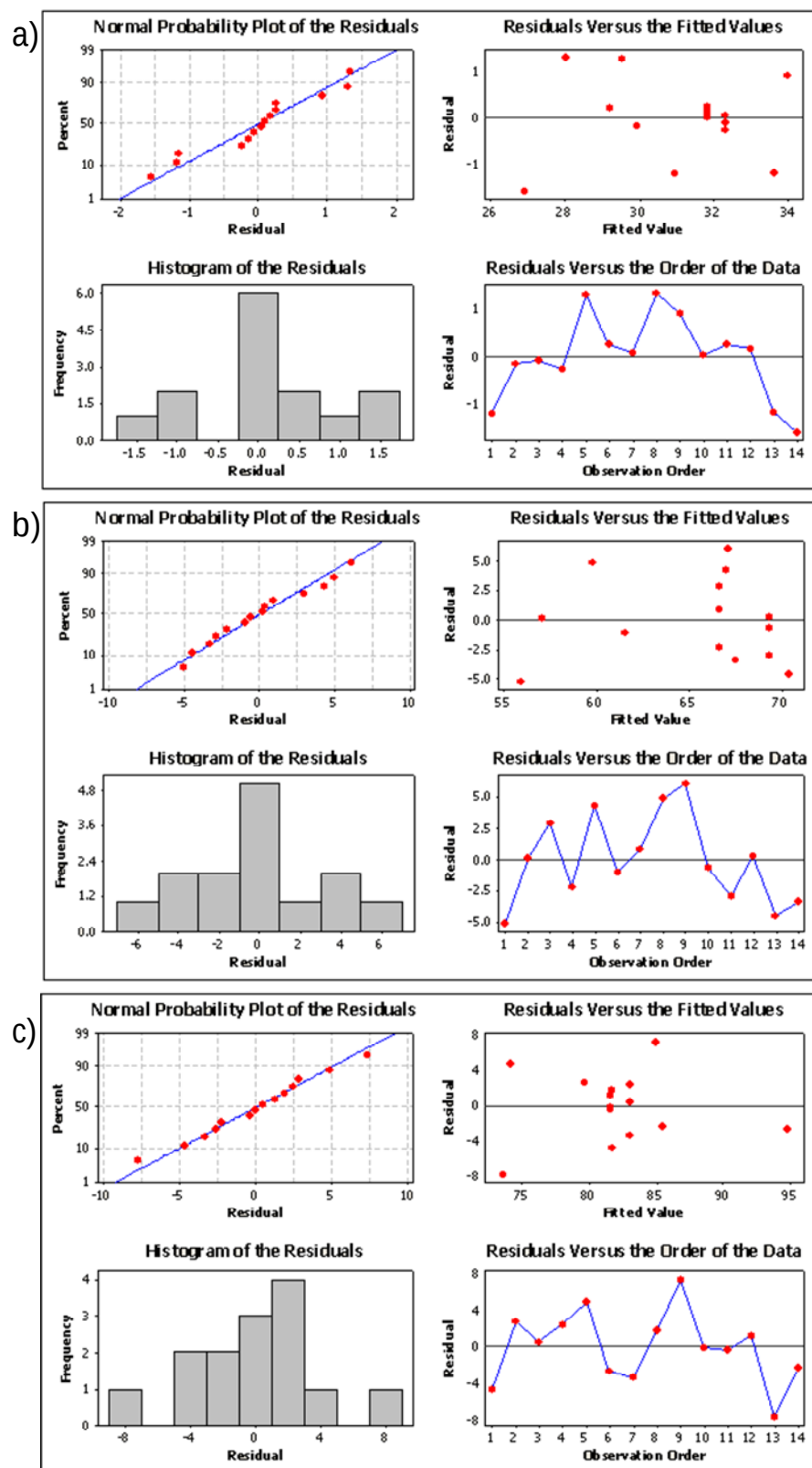


Figure 4.3: Residual Plots generated for a)  $MDT_{12}$  b) DEE and c) microparticle yield.

The complete regression equations generated for MDT<sub>12</sub>, DEE and yield are indicated below:

$$MDT_{12} = 27.6459 - 16.5178[CHT] + 19.2890[EUD] + 7.88542[CHT*CHT] - 7.78354[EUD*EUD] + 0.890252[CHT*EUD]$$

[Equation 4.1]

$$DEE = 63.1551 - 2.137[CHT] + 17.2748[EUD] - 2.35733[CHT*CHT] - 10.1321[EUD*EUD] + 2.87333[CHT*EUD]$$

[Equation 4.2]

$$Yield = 54.5488 + 45.8487[CHT] + 1.12296[EUD] - 9.50267[CHT*CHT] + 3.48326[EUD*EUD] - 12.3467[CHT*EUD]$$

[Equation 4.3]

#### 4.3.5. Surface response analysis

##### 4.3.5.1. Mean Dissolution Time

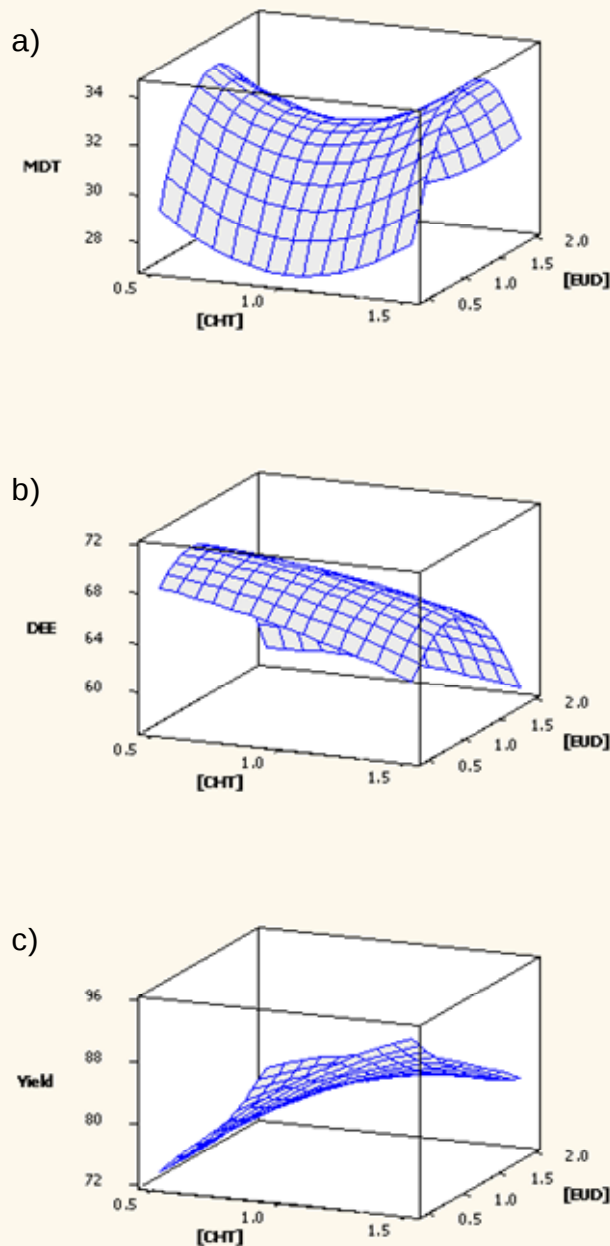
The effect of factor CHT and EUD on response MDT<sub>12</sub> is shown in Figure 4.4(a). Over the entire range of factor CHT, an increase in factor EUD from 0.5 to 1.5% w/v resulted in an increase of MDT<sub>12</sub>, although a further increase to 2% w/v resulted in the reduction of MDT<sub>12</sub>. Over the entire range of factor EUD, an initial increase in factor CHT from 0.5 to 1% w/v resulted in a reduction of MDT<sub>12</sub>, although an increase in MDT<sub>12</sub> was observed when factor CHT was increased from 1 to 1.5% w/v. Optimal MDT<sub>12</sub> is seen when Eudragit® RL 100 30D, which possesses high swelling and permeability, is used in moderate concentrations. The influence of Eudragit® on drug release can be explained as follows: A low concentration of Eudragit® may lead to the formation of a less dense polymer complex resulting in the loosening of chains, making the hydrogel matrix more porous. In the case of high polymer content, there is a greater hydrophilic polymer content in the matrix, which may in turn lead to polymer dissolution and water penetration causing a rapid drug release. For a given amount of Eudragit® and TPP, an increase in chitosan concentration may cause an increase in MDT due to enhanced complexation with Eudragit® and the formation of more intermolecular crosslinks with TPP as explained further in molecular mechanics simulations.

#### **4.3.5.2. Drug Entrapment Efficiency**

The effect of factor CHT and EUD on response DEE is shown in Figure 4.4(b). At low levels of factor CHT, DEE is high. An increase in factor EUD from 0.5 to 1%<sup>w/v</sup> results in the increase in DEE, although a further increase to 2%<sup>w/v</sup> resulted in the reduction of DEE. At high levels of factor CHT, DEE was low. The same effect was observed with the gradual increase in factor EUD. At low levels of factor EUD, DEE was high, decreasing as the level of factor CHT increases. At high levels of factor EUD, DEE was low. Minimal change in DEE was observed as the level of CHT is increased. A low concentration of Eudragit® may form a loose network resulting in leaching out of drug from the polymer matrix during microparticle formation. Additionally, higher concentrations of Eudragit® may lead to formation of too dense a network making it difficult for the drug molecules to get into the polymer matrix. Furthermore, this may be due to the premature swelling of the microparticle matrix, resulting in excessive loss of drug in the crosslinking solution.

#### **4.3.5.3. Microparticle Yield**

The effect of factor CHT and EUD on response yield is shown in Figure 4.4(c). At low levels of factor CHT, yield is low. An increase in factor EUD resulted in an increase in yield. At high levels of factor CHT, yield was high, reducing with the increase in factor EUD. At low levels of factor EUD, yield was high, reducing as the level of CHT decreases. At high levels of factor EUD, yield was moderate. Not much change in yield was observed over the range of factor CHT. The increased yield can be associated with the increase in viscosity of the polymer solution with an increase in chitosan concentration. As the polymer solution becomes more viscous it becomes more resistant to fragmentation into small droplets which, in turn, may leads to the generation of larger microparticles having less surface area and more drug entrapment. A lesser surface area may utilise the crosslinking more efficiently leading to formation of more and denser microparticles ultimately increasing the % yield.



**Figure 4.4:** Response surface plots depicting the effect of polymer concentrations on: a)  $MDT_{12}$ , b) DEE, and c) microparticle yield.

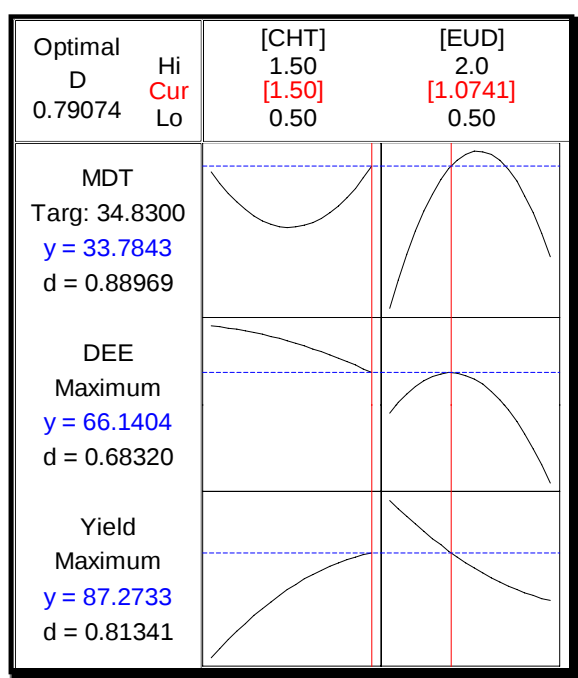
#### 4.3.6. Response optimization

Response optimization (MINITAB®, V14, Minitab, USA) was used to obtain the optimized levels of chitosan and Eudragit®. One optimal formulation was developed following constrained optimization of  $MDT_{12}$ , DEE and microparticle yield. An  $MDT_{12}$  value representing first order drug release over a period of 12 hours was targeted ( $MDT_{12}=34.883$ ). Figure 4.5 shows the desirability plots of each constraint for the optimized formulation. The optimized levels of the independent variables that would

achieve the desired drug entrapment, yield and dissolution are represented in Table 4.5. Table 4.6 shows the optimized levels of the independent variables, the predicted response, the desirability score as well as the correlation co-efficient for each response. Based on the statistical desirability function, it was found that the desirability for the formulations was > 0.9. The constrained settings utilized are outlined in Table 4.4.

**Table 4.4:** Formulation constraints employed for response optimization

| Responses         | Limits  |
|-------------------|---------|
| DEE               | Maximum |
| Yield             | Maximum |
| MDT <sub>12</sub> | 34.883  |



**Figure 4.5:** Desirability plots depicting the requisite variables for producing microparticles with the desired targeted responses.

**Table 4.5:** Optimized formulations obtained via the surface response method

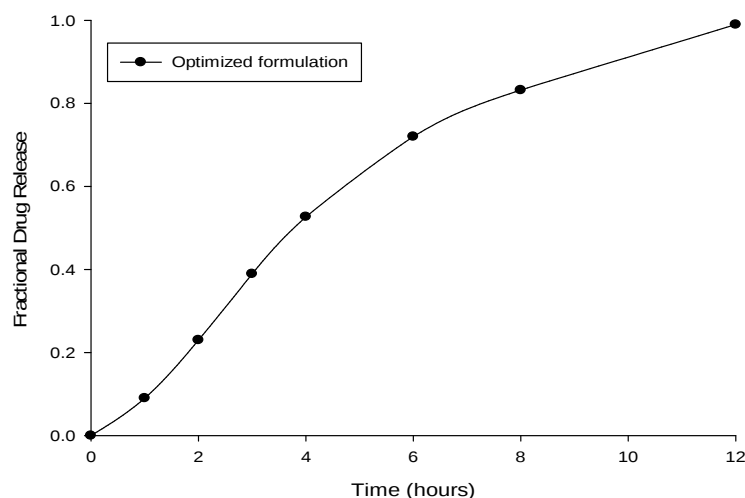
| Microparticle Formulation | Chitosan (%) | Eudragit® RL 100 30D (%) |
|---------------------------|--------------|--------------------------|
| 1                         | 1.5          | 1.0741                   |

The ideal formulation was prepared according to the optimal predicted settings. The experimentally derived values for MDT<sub>12</sub>, DEE and yield of the optimized formulation were in close agreement with the predicted values (Table 4.6), demonstrating the reliability of this optimisation procedure.

**Table 4.6:** Experimental and predicted response values for the optimised

|                   | Desirability | Predicted response | Actual Response |
|-------------------|--------------|--------------------|-----------------|
| MDT <sub>12</sub> | 0.88969      | 33.7843            | 32.2048         |
| DEE               | 0.68320      | 66.1404            | 59.23           |
| Yield             | 0.81341      | 87.2733            | 86.743          |

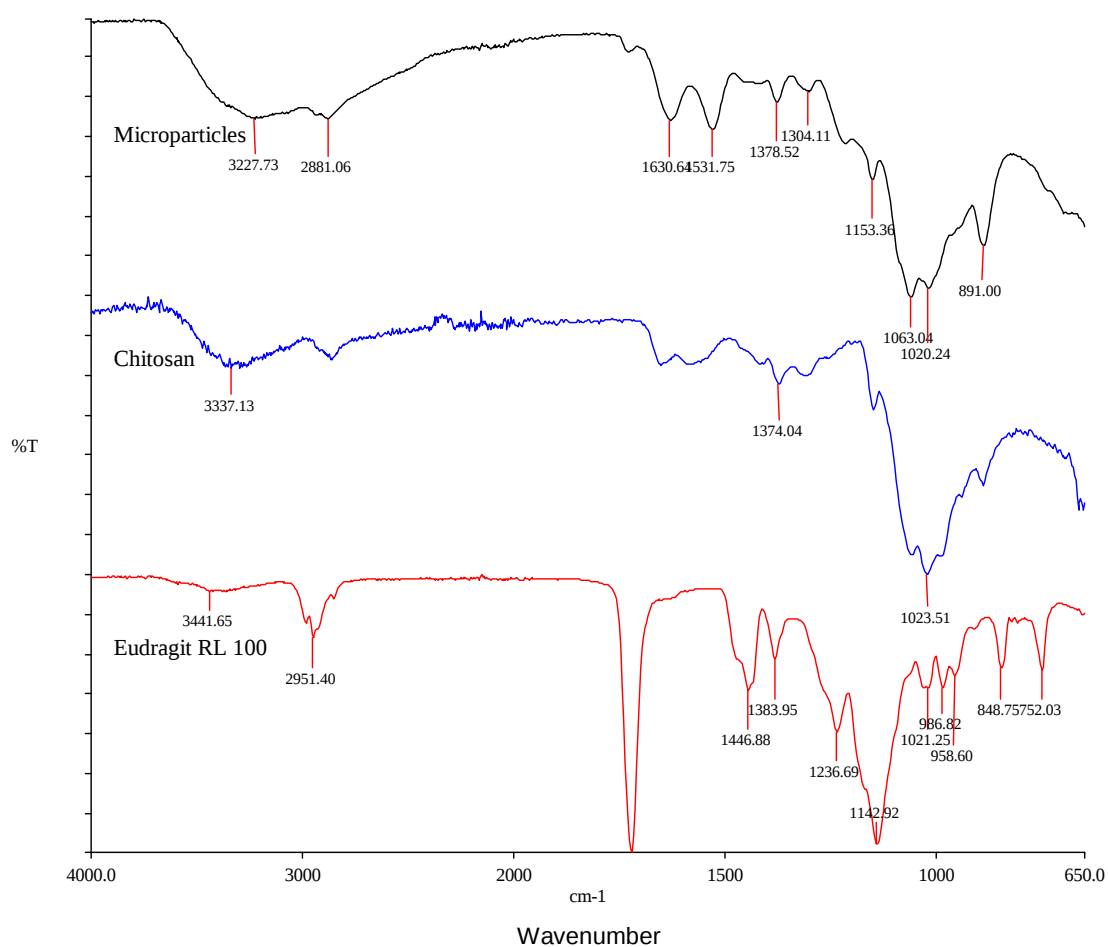
Simultaneous optimisation of MDT<sub>12</sub>, DEE and yield resulted in the production of an optimum microparticle loaded gastrosphere. The dissolution profile (Figure 4.6) of the optimum gastric retentive formulation depicts the control of drug release in a near zero order manner as was required.



**Figure 4.6:** Drug release profile for ciprofloxacin for optimized formulation (N=3, SD<0.031).

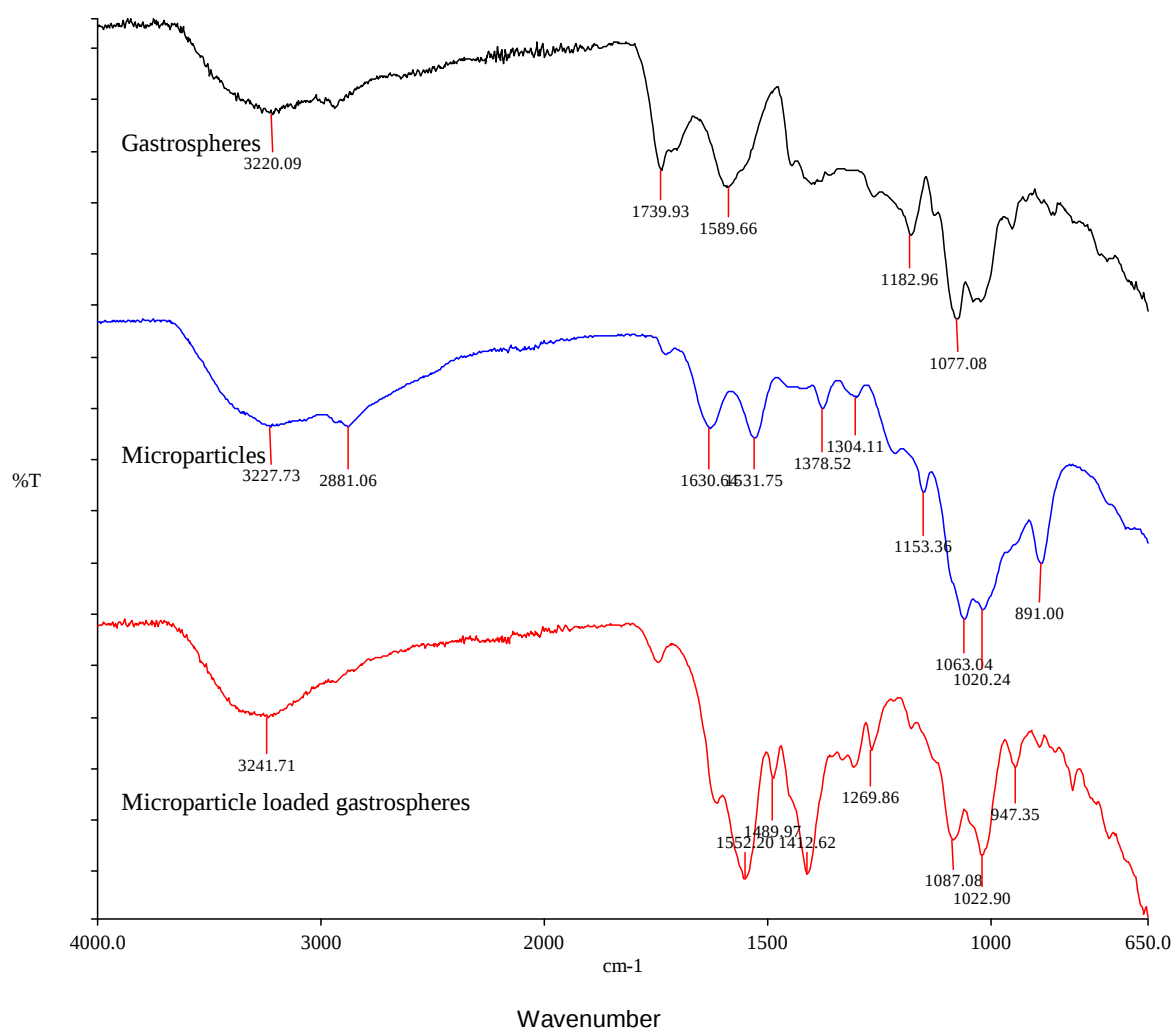
#### 4.3.7. FTIR characterisation

Figure 4.7 shows a comparison of the structural patterns between the microparticle and its component polymers. NH stretching vibrations ( $3300\text{--}3500\text{cm}^{-1}$ ) present in the chitosan and Eudragit<sup>®</sup> is still present in the formed microparticle. C=O stretching vibrations visible in the Eudragit<sup>®</sup> between  $1700$  and  $1900\text{cm}^{-1}$  are still present in the microparticle formulation, although to a lesser degree. C-H bands ( $2800\text{--}2950\text{cm}^{-1}$ ;  $1355\text{--}1395\text{cm}^{-1}$ ;  $1405\text{--}1465\text{cm}^{-1}$ ;  $1430\text{--}1470\text{cm}^{-1}$ ) are present in all spectra. The C=O ester ( $1730\text{cm}^{-1}$ ) visible in Eudragit<sup>®</sup> is no longer present in the microparticle, evidence of the breakage of this bond.



**Figure 4.7:** FTIR spectra of the component polymers and microparticles.

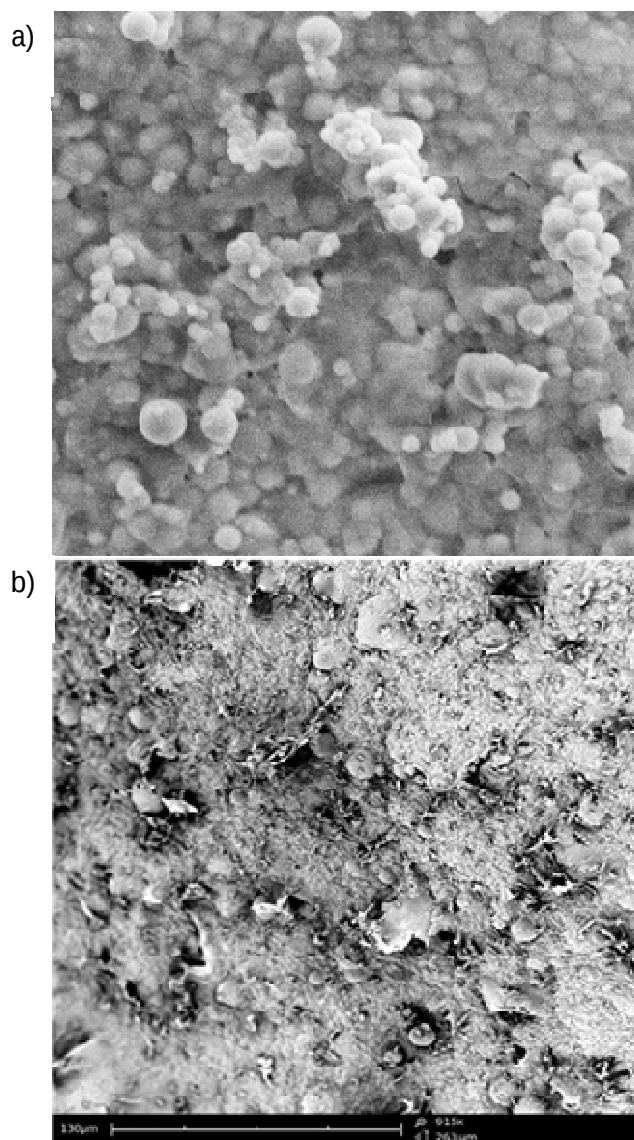
It can be seen in Figure 4.8 that the strong bands observed at  $\pm 3200\text{cm}^{-1}$  in both the microparticles and gastrosphere is still present when the microparticles are loaded within the gastrosphere. The numerous peaks seen between  $900$  and  $1600\text{ cm}^{-1}$  show an accumulative effect when combined. This proves that no interaction occurred between the microparticles and gastrospheres thereby implying that drug release follows a two phase diffusion model.



**Figure 4.8:** FTIR spectra of the gastrospheres and microparticles individually and in combination.

#### 4.3.8. Surface characterisation by SEM analysis

A scanning electron microscope image of the optimized microparticle formulation is shown in Figure 4.9(a). Smooth-edged, spherical microparticles ranging from 1 to 4  $\mu\text{m}$  in diameter are visible. The shape of these microparticles are ideal as the forces of friction between particles is minimized, resulting in good flow properties. Surface morphology of the microparticle loaded gastrospheres is shown in Figure 4.9(b). Air pockets or voids are visible just below the gastrosphere surface. These pockets are due to the sublimation of water crystals during the lyophilisation process and result in the buoyancy of the drug delivery system.



**Figure 4.9:** SEM images of a) microparticle (magnification: 2640x); and b) microparticle loaded gastrosphere (magnification: 215x).

#### **4.3.9. Molecular mechanics assisted model building and energy refinements**

The performance of the  $\text{Ca}^{2+}$  crosslinked Alg-Pec-PAA-PLGA and the interaction of Alg-Pec-PAA-PLGA- $\text{Ca}^{2+}$  with glycosylated gastric mucopeptide analogue, has been discussed in Chapter 3.

A molecular mechanics conformational searching procedure was employed to acquire the data employed in the statistical mechanics analysis, and to obtain differential binding energies of a Polak–Ribiere algorithm and to potentially permit application to complexation and crosslinking of chitosan with/by EUD and TPP respectively. MM+ is a HyperChem modification and extension of Norman Allinger's Molecular Mechanics program MM2 (Warhurst et al., 2003) whereas AMBER, is a package of computer programs for applying molecular mechanics, normal mode analysis, molecular

dynamics and free energy calculations to simulate the structural and energetic properties of molecules.

#### 4.3.10. Molecular mechanics energy relationship analysis

Molecular mechanics energy relationship (MMER), a method for analytical-mathematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, noncovalent Coulombic terms, and noncovalent van der Waals interactions for the complexed/crosslinked morphologies. The MMER model for the potential/steric energy factors in various molecular complexes can be written as:

$$E_{\text{molecule/complex}} = V_{\Sigma} = V_b + V_{\theta} + V_{\phi} + V_{ij} + V_{hb} + V_{el} \quad [\text{Equation 4.4}]$$

$$E_{\text{CHT}} = 35.555V_{\Sigma} = 3.120V_b + 18.035V_{\theta} + 25.774V_{\phi} + 13.323V_{ij} - 24.697V_{el} \quad [\text{Equation 4.5}]$$

$$E_{\text{EUD}} = 45.892V_{\Sigma} = 4.198V_b + 30.278V_{\theta} + 9.398V_{\phi} + 2.017V_{ij} \quad [\text{Equation 4.6}]$$

$$E_{\text{EUD-CHT}} = 94.699V_{\Sigma} = 11.435V_b + 72.059V_{\theta} + 57.228V_{\phi} - 21.148V_{ij} - 0.597V_{hb} - 24.277V_{el} \quad [\text{Equation 4.7}]$$

$$[\Delta E_{\text{BINDING}} = -78.532 \text{ kcal/mol}]$$

$$E_{\text{TPP}} = 199.744V_{\Sigma} = 1.927V_b + 93.088V_{\theta} + 1.599V_{\phi} + 0.046V_{ij} + 103.082V_{el} \quad [\text{Equation 4.8}]$$

$$E_{\text{CHT-TPP}} = 901.408V_{\Sigma} = 18.542V_b + 514.621V_{\theta} + 54.501V_{\phi} + 28.920V_{ij} - 1.065V_{hb} + 285.889V_{el} \quad [\text{Equation 4.9}]$$

$$[\Delta E_{\text{BINDING}} = -132.867 \text{ kcal/mol}]$$

where,  $V_{\Sigma}$  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),  $V_{\phi}$  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.

#### 4.3.11. 3D-computational modelling for polymer-polymer complexes

The observed sustained drug release behaviour of the microparticulate matter consisting of chitosan and Eudragit<sup>®</sup> forming a polymeric matrix may depend on confound geometry and the extent of network innervations. We hereby enquire, using molecular mechanics simulations, the requirement of geometrically proportional

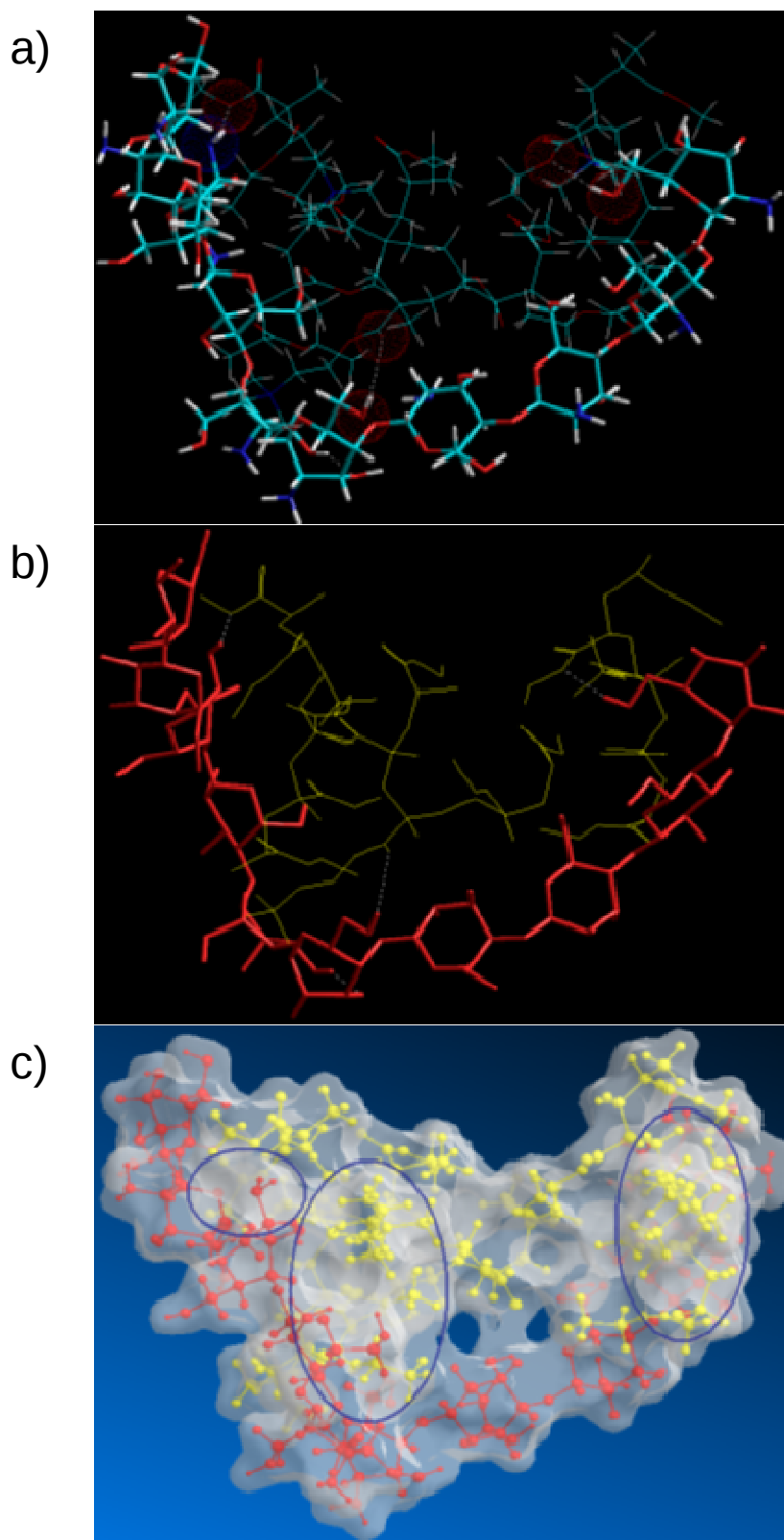
networks for all simulations to systematically control and isolate the effects of a given parameter. Therefore, for carrying out the static lattice atomistic simulations for the polymer-polymer complexes in vacuum, we used the three representative networks shown in Figure 4.10 for our measurements and randomly disposed EUD around CHT to form EUD-CHT polymeric complex.

It is evident from Equations 4.5-4.7, that the formation of EUD-CHT (in vacuum) was accompanied by energy stabilization of -78.532kcal/mol. Molecular modelling studies can account for specific interactions between polymer segments and may provide an estimate of whether two polymers will form a compatible blend and a necessary condition for the miscibility of a mixture of two polymers is a negative free energy of mixing (Tiller and Gorella, 1994). This confirms the compatibility of CHT and EUD and stability of the microparticulate system in dried state. The energy data displayed very interesting results as follows: the total electrostatic energy of the complex (Equations 4.5-4.7) remained approx. the same ruling out the possibility of any electrostatic attraction or repulsion. However, energy of H-bonding of the complex was stabilized with -0.598kcal/mol as compared to no H-bonding energy in case of both the polymers. The possible H-bonding between CHT and EUD is evident from Figure 4.10 where it is demonstrated to be forming -C=O...H-O-C, C-O-C...H-O-C and C-O-C...H-N between EUD and CHT, respectively. But the main energy stabilization was contributed by van der Waals forces in the form of hydrophobic interactions where an initial combined van der Waals energy of ~20kcal/mol (CHT + EUD<sub>3</sub>) was minimized to -21.148kcal/mol (CHT-EUD<sub>3</sub>) leading to  $\Delta E_{\text{vdw-binding}}$  of 40.5201kcal/mol. This confirms the heavy involvement of non-bonding interactions in form of H-bonding and van der Waals forces. These underlying chemical interactions may cause conformational changes responsible for mechanical strength and drug release characteristics of the multiparticulate polymer composite. The stabilization of the structure is clearly obvious from rotation of the glucosamine residues of chitosan producing strain due to steric interactions which in turn are relieved by the inclusion of bond length and angle adjustment after interaction with Eudragit® molecules. These steric adjustments were appeared to be responsible for the formation of H-bonds between CHT and EUD as explained above. The aliphatic/hydrophobic groups of EUD moved along with hydroxyl and NH<sub>2</sub> groups of HPMC to their nearest minimum from the starting point during minimization, driving the molecule through unfavourable regions. Additionally, the resulting large steric interactions may cause the non-interacting groups to overcome torsional barriers presenting a highly dense polymeric matrix (Figure 4.10c) causing a prolonged release of the drug from microparticles.

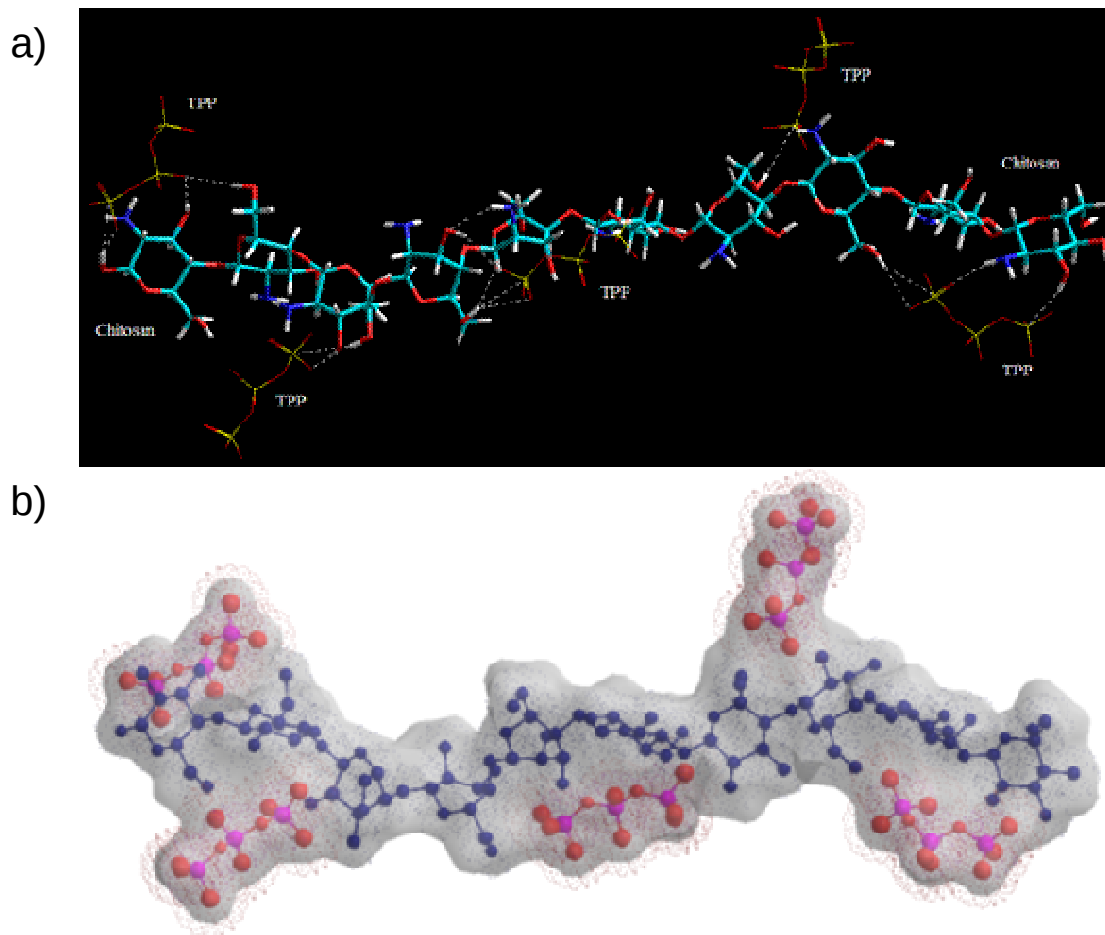
Although the CHT-EUD polymeric complex was stabilized by non-bonding interactions, the inherent bonding interaction in the form of high bond energy (11.435kcal/mol), high angle energy (72.06kcal/mol) and high dihedral energy (57.228kcal/mol) may induce degradation of the polymeric matrix in a quest to attain further energy stabilization on hydration. This makes the hydrophilic polymers, such as chitosan, containing complexes vulnerable to hydration leading to early release of drug due to diffusion of water molecules inside the torsional restraints. Additionally, this may cause loosening of the network structure (Figure 4.10c) that may allow for the leaching out of drug particles during microparticles preparation eventually affecting the encapsulation efficiency (Kumar and Bhatia, 2010). These observations lead to the postulation that the chitosan (the hydrophilic polymer) should be crosslinked in order to get a dense and rigid network causing retention of more drug particles during microparticles preparation and also for prolonged drug release as explained further in this discussion.

#### **4.3.12. 3D-computational modelling for crosslinked-polymer complexes**

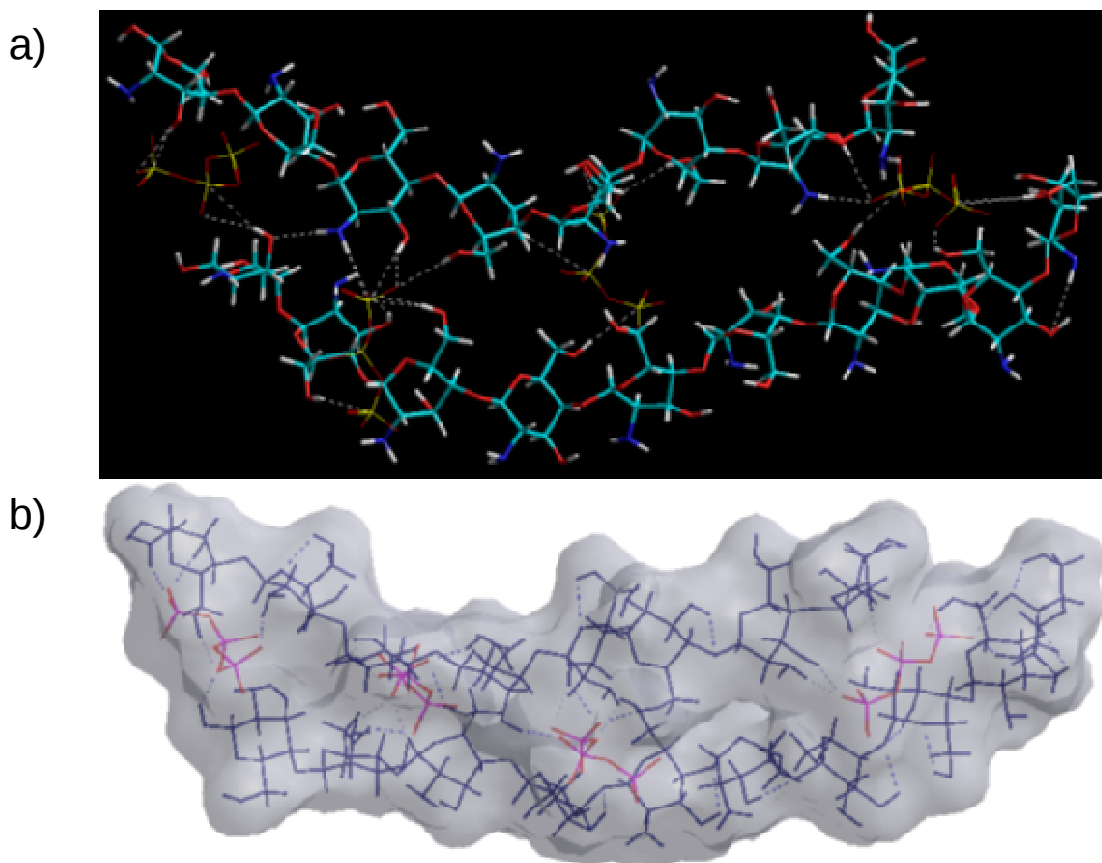
The chitosan present in the microparticulate polymeric matrix in particular was further stabilized and crosslinked due to the addition of sodium tripolyphosphate (TPP) as shown in Figure 4.11 and Equations 4.6, 4.8 and 4.9. The CHT-TPP complex was mainly stabilized by nonbonding interactions in terms of London dispersion forces, H-H bonding and ion pair-ion pair electrostatic interactions where the molecular complex demonstrated a  $\Delta E_{\text{BINDING}}$  of -132.867kcal/mol (Equations 4.6, 4.8 and 4.9). The direct linking of adjacent glucosamine units could have been responsible for bringing about the definitive change in inherent energy attributes relative to uncrosslinked state. The crosslinking of CHT by TPP was evident from the formation of  $\text{PO}_4^- \dots \text{O-H}$  crosslinks,  $\text{PO}_4^- \dots \text{N-H}$  crosslinks,  $\text{O-H} \dots \text{PO}_4^- \dots \text{O-H}$  crosslinks,  $\text{N-H} \dots \text{PO}_4^- \dots \text{N-H}$  crosslinks and  $\text{O-H} \dots \text{PO}_4^- \dots \text{N-H}$  crosslinking. Furthermore, this may also initiate an intermolecular crosslinking leading to a significant axial stress due to buildup of the adjacent crosslinks (Figure 4.12). This provides a reasonable explanation for the experimentally observed controlled release behaviour of the microparticle formulations due to formation of a dense polymeric matrix owing to this very crosslinking mechanism of TPP and the complexation of CHT and EUD.



**Figure 4.10:** a and b) Visualization of geometrical preferences of EUD (stick rendering - yellow) in complexation with CHT (tube rendering - red) after molecular mechanics simulations; c) The dense polymeric matrix is encircled in the Connolly molecular electrostatic potential surfaces in translucent display mode. Colour codes: C (cyan), O (red), N (blue) and H (white).



**Figure 4.11:** a) Visualization of geometrical preferences of TPP (stick rendering) in complexation with CHT (tube rendering) after molecular mechanics simulations; and b) The Connolly molecular electrostatic potential surface of crosslinked polymeric matrix in translucent display mode. Colour codes: C (cyan), O (red), N (blue), P (yellow) and H (white).



**Figure 4.12:** a) Visualization of geometrical preferences of intermolecular crosslinking of CHT (tube rendering) by TPP (stick rendering) after molecular mechanics simulations; b) The Connolly molecular electrostatic potential surface of intermolecularly crosslinked polymeric matrix in translucent display mode. Colour codes: C (cyan), O (red), N (blue), P (yellow) and H (white).

#### 4.4. CONCLUDING REMARKS

In this chapter, the Central Composite Design was applied for the development and optimisation of a novel microparticle entrapped gastrosphere to deliver ciprofloxacin in a gastric retentive manner. The design generated 14 microparticulate formulations, which varied in their drug entrapment, yield and release characteristics. Regression analysis demonstrated the agreement between the predicted and observed responses obtained, indicating the applicability of the models generated by the Central Composite Design. Optimisation of the design resulted in the production of an optimal formulation having a DEE of 59.23%, yield of 86.743% and a  $MDT_{12}$  of 32.2058, which was capable of obtaining a near zero-order drug release over a period of 12 hours. Overall, the experimental findings were well corroborated by the chemometric modelling.

## CHAPTER 5

### *In Vivo* Analysis of Drug Delivery Systems

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#### **5.1. ANIMAL STUDIES**

##### **5.1.1. The swine animal model**

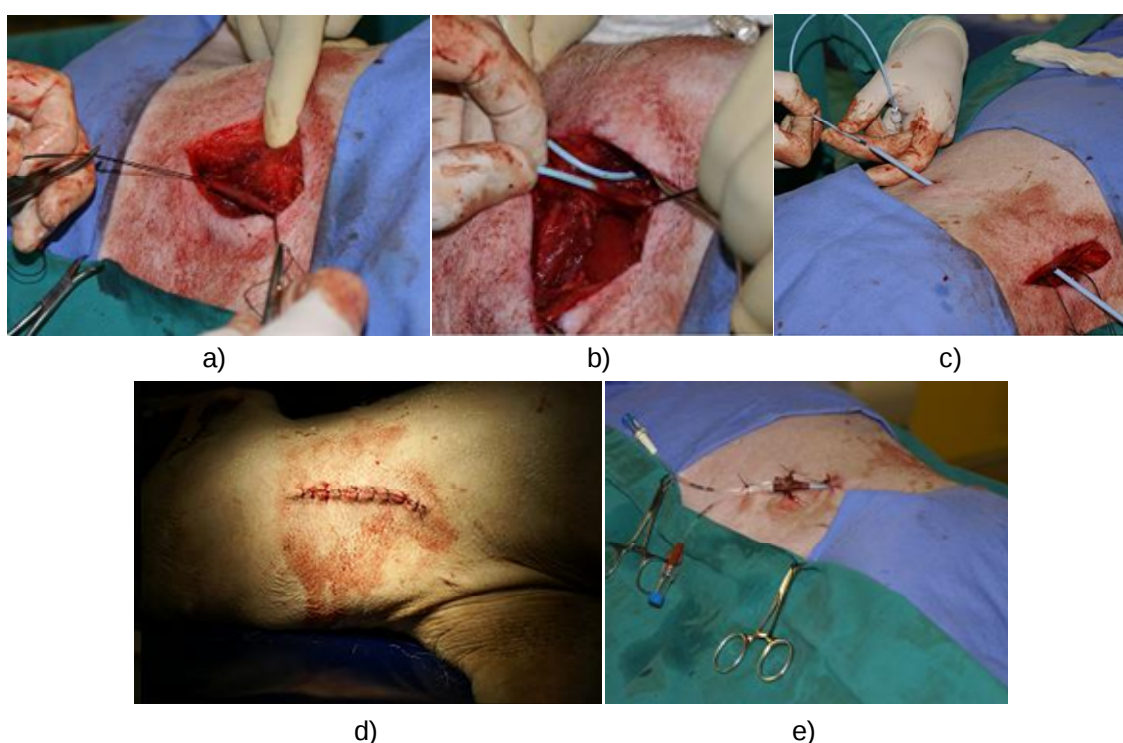
There are several factors to take into account when selecting an appropriate animal model for biomedical research, these include: the cost of the animal, ease of handling and housing, breeding time, longevity and whether it meets size requirements (Mullen et al., 1992; Pennington, 1992; Sachs, 1992; White et al., 1992). There is no animal which perfectly satisfies all the requirements of an ideal animal model as a replacement for human studies (Pennington, 1992; White et al., 1992). Although dogs, cats and primates have been used in the past in biomedical research, there is a growing resistance to this due to high cost and pressure from special interest groups (Mullen et al., 1992). The use of pigs in this field has increased steadily over the past decade due to the great similarities between human and swine anatomy and physiology, as well as meeting many of the above mentioned factors (Sachs, 1992). It is however, important that all personnel are knowledgeable in the proper care and handling techniques of swine, as well as the irregularity of anaesthesia induction (Bloor et al., 1992). The majority of porcine biomedical research is conducted on immature domestic pigs, with researchers extrapolating the results to the human adult (Hannon, 1992). With particular importance to this study, basal and histamine-stimulated release of pepsin and gastric acid is exhibited by pigs (Hannon, 1992).

##### **5.1.2. Methods**

###### **5.1.2.1. Implantation of chronic catheter**

A pig weighing 35-45kg was anaesthetised with ketamine (11mg/kg I.M.) and midazolam, (0.3mg/kg I.M.). Buprenorphine (0.05mg/kg I.M.) and carprofen (4mg/kg I.M.) were administered for analgesia and inflammation. The pig was then intubated and anaesthesia was maintained with 2% isoflurane in 100% oxygen. Under aseptic conditions, a 7 french gauge double lumen 35cm catheter (CS-28702) (Arrow Deutschland GmdH, Erding, Germany) was surgically inserted into the left jugular vein as depicted in Figure 5.1. 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 was inserted 10cm into the lumen of the vein. The lumen of the catheter was 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 trocar, to an exit point cranial to the dorsal aspect of the scapular. Both the incision and exit points were sutured. The externalised injection ports of the catheter were sutured to the skin of the pig so as to limit excessive movement and bending. Blood was removed via the catheter and the catheter was flushed with heparin saline (1000 i.u. of heparin in 1L of 0.9% saline). Thereafter the animal was allowed 8 days to recover from the surgical procedure. During this time, it was habituated to the process of blood sampling. Throughout the study, the catheter was flushed with heparinised saline three times a day.



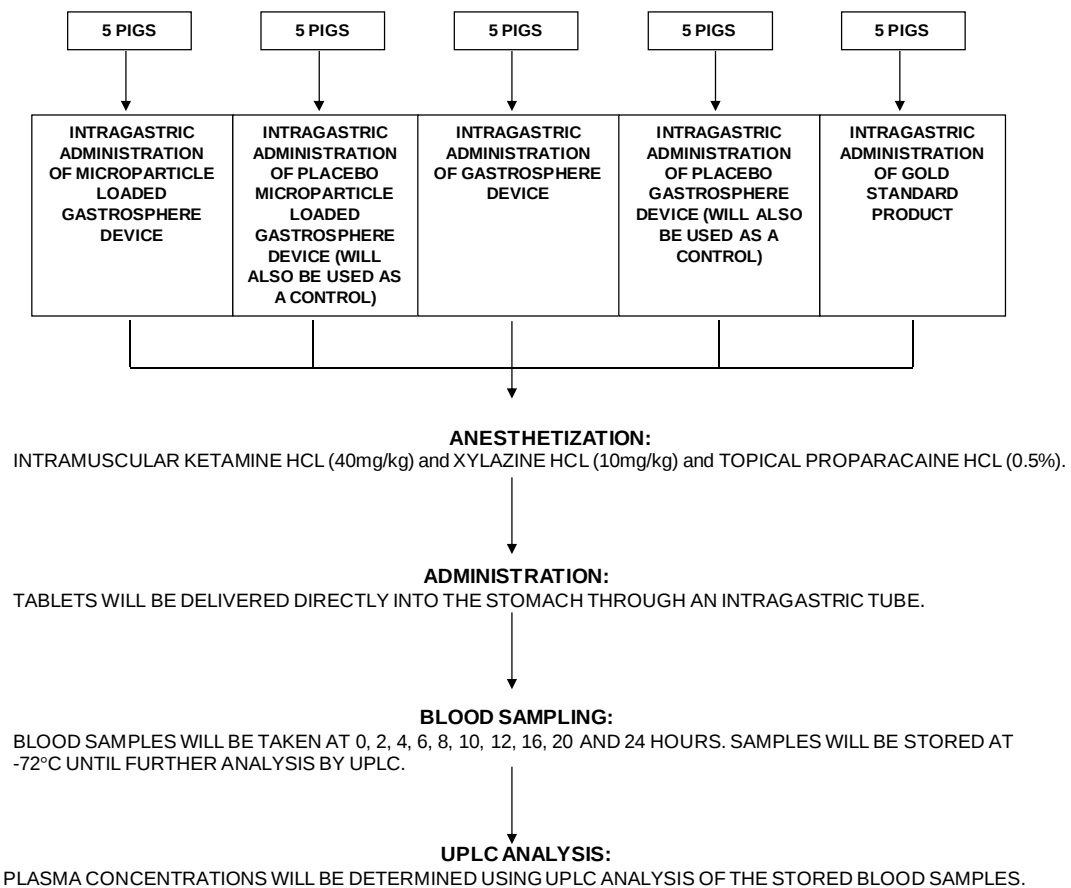
**Figure 5.1:** Digital images depicting the surgical procedure for the implantation of the chronic catheter a) isolation of jugular vein, b) insertion of the catheter through the wall of the vein, c) subcutaneous tunneling of catheter through a trocar, d) sutures of the incision and e) suturing of catheter ports and exit point.

#### 5.1.2.2. Administration of drug delivery system

Dormicum® and Anaket® was injected directly into the jugular vein catheter. Once sedated, the pig was anaesthetised with 2% isoflurane in 100% oxygen. An intragastric tube was inserted into the stomach of the pig, and the delivery system was washed down the tube with 50mL water as depicted in Figure 5.2. While under sedation, all wounds were checked and sutures were repaired. The pig was returned to its pen to recover under observation. A summary of the *in vivo* study is illustrated in Figure 5.3.



**Figure 5.2:** Digital images depicting the administration of the drug delivery system a) anesthetisation of the sedated pig using isoflurane gas and b) administration of capsules through an intragastric tube.



**Figure 5.3:** Summary of the *in vivo* study.

### **5.1.2.3. Sampling of blood**

Blood samples were taken over a 24 hour period. Two hour intervals were utilised for the first 12 hours, thereafter, samples were taken every four hours for the remaining 12 hours.

The catheter was disinfected and an aseptic technique was utilised to prevent the introduction of foreign organisms. Before blood was sampled, the catheter was flushed with heparinised saline in order to clear any clots and remove old blood. Thereafter, blood was drawn and placed in a lithium heparin Vacutainer<sup>®</sup>. The catheters were once again flushed with heparinised saline. Blood samples were centrifuged at 5000rpm for 15 minutes. The plasma was removed and frozen at -72°C until required.

## **5.2. ANALYSIS OF BLOOD SAMPLES**

### **5.2.1. Liquid Chromatography**

Liquid chromatography is a technique which is widely used in order to control and evaluate the quality and consistency of active pharmaceutical compounds in drug delivery systems (Dongre et al., 2008).

Chromatography involves the separation of compounds based on their polarity. This is achieved through the principle of 'like attracts like'. Two phases are required, namely the mobile phase and the stationary phase, one of which must be polar, while the other is non-polar. Migration of compounds occurs when that compound is in the mobile phase. Therefore, compounds which are 'most like' the mobile phase in polarity will be eluted first, whereas compounds which are 'least like' the mobile phase will be eluted last.

Selection of the correct mobile and stationary phases is of the utmost importance in order to achieve a successful separation. Most commonly, the polarity of the stationary phase is matched to that of the compound, and the mobile phase is of opposite polarity. This is done in order to ensure that the compound and mobile phase are not too similar in polarity, which would result in very little retention, causing the compound to become eluted with the void volume of the mobile phase. When the stationary phase is more polar than the mobile phase, it is said to be 'normal phase chromatography', whereas if the mobile phase is more polar than the stationary phase, it is termed 'reverse phase' (Hamilton and Sewell, 1982; Lim, 1986). Reverse phase chromatography is the most commonly used mode of liquid chromatography due to its versatility (Lim, 1986).

In reverse phase chromatography, the stationary phase consists of silica which is chemically bonded with an alkylsilyl compound in order to obtain a non-polar, hydrophobic surface (Lim, 1986) and named according to the number of carbon groups which have been attached. The most common packaging is the C<sub>18</sub> type, although they are available from C<sub>1</sub> up to C<sub>22</sub> (Lim, 1986). The retention of compounds is usually proportionate with the length of the bonded carbon group.

The mobile phase may be a single solvent, or a combination of numerous solvents (Hamilton and Sewell, 1982). Besides the polarity, other characteristics such as viscosity, volatility, refractive index and UV absorption may influence the scientist's decision in the selection of an appropriate mobile phase (Hamilton and Sewell, 1982). Separations may be run isocratically (the composition of the mobile phase is kept constant) or in a gradient method (the mobile phase composition changes during the elution, including changes in pH or ionic strength) (Pryde and Gilbert, 1979).

Internal standards, which are added to the solution before it is analysed, are commonly used in liquid chromatography in order to eliminate apparatus and procedural errors (Brown, 1973). There are certain requirements which make an internal standard suitable for use (Brown, 1973). These are:

- It must elute near the compound being tested
- It must be completely resolved from the compound of interest
- It should be at a similar concentration to the compound of interest
- It must be chemically inert

### **5.2.2. Ultra Performance Liquid Chromatography (UPLC)**

UPLC is a relatively new separation technique based on liquid chromatography (Dongre et al., 2008). UPLC instruments can run at higher operating pressures (up to 1000 bars as opposed to a maximum of 400 bars associated with HPLC), and the columns are packed with sub-2µm particles. These changes allow for much greater resolution in a significantly reduced separation time with high peak capacities (Dongre et al., 2008; Wren and Tchlitcheff, 2006). It is because of these improvements in speed and sensitivity that UPLC has received much attention from the pharmaceutical industry in recent years (Dongre et al., 2008; Wren and Tchlitcheff, 2006)

### **5.2.3. UPLC analysis of metformin loaded gastrospheres**

#### **5.2.3.1. Materials and Methods**

##### **5.2.3.1.1. Reagents**

Metformin (MET) and the internal standard, diphenhydramine (DPH) were purchased from Aldrich Chemistry (Sigma-Aldrich, Steinheim, Germany). Acetonitrile (ACN) and methanol (MeOH) were purchased from Romil Ltd (Cambridge, UK), formic acid, ammonium solution 25%, NaOH and  $\text{KH}_2\text{PO}_4$  were purchased from Rochelle Chemicals (Johannesburg, South Africa). Water was deionised and filtered on Millipore water purification system (Milli-Q gradient, Mass, USA).

##### **5.2.3.1.2. Preparation of calibration standard solutions and determination of the limit of quantification**

Stock solutions of MET (500 $\mu\text{g}/\text{mL}$ ) and internal standard (DPH) (200 $\mu\text{g}/\text{mL}$ ) were prepared in deionized water. The MET solution was diluted with deionized water in order to prepare spiking solutions of metformin concentrations ranging from 1 to 500 $\mu\text{g}/\text{mL}$ . In order to prepare the calibration standards, 50 $\mu\text{L}$  aliquots of the spiking solutions were added to 150 $\mu\text{L}$  blank plasma. The concentrations of plasma calibration standards ranged between 25 $\text{ng}/\text{mL}$  and 125 $\mu\text{g}/\text{mL}$ . Each standard was subjected to the solid phase extraction (SPE) procedure as described in 5.2.3.1.4 and was spiked with the internal standard (50 $\mu\text{L}$ ). The samples were then placed in Waters certified UPLC injection vials for analysis. A 5 $\mu\text{L}$  sample was injected into the UPLC column for analysis of metformin content.

The peak area ratio of MET and the internal standard (DPH) were plotted against the concentration for the calibration standards. The means of the least square method, the linearity equation and correlation coefficient, was obtained.

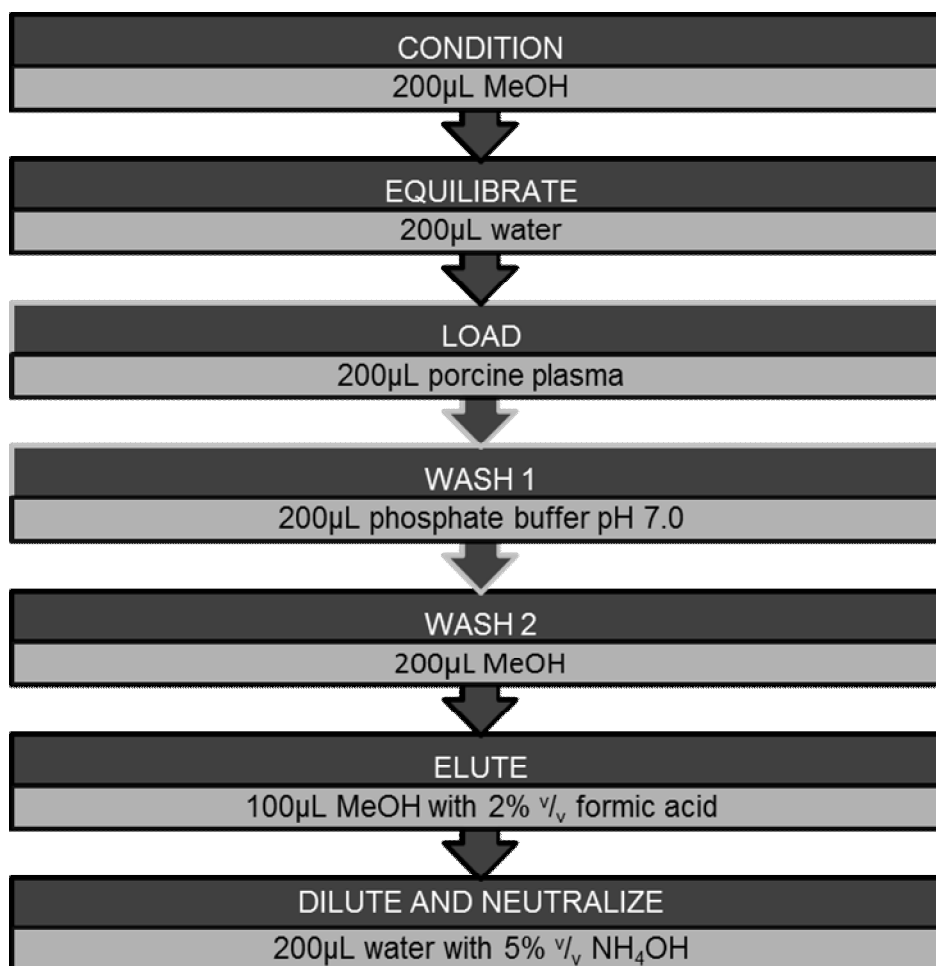
The limit of quantification (LOQ) is determined by calculating the concentration of metformin at which the chromatographic peak was equal to 10 and 3 times of the baseline noise.

##### **5.2.3.1.3. Sample preparation**

Blood samples were removed from the freezer (-70°C) and thawed. A 150 $\mu\text{L}$  aliquot of each blood sample was transferred to a centrifuge tube. ACN (150 $\mu\text{L}$ ) and deionised water (50 $\mu\text{L}$ ) was added to each vial and vortexed. Each tube was subsequently centrifuged at 3200rpm for 15 minutes (Optima® LE-80K, Beckman, USA). The supernatant was removed and subjected to the SPE procedure described in 5.2.3.1.4 and spiked with internal standard (DPH) (50 $\mu\text{L}$ ). The samples were then placed in Waters certified vials for analysis.

#### 5.2.3.1.4. Extraction of Metformin from porcine plasma samples

Waters Oasis® WCX 1cc cartridges (Millipore Corporation, Mass, USA) were used in order to separate metformin from porcine plasma under vacuum. Figure 5.4 shows the procedure that was followed in order to achieve extraction.



**Figure 5.4:** Procedure followed in order to extract metformin from porcine blood plasma.

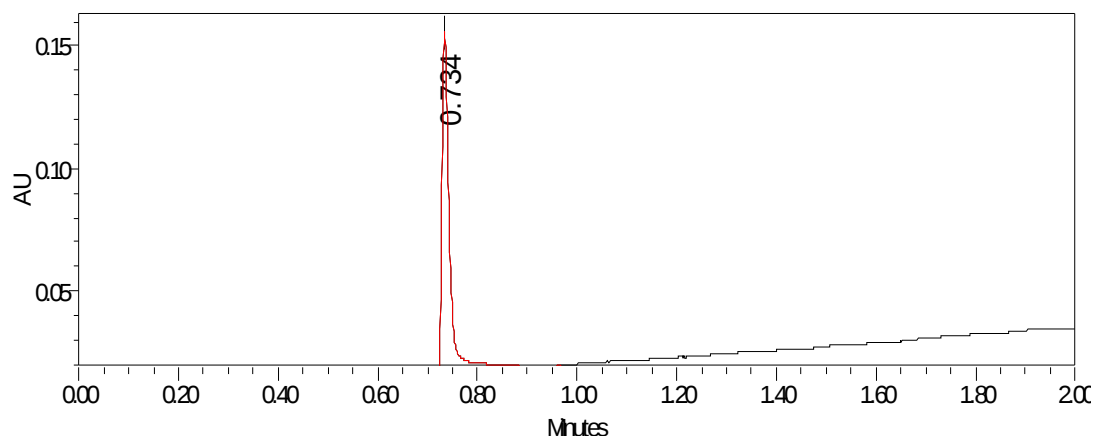
#### 5.2.3.1.5. Chromatographic system and conditions

All analyses were performed on a Waters Acquity® UPLC system (Waters Corp., Milford, MA, USA) including binary solvent manager, sample manager and PDA detector, connected to a Waters Empower 2 data station. UPLC separation was achieved on an Acquity® UPLC HSS T3 column (2.1mmx150mm I.D. 1.8µm), column temperature was set at 30°C. The mobile phase consisted of water and ACN under isocratic conditions (ACN-H<sub>2</sub>O 65:35 v/v) at a flow rate of 0.40mL/min. The total run time was 3 min with an injection volume of 5µL. The system was allowed to equilibrate for 5 minutes between injections. The photodiode array (PDA) detector was set at 241nm.

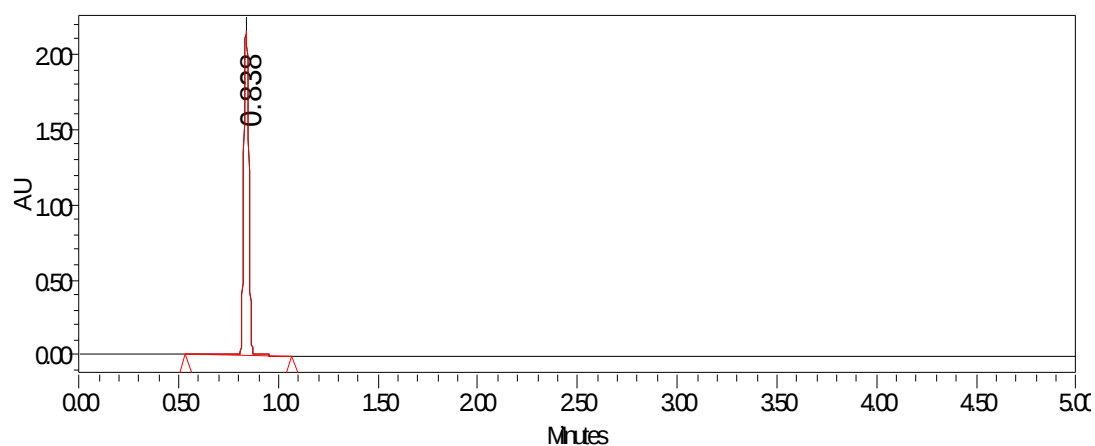
### 5.2.3.2. Results and discussion

#### 5.2.3.2.1. Chromatograms for standard solutions

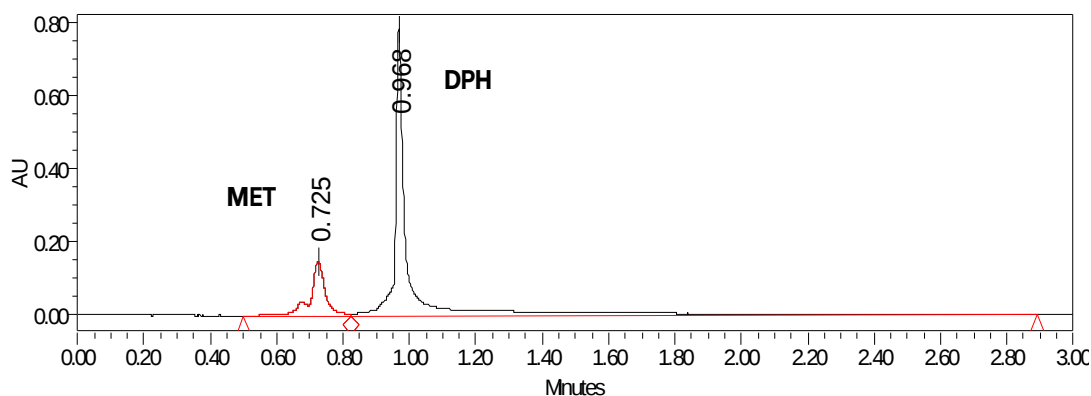
A typical chromatogram of standard solution of MET (Figure 5.5), DPH (Figure 5.6) and a plasma sample post-administration of the gastrospheres is displayed in Figure 5.7. These chromatograms prove that the methods employed for the elution of both MET and DPH were successful.



**Figure 5.5:** A typical chromatogram depicting the peak ( $R_t=0.734$ ) for MET employing UPLC at 241nm in water.



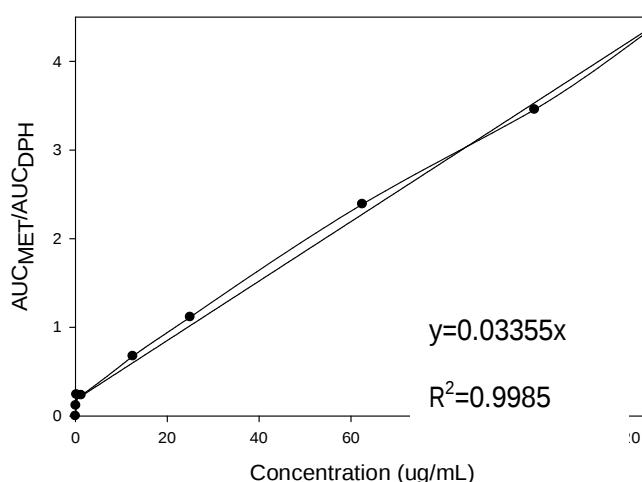
**Figure 5.6:** A typical chromatogram depicting the peak ( $R_t=0.838$ ) for DPH (internal standard) employing UPLC at 241nm in water.



**Figure 5.7:** A typical UPLC chromatogram depicting the distinct separation of MET ( $R_t=0.725$ ) and DPH ( $R_t=0.968$ ) at 241nm from the porcine plasma samples.

#### 5.2.3.2.2. Calibration curve

Figure 5.8 displays the standard linear curve constructed for the determination of MET concentrations in porcine plasma. Good linearity was achieved ( $R^2=0.9985$ ).



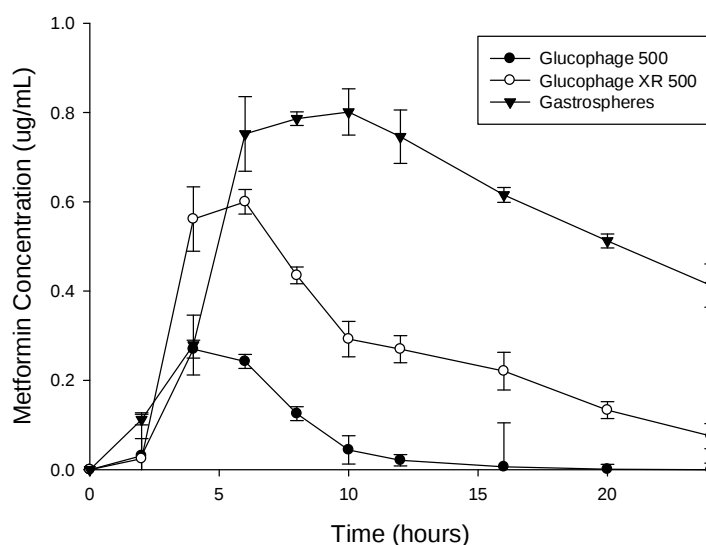
**Figure 5.8:** Calibration curve of metformin concentrations in blank porcine plasma.

The lower limit of quantification for this study was determined to be 0.742ng/mL.

#### 5.2.3.2.3. *In vivo* metformin release profile from gastrosppheres

A comparison is made in Figure 5.9 between the metformin plasma concentrations obtained after the administration of a single dose of Glucophage® 500, Glucophage® XR 500 and the gastrosppheres designed in Chapter 3. It can be noted that the gastrosppheres not only obtain a higher peak plasma concentration, but also results in an improvement in the maintenance plasma concentration.

The original Glucophage® 500 displays the lowest plasma concentrations over the entire 24 hours. This results in the requirement for repeated doses every 8 hours.



**Figure 5.9:** *In vivo* profile for metformin release from Glucophage® 500mg (N=5, SD<0.03514 in all cases), Glucophage® XR 500 (N=5, SD<0.03584 in all cases) and the gastrosphere drug delivery system (N=5, SD=0.04113 in all cases).

## 5.2.4. UPLC analysis of ciprofloxacin loaded microparticles

### 5.2.4.1. Materials and Methods

#### 5.2.4.1.1. Reagents

Ciprofloxacin (CIPRO) was sourced from Fluka Analytical (Sigma-Aldrich, Steinheim, Germany) and the internal standard, ranitidine hydrochloride (RAN) was purchased from Sigma RBI (Steinheim, Germany). Acetonitrile (ACN) and methanol (MeOH) were purchased from Romil Ltd (Cambridge, UK), formic acid, ammonia solution 25% and potassium dihydrogen orthophosphate was purchased from Rochelle Chemicals (Johannesburg, South Africa). Water was deionised and filtered on Millipore water purification system (Milli-Q gradient, Mass, USA).

#### 5.2.4.1.2. Preparation of calibration standard solutions and determination of the limit of quantification

Stock solutions of CIPRO (1µg/mL) and internal standard (RAN) (40µg/mL) were prepared. The CIPRO solution was diluted with deionised water in order to prepare spiking solutions of CIPRO concentrations ranging between 1.125 and 9µg/mL. In order to prepare the calibration standards, 800µL aliquots of the spiking solutions were added to 1mL blank plasma. The concentrations of plasma calibration standards ranged between 0.5 and 4µg/mL. Each standard was subjected to the SPE procedure described in 5.2.4.1.4 and was spiked with the internal standard (20µL). The samples were then placed in Waters certified UPLC injection vials for analysis. A 5µL sample was injected into the UPLC column for analysis of ciprofloxacin content.

The peak area ratio of CIPRO and the internal standard RAN were plotted against the concentration for the calibration standards. The means of the least square method, the linearity equation and correlation coefficient was obtained.

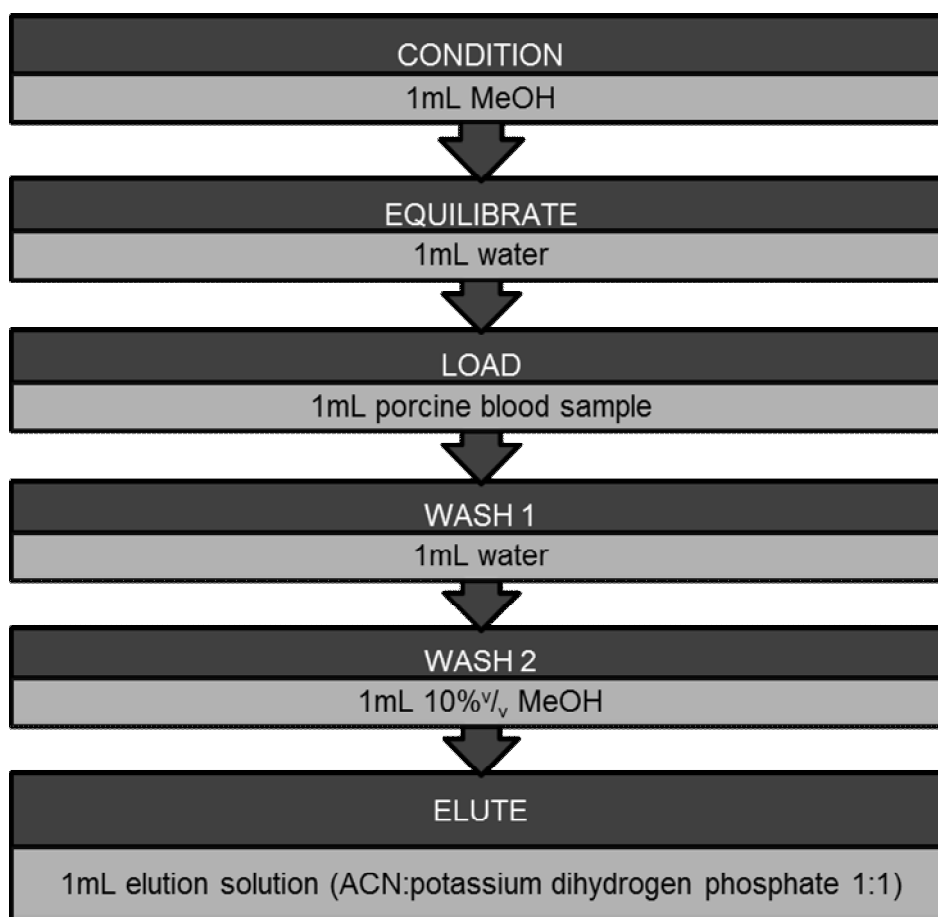
The limit of quantification (LOQ) is determined by calculating the concentration of ciprofloxacin at which the chromatographic peak was equal to 10 and 3 times of the baseline noise.

#### **5.2.4.1.3. Sample Preparation**

Blood samples were removed from the freezer (-70°C) and thawed. A 1mL aliquot of each blood sample was transferred to a centrifuge tube. ACN (0.2mL) and deionised water (0.8mL) were added to each tube and vortexed and subsequently centrifuged at 3200rpm for 15 minutes (Optima® LE-80K, Beckman, USA). The supernatant was removed and subjected to the SPE procedure described in 5.2.4.1.4 and spiked with internal standard (RAN) (20µL). The samples were then placed in Waters certified vials for analysis.

#### **5.2.4.1.4. Extraction of ciprofloxacin from plasma samples**

Waters Oasis HLB 3cc cartridges (Millipore Corporation, Mass, USA) were used in order to separate ciprofloxacin from plasma under vacuum. Figure 5.10 shows the method which was carried out in order to achieve this.



**Figure 5.10:** Procedure followed to extract ciprofloxacin from porcine blood plasma.

#### 5.2.4.1.5. Chromatographic system and conditions

All analyses were performed on a Waters Acquity® UPLC system (Waters Corp., Milford, MA, USA) including binary solvent manager, sample manager and PDA detector, connected to a Waters Empower 2 data station. UPLC separation was achieved on an Acquity® UPLC BEH shield Reverse Phase C18 column (2.1mmx100mm I.D. 1.7µm), column temperature was set at 25°C. The mobile phase consisted of buffer and ACN under gradient conditions as shown in Table 5.1 at a flow rate of 0.40mL/min. The total run time was 5 min with an injection volume of 5µL. The photodiode array (PDA) detector was set at 280nm.

**Table 5.1:** Gradient UPLC methodology for the detection of CIPRO and the IS (RAN).

| Time (min) | Flow (mL/min) | % Buffer | % ACN |
|------------|---------------|----------|-------|
| 0          | 0.4           | 90       | 10    |
| 0.5        | 0.4           | 80       | 20    |
| 3          | 0.4           | 90       | 10    |

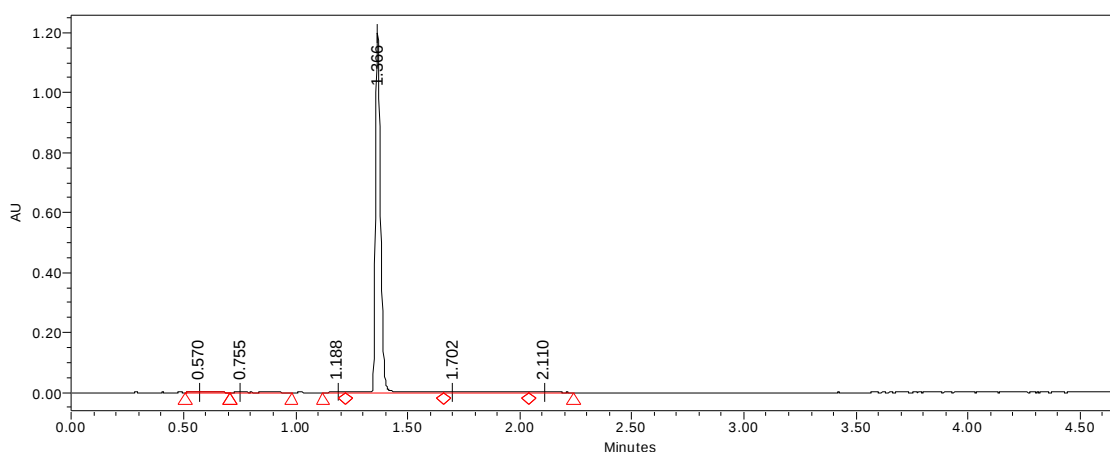
#### 5.2.4.1.6. Preparation of mobile phase

A buffer concentrate was prepared by pouring 200mL deionised water into a 1L volumetric flask and adding ammonium solution 25% (3mL) and formic acid (10mL). The solution was made up to 1L and shaken. The mobile phase is prepared by taking 100mL buffer concentrate and making it up to 1L with ionised water.

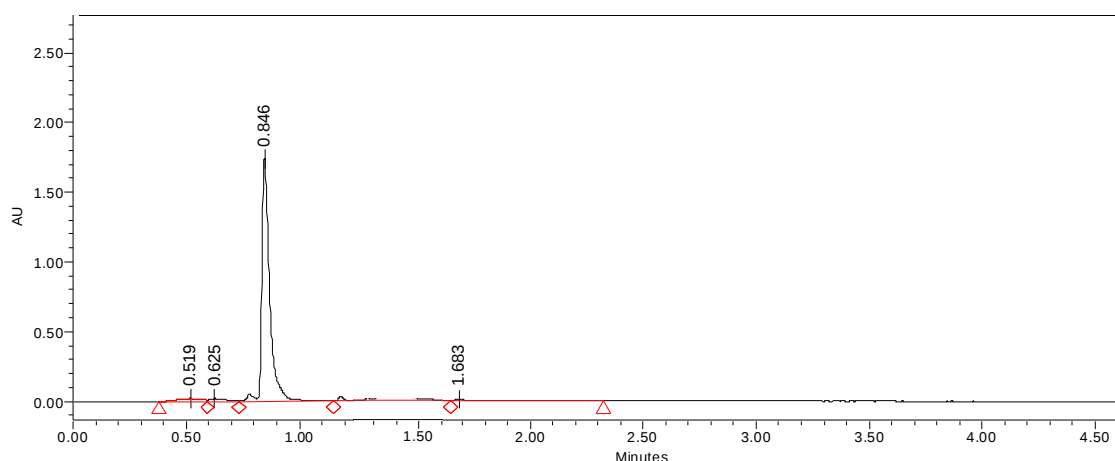
#### 5.2.4.2. Results and discussion

##### 5.2.4.2.1. Chromatograms for standard solutions

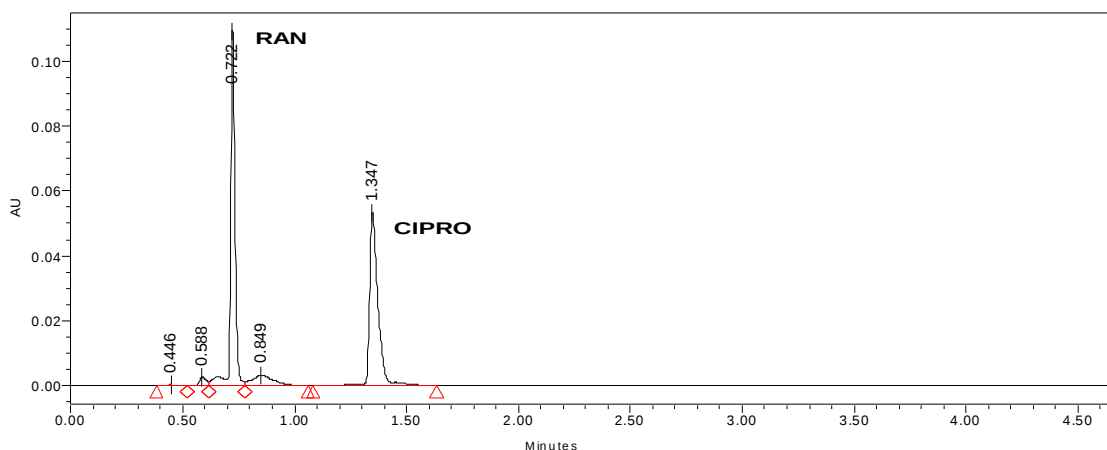
A typical chromatogram of standard solution of CIPRO (Figure 5.11), RAN (Figure 5.12) and a plasma sample post-administration of the microparticle loaded gastrospheres is displayed in Figure 5.13. These chromatograms prove that the methods employed for the elution of both CIPRO and RAN was successful.



**Figure 5.11:** A typical chromatogram depicting the peak ( $R_t=1.366$ ) for CIPRO employing UPLC at 280nm in water.



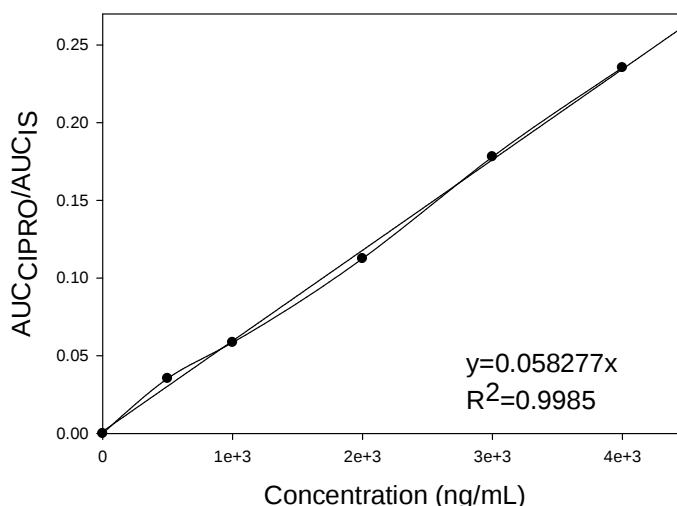
**Figure 5.12:** A typical chromatogram depicting the peak ( $R_t=0.846$ ) for RAN (internal standard) employing UPLC at 280nm in water.



**Figure 5.13:** A typical UPLC chromatogram depicting the distinct separation of CIPRO ( $R_t=1.347$ ) and RAN ( $R_t=0.722$ ) at 280nm from the porcine plasma samples.

#### 5.2.4.2.2. Calibration curve

Figure 5.14 displays the standard linear curve constructed for the determination of CIPRO concentrations in porcine plasma. Good linearity was achieved ( $R^2=0.9984$ ).



**Figure 5.14:** Calibration curve of ciprofloxacin concentrations in blank porcine plasma.

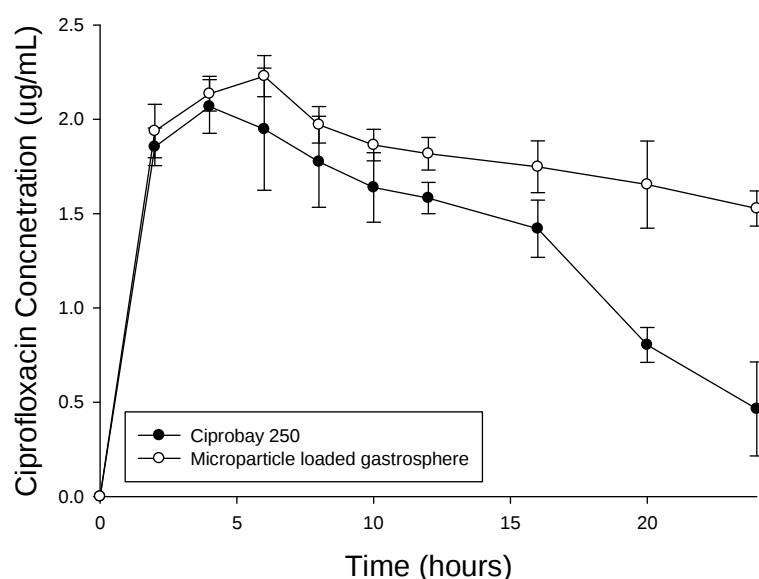
The lower limit of quantification for this study was determined to be 0.317ng/mL.

#### 5.2.4.2.3. *In vivo* ciprofloxacin release profile from microparticle loaded gastrospheres

The extraction procedure followed in order to remove ciprofloxacin from the porcine plasma is obtained from Vybiralova et al. (2005). The chromatographic conditions were adapted from Pearce et al. (2009) for the quantitative determination of ciprofloxacin. The adaptation has resulted in the easier preparation of mobile phases and thus a change in the mobile phases utilised. This method is simple, quick and effective,

showing good separation of peaks and complete elution is achieved in less than 2 minutes under the chromatographic conditions described.

Drug plasma concentrations can be seen in Figure 5.15. A single dose of Ciprobay<sup>®</sup> 250mg and a single dose of microparticle loaded gastrospheres are both represented by a curve. It can be seen that the drug concentration is sustained in a controlled manner after administration of the microparticle loaded gastrospheres whereas ciprofloxacin concentrations steadily decrease after peak concentration is reached following administration of Ciprobay<sup>®</sup>.



**Figure 5.15:** *In vivo* profile for ciprofloxacin release from Ciprobay<sup>®</sup> 250 (N=5, SD<0.1741 in all cases) and microparticle loaded gastrospheres (N=5, SD<0.1189 in all cases).

### 5.3. CONCLUDING REMARKS

This study sought to address the *in vivo* release of metformin and ciprofloxacin with the use of SPE and UPLC techniques.

UPLC analysis was undertaken in order to determine plasma concentrations of metformin and ciprofloxacin after the administration of the drug delivery systems designed and optimized in chapters 3 and 4 respectively.

Drug release profiles illustrated superior plasma concentrations of both metformin and ciprofloxacin in comparison the gold standard products currently on the market, thereby reducing the dosing intervals and ultimately improving patient compliance.

## CHAPTER 6

### Conclusion and Recommendations

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#### 6.1. CONCLUSIONS

Extensive *in vitro* testing has resulted in the development of two novel gastroretentive drug delivery systems.

The gastropheres were optimised employing the Box-Behnken experimental design. 15 formulations were investigated with respect to yield, buoyancy, mucoadhesion and MDT<sub>12</sub>. A Central Face-Centred design was utilised in order to optimise the microparticle formulation. This entailed the investigation of nine formulations with respect to drug entrapment, yield and MDT<sub>12</sub>.

Extensive tests including water uptake and swelling, surface area and porosity analysis, FTIR and SEM were undertaken in order to determine the physicochemical characteristics of the developed drug delivery systems.

Both the gastrophere delivery system and microparticle loaded gastrophere delivery system displayed effective control over drug release in an *in vitro* environment. Both delivery systems were tested *in vivo* in the pig model in order to prove this controlled drug release.

The development of effective UPLC and SPE techniques allowed for the testing of *in vivo* porcine plasma samples. Results for both drug delivery systems provide evidence that confirms the effective control which has been achieved over the rate of drug release.

#### 6.2. RECOMMENDATIONS

With these two novel drug delivery systems, I have been able to improve the bioavailability of two drugs with opposing aqueous solubilities. A drug delivery platform has therefore been developed which would ultimately have the ability to deliver any drug possessing a narrow absorption window and low bioavailability. Resulting in single daily dose regimens with fewer side effects, improved patient compliance and a greater overall rate of therapeutic success.

These same two platforms may also be adapted for the treatment of specific disease states. Peptic ulcers, especially with the presence of *H. pylori*, may benefit from local treatment. It is envisaged that traditional 'Triple Therapy' which involves the use of a PPI (proton pump inhibitor) and two antibacterials namely, amoxicillin and clarithromycin be incorporated into a single dose. This would enable both systemic and local eradication of the bacterium.

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# APPENDICES

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## APPENDIX A

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### PUBLICATIONS

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## APPENDIX A1

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### Gastroretentive Drug Delivery Systems: Current Developments in Novel System Design and Evaluation

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**ABSTRACT:** The task of achieving efficient delivery of drugs that have poor bioavailability or narrow absorption windows have plagued the pharmaceutical industry for decades. Thus, much research has been dedicated to the development of novel polymeric-based gastroretentive drug delivery technologies that may optimize the bioavailability and subsequent therapeutic efficacy of such drugs. An effective approach of achieving this is through the prolongation of the gastric residence time employing several gastroretentive drug delivery mechanisms such as the use of buoyant systems, high density systems, magnetic systems, mucoadhesive systems, swelling/expanding systems, superporous hydrogels and the inclusion of gastric motility retarding agents with biocompatible polymeric materials. It is known that variations in the gastric physiology such as, gastric pH, and motility exhibit both intra- as well as inter-subject variability demonstrating a significant impact on the gastric retention time and drug delivery behavior. Nevertheless, gastroretentive drug delivery systems have shown promising results. Therefore, in this mini-review, current research and development in this field (i.e. over the last 3-5 years), the polymeric material used for the design of gastroretentive drug delivery systems and techniques employed for the pharmaceutical evaluation of gastroretentive technologies are comprehensively revealed and discussed in an assimilatory manner.

**KEY WORDS:** Gastric retention, biocompatible polymers, drug delivery, effervescent, mucoadhesion, multi-units, expanding, buoyancy, high density, bioavailability

## APPENDIX A2

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### Optimization of a Dual Mechanism Gastrofloatable and Gastroadhesive Delivery System for Narrow Absorption Window Drugs

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#### Abstract

In order to overcome poor bioavailability of narrow absorption window drugs, a system comprising two mechanisms of gastric retention, namely buoyancy and gastroadhesion has been investigated employing poly-lactic-co-glycolic acid (PLGA), polyacrylic acid (PAA), alginate and pectin and a model drug metformin hydrochloride. Fifteen formulations were obtained using the design of experiments Box-Behnken approach. The yield was found to be above 80% in all cases, although due to the high water solubility of metformin, drug entrapment efficacy was only between 18 and 54%. Mean dissolution time (MDT) and gastroadhesive strength were used as the formulation responses in order to optimize the formulation. Furthermore, the molecular mechanics (MM) force field simulations were performed to corroborate the experimental findings. Drug release profiles revealed three different release kinetics, namely burst; first-order and zero-order release. Varying gastroadhesive results were obtained, and were highly sensitive to changes in polymer concentrations. FTIR revealed that strong bonds of PAA and PLGA were retained within the gastrosphere. Surface area and porosity analysis provided supporting evidence that the lyophilization process resulted in a significant increase in the porosity. Analysis of the surface morphology by SEM revealed that air pockets were spread over the entire surface of the gastrosphere, providing a visual proof of the high porosity and hence low density of the gastrosphere. The spatial disposition and energetic profile of the sterically constrained and geometrically optimized multi-polymeric complex of alginate, pectin, PAA and PLGA corroborated the experimental results in terms of *in vitro* drug release and gastroadhesive strength of the fabricated gastrospheres.

**Keywords:** Gastrofloatable; Gastroadhesive; Narrow Absorption Window; Buoyant; Gastrospheres; PLGA; Box-Behnken

## APPENDIX A3

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### Formulation and Development of Gastroretentive Microparticle Loaded Gastrospheres for the Delivery of Narrow Absorption Window Drugs

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#### Abstract

In order to develop a drug delivery system which will overcome the shortfalls commonly associated with drugs possessing a narrow absorption window, a microparticulate loaded gastroretentive drug delivery system has been investigated. This study deals with the development of a novel microparticle formulation loaded within a pre-developed gastrosphere design which is both gastrofloatable and gastroadhesive. A combination of chitosan and polymethacrylate were used in order to form the ionically crosslinked microparticles, while the gastrospheres were formed from alginate, pectin, poly(acrylic) acid and poly(lactic-co-glycolic) acid. A face-centered central composite design was constructed for this study which resulted in 14 statistically derived formulations. In order to identify the optimal polymeric concentrations, microparticle drug entrapment, microparticle yield and mean dissolution time (MDT) were utilized as formulation constraints. Physicochemical and morphological characterisation was conducted on the optimized formulation, including Fourier Transition Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM) characterization. Regression analysis and constraint optimization was utilized in order to obtain the optimal formulation. Surface response analysis was utilised in order to determine the interactions between chitosan and Eudragit<sup>®</sup> RL100 30D. To mechanistically elucidate the complexation and crosslinking mechanism of Eudragit<sup>®</sup> and sodium tripolyphosphate with respect to chitosan, we employed computer-aided modelling of the three-dimensional structure of the active residues of the guest molecules with the respective substrate, to predict the possible orientation of residues most likely affecting the drug delivery system's performance. Microparticle yield was relatively high, ranging from 77 to 92%, while drug entrapment ranged between 50 to 73%. Drug release profiles displayed an initial burst release followed by zero order release over a 12 hour period. FTIR analysis showed no chemical interaction occurring between the microparticle and gastrosphere. SEM imaging showed that microparticles were uniform and spherical with diameters of between 3 and 5µm. *In vivo* drug release was performed a porcine model and displayed more controlled release over a 24 hour period when compared to that of Ciprobay<sup>®</sup>. Overall, the developed delivery system may be suitable for the delivery of narrow absorption window drugs.

**Keywords:** Gastroretentive microparticles, Gastroadhesive drug delivery system, Gastrofloatable drug delivery system, Narrow absorption window drugs, Face-centered central composite design.

## APPENDIX B

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### CONFERENCE PROCEEDINGS

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## APPENDIX B1

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### **Effect of poly(lactic-co-glycolic acid) on drug release, adhesion and buoyancy of a gastroretentive gastrosphere**

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#### **Purpose**

Gastroretentive drug delivery systems are intended for the delivery of drugs which display a low bioavailability due to a narrow absorption window (eg. metformin). Poly(lactic-co-glycolic acid) (PLGA) is an aqueous insoluble polyester which is known to control the release of drug over extended periods of time via diffusion, erosion or a combination thereof.

The purpose of this study is to therefore investigate what effect the addition of PLGA has on the buoyancy, adhesion and drug release of an alginate/pectin/poly(acrylic acid) (PAA) gastrosphere.

#### **Methods**

*Preparation of gastrospheres:* Three formulations were prepared. All three formulations contained 2% w/v pectin, alginate and metformin, in addition to which formulation i) contained 1% w/v PAA, formulation ii) contained 2% w/v PAA and PLGA and formulation iii) contained 2% w/v PAA.

The polymer solutions were homogenised and added drop-wise with an 18G needle into a 2% w/v zinc gluconate solution and left to cure for 24 hours. Each formulation was frozen at -72°C for 24 hours and lyophilised at -60°C and 25mmtor for 24 hours.

*Determination of buoyancy:* Buoyancy was determined visually, by observing gastrosphere characteristics in simulated gastric fluid (pH 1.2; 37°C) agitated in a shaker bath (SBS40 Shaking Water Bath, Stuart, New York, USA).

*In Vitro drug release studies:* Release studies were conducted in a rotating paddle apparatus in simulated gastric fluid (SGF) (pH 1.2; 37°C). 5mL samples were drawn at pre-determined intervals over a 12 hour period and analysed by UV spectrophotometry.

*Determination of bioadhesion:* Bioadhesivity testing was performed using a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK) employing a simulated GIT membrane surrounding both the textural probe and platform stage. Samples were tested using an applied force of 2.00N, a contact time of 15 seconds and a trigger force of 0.05N. The pre-test and test speed was set at 0.50mm/sec while the post-test speed was maintained at 10mm/sec.

#### **Results**

The studies revealed that the inclusion of PLGA into gastroretentive gastrospheres results in a decreased, although more controlled, drug release over the entire 12 hour period, while only a minimal decrease in bioadhesivity was noted with no alteration in buoyancy.

It can therefore be concluded that the inclusion of PLGA results in a gastrosphere which displays an improved and more desirable controlled drug release.

## APPENDIX B2

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### Assessment of the buoyancy and retention of polyacrylic acid blended gastropheres

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#### **Purpose**

In order to extend gastric retention of the drug delivery system, the influence of incorporating a bioadhesive polymer, such as poly(acrylic) acid, was assessed. This mechanism it intended for the delivery of narrow absorption window drugs that display poor bioavailability. Therefore, the purpose of this study is to develop a novel buoyant and gastroadhesive drug delivery system for the delivery of narrow absorption window drugs.

#### **Methods**

*Preparation of the gastropheres:* Varying concentrations of PAA solutions, ranging from 0.5–1.5% w/v, were mixed with a 2% w/v alginate-pectinate solution. The polymeric solution was then added dropwise into a 2% w/v zinc gluconate solution and left for 24 hours to cure. Samples were frozen at -72°C for 24 hours and lyophilised at -60°C condensation phase and a sublimation phase of 24 hours at 25 mmHg.

*Determination of buoyancy:* Buoyancy time was determined visually, by observing gastrophere characteristics in simulated gastric fluid (pH 1.2) while agitated in a shaker bath (SBS40 Shaking Water Bath, Stuart, New York, US) maintained at 37.5°C. A timer was used in order to measure the duration of buoyancy.

*Determination of bioadhesion:* Bioadhesivity testing was conducted using a TA.XT.plus Texture Analyser (Stable Micro Systems, Surrey, UK) with a simulated membrane covering both the probe and platform stage. Samples were tested using an applied force of 2N, a contact time of 15 seconds and a trigger force of 0.04903N. The pre-test and test speed was 0.50 mm/sec while the post-test speed was 10 mm/sec.

#### **Results**

The gastropheres, both with and without PAA, were immediately buoyant and remained buoyant for greater than 72 hours.

Bioadhesion results indicated that the addition of PAA into the alginate-pectin gastro-sphere formulation resulted in a more favourable bioadhesion profile. Samples containing PAA displayed a gradual increase in bioadhesion, showing an initial peak after 4 hours, thus revealing that adhesion increased over a factor of time. It was observed that samples containing 1% w/v PAA demonstrated optimal bioadhesivity.

This study displayed that incorporation of PAA into alginate-pectinate gastropheres successfully improved bioadhesion, without altering buoyancy. These gastropheres may therefore be utilised as a gastroretentive drug delivery system for the delivery of narrow absorption window drugs.

## APPENDIX B3

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### Development of Low Density Gastrospheres for Enhanced Bioavailability in Iliac-Specific Drug Absorption

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#### **Purpose**

To develop low density gastrospheres for improving the bioavailability of drugs absorbed in the ileum by increasing the gastric retention through floatation and mucoadhesion.

#### **Methods**

PLGA dissolved in 5mL dichloromethane was added to a solution of alginate, pectin, polyacrylic acid and model drug metformin. The blend was added drop-wise into a 1.5%<sup>w/v</sup> zinc gluconate solution and crosslinked for 8 hours. Gastrospheres were collected, washed, frozen at -72°C for 24 hours and lyophilised (-60°C; 25mmtor) for 24 hours. A rotating paddle apparatus in 700mL SGF (pH 1.2, 37°C) was used for drug release studies. Samples were analysed with UV at 241nm. For mucoadhesion analysis gastrospheres were immersed in SGF (pH 1.2, 37°C) and assessed with a Texture Analyser. Adhesion was determined by measuring the force of detachment.

#### **Results**

Zero-order release profiles were obtained that ensured a constant quantity of drug would be present at the absorption window over a 12 hour period. Mucoadhesion was found to be highly sensitive to the concentrations of polyacrylic acid used. Optimum adhesion was represented by the maximum force attained from 0-2 hours and over 2-12 hours.

#### **Conclusions**

The gastrospheres demonstrated a desired zero-order release as well as sufficient mucoadhesion in order to be retained within the gastric region.

### Design and Development of Retentive Gastrospheres for the Delivery of Narrow Absorption Window and Low Bioavailable Drugs

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#### ABSTRACT SUMMARY:

Retentive gastrospheres were fabricated using an ionic gelification approach with subsequent lyophilisation for the design and evaluation of a drug delivery system intended for the delivery of narrow absorption window and low bioavailable drugs.

#### INTRODUCTION:

Several disease states exist for which drug treatment is complicated by the physicochemical nature of the drug<sup>1</sup>. The absorption of these drugs, such as metformin, acyclovir, ciprofloxacin and levodopa, are limited to a narrow specific site, usually within the duodenum<sup>1</sup>. This results in decreased bioavailability of the drug, requiring frequent dosing and ultimately poor patient compliance and therapeutic failure.

In order to increase bioavailability, the transit time of the delivery system within the gastrointestinal tract requires retardation. The most logical approach of achieving this is to prolong the gastric residence time of the drug delivery system<sup>1,2</sup>. Numerous such systems have been investigated employing various techniques.

A multi-particulate gastrofloatable and gastroadhesive drug delivery system, termed gastrospheres, using the model drug metformin has been developed in order to retain the drug delivery system within the gastric region for at least 12 hours, delivering small quantities of drug over an extended period of time, thus, resulting in a constant flow of drug being in contact with the specific site of absorption in the duodenum over the duration of drug therapy.

#### EXPERIMENTAL METHODS:

Preparation of gastrospheres: An aqueous solution of metformin, alginate, poly (acrylic acid) (PAA) and PLGA was prepared and homogenised. The polymeric solution was injected drop-wise into 500mL of a crosslinking solution. The gastrospheres were allowed to crosslink and cure under constant rotation overnight.

Crosslinked gastrospheres were collected and washed thoroughly with distilled water and stored at -72°C for 24 hours. Frozen gastrospheres were lyophilised with a 2 hour condensation phase at -60°C and a 24 hour sublimation phase at 25mmHg.

Gastrosphere yield: The dry mass of gastrospheres was measured and compared to the mass of the initial dry powders utilized during formulation. The yield of gastrospheres was determined by the following equation:

$$\% \text{ Yield} = (\text{Final mass} / \text{Initial dry mass}) \times 100$$

Buoyancy studies: A sample of 50 particles were immersed in 100mL simulated gastric fluid (SGF) and placed in an orbital shaking incubator for 12 hours. Each sample was observed at predetermined time intervals, noting the number of particles that were no longer buoyant.

Swelling studies: A sample of 50 particles were weighed and immersed in 100mL SGF and placed in an orbital shaking incubator for 12 hours. The particles were dried and weighed at predetermined time intervals. The hydrated mass was then compared to the dehydrated mass in order to determine the swelling (%).

Drug entrapment efficacy (DEE): Gastrospheres were weighed and dissolved in PBS (pH 7.4, 37°C). Drug content was determined by UV spectrophotometry at 241nm.

*In vitro* drug release studies: A USP XXIII type 2 dissolution test apparatus was used (Erweka DT 700, Heusenstamm, Germany). Samples were immersed under a wire mesh in 700mL SGF (pH 1.2, 37°C) at 50rpm. 5mL samples were removed at predetermined time intervals. Equal volumes of drug free SGF were replaced in order to maintain sink conditions. Samples were analysed with UV spectrophotometry at 241nm.

*In vitro* bioadhesion studies: Samples of gastrospheres were immersed in SGF (pH 1.2, 37°C) for predetermined time intervals. Bioadhesion was measured using a TA.XT.plus Texture Analyser (Stable Micro Systems, Surrey, UK) with a simulated gastric membrane covering both the probe and platform stage. Samples were tested using an applied force of 2N and a trigger force of 0.049N. Bioadhesion was determined by comparing the force of detachment.

#### RESULTS AND DISCUSSION:

Gastrosphere yield: An average yield of 96.84% was obtained. As PLGA does not undergo crosslinking, a slightly lower yield is obtained from formulations in which PLGA had been included (66.43%).

Buoyancy studies: It was observed that the gastrospheres displayed immediate buoyancy. The average buoyancy after the 12 hour period in SGF was calculated to be 96%. This period was sufficient to maintain drug release prior to the absorption window.

Swelling studies: Figure 1 shows the effect of PLGA on the swelling properties of the gastrospheres. It was evident that greater swelling occurred after the first 2 hours. Figure 2 shows the significant influence exhibited by alterations in PAA concentration. It was noted that due to the

hydrophilicity of PAA, a higher concentration resulted in a greater degree of swelling.

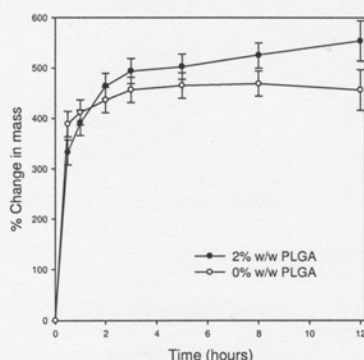


Figure 1: Effect of PLGA on the swelling ability of the gastrosppheres (N=3).

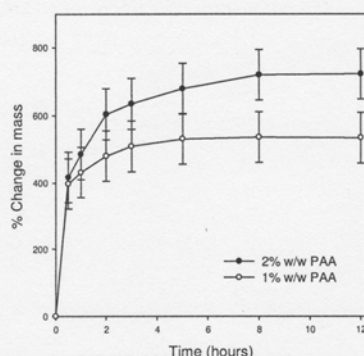


Figure 2: Effect of PAA concentration on the swelling ability of the gastrosppheres (N=3).

Drug entrapment efficacy (DEE): Results revealed that metformin was efficiently entrapped within the gastrosppheres and ranged between 66 - 98%.

*In vitro* drug release studies: Drug release profiles revealed an initial rapid release of 47% within the first 3 hours, followed by a controlled diffusional release phase over the subsequent 9 hours.

It is apparent from Figure 3 that an increase in PAA concentration resulted in a more rapid drug release, while Figure 4 provided evidence that the inclusion of PLGA restricted drug release over the entire 12 hour period.

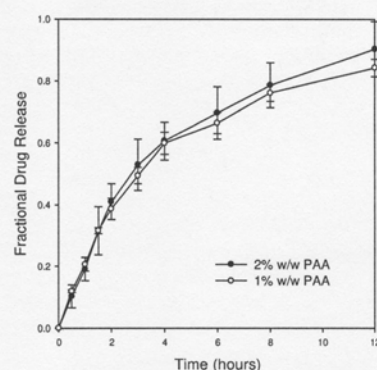


Figure 3: Drug release profile displaying the effect of PAA concentration (N=3).

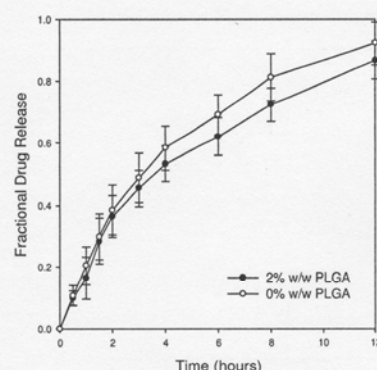


Figure 4: Drug release profile displaying the effect of PLGA (N=3).

*In vitro* bioadhesion studies: It was observed that PAA was primarily responsible for the bioadhesiveness of the gastrosppheres. The degree of bioadhesion therefore correlated with the concentration of PAA incorporated within the gastrosppheres. The inclusion of PLGA resulted in a lowering of bioadhesion, due to its hydrophobic nature (0.1042 vs 0.2324 N/mm/sec).

#### CONCLUSION:

Preliminary studies have shown that this method is suitable for the delivery of narrow absorption window and low bioavailable drugs. *In vivo* animal studies are currently underway.

#### REFERENCES:

1. S.S Davis. Drug Discovery Today, 10; 4 (2005)
2. P.L. Bardonett, *et. al.*, Journal of Controlled Release, 111 (2006)

#### ACKNOWLEDGEMENTS:

This work is supported by South Africa's Biotechnology Partnerships and Development (BioPAD).

## APPENDIX B5

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### **Gastrospheric multi-units for improving the bioavailability of Narrow Absorption Window bioactives through matrix pore mediation**

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#### **Introduction**

This study evaluated three gastrosphere formulations containing varying concentrations of polymers in order to assess the effects of these polymers have on the physicochemical and physicomachanical characteristics of the drug delivery system.

#### **Methods**

An aqueous solution of metformin, alginate, pectin, PAA and PLGA was prepared and homogenized. The polymeric blend was injected drop-wise into 500mL of a crosslinking solution and allowed to crosslink over 8 hours. Crosslinked gastrospheres were collected, washed and frozen at -72°C for 24 hours and subsequently lyophilized at -60°C and 25mmtor for 24 hours. A sample of gastrospheres were immersed in 100mL simulated gastric fluid (SGF) and placed in an orbital shaking incubator for 12 hours. Each sample was observed at predetermined time intervals and observing the number of particles that were no longer buoyant. A sample of gastrospheres were weighed and immersed in 100mL SGF and placed in an orbital shaking incubator for 12 hours. The particles were dried and gravimetrically analyzed at predetermined time intervals. The hydrated mass was then compared to the non-hydrated mass in order to determine the swelling (%). For bioadhesion studies samples of gastrospheres were immersed in SGF (pH 1.2, 37°C) for predetermined time intervals. Bioadhesion was measured using a TA.XTplus Texture Analyzer (Stable Microsystems, Surrey, UK) by measuring the force of detachment. A USP25 type 2 dissolution apparatus was used. Samples were immersed under a wire mesh in 700mL SGF (pH 1.2, 37°C) at 50rpm. 5mL samples were removed, filtered and analysed under UV at predetermined time intervals. Equal volumes of drug-free SGF were replaced in order to maintain sink conditions.

#### **Results**

Gastrospheres were immediately buoyant, and remained buoyant for the entire 12 hour period which may be attributed to the highly porous structure as a result from the lyophilization step. Water uptake and bioadhesion of the gastrospheres are due to the highly hydrophilic PAA, which strongly attracted water molecules apposing the inverse effect of the hydrophobic PLGA. Drug release profiles revealed the significant influence of PLGA retarding drug release. PLGA-free formulations first-order release kinetics was achieved. When 1%<sub>w/v</sub> PLGA was included, an initial rapid release phase was noted at  $t_{3\text{hours}}$  followed by a subsequent diffusional phase of drug release. PLGA (2%<sub>w/v</sub>) resulted in zero-order release kinetics due to hydrophobic nature of PLGA that resulted in the retardation of swelling and therefore the mechanism of drug release was erosion-dependant.

#### **Conclusions**

Results have revealed that the three gastrosphere formulations containing varying concentrations of polymers affected the buoyancy and the mechanism of drug release from the gastrospheres. Higher concentrations of PLGA resulted in more consistent drug release.

## APPENDIX B6

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### **Gastroretentive micro-beads for the localized treatment of gastric disorders**

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#### **Introduction**

The purpose of this study was to develop an approach for the design and formulation of microbeads intended for local gastric drug delivery of antimicrobials for the treatment of *Helicobacter pylori* associated peptic ulcers.

#### **Methods**

An aqueous polymeric blend, comprising 1%<sup>w/v</sup> sodium alginate (SA) and 1%<sup>w/v</sup> poly(acrylic acid) (PAA) was prepared at 40°C for 45 minutes until homogenous. A 0.5%<sup>w/v</sup> chitosan (CHT) solution was prepared and added to a 2%<sup>w/v</sup> crosslinking solution of NaSO<sub>4</sub> and CaCl<sub>2</sub>. The polymeric solution was added drop-wise into the crosslinking solution, while stirring at 700rpm under a top stirrer. The microbeads were left to cure for 4 hours and then collected and washed thoroughly before frozen at -72°C for 24 hours and subsequently lyophilised at -60°C and 25mmtor. To assess buoyancy a sample of microbeads were immersed in 100mL simulated gastric fluid (SGF) and placed in an orbital shaking incubator for 12 hours. Each sample was observed at predetermined time intervals, noting the number of particles that were no longer buoyant. A USP25 type 2 dissolution apparatus was used (Erweka DT 700, Heusenstamm, Germany). Samples were immersed under a wire mesh in 700mL SGF (pH 1.2, 37°C) at 50rpm. 5mL samples were removed, filtered and analysed with UV at predetermined time intervals. Equal volumes of drug-free SGF were replaced in order to maintain sink conditions.

#### **Results**

The microbeads were immediately buoyant and remained buoyant for up to 12 hours. The buoyancy may be attributed to the highly porous structure resulting from the lyophilisation step. Drug release studies revealed that NaSO<sub>4</sub> was the superior crosslinking reagent due to the additional retardation in drug release. An initial burst release was achieved over the first 3 hours, followed by a controlled diffusional release phase.

#### **Conclusions**

Drug release profiles obtained from this study revealed that the gastroretentive micro-bead system may be suited enhancing gastric residence time and drug concentration is kept constant over the entire duration of treatment.

## APPENDIX B7

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### Optimisation of gastroretentive drug delivery system by way of Box-Behnken statistical design

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Department of Pharmacy and Pharmacology

Keywords: Gastroretention, Drug delivery system

#### Introduction

Narrow absorption window drugs possess many pitfalls which may be overcome through the formulation of gastroretentive drug delivery systems.

#### Aim of study

To obtain an optimised formulation through the use of a Box-Behnken design, taking into account particular formulation parameters.

#### Method

A polymeric blend of alginate, pectin, PAA, metformin and PLGA was added drop wise into 2% calcium hydroxide. The gastro-spheres cured for 30 minutes, after which they were collected, washed, frozen and lyophilised.

A randomized Box-Behnken statistical experimental design (Minitab® V15, Minitab Inc., PA, USA) was constructed in order to model the number of formulations required for optimization. The design consists of 15 statistically derived formulations.

Drug release studies were performed using a USP XXIII type 2 dissolution test apparatus in 900mL SGF (pH 1.2, 37°C) at a rotation speed of 50rpm.

Bioadhesion was determined using a TA.XT.*plus* Texture Analyser with a simulated gastric membrane covering both the probe and platform stage. Adhesion was determined by comparing the force of detachment.

#### Results

Three formulations achieved the appropriate zero order drug release profile, of which, one had the highest force of detachment.

#### Conclusion

An optimised formulation has been determined using the design.

### **Formulation and Development of a Gastroretentive Drug Delivery System for the Delivery of Narrow Absorption Window Drugs**

**Caragh S. Murphy, Viness Pillay\*, Yahya E. Choonara and Lisa C. du Toit**

University of the Witwatersrand, Department of Pharmacy and Pharmacology, 7 York Road, Parktown, 2193, Johannesburg, South Africa

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#### **Purpose**

A gastroretentive drug delivery system has been developed in order to deliver metformin over a 12 hour period. The system comprised ionically crosslinked mucoadhesive gastropheres that are also buoyant within the stomach.

#### **Methods**

A polymeric emulsion was prepared by blending an aqueous solution of alginate, pectin, PAA and metformin with an organic solution of PLGA. The emulsion was then added drop-wise through an 18G needle into a crosslinking solution of 2% Ca(OH)<sub>2</sub>. The gastropheres were allowed to cure for 30 minutes, after which they were collected, washed and frozen at -72°C for 24 hours. Lyophilisation was conducted on the gastropheres at -60°C and 25mtorr for 24 hours. A randomized Box-Behnken statistical experimental design was constructed in order to model the number of formulations required for optimization. The design consisted of 15 statistically derived formulations of various polymer combinations. Drug entrapment was determined by immersing gastrophere samples in 100mL PBS (pH 7.4, 37°C) and allowing complete drug release. Drug release studies were performed using a USP 32 type 2 dissolution apparatus. Samples were immersed under a wire mesh in 900mL SGF (pH 1.2, 37°C) at 50rpm and dissolution media was removed at predetermined intervals and analysed by UV. Buoyancy studies were conducted by immersing the gastropheres in simulated gastric fluid (SGF) and observed. Bioadhesion was determined using a TA.XTplus Texture Analyser (Stable Microsystems, UK) with a simulated gastric membrane covering both the probe and platform stage. *In vivo* drug release studies have been conducted on pigs which have surgically implanted chronic jugular catheters. The drug delivery system was administered via an intragastric tube while under sedation. Blood samples were extracted via the jugular catheter Metformin concentrations were determined by UPLC.

#### **Results and Discussion**

Drug release profiles were classified into three distinct types comprising zero-order release, burst release and first-order release. Drug entrapment ranged between 25-53%. It was observed that the gastropheres displayed immediate buoyancy. The average buoyancy after 8 hours was 98% and 96% after the full 12 hour period in SGF. Bioadhesion was primarily due to PAA, while the inclusion of PLGA lowered bioadhesion. The Box-Behnken design resulted in the optimisation of the gastrophere formulation, achieving maximum bioadhesion as well as zero-order drug release. *In vivo* studies revealed that the gastropheres resulted in drug release over a 12 hour period, the profile of which depicted a much more controlled release than the gold standard (Glucophage<sup>®</sup> 500).

#### **Conclusions**

The gastrophere drug delivery system has proven to be retained within the stomach, resulting in a more desirable drug release profile, which will reduce dosing frequencies and side-effects and improve the bioavailability of NAW drugs such as metformin, thus improving patient compliance.

## APPENDIX B9

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### Design and Development of Chitosan-Polymethacrylate Microparticles for Rate-Modulated Drug Delivery

C. S. Murphy<sup>1</sup>, V. Pillay<sup>1</sup>, Y. E. Choonara<sup>1</sup>, L. C. du Toit<sup>1</sup>

<sup>1</sup>University of the Witwatersrand

#### Purpose

To develop a microparticulate system that will enable the controlled release of drug over an extended period of time employing a novel combination of chitosan and polymethacrylate polymers.

#### Methods

Microparticles were prepared by a W/O emulsion using a polymethacrylate (PMC) solution (organic phase) and chitosan (CHT) in acetic acid (aqueous phase). In addition, metformin (model drug) and Span 80 (stabilizer) was added. The emulsion was ultra-sonicated at 10kHz for 1 hour and thereafter 2mL of sodium tripolyphosphate (TPP) was added in order to ionically crosslink the CHT. The system was left to sonicate for 30 minutes and the resultant microparticles were filtered, washed with deionized water and allowed to air dry. Drug release data was obtained by immersing the microparticles in 100mL of SGF (pH 1.2; 37°C) contained in 150mL vessels and stored in a orbital shaking incubator (20rpm; 37°C). Samples (10mL) were removed at predetermined intervals, centrifuged and 3mL of the supernatant was extracted for UV analysis to ascertain metformin content. Drug-free SGF (3mL) was replaced into the centrifuged tube, re-dispersed and placed back into the dissolution vessel. Drug entrapment efficacy (DEE) was determined by placing microparticle samples in 100mL PBS (pH 7.4, 37°C) and left overnight to ensure complete drug solubilization. Samples (10mL) were centrifuged, filtered and analysed using UV spectroscopy. Microscopic images were taken at 50x magnification.

#### Results

Digital images revealed that the microparticles varied in shape from circular to elliptical and appeared to have smooth surface morphologies with measured diameters of the microparticles ranging between 80-100µm. The cationic CHT polymer interacted with the PMC (anionic) resulting in the formation of a microparticulate polyelectrolyte complex. Excess CHT was crosslinked with TPP in solution that resulted in microparticles that were able to superiorly control the release of metformin. Drug release studies revealed that 40% of metformin was released after 6 hours and 80% after 12 hours with pseudo zero order drug release kinetics. DEE values averaged 63±1.5% (N=3).

#### Conclusions

A combination of chitosan and polymethacrylate polymers produced microparticles that were able to control drug release over a period of 12 hours with a >50% drug entrapment efficacy.

## APPENDIX C

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### ADDITIONAL OUTPUTS FOR COLLABORATIVE RESEARCH

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## APPENDIX C1

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### Pharmaceutical Applications of Electro-Spinning

Clare Dott, Viness Pillay\*, Yahya E. Choonara, Lisa C. du Toit, Valence M.K. Ndesendo  
and Caragh S. Murphy

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Road, Parktown, 2193, Johannesburg, South Africa

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#### Abstract

Electro-spinning of polymers is a unique technology which produces very fine fibres using electrostatic repulsive forces obtained from applying an electrical potential to a liquid. The resultant fibres have much smaller diameters in comparison with fibres obtained using different methods. Electro-spun fibres have found application in many and varied fields such as drug and gene delivery, tissue engineering, wound dressing, electronics, filtration as well as absorption and adsorption. This review will cover the various processing parameters which play a role in electro-spinning, including the applied voltage, solution flow rate, solution viscosity, solvents, solution conductivity, capillary-to-collector distances and the influence of surfactants. A main focus will be on the application of electro-spun fibres in drug delivery, where researchers have already investigated the use of these fibres in transdermal delivery systems, long-term implants and grafts. Different methods of drug incorporation will be discussed as well as numerous polymers which have been successfully utilised in this field, including cellulose acetate, poly( $\epsilon$ -caprolactone), poly(ethylene oxide), poly(vinyl alcohol), gelatin, poly(D,L-lactide-co-glycolide), poly(lactic acid), poly(L-lactic acid), polyurethane, poly(ethylene-co-vinyl acetate), poly(ethylene glycol), and poly(acrylic acid).

**Keywords:** Electro-spinning, drug delivery, electrospun fibres, electrospun scaffolds, Parameters.

## APPENDIX C2

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### **Review Article: Recent Advances in the Design of Drug-loaded Polymeric Implants for the Treatment of Solid Tumors**

Ameena Wadee<sup>1</sup>, Viness Pillay\*<sup>1</sup>, Yahya E. Choonara<sup>1</sup>, Lisa C. du Toit<sup>1</sup>, Clement Penny<sup>2</sup>, Valence Ndesendo<sup>1</sup>, and Caragh Murphy<sup>1</sup>

<sup>1</sup>University of the Witwatersrand, Department of Pharmacy and Pharmacology, 7 York Road, Parktown, 2193, Johannesburg, South Africa.

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#### **Abstract**

The effective treatment of solid tumors still continues to be a great challenge to clinicians despite the development of novel drugs. In order to improve the clinical efficacy of the existing chemotherapeutics agents, researchers have considered the possibility of local treatment at the site of the solid tumor. The greatest advantage of this localised delivery is the significantly fewer side effects experienced by the patient. In the past, the peri- or intra- tumoral delivery of chemotherapeutics agents was mainly based on implants that used to be inserted surgically into the affected region. In the recent years, *in situ* forming implants have attracted considerable interest. These are polymeric systems which are injected as solutions into the tumor site using commercially available syringes and needles. The injected solution forms an implant at the tumor site as a result of local environmental stimuli and hence removes the need for surgical implantation. However, while these implants have been shown to improve the treatment of various solid tumors, the ideal implant is yet to be formulated. To date, it is only a few implants that are biodegradable and able to deliver the chemotherapeutic agent over a prolonged period of time. Many of these implants also have an undesirable initial burst release effect. This review summarises the attempts that have so far been made in the development of polymeric implants for the treatment of solid tumors.

**Keywords:** Chemotherapy, Environmental stimuli, Implant, Polymeric systems, Solid tumors.

### **The simultaneous *in vitro* characterization of poly(lactic co-glycolic acid) and poly(glucuronide)-rich nanoparticles employing various sol-gel synthetic wet chemical processing strategies**

Yahya E. Choonara<sup>1</sup>, Viness Pillay<sup>\*1</sup>, Valence M.K. Ndesendo<sup>1</sup>, Lisa C. du Toit<sup>1</sup>, Riaz A. Khan<sup>2</sup>, and Caragh S. Murphy<sup>1</sup>

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#### **Abstract**

This study focussed on the formulation and evaluation of polymeric nanoparticles adopting various preparation approaches and attempts to explicate the mechanisms of nanoparticle formation employing molecular modeling and chemometrical computations. Nanoparticles were formulated using three different approaches such as an Emulsification/Solvent Evaporation (ESE), Emulsification/Surfactant/Solvent Evaporation (ESSE) and Ionic Gelification (IG) approaches. The ESE approach comprised the emulsification of an aqueous and organic solution, while sorbitan monooleate was added as a surfactant during the ESSE approach. Cation-induced crosslinking of hydrophilic alginate was employed for the IG approach. Fourier Transform Infrared (FT-IR) analysis was performed to elucidate any changes in the structural backbone of the native polymers due to nanoparticle formation. The size and morphology of nanoparticles were analysed by Zetasize analysis and Scanning Electron Microscopy (SEM) with photomicrographs taken at several magnifications. Step-wise molecular simulation models revealed the mechanisms of nanoparticle formation to occur via solvation, surface interactions, crosslinking/precipitation initiation and surface-volume minimization with sphericalization and interlaced network formation. The size distribution of the nanoparticles were manipulated by the surfactant introduction. The addition of sorbitan monooleate prevented coalescence of particles, resulting in stable nano-emulsions with distinct particle morphologies and particle sizes and zeta potential values in the region of 200nm and -40mV, respectively. The IG approach also produced stable nano-emulsion with a higher yield of nanoparticles with superior size and stability control of confined nanoparticles. Both the ESSE and IG approaches were found to be suitable for producing stable nano-emulsions that may potentially be employed for the novel delivery of various drug molecules.

**Keywords:** Polymeric nanoparticles, alginate, poly(lactide-co-glycolide), polymeric characterization, surfactant, crosslinking, emulsification, drug delivery

## APPENDIX D

### ANIMAL ETHICS CLEARANCE CERTIFICATE

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 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/In0015/AESCCert.wps