DESIGN AND EVALUATION OF A GASTRORETENTIVE DEVICE FOR DRUGS WITH A NARROW ABSORPTION WINDOW

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DECLARATION

I, Sarashnee Moonisami declare that this research report is my own work. It has been submitted for the degree of Master of Science (Pharmaceutical Affairs) in the Faculty of Health Sciences in the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University

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RESEARCH OUTPUTS

A poster entitled, "A Novel Multi-Unit Gastrofloatable Device for Delivery of 'Narrow Absorption Window (NAW)' Bioactives, by Viness Pillay, Sarashnee Moonisami, Yahya E. Choonara and Michael P. Danckwerts, 4th International Conference on Pharmaceutical and Pharmacological Sciences, Riverside Hotel, Vanderbijlpark, South Africa, 20-23 September, 2006.

A poster entitled, "Configuration of a Gastroretentive Device for Drugs with a Narrow Absorption Window, by Viness Pillay, Sarashnee Moonisami, Michael P. Danckwerts, Annual Conference of the Academy of Pharmaceutical Sciences, Port Elizabeth, South Africa, 29 September - 02 October, 2005.

A patent filed as "A gastroretentive drug delivery system", by Sarashnee Moonisami, Viness Pillay, Yahya E. Choonara, and Caragh S. Murphy, SA Provisional Patent Application 2007/10997 filed on December 19, 2007.

SUMMARY

Narrow Absorption Window ("NAW") drugs often display poor oral bioavailability as a result of their site-specific absorption. Such drugs are primarily absorbed from specific sites within the upper part of the gastro-intestinal tract i.e. stomach and small intestine. Consequently, low absorption, fluctuating plasma levels and frequent dosing characterize several important drugs from various pharmacological categories as a result of their low oral bioavailability.

Over the last two decades, a variety of controlled release drug delivery systems targeting drug delivery to the gastrointestinal tract (GIT) have been employed to improve the bioavailability of drugs that display site specific absorption following oral administration. A gastroretentive drug delivery device based on the rationale that retaining the device in the stomach can significantly extend the period of time over which the drug is released at or above its primary absorptive site, has been the focus of interest and has led to the development of novel drug delivery systems.

To address some of the challenges associated with current gastroretentive drug delivery systems, a novel gastroretentive multiple-unit system using various combinations of swellable, lyophilized polymers was developed. The polymers employed were alginate, pectin and Poly (lactide-co-glycolide). Riboflavin was employed as the model drug.

Twenty seven different statistically planned gastroretentive multi-units were prepared. The floatability, physico-mechanical and physico-chemical properties and drug encapsulation efficiency was assessed. *In vitro* drug release in simulated gastric fluid was subsequently determined.

Excellent floatability of the gastroretentive multi-units was demonstrated for an extended period of time. No significant erosion of the polymeric matrices in simulated gastric fluid occurred. Constant, different release of riboflavin occurred over a period of 24 hours. It was concluded that the polymer concentration and the period of lyophilization had a significant effect on the drug release and encapsulation efficiency of the various formulations.

The gastroretentive multi-units developed in this study present a novel approach in the design of gastroretentive drug delivery devices using lyophilization as a technique for achieving prolonged buoyancy and biodegradable polymers to attain constant drug release over a period of time. This approach may have significant therapeutic benefits for "NAW" drugs.

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To my Parents, Brother and Sister to whom I wish to express my deepest gratitude for their encouragement, continued support and enduring strength.

DEDICATION

This Research Report is dedicated to my Mother and Father for their unwavering support, love and motivation which shall always be my driving force in life.

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SECTION 1

LITERATURE REVIEW AND MOTIVATION FOR STUDY

1.1. Introduction

Oral administration of drugs still remains the most widely preferred route of drug delivery for the majority of clinical applications (Davis, 2005; Streubel et al., 2006). Some drugs have ideal characteristics for superior absorption to occur throughout the gastrointestinal tract (GIT) while others present challenges (Davis, 2005). In the latter case, important drugs from various pharmacological categories have poor oral bioavailability due to incomplete absorption and/or degradation in the GIT (Hoffman et al., 2004).

Some of these drugs are characterized by a narrow absorption window (NAW) in the upper part of the GIT i.e. the stomach and small intestine (Klausner et al., 2002). This is because the proximal part of the small intestine exhibits extended absorption properties. Despite the extensive absorption properties of the duodenum and jejunum, the extent of absorption at these sites is limited because the passage through this region is rapid (Hoffman et al., 2004). As a consequence, their oral bioavailabilities can be affected by the limited absorptive sites in the upper GIT (Davis, 2005).

Several scientific advances have been made in the research and development of rate-controlled drug delivery systems (Singh and Kim, 2000). However, formulating "NAW" drugs into conventional controlled release drug delivery systems does not adequately improve absorption due to the relatively short transit time in the crucial

absorbing region i.e. stomach or proximal small intestine of the GIT (Streubel et al., 2006). After a short period of time, the controlled release drug delivery system leaves the upper GIT and releases the "NAW" drug in the non-absorbing distal segments of the GIT (Chavanpatil et al., 2005).

Gastroretentive drug delivery systems may provide increased bioavailability for drugs that act locally in the stomach or that may be absorbed in the upper region of the GIT i.e. "NAW" drugs (Ahmed and Ayres, 2007). Most absorption windows are located in the proximal small intestine. By retaining the drug in the stomach and controlling the rate of release prior to reaching the absorption window, free drug can be continuously supplied to its absorption site in the upper GIT (Hwang et al., 1998). This mode of administration would best achieve the known pharmacokinetic and pharmacodynamic advantages of sustained release drug delivery systems for these drugs (Hwang et al., 1998; Hoffman and Stepensky, 1999). There are several important drugs with low oral bioavailability currently in clinical use that would benefit from an increased residence time in the stomach or small intestine such as furosemide (33%) (Singh and Kim, 2000), bromocriptine (30%) (Arora et al., 2005) and riboflavin (15%) (Davis, 2005).

Some of the approaches explored to achieve gastroretention involved the use of swellable and/or biodegradable polymers such as hydroxypropylmethycellulose (HPMC), alginate and superporous hydrogels (Qiu and Park, 2003). Among the various classes of biodegradable polymers, the thermoplastic, aliphatic poly(esters) such as poly(lactic acid) (PLA) and the copolymer poly(lactide-co-glycolide) (PLGA) have generated tremendous interest due to their excellent bio-compatibility, biodegradability, and mechanical strength (Huh et al., 2005). They are also easy to

formulate into various devices for carrying a variety of drug classes. Polymer mediated drug delivery systems appear to be an effective and rational approach to modulation of controlled, oral drug delivery (Singh and Kim, 2000). Biodegradable, swellable polymers have been used in several drug delivery devices thus far to control drug release and target drugs to specific sites.

To develop an efficient gastroretentive drug delivery system has proven to be challenging since the system should possess in addition to controlled release properties, an ability to withstand physiological adversities such as repeated peristaltic contractions in the stomach whilst achieving extended gastric retention (Kagan et al., 2006). One of the methods to improve the mechanical strength of such drug delivery systems involves the use of various types of biodegradable polymers. Recent advances have shown that due to their unique properties, certain biodegradable polymeric materials may be used to extend the gastric residence time of drugs thereby achieving long-term oral controlled drug delivery (Qui and Park, 2003).

In order to overcome the above challenges, this study employed a lyophilized, swellable composite, polymeric system comprising of alginate, pectin and PLGA, strengthened with matrix consolidators to develop a gastroretentive multi-unit device. Riboflavin (Vitamin B2) was selected as the model compound for this study since it demonstrates absorption mainly in the proximal segment of the small intestine (Sato et al., 2004), it is a compound classified as a "NAW" drug, it undergoes negligible metabolism, lacks side-effects, has no pharmacological effect on gastric motility and its pharmacokinetics can be measured by analysis of urinary excretion following oral administration in humans.

1.2. Specialized drug delivery to the gastrointestinal tract

Conventional controlled release drug delivery systems have only limited use for drugs with a narrow absorption window (NAW), for those drugs acting topically in the gastric region, drugs which degrade in the colon, drugs that are poorly soluble in a high pH environment and those with a major absorption site in the upper GIT since transit through these crucial absorption regions is relatively rapid lasting approximately 2-6 hrs (Klausner et al., 2003; Friedman et al., 2004; Chavanpatil et al., 2005).

Oral drug absorption from the GIT is a complex process and is highly variable despite the drug exhibiting excellent *in vitro* release patterns. The reason for unsatisfactory drug absorption is mainly physiological and is usually affected by gastrointestinal transit time of the drug delivery system (Moes, 1993). Consequently, poor drug absorption due to transit variability following oral administration led to multiple daily dosing, fluctuating plasma drug levels and decreased patient compliance (Koner et al., 2007). This led to a significant increase in strategies for site-specific delivery in the GIT both to maximize a therapeutic response and improve bioavailability (Koner et al., 2007). One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GIT is to control the gastric residence time (Garg and Sharma, 2003). Gastroretentive drug delivery systems have generated enormous interest in recent years owing to its potential application to improve the oral delivery of some important drugs for which prolonged retention in the upper GIT can greatly improve their oral bioavailability and/or therapeutic outcome (Hoffman et al., 2004).

1.2.1. Gastroretentive drug delivery systems

Gastroretentive drug delivery systems are able to be retained in the stomach for a prolonged period of time after oral administration and release the drug in a controlled manner (Friedman et al., 2004). In the case of "NAW" drugs, it would be advantageous to hold these drugs which display less than ideal absorption behaviour from the small intestine, in the stomach above the main absorption site for extended periods of time in a gastroretentive drug delivery system (Illum and Ping, 2001). Adequate control of the gastric residence time combined with time-controlled drug release patterns can significantly increase the bioavailability of the drug (Streubel et al., 2006). Another group of drugs that could benefit from retained and controlled release in the stomach are those indicated for the treatment of pathologies located locally in the stomach, the duodenum or the small intestine (Hoffman et al., 2004; Hamdani et al., 2006). Over the past three decades, the pursuit and exploration of drug delivery systems designed to be retained in the upper GIT have advanced consistently in terms of technology and diversity encompassing a variety of systems such as bio-adhesive, floating, high density and expandable systems (Rocca et al., 2003). Based on these mechanisms, categories of controlled release delivery systems have been described (Arora et al., 2005).

1.2.1.1. Bio-adhesive drug delivery systems

Bioadhesive drug delivery systems (BDDS) may provide an enhanced gastrointestinal transit time through adhesion to the gastro-intestinal mucosal surface enabling the BDDS to resist gastric emptying (Rocca et al., 2003). Various approaches have been explored such as the use of mucoadhesive polymers (Bardonnet et al., 2006). Chitosan has been a popular choice because it has superior mucoadhesive properties (Illum and Ping, 2001; Davis, 2005). Adherence of these

systems may also be facilitated by the hydration and swelling of the polymer in the system upon contact with gastric fluid. Bioadhesion is usually achieved through the interaction of either a synthetic or natural mucosal membrane. Other novel approaches used in bioadhesive drug delivery systems include the use of adhesive material derived from fimbriae of bacteria or synergistic analogues conjugated to the drug to provide for attachment to the GIT (Garg and Sharma, 2003).

1.2.1.2. Floating drug delivery systems

Floating drug delivery systems may be particularly suitable for acid soluble drugs, drugs poorly soluble or unstable in intestinal fluids and drugs which display significant changes in their pH dependant solubility (Singh and Kim, 2000). They are also particularly advantageous for drugs that are specifically absorbed from the stomach or the small intestine (Koner et al., 2007). As sustained release systems, floatation offers various potential advantages. Drugs that have poor bioavailability due to their absorption being restricted to the upper GIT can be delivered efficiently thereby maximizing their absorption and improving their absolute bioavailability without affecting the intrinsic rate of gastric emptying (Singh and Kim, 2000, Arora et al., 2005). Floating drug delivery systems have been classified into low density, non-effervescent, effervescent and raft forming systems (Rocca et al., 2003; Arora et al., 2005).

1.2.1.2.1. Low density drug delivery systems

These systems are designed to have a bulk density lower than the density of the gastric fluid i.e. < 1g/cm^3 and therefore remain buoyant without affecting the gastric emptying rate for a prolonged period of time. The drug is gradually released at a desired rate from the system. After the release of the drug, the residual system is

emptied from the stomach (Koner et al., 2007). Some of the technologies based on the buoyancy mechanism include effervescent and non-effervescent systems.

1.2.1.2.1.1. Effervescent drug delivery systems

These systems are typically matrix type systems prepared with swellable polymers such as methylcellulose and various effervescent compounds such as sodium bicarbonate and citric acid (Arora et al., 2005). Upon contact with the gastric fluid, carbon dioxide bubbles are generated and are entrapped within the matrix of the polymer providing buoyancy to the delivery system. In addition to imparting buoyancy, incorporating carbonates into the formulation also provides an alkaline environment for polymers to gel (Singh and Kim, 2000).

1.2.1.2.1.2. Non-effervescent drug delivery systems

One of the approaches used in this type of floating drug delivery system involves the mixing of drug with a gel forming hydrocolloid which swells upon contact with the gastric fluid and maintains a relative integrity of shape. The air entrapped by the swollen polymer confers buoyancy and the gel structure acts as a reservoir for sustained drug release since the drug is released in a controlled diffusion manner (Tayade, 2004). Commonly used excipients are highly swellable cellulose type hydrocolloids and matrix forming polymers.

1.2.1.2.2. Raft-forming drug delivery systems

These systems incorporate alginate gels which have a carbonate or bicarbonate component. Upon contact with the gastric fluid, the gel forming solution swells and forms a viscous cohesive gel containing entrapped CO_2 bubbles enabling system to

float. These systems are called rafts as the viscous components float above the gastric fluid (Bardonnet et al., 2006).

Both single-unit and multiple-unit floating drug delivery systems have been developed (Whitehead et al., 1998). Single-unit systems tend to be affected by the high variability in GIT transit time which is a disadvantage for this type of system. Multiple-unit floating formulations have shown greater potential since they avoid the "all or nothing" gastric emptying process which is characteristic of single-unit systems and tend to be eliminated in a linear profile (Jain et al., 2005). Multiple-unit systems also appear to be better suited to reduce variability in absorption and lower the probability of the "all or nothing gastric emptying time" (Streubel et al., 2003).

1.2.1.3. High density drug delivery systems

Gastric contents have a density close to water (approx 1.004 g/cm ^{3}) (Whitehead et al., 1998; Bardonnet et al., 2006). In this type of system, the entire formulation exceeds the density of the gastric fluid. The dosage form becomes entrapped in the antrum of the stomach and withstands the peristaltic waves of the stomach wall. One of the methods used includes coating the drug with heavy inert materials. It was found that a density close to 2.5 g/cm ^{3} is necessary for significant prolongation of gastric residence time (Bardonnet et al, 2006) and the gastrointestinal time may be extended up to 25 hours. However, the safety and non-toxicity of such systems have still to be proven.

1.2.1.4. Expandable drug delivery systems

This type of drug delivery system has explored retention in the stomach by increasing the size of the system above the diameter of the pylorus thereby retarding

the exit of the system through the pyloric sphincter of the stomach (Streubel et al., 2006). The expandable drug delivery systems are usually based on 3 configurations: a small configuration which enables convenient intake, an expanded form that is achieved in the stomach preventing passage through the pyloric sphincter and finally another small form that is achieved in the stomach when retention is no longer required i.e. after the delivery system has released the active ingredient (Klausner et al., 2003). Expansion can be achieved through swelling or unfolding (Davis, 2005). In previous studies, swelling was achieved through the use of superporous hydrogels and enzyme-digestible hydrogels (Streubel et al., 2006). Super-porous hydrogel composites have a very high swelling capacity because of the presence of numerous large pores (Davis, 2005). Upon exposure to gastric fluid, these hydrogels demonstrate fast swelling and a large swelling ratio (Qui and Park, 2003).

Unfolding gastroretentive drug delivery systems are characterized by various erodibilty, mechanical properties, sizes and geometrics (Klausner et al., 2003). Thus far several different geometric configurations have been explored such as ring, tetrahedron and planar disc (Badonnet et al., 2006). In a previous study the ring and tetrahedron configurations have shown to display enhanced gastroretention for a prolonged period of time i.e.> 24 hours (Klausner et al., 2003). Despite several advances in the development of expandable delivery systems, there are several limitations with this design that has prevented its use in the clinical setting such as the potential hazard of permanent retention in the stomach, the mechanical shape memory for the unfolding types is relatively short-lived and rapid change in dimensions of the delivery system would need to be achieved in a fail-safe manner (Davis, 2005; Bardonnet et al., 2006; Streubel et al., 2006).

1.2.2. Other novel gastroretentive drug delivery systems

Other novel drug delivery systems have also been designed to prolong the gastro-intestinal residence time and/or induce greater levels of absorption such as magnetic systems and gastrointestinal patch systems.

1.2.2.1. Magnetic Systems

Magnetic systems are based on a novel concept where the drug delivery system contains a small internal magnet and a magnet is placed on the abdomen over the position of the stomach (Bardonnet et al., 2006). The internal magnet within the drug delivery system is guided with the use of the external magnet to the region of absorption (Bardonnet et al., 2006). There has been some reported success with this technique thus far though such systems are dependant on a great degree of precision.

1.2.2.2. Gastrointestinal patch systems

One of the proposed approaches for inducing greater levels of absorption has been the use of a multi-layered patch system (Tao and Desai, 2005). When the patch is applied, the drug is released into the systemic circulation at a regulated rate which maintains effective drug plasma levels. Several patch systems such as an insulin patch system for oral drug delivery have been developed. The key attributes of these systems include bioadhesive properties for gastric retention, controlled drug release and unidirectional release towards the intestinal epithelium (Tao and Desai, 2005). Gastrointestinal patch systems present a unique approach to improving oral bioavailability through the combination of bioadhesion, drug protection and unidirectional release.

1.3. Advantages offered by gastroretentive drug delivery

Gastroretentive, controlled release drug delivery systems offer the following potential advantages (Singh and Kim, 2000; Hoffman et al., 2004; Arora et al., 2005; Hamdani et al., 2006).

I. Enhanced absolute bioavailability of drugs with a narrow absorption window through an extended absorption phase

II. Delivery of drug directly to a specific site of absorption

III. Optimum drug delivery for drugs acting locally in the stomach or small intestine

IV. Reduced fluctuations in plasma drug concentrations due to delayed gastric emptying especially for drugs with a narrow therapeutic index

V. Prolonged drug levels above the minimum effective concentration

VI. Improved solubility of drugs that are less soluble at a high pH

VII. Improved selectivity in receptor activation through the minimization of fluctuations in drug concentrations

VIII. Improved pharmacokinetic and pharmacodynamic profiles due to less variation in gastric transit profiles

IX. Enhanced therapeutic efficacy of several important drugs such as bromocriptine which is used in the treatment of Parkinson's disease

X. Reduced dosing frequency and increased patient compliance

1.4. Statement of the problem

1.4.1. Understanding Narrow Absorption Window ("NAW") Drugs

"NAW" drugs have distinct absorption characteristics since they are absorbed in specific regions of the upper GIT. Region specific absorption can be attributed to several factors such as the permeability of a drug being restricted to a particular region of the GIT, low mucosal permeability of the drug, a drug being unstable in the GI environment or stability in different regions of the intestine as a result of changes in environmental pH (Hoffman et al., 2004; Davis, 2005; Tao and Desai, 2005). Since the absorptive site of such drugs is limited, drug that is released in the region preceding or close to the absorption window is only available for absorption. After a short period of less than 6 hours, the drug has already left the upper GIT and is released in the non-absorbing distal segments of the GIT. This short absorption phase is often accompanied by a reduced bioavailability (Chavanpatil et al., 2005). Once the absorption window is passed, a large and undefined portion of the dose goes to waste with negligible or no absorption leading to diminished efficacy of the administered dose and negating the beneficial effects of a once a day dose (Singh and Kim, 2000; Rocca et al., 2003). Multiple doses need to be administered to compensate for the narrow absorption window which may result in decreased patient compliance and/or a plethora of side-effects. There are several clinically important drugs that would benefit from an increased residence time in the upper GIT as depicted in Table 1.1.

Table 1.1: Examples of Narrow Absorption Window ("NAW") drugs.

"NAW" Drug	Location of Absorption Window	Bioavailability (%)
Levodopa	Upper Small intestine	23
Riboflavin	Proximal small intestine	15
Gabapentin	Upper small intestine	27
Metformin	Upper small intestine	50
Acyclovir	Small intestine	23
Furosemide	Stomach	29
Repaglinide	Proximal small intestine	50

1.4.2. Challenges associated with current drug delivery systems for "NAW" drugs

A review of current literature has described the following limitations of current drug delivery systems for ""NAW "drugs. One of the limitations of bioadhesive drug delivery systems is that the gastric bioadhesive force tends to be weak and may not impart an adequate ability of the drug delivery system to resist the strong propulsive forces of the stomach wall (Garg and Sharma, 2003). The electrostatic and H-bond formation responsible for the adhesion between polymer and mucus can also be prevented by the acidic environment and thick mucus present in the stomach (Koner et al., 2007). In addition, the high turnover rate of the gastric mucous and the dilution of the stomach content also limit the potential of bioadhesion as a gastroretentive force (Garg and Sharma, 2003; Streubel et al., 2006). Expandable drug delivery systems are designed to be larger than the pyloric opening which prevents their exit from the stomach. Drawbacks associated with this approach include bowel obstruction, life threatening consequences may result from permanent retention in the stomach and these systems also need to be sufficiently rigid to withstand the powerful mechanical contractions within the stomach (Streubel et al., 2006).

Floatation as a gastroretention mechanism requires the presence of a high level of fluids in the stomach for the drug delivery system to float and work efficiently. However this limitation can be overcome by coating the drug delivery system with bioadhesive polymers thereby enabling them to adhere to the mucosal lining of the stomach. Alternatively, the dosage form may be administered with approximately 200-250mL of fluid (Singh and Kim, 2000). Floating drug delivery systems may not be suitable for drugs that have solubility or stability problems in the GIT. Most of the floating systems reported in the literature are single-unit systems (Koner et al., 2007). The limitations associated with this type of drug delivery systems include high variability in bioavailability, local irritation due to a large amount of drug delivered at a particular site and these systems tend to be unreliable in prolonging the gastric residence time owing to their "all or none emptying process" (Jain et al., 2005). There are several other factors such as density, size and shape of the drug delivery systems and biological factors such as age, gender and posture that also present challenges for current "NAW" drug delivery systems (Bardonnet et al., 2006, Koner et al., 2007).

1.5. Outline of the problem

To overcome the above challenges of single-unit floating drug delivery systems, a lyophilized, swellable polymeric system strengthened with matrix consolidators has been employed to develop a gastroretentive multi-unit device. Different combinations of the polymers alginate, pectin and poly (lactide-co-glycolide) (PLGA) have been used to maintain the structural integrity of the system against the peristaltic force within the GIT whilst providing a constant release of drug. In addition, these swellable polymers and lyophilization are employed to provide floatability of the system on gastric fluid preventing it from being expelled early from the stomach.

1.5.1. Desirable criteria for a gastroretentive drug delivery system

- The system should possess an ability to control the gastric residence time combined with a time controlled release pattern of the drug (Streubel et al., 2006).
- Due to physiological factors in the stomach, the system must be able to withstand peristaltic forces in the stomach.
- It should be able to load drugs with different physicochemical properties (Klausner et al., 2003).
- The system should have no effect on gastric motility or the rate of gastric emptying.
- The delivery system should resist premature gastric emptying.
- It should not induce any local gastric mucosal damage through its prolonged retention in the GIT.
- The materials used in the formulation of the delivery system should be biodegradable and non-toxic and the system should disintegrate completely (Klausner et al., 2003; Friedman et al., 2004).
- The two most important features of the systems are size and density. Size is especially important as it has been proven that generally particles with a diameter of more than 15mm are less easily evacuated from the stomach which is necessary for prolonging retention (Bardonnet et al., 2006).
- Convenient intake of the system should also be considered and whether the kinetics of drug absorption is independent of a fasting or "fed" state of the patient.

1.6. Aim and Objectives of this study

The aim of this study was to develop a gastroretentive muti-unit device that would provide prolonged release rate of the model drug, riboflavin as a gastroretentive drug delivery system. To accomplish this, the following objectives were outlined:

(i) Gelification was employed as a technique to formulate the different statistically-planned combinations of cross-linked polymeric multi-unit matrices.

(ii) Optimizing the conditions of lyophilization was used to reduce the density and size of the polymeric matrices to achieve floatability of the multi-units and improve the entrapment efficiency.

(iii) The buoyancy time was maximized for controlled drug release prior to the absorption window.

(iv) The physicomechanical properties of the multi-units were assessed to determine the structural integrity of the multi-units

(v) A matrix consolidator was added to improve the resistance of the multi-units against the physiological conditions of the stomach

(vi) Dissolution studies were performed to determine the release pattern of riboflavin from the multi-units

1.7. Overview of the research report

Section 1 provides an overview of the rationale and motivation for the study. It describes the need for specialized drug delivery to the GIT within the context of modern drug delivery and outlines the various mechanisms explored to achieve this. It defines narrow absorption window ('NAW") drugs and describes the potential of gastroretentive delivery systems in overcoming the obstacles associated with drug delivery for "NAW" drugs. The advantages and limitations of existing gastroretentive drug delivery systems and the desirable criteria for effective gastroretention have also been described. This section concludes with the aims and objectives of the current study.

In Section 2, the development of the gastroretentive multi-units and the outcome of the study are described. The materials and approach used in the formulation of the multi-units and the variables employed to optimize the formulation process using a Box-Behnken statistical design are also described. The multi-units were assessed for their drug encapsulation efficiency, floatability, effect of lyophilization time, textural profiling and *in vitro* drug release. The methods used to evaluate these properties are described. This section also describes the influence of the various process variables on the performance behaviour of the multi-units with respect to *in vitro* drug release, drug encapsulation efficiency and floatability.

Section 3, describes the recommendations for the improvement of the gastroretentive multi-unit system and its future potential.

SECTION 2

DEVELOPMENT OF THE GASTRORETENTIVE MULTI-UNITS

2.1. Introduction

This section aims to describe the development of the gastroretentive multi-units. One of the major scientific challenges in the development of gastroretentive drug delivery systems is overcoming the physiological conditions of the stomach which consist of strong gastric contractions every few hours especially in the fasted state (Rocca et al., 2003). In order to deliver riboflavin to its maximum site of absorption i.e. proximal small intestine, it was essential to ensure that the drug delivery system retains in the stomach which is the region preceding the target site. The most active area of research using biodegradable polymers is in controlled drug delivery. Biodegradable, aliphatic polyesters such as poly (D, L-lactide) (PLA) and poly (D, L-lactide co-glycolide) (PLGA) have been used for a wide variety of biomedical and pharmaceutical applications (Huh et al., 2005). In addition to their biocompatibility, predictability of degradation kinetics, ease of fabrication and non-toxicity other properties such as their mechanical strength, hydrophilicity / hydrophobicity and degradation make them suitable for controlled drug delivery. When these polymers are fabricated into controlled drug delivery systems, additional qualities such as surface area, bulk density and particle size are introduced and many affect both degradation of and drug release from the polymeric system

In addition, plant polysaccharides such as alginate and pectin retain their integrity because they are resistant to the digestive action of the gastrointestinal enzymes and have an appeal in the area of drug delivery because of their versatility,

biodegradability and low toxicity (Chourasia and Jain, 2003). The physicomechanical and biological aspects of biodegradable polymers have proved to be successful in the control of drug release and represent vital areas in the reliable and efficacious functioning of controlled release drug delivery systems. In addition, polymers offer substantial mechanical strength to control the gastroretentivity by maintaining the system in its desired configuration for a pre-determined time.

The gastroretentive multi-units employed crosslinking of various combinations of the hydrophilic polymers, alginate and pectin with the hydrophobic polymer, PLGA. Crosslinking of polymers created a polymeric matrix which is not instantly soluble in the gastric fluids and which gradually dissolves thereby prolonging gastroretention and providing improved physical resistance to gastric acidity. In addition, PLGA was used as a matrix consolidator to improve the mechanical strength of the gastroretentive multi-units. A Box-Behnken statistical, experimental design was employed to derive the 27 experimental, formulation combinations of the independent and dependant formulation variables.

2.2. Materials and Methods

2.2.1. Materials

Riboflavin-5-phosphate sodium, also known as vitamin B_2 which is a water soluble vitamin was used as the model compound of narrow absorption drugs. Poly-lactide-co glycolide (PLGA), (Resomer® RG502, Boehringer Ingelheim, Germany) was utilized. The biodegradable polymers, Protanal LF 10/60 (FMC Biopolymer, USA) and Pectin Classic (Hebstreith and Fox, Germany) were used. $2\%^{w}/_{v}$ zinc gluconate was used as the crosslinking solution. The buffer comprised of simulated gastric fluid (pH 1.2; 37°C) USP.

2.2.2. Methods

2.2.2.1. Building the experimental design

Design of Experiment (DOE) is an efficient statistical tool for planning formulations so that data obtained can be analyzed to yield valid and objective conclusions. The primary objectives of this study were to achieve floatability of the gastroretentive multi-units in gastric simulated fluid (pH 1.2; 37°C) and achieve prolonged release. Response Surface Methodology (RSM) was employed to determine the optimal process settings to achieve the study objectives and to make the process more robust against non-controllable influences. In addition, RSM was employed to maximize the responses to achieve prolonged drug release and extended floatability of the gastroretentive multi-units. RSM is used when only a few significant factors are used in optimization. Different types of RSM designs include 3-level factorial design, central composite design and the Box-Behnken design (Palamakula et al., 2004).

A Box-Behnken statistical design composed of 4 factors, 27 random experimental runs and 3 centre-points was built using Minitab® V15 software (Minitab® Inc., PA, USA). A series of 27 different formulations were carefully chosen from a fractional factorial design to formulate and analyse. The samples were selected so as to give the maximum effective interpretation of results without using an excessive number of experiments which would have been 81 in total. Four different process variables namely alginate, pectin, poly-D-L-lactide-co glycolide (PLGA) and the lyophilization time were optimized using this design and were varied in a series of 27 different formulations. The effect of the variations within each formulation on drug release, drug entrapment and floatability were observed.

2.3. Formulation of the gastroretentive multi-units

Various formulations of alginate and pectin as listed in Table 2.1 were dissolved in 100mL de-ionized water. The concentration of the model compound, Riboflavin was maintained in a constant 2:1 polymer to drug ratio. The polymers were tested in a concentration of 0.5-2%$^{w}/_{v}$. The dispersion was covered with aluminium foil to prevent exposure to light and stirred for 1 hour. Thereafter the PLGA was added to the dispersion as a matrix consolidator. The hydrophobic polymer, PLGA was homogenized to form a multi-polymeric dispersion. The solution for the gelification and crosslinking of the drug polymer dispersion comprised of 2%$^{w}/_{v}$ zinc gluconate solution. The gastroretentive multi-units were prepared from a 100mL solution of polymers and riboflavin which was dropped via a syringe (internal diameter: 0.5mm) into a stirred 2%$^{w}/_{v}$ zinc gluconate solution. Instantaneous gelation of the outer surface of the multi-units occurred. The multi-units were gently stirred in this solution for 40 minutes and remained in the zinc gluconate solution for an additional 24 hours. Therafter, the multi-units were filtered and washed (3x100mL) with de-ionized water. In their hydrated state, the multi-units were introduced into plastic trays and subjected to a variable period of lyophilization. The lyophilization times ranged from 2 hours to 24 hours. The general set of operating conditions maintained for the 27 formulations were an average temperature of -60°C and vacuum pressure = 25mtorr. Table 2.1 depicts the independent formulation variables tested in the 27 experiments.

Table 2.1: *Box-Behnken design for synthesis of the gastroretentive multi-units*

Experimental Runs	Alginate (g/100mL)	Pectin (g/100mL)	PLGA[a] (g/100mL)	Lyophilization Time (hrs)
1	1.25	1.25	0.50	24
2	0.50	0.50	1.25	13
3	1.25	2.00	2.00	13
4	1.25	1.25	1.25	13
5	1.25	0.50	1.25	24
6	2.00	2.00	1.25	13
7	0.50	1.25	2.00	13
8	0.50	1.25	1.25	24
9	2.00	1.25	0.50	13
10	2.00	1.25	1.25	24
11	0.50	1.25	1.25	2
12	0.50	1.25	0.50	13
13	2.00	1.25	1.25	2
14	1.25	2.00	1.25	2
15	1.25	0.50	2.00	13
16	0.50	2.00	1.25	13
17	1.25	0.50	0.50	13
18	1.25	1.25	1.25	13
19	1.25	1.25	1.25	13
20	1.25	0.50	1.25	2
21	2.00	0.50	1.25	13
22	2.00	1.25	2.00	13
23	1.25	1.25	2.00	24
24	1.25	2.00	1.25	24
25	1.25	1.25	2.00	2
26	1.25	2.00	0.50	13
27	1.25	1.25	0.50	2

[a]*Poly(lactic-co-glycolic acid)*

The RSM, Box-Behnken statistical design described in Section 2.2.2.1 was employed to optimize and evaluate the main effects and interaction effects of the formulation ingredients on the *in vitro* performance of the gastroretentive multi-units. The concentrations of the independent variables which were used to prepare the design generated experimental formulations are illustrated in Table 2.1. The dependent variables included drug entrapment efficiency, matrix deformability gradient, floatability and cumulative percentage drug release.

2.4. UV spectrophotometry analysis of riboflavin content

2.4.1. Preparation of calibration curves and assay procedure

Stock solutions were prepared by separately dissolving variable quantities of riboflavin in 100mL simulated gastric fluid (SGF) buffer (pH 1.2; 37°C).The following concentrations were prepared: 0.005mg/mL, 0.004mg/mL, 0.003mg/mL and 0.002mg/ml. The UV absorbance of each standard solution was determined at the maximum wavelength of absorption (λ_{max}) of 444nm for Riboflavin. SGF was used as the blank. No other ingredients are absorbed in this range i.e. alginate, pectin or PLGA. A calibration curve (correlation coefficient; R^2=0.96) was constructed as depicted in Figure 2.1.

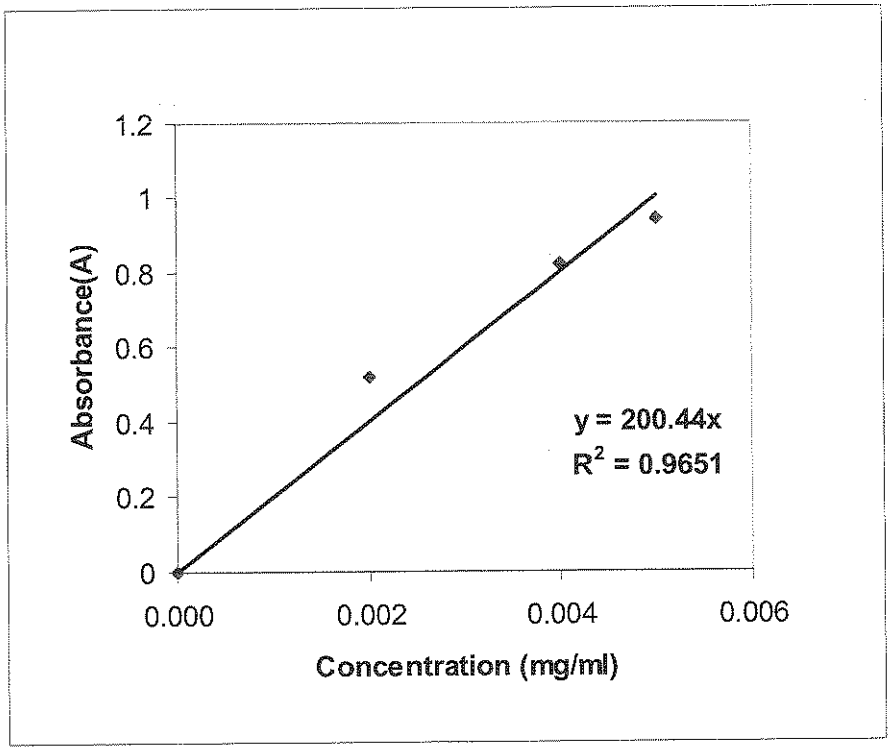


Figure 2.1: *Calibration curve of riboflavin-5-phosphate in simulated gastric fluid (pH 1.2; 37°C).*

2.5. Determination of drug entrapment efficiency

A total of 27 samples comprising an equivalent of 50mg of riboflavin gastroretentive multi-units were placed in a 100mL volumetric flask and diluted to volume with simulated gastric fluid (pH 1.2; 37°C). Dissolution of the multi-units was facilitated by thoroughly triturating the gastroretentive multi-units before addition to the buffer. Samples of 5mL were drawn and the concentration of riboflavin was spectrophotometrically (Specord 40, Analytik Jena, AG) determined on a UV spectrum at an absorption wavelength of 444nm. The drug entrapment (%) for each of the 27 formulations was subsequently determined using the standard calibration curve. The drug entrapment efficiency was calculated using Equation 1

$$DEE = \frac{Q_a}{Q_t} \times 100 \qquad \text{Equation 1}$$

2.6. Textural profile analysis

A calibrated Texture Analyzer (TA.XT*plus*, Stable Microsystems, England) fitted with a steel probe (2mm diameter) was employed in the determination of the textural characteristics of the gastroretentive multi-units. Analysis was conducted on samples of unhydrated multi-units from each of the 27 different formulations. The parameter settings employed in this analysis are outlined in Table 2.2. Samples were analyzed for matrix hardness (N/mm), fracture gradient (N/mm) and deformation energy (J).

2.6.1. Determination of matrix deformability gradients

The matrix deformability gradient was used to measure the hardness of the matrix. Figure 2.2a depicts an example of a Force-Distance profile for the determination of

the matrix hardness (N/mm), which is represented by the gradient between the initial force (anchor 1) and the maximum force attained (anchor 2).

2.6.2. Determination of matrix deformation energy

The deformation energy (J) was determined by calculating the area under the curve (AUC_{FD}) of a Force–Distance profile as depicted in Figure 2.2b.

2.6.3. Determination of matrix fracture gradients

The matrix fracture gradient was calculated as the gradient formed between the initial force (anchor 1) and force attained to rupture the matrix anchor 2 as depicted in Figure 2.2c

Table 2.2:*Textural parameter settings employed in the determination of deformability gradients, fracture gradients and deformation energy.*

Parameters	Settings
Pre-test speed	1mm/sec
Test Speed	0.5mm/sec
Post-Test Speed	1mm/sec
Compression Force	40N
Load Cell	50kg
Trigger Type	Auto
Trigger Force	0.5 N
Return Distance	20mm

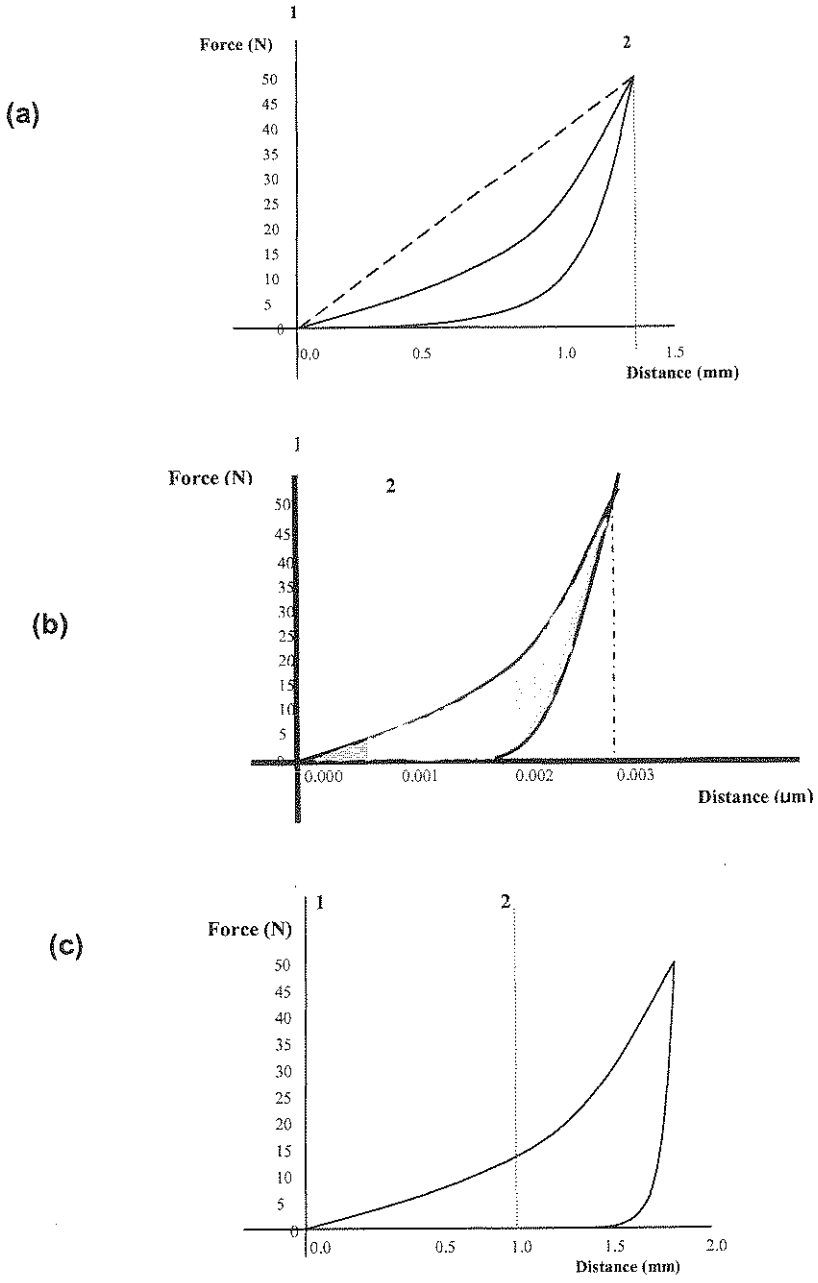


Figure 2.2:*a) Typical Force-Distance profile in the determination of matrix deformability gradients, b) Typical Force-Distance profile in the determination of matrix deformation energy, c) Typical Force-Distance profile in the determination of matrix fracture gradient*

2.7. Floatability of the gastroretentive multi-units

2.7.1. Determination of the buoyancy lag-time

In vitro buoyancy was characterized by floating lag time and total floating time. The determination of floating lag-time was performed for each of the 27 formulations. Each formulation was placed in a calibrated six-station dissolution testing apparatus (Caleva Dissolution Apparatus, model 7ST) using a USP25 rotating paddle method at 50rpm with 900mL of simulated gastric fluid (pH 1.2; 37°C). The time interval between the introduction of multi-units into the dissolution media and its rise to the surface of the media was noted as the buoyancy lag-time.

2.7.2. Determination of floatation of the system

The floatation within the dissolution medium was considered as the time of total system floatation which was determined for the 27 gastroretentive multi-units over a period of 24 hours. Each formulation was placed in a calibrated six-station dissolution testing apparatus (Caleva Dissolution Apparatus, model 7ST) using the USP25 rotating paddle method at 50rpm with 900mL of simulated gastric fluid (pH 1.2; 37°C).

2.8. Assessment of the surface morphology of the gastroretentive multi-units

The external morphology of the 27 lyophilized multi-unit samples were visually characterized according to their shape, size and texture. Samples were prepared for photomicrographs by applying a thin layer of colloidal graphite on aluminum stubs and mounting the multi-units on graphite to hold them in place during microscopic examination. The multi-units were then coated with a thin layer of gold-platinum using a sputter coater under an electrical potential of 15 kV. Several

photomicrographs were produced by scanning fields, selected at different magnifications using a Jeol JSM-840 scanning electron microscope (Tokyo, Japan).

2.9. *In vitro* drug release studies

2.9.1. Preparation of simulated gastric fluid

Simulated gastric fluid was prepared according to the USP method using 2.0g of sodium chloride and 3.2g of pepsin which was dissolved in 7mL of hydrochloric acid. Sufficient distilled water was added to 1000mL.The pH of the test solution used was 1.2.

2.9.2. Dissolution of the gastroretentive multi-units

In vitro drug release studies were performed on the 27 gastroretentive multi-units. Each formulation was placed in a calibrated six-station dissolution testing apparatus (Caleva Dissolution Apparatus, model 7ST) using a USP25 rotating paddle method at 50rpm with 900mL of simulated gastric fluid (pH 1.2; 37°C). For the determination of the riboflavin concentration, 5mL samples were manually withdrawn and replaced with an equivalent volume of simulated gastric fluid to maintain sink conditions. These studies were performed at specific time intervals over a period of 24 hours.

2.9.3. Riboflavin analysis

The concentration of riboflavin was spectrophotometrically determined for the 27 formulations. All analyses were conducted in triplicate. 5mL samples were withdrawn at specific time intervals over a period of twenty four hours. Samples were analyzed by ultraviolet spectroscopy (Specord 40, Analytik Jena, AG) at 444nm.

2.10. Results and discussion

2.10.1. Morphology and yield of gastroretentive multi-units

The yellow colour of the Riboflavin salt appeared to be dispersed evenly within each of the formulations. The samples visually appeared yellow in colour and were predominantly spherical in shape. There appeared to be some variations in the dimensions and the darkness of the gastroretentive multi-units between the different formulations. This may be due to varying the concentrations of polymer and drug used in each formulation. The texture of the multi-units appeared porous since each formulation was exposed to varied periods of lyophilization.

It was also visually observed that none of the 27 formulations disintegrated in simulated gastric media, pH 1.2 or in alkaline pH 6.8 demonstrating that the concentration ratio and type of polymers used in the dosage form played a vital role in retaining the dosage form intact. These results correlate with findings in a previous study where those gastroretentive dosage forms made of naturally occurring carbohydrate polymers retained their structural integrity for 24 hours in simulated gastric fluid (Ahmed and Ayres, 2006).

The morphology of Riboflavin multi-units after various periods of lyophilization is depicted below in Figure 2.3(a-c).

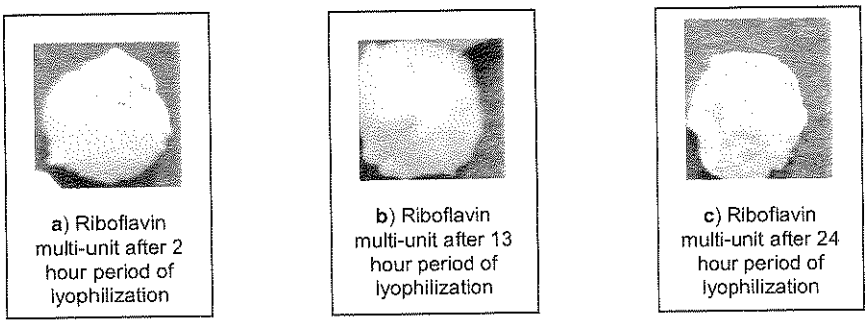


Figure 2.3: *Surface morphology of gastroretentive multi-units after varying lyophilization times of a) 2 hour lyophilization, b) 13 hour lyophilization, c) 24 hour lyophilization.*

2.10.2. Drug entrapment efficiency

The effects of the various formulation variables on the drug entrapment efficiency (DEE) of the gastroretentive multi-units are depicted in Table 2.3. DEE range was between 2.8-54%. Superior DEE values were obtained when the concentration of the polymers, Alginate, Pectin, and PLGA were maintained in a ratio of 0.5:1.25:1.25 irrespective of the polymer i.e. total polymer concentration of 3g/100mL. This polymer to drug ratio reflected the optimum capacity for drug encapsulation. Further increases in the polymer to drug ratio did not have a significant change on entrapment efficiency. The finding was consistent with a previous study where encapsulation of a water soluble drug by cross linking was assessed. It was found that no significant change was observed when the polymer: drug ratio was increased above the optimum capacity for drug encapsulation (Tayade and Kale, 2004). A constant polymer to drug ratio of 2:1 was employed for each formulation.

Variable DEE values were obtained for different polymer concentrations as depicted in Figure 2.4(a-c). The results obtained indicate that the lyophilization time and polymer concentration determined the entrapment efficiency of the gastroretentive multi-units. Superior DEE values were obtained when the total polymer concentration was 3g/100mL and the period of lyophilization was 24 hours. These results conform to a previous study where it was concluded that there was a strong correlation between polymer ratio and release of riboflavin when the riboflavin microballoons were evaluated as a floating drug delivery system (Sato et al., 2004).

An increase in the DEE (%) as the polymer concentration was increased to 3g/100mL may be due to the increased viscosity of the polymeric matrix preventing diffusion of the drug from the inner aqueous phase resulting in increased entrapment of riboflavin. In addition, the multi-units formed at higher polymer concentrations will be denser than those formed at lower polymer concentrations. This may increase the entrapment of the Riboflavin. An increase in the size of the riboflavin particles after the 24 hour lyophilization period may have resulted in the increased DEE (%) values. In addition, the hydrophobic PLGA layer may have become less permeable to the riboflavin particles following a 24 hour lyophilization period resulting in a greater percentage of entrapped drug.

Figure 2.5 depicts an increase in DEE (%) with an increase in riboflavin concentration. An increase in DEE (%) as the riboflavin concentration is increased can be ascribed to increased drug solubility in the external media. In a previous study which investigated the effects of drug loading on drug entrapment efficiency, the percentage drug entrapment was approximately 75% at higher drug loading levels due to the formation of larger particles (Jain et al., 2005). Since the maximum

drug entrapment efficiency achieved in this study was approximately 54%, drug loading may be explored in future to improve the drug entrapment efficiency of the multi-units.

The results of these studies show that DEE of the gastroretentive multi-units can be increased by optimizing the concentration of the polymers alginate, pectin and PLGA and lyophilization times. In addition, polymer to drug ratio may play a significant role in the determination of the DEE of the gastroretentive multi-units. Higher polymer concentrations can result in increased polymer viscosity which may lead to a decrease in the diffusion of the drug into the external media. Consequently, a higher percentage of the drug will remain entrapped in the polymeric matrices.

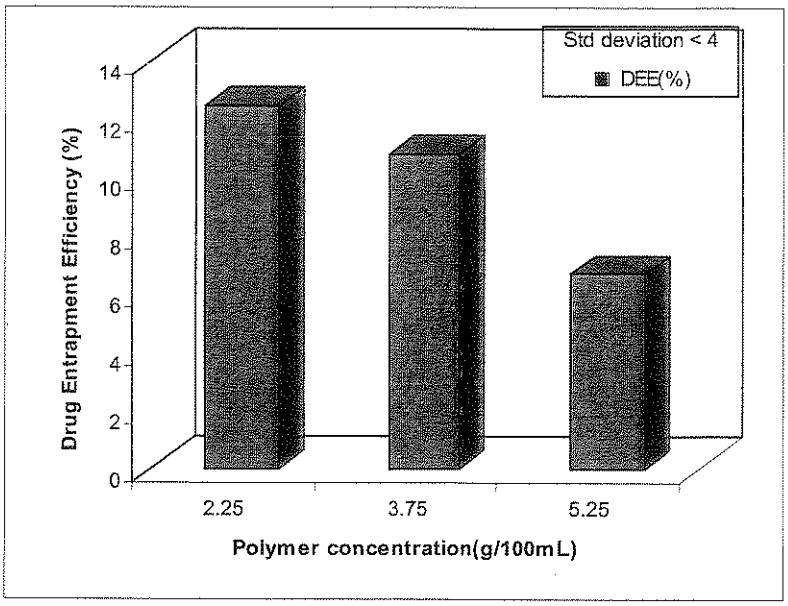


Figure 2.4a: *Effect of polymer concentration on drug entrapment efficiency for a lyophilization time of 13 hours*

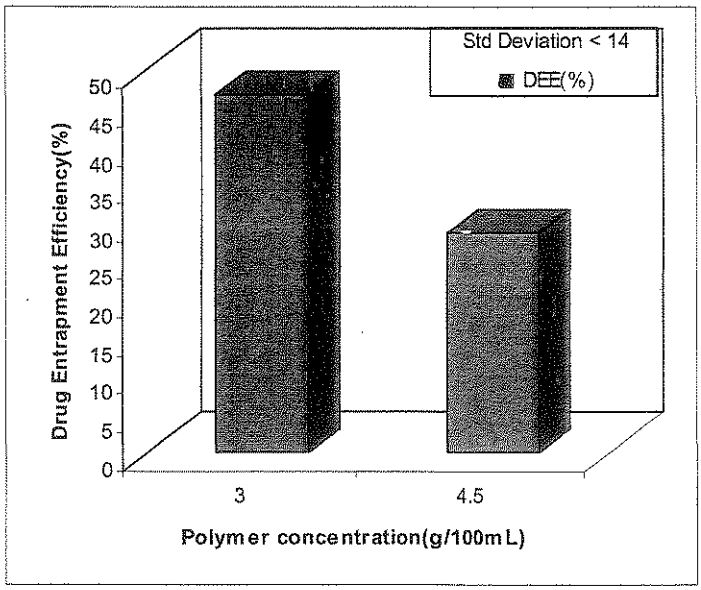


Figure 2.4b: *Effect of polymer concentration on drug entrapment efficiency for lyophilization time of 24 hours*

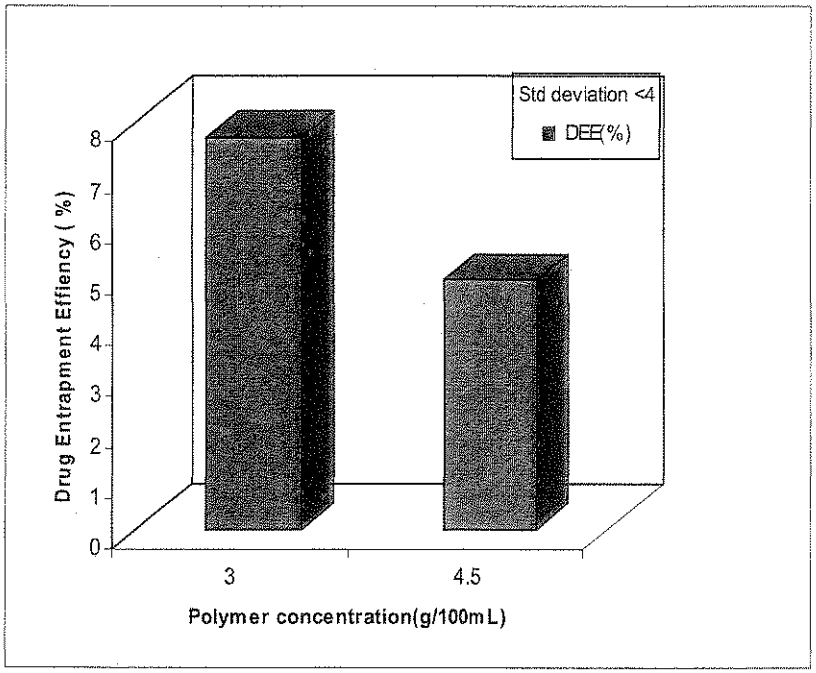


Figure 2.4c: *Effect of polymer concentration on drug entrapment efficiency at lyophilization time of 2 hours*

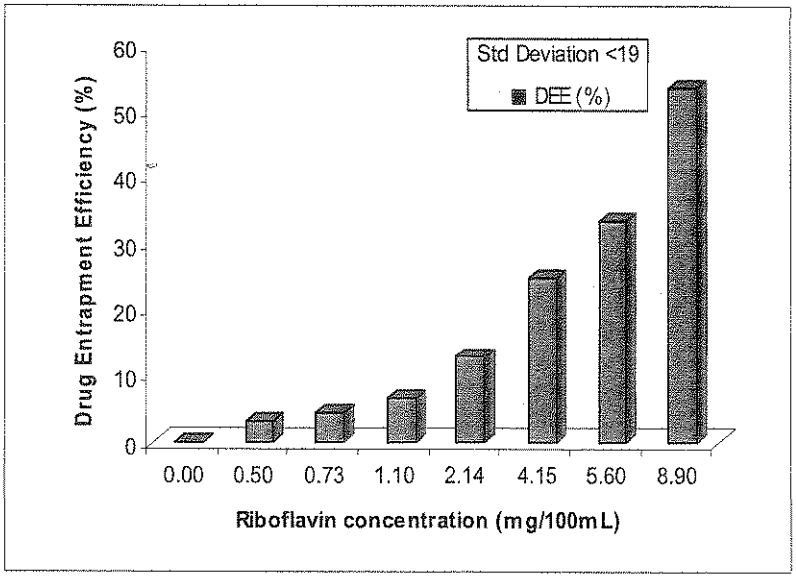


Figure 2.5: *Effects of riboflavin concentration on drug entrapment efficiency*

Table 2.3: *Drug entrapment efficiency (%) of the gastroretentive multi-units*

Formulation	[Alginate] % $^{w}/_{w}$	[Pectin] % $^{w}/_{w}$	[PLGA] % $^{w}/_{w}$	Lyophilization Time (hrs)	Drug in 50mg sample (mg)	DEE %
1	1.25	1.25	0.50	24	5.57	33.0
2	0.50	0.50	1.25	13	2.29	14.0
3	1.25	2.00	2.00	13	0.47	2.80
4	1.25	1.25	1.25	13	1.87	11.2
5	1.25	0.50	1.25	24	8.90	53.3
6	2.00	2.00	1.25	13	1.08	6.5
7	0.50	1.25	2.00	13	1.60	9.8
8	0.50	1.25	1.25	24	9.00	54.0
9	2.00	1.25	0.50	13	0.73	4.3
10	2.00	1.25	1.25	24	4.15	25.0
11	0.50	1.25	1.25	2	1.30	7.8
12	0.50	1.25	0.50	13	1.74	10.4
13	2.00	1.25	1.25	2	0.50	3.0
14	1.25	2.00	1.25	2	0.63	3.8
15	1.25	0.50	2.00	13	2.50	15.0
16	0.50	2.00	1.25	13	2.10	12.8
17	1.25	0.50	0.50	13	2.20	13.5
18	1.25	1.25	1.25	13	1.95	11.7
19	1.25	1.25	1.25	13	2.03	12.2
20	1.25	0.50	1.25	2	1.97	11.8
21	2.00	0.50	1.25	13	2.10	12.8
22	2.00	1.25	2.00	13	1.09	10.9
23	1.25	1.25	2.00	24	3.80	23.0
24	1.25	2.00	1.25	24	6.46	38.7
25	1.25	1.25	2.00	2	1.30	8.0
26	1.25	2.00	0.50	13	1.23	7.4
27	1.25	1.25	0.50	2	0.60	3.6

2.10.3. Textural analysis of gastroretentive multi-units

The deformability gradient is a measure of the hardness of the gastroretentive multi-units. The deformation energy is measured as the work performed to overcome the inherent force within the matrix of the multi-units and the fracture gradient measures the minimum in-situ stress required to disentangle the matrix of the multi-units. The values obtained for the deformation energy were low with all samples producing results below 1 Joule. This indicated that a relatively small force would be required to deform the matrix of the gastroretentive multi-units. It was observed that the values for matrix hardness were higher in those samples obtained from formulations where the total polymer concentration was = 3g/ml. This may be a result of the increased polymer viscosity when the polymer concentration was increased. In addition, polymers possess mechanical strength which can improve the rigidity of the multi-unit matrices. The numerical values of the deformability gradient, deformation energy and fracture gradients are listed in Table 2.4.

Table 2.4: *Textural profiling results of gastroretentive multi-units*

Formulation	Lyophilization Time	Matrix Deformation Energy (J)	Matrix Deformability Gradient (N/mm)	Matrix [1] Fracture Gradient (N/mm)	Matrix [2] Fracture Gradient (N/mm)	Matrix [3] Fracture Gradient (N/mm)
1	24	0.009	7.22	0.465	-	-
2	13	0.028	13.19	1.210	-	-
3	13	0.021	37.89	28.51	-	-
4	13	0.014	10.24	1.450	-	-
5	24	0.023	5.14	0.182	-	-
6	13	0.098	15.47	18.97	3.74	-
7	13	0.015	10.66	6.495	4.87	4.04
8	24	0.003	6.22	0.492	-	-
9	13	0.033	10.22	4.960	2.66	-
10	24	0.015	10.03	0.691	-	-
11	2	0.029	18.06	12.69	4.29	-
12	13	0.006	9.20	0.922	-	-
13	2	0.008	14.17	8.540	4.19	1.51
14	2	0.043	1.49	0.014	-	-
15	13	0.066	12.56	2.250	-	-
16	13	0.014	10.45	3.210	2.83	-
17	13	0.016	8.84	0.490	-	-
18	13	0.009	11.33	2.900	-	-
19	13	0.014	9.99	6.780	4.25	-
20	2	0.021	23.38	10.59	6.67	-
21	13	0.033	11.46	7.840	0.61	-
22	13	0.038	9.77	2.220	-	-
23	24	0.008	9.88	1.120	-	-
24	24	0.022	7.04	2.180	0.74	-
25	2	0.000	0.00	0.000	0.00	-
26	13	0.082	19.73	2.590	1.69	-
27	2	0.015	29.92	12.59	-	-

[1] indicates fracture gradient caused by minimum force required to fracture the sample
[2, 3] Applied force is higher that minimum in-situ stress resulting in multiple fracture gradients

The relationship between the independent variables and dependant variables were further elucidated using response surface plots as shown in Figures 2.6 and 2.7.

2.10.4. Effect on formulation components on drug entrapment efficiency

Alginate, pectin and PLGA concentrations between 1.2-1.8%$^{w}/_{v}$ resulted in an increase in the drug entrapment efficiency (DEE) (Figures 2.6a, b and f) while higher alginate and pectin concentrations (>1.8%$^{w}/_{v}$) (Figures 2.6c and e) did not have any significant increase in DEE. This may result due to the unavailability of excess polymer for encapsulation of riboflavin. In addition, changes in the solubility of the polymer network at higher polymer concentrations (>1.8%$^{w}/_{v}$) may occur, which can prevent further entrapment of riboflavin. Higher PLGA concentrations (>1.8%$^{w}/_{v}$) dominated the increase in riboflavin entrapment to greater than 40% (Figure 2.6e). This can result from an increased viscosity of the PLGA gel network which can retard the release of the drug. The results showed that lyophilization periods between 10-20 hours had a less significant impact on the DEE (Figures 2.6c, e and f). The results demonstrate that polymer concentration play a major role in the determination and improvement of drug entrapment efficiency within the multi-unit matrices

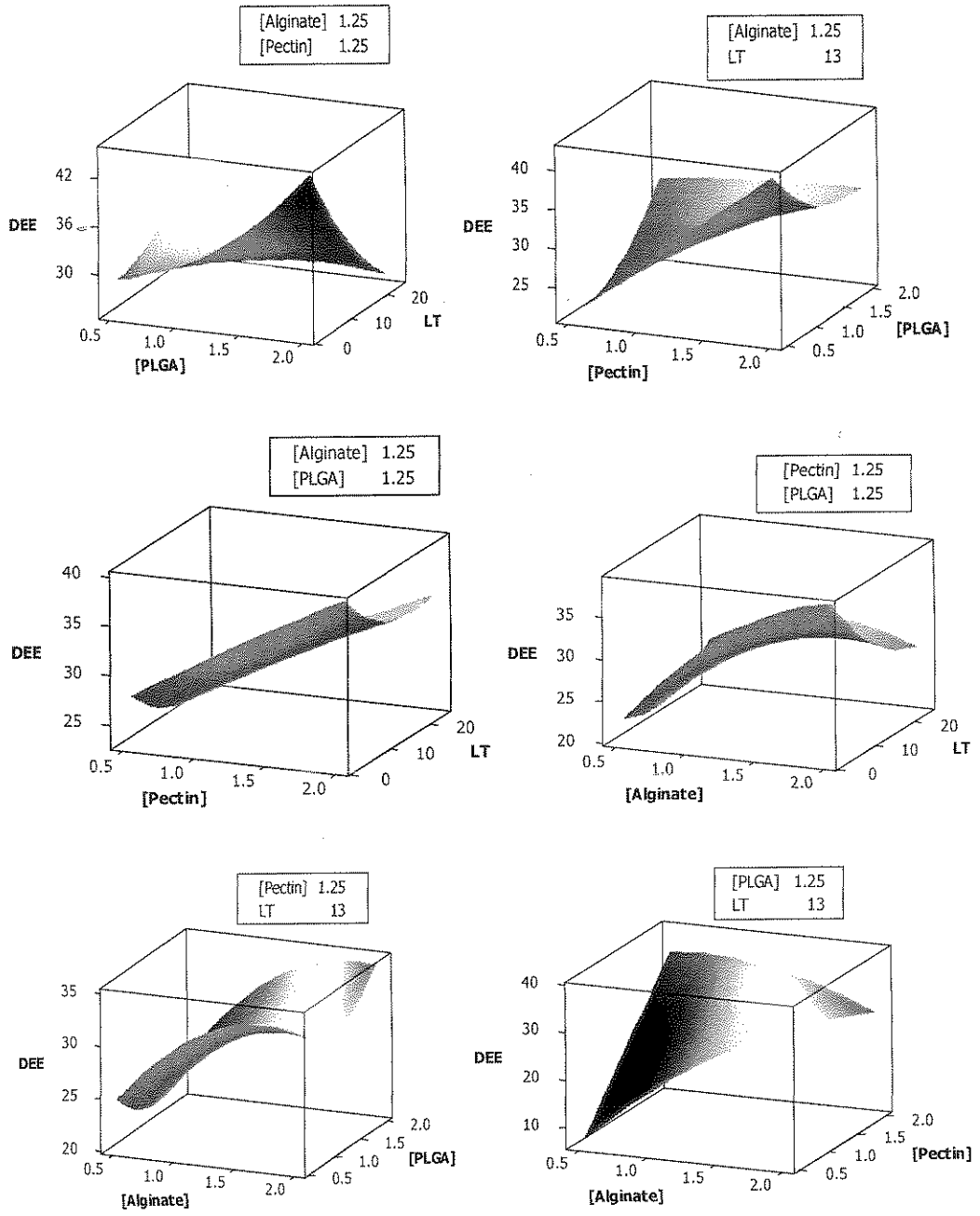


Figure 2.6(a-f): *Response surface plots depicting the effect of independent variables on the Drug Entrapment Efficiency (DEE) of riboflavin within the salted-out PLGA scaffolds.*

2.10.5. Effect on formulation components on the matrix deformability gradient

Higher alginate concentrations (>1.8%$^{w}/_{v}$) resulted in an increase in the matrix deformability gradient (MDG) (Figure 2.7a) while lower alginate concentrations (0.6%-1.2%$^{w}/_{v}$) resulted in a decrease in the MDG (Figures 2.7b-c). Higher pectin concentrations (>1.8%$^{w}/_{v}$) resulted in an increase in the MDG (Figure 2.7d) while a lower concentration resulted in a slight decrease (0.2%) in the MDG (Figure 2.7e). PLGA concentrations (1.2-1.8%$^{w}/_{v}$) produced a significant increase in the MDG (Figures 2.7d and f) whereas lyophilization time between 10-20 hours resulted in higher MDG values. These results support the theory that crosslinking of the biodegradable polymers, alginate, pectin and PLGA during the formulation process, can increase the mechanical strength of the polymers. By improving the mechanical integrity of the polymers, the overall hardness of the matrices increased with a resultant increase in the MDG values. Increasing lyophilization times result in a decrease in MDG values due to the increased porosity of the matrices with longer lyophilization periods. These results have suggested that alginate and pectin are significant for the controlled and sustained delivery of riboflavin when incorporated in the salted-out PLGA scaffolds.

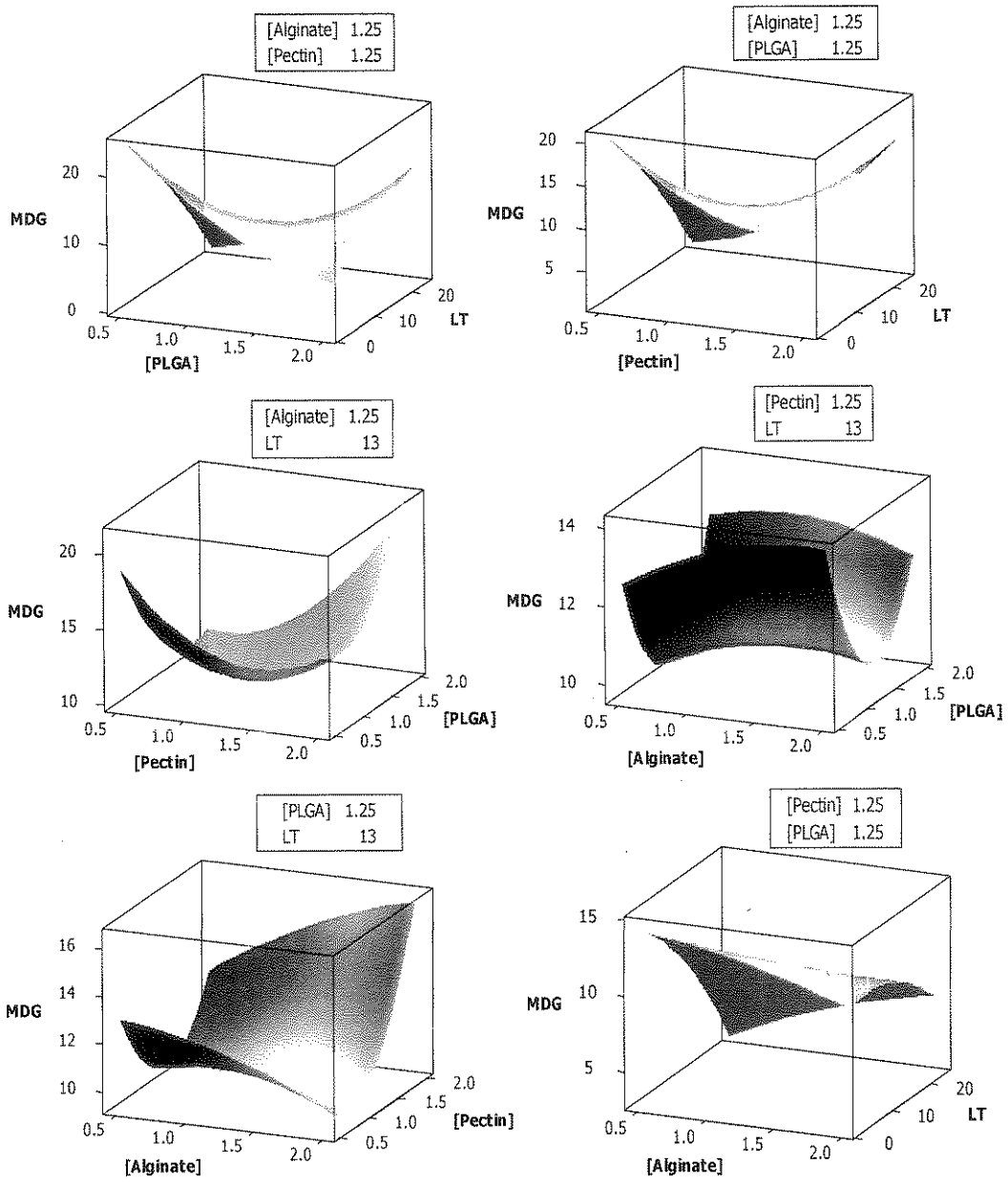


Figure 2.7(a-f): *Response surface plots depicting the effects of the independent variables on the Matrix Deformability Gradient of the salted-out PLGA scaffolds*

2.10.6. Buoyancy lag-time and floatation of the system

Gastrofloatability was achieved through the use of swellable polymers. The multi-units were exposed to various periods of lyophilization which was successful in achieving a system that is less dense than the gastric fluid ensuring that the system floated on the gastric fluid. Samples of the 27 formulations demonstrated superior floating performances in simulated gastric fluid (pH 1.2; 37°C). The floating lag-time for all 27 formulations was less than 30 seconds. The duration of floatation for the 27 formulations exceeded 12 hours and was still afloat at 24 hours. In previous studies where floatation was assessed in a similar method, floatation of the formulations ranged between 8-10 hours with floating lag times below 2 minutes (Jain et al., 2005; Patel and Patel, 2006). In comparison, the gastroretentive multi-units demonstrated a superior floatation ability which can be attributed to the optimization of the formulation variables. No difference in floating lag-times and system floatation was observed when the period of lyophilization was varied for the different formulations. No disentanglement of the polymeric matrices was observed in the 27 formulations. *In vitro* floatability was achieved through optimization of the following process variables:

1. During lyophilization, the porosity of the multi-units increased, reducing the overall density of the multi-units.
2. The air entrapped by the swollen polymer conferred buoyancy to these dosage forms.
3. The hydrophilic polymers, sodium alginate and pectin were crosslinked with the polymer PLGA. This produced a highly swellable, elastic multi-polymeric system within the multi-units. The overall mass of the multi-units was further reduced resulting in floatation upon introduction to the simulated gastric fluid (pH 1.2).

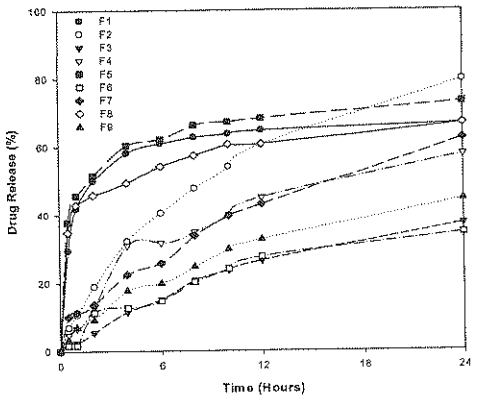
2.10.7. *In vitro* riboflavin release

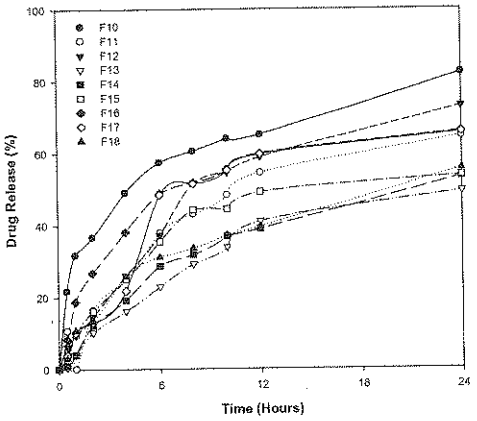
The dissolution profiles for the 27 gastroretentive multi-units are depicted in Figure 2.8(a, b and c). It was found that the drug release (%) over 24 hours decreased when the total polymer concentration of a sample formulation exceeded 5g/100mL. This was probably due to the formation of the highly swollen polymeric matrix which retarded release of drug contained within the polymeric matrix.

Release profiles for samples of formulations 1, 5, 8, 10, 23 and 24 were biphasic with an initial rapid release of riboflavin followed by a slower diffusional drug release phase. Samples of the aforementioned formulations comprised of different concentrations of the polymers which were subjected to the same lyophilization period of 24 hours at 25_mtorr. Release profiles for the remaining formulations showed a steady release of riboflavin without the initial burst effect. An increase in the riboflavin release (%) slowed after approximately 10 hours followed by constant drug release. These results conform to a previous study which found that after an initial fast release of drug content, the release of Riboflavin-5-phosphate from a gastroretentive dosage form was constant and followed a zero-order kinetics throughout the release process. It was concluded that the the slow and continuous drug release obtained after administration of the gastroretentive dosage form enabled prolongation of the time period in which the drug levels were above baseline concentrations (Klausner et al., 2002)

There are several mechanisms affecting drug release from polymeric formulations. In the case of formulations containing swellable polymers, other processes in addition to diffusion play an important role in exploring the drug release mechanisms (Chavanpatil et al., 2006).

These processes include relaxation of polymer chains, imbibition of water causing polymers to swell and changing them from the initial glassy to rubbery state (Chavanpatil et al., 2006). The decrease in riboflavin release from the gastroretentive multi-units after 10 hours may have resulted from the swollen polymeric matrices caused by the imbibition of gastric fluid. These results indicate that the dissolution of the gastroretentive multi-units were predominantly dependant on polymer concentration and the period of lyophilization.





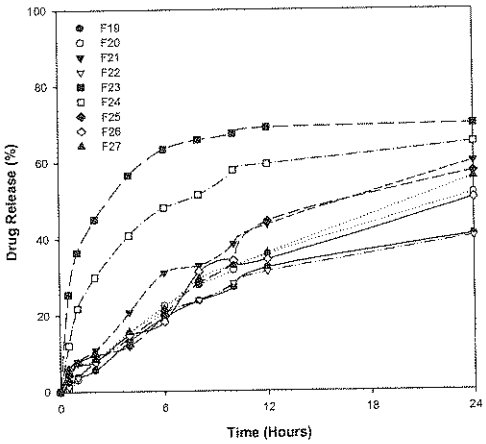


Figure 2.8: *Dissolution profiles for the gastroretentive multi-units a) formulations 1-9 b) formulations 10-18 and c) formulations 19-27.*

SECTION 3

CONCLUSION AND RECOMMENDATIONS

3.1. Conclusion

This study explored the formulation of gastroretentive multi-units which were successfully prepared through gelification of different statistically planned combinations of cross-linked polymers. The statistical box-behnken design was employed to optimize the conditions of lyophilization and each formulation was lyophilized for pre-determined periods of 2 hours, 13 hours or 24 hours. The results of the DEE (%) study showed that the varied lyophilization period had an effect on riboflavin entrapment within the polymeric matrices of the multi-units. The 27 formulations displayed prolonged buoyancy in the simulated gastric fluid and the formulations remained intact and buoyant at 24 hours. Floatation of multi-units was achieved through optimizing combinations of polymer concentrations and the duration of lyophilization. The aliphatic polymer, PLGA which was added as a matrix consolidator retained the structural integrity of the multi-units for a period of 24 hours. *In-vitro* drug release varied depending on the formulation variables e.g. concentration of the polymers PLGA, pectin and alginate and the period of lyophilization. The DEE (%) study showed that the modulation of the polymer concentration, riboflavin concentration and the period of lyophilization affected the entrapment efficiency of the multi-units. The combination of different types of polymers played a role in extending the period of floatability, drug release and retaining the polymeric matrices intact without disentanglement. The formulation strategy used in this study where the model drug riboflavin was dispersed in a multi-polymeric delivery system, showed

promising results for achieving both extended release and extended retention of the delivery system in simulated gastric fluid.

3.2. Recommendations

The gastroretentive multi-units developed in this study suggests the potential for increasing the bioavailability of riboflavin and similar drugs with a narrow absorption window ("NAW") in the upper small intestine thereby increasing the effectiveness of treatment for several medical conditions. Further studies would need to be conducted to determine the optimum combination of polymers, lyophilization time and release profile. *In vivo* studies comparing the release of riboflavin from the optimized formulation and the currently marketed riboflavin formulations may also be conducted to determine differences in the bioavailability of the drug. The effects of increasing the drug to polymer ratio should be investigated to determine the effect on the drug entrapment efficiency. Increasing the concentration of the outer hydrophobic polymeric layer may also be explored to determine its impact on the improvement of drug entrapment efficiency. Further methods such as coating the multi-units with a gelatin layer may be investigated for its effect on drug entrapment efficiency. The effects of drug loading on entrapment efficiency should also be evaluated since it has been shown in previous studies (Tayade and Kale, 2004; Jain et al., 2005; Strubel et al., 2006) that an increased polymer viscosity is responsible for the formation of large particles resulting in improved entrapment efficiency.

The addition of a mucosal adhering layer to the outer surface of the gastroretentive multi-units may also be investigated to determine the effect on the prolongation of the gastric residence time of the multi-units.

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