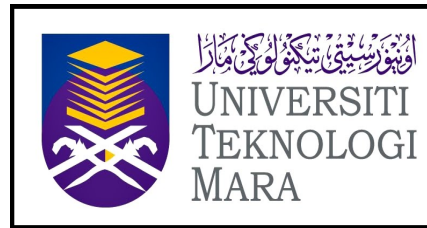




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DIPARTIMENTO DI FARMACIA



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NON DESTRUCTIVE BIOMEDICAL AND PHARMACEUTICAL RESEARCH CENTER

CO-TUTORSHIP DOCTORATE IN BIOPHARMACEUTICS- PHARMACOKINETICS

CICLE XXVII

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**NEW MODULES AND ASSEMBLED SYSTEMS FOR THE
CONTROLLED RELEASE OF DRUGS IN COMBINATION**

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Thesis

Introduction

1 Introduction

Oral administration is the most viable route to deliver drugs and it is also the method which shows the highest patient compliance.

Unfortunately some drugs show a low oral bioavailability or they have a short half-life which require taking several doses in one day.[1]

Modified release drug delivery systems can overcome such issues through changing the kinetics of drug release which then:

- modify drug bioavailability and plasma drug concentration
- reduce the number daily dosing and increase patient compliance
- minimize adverse effects
- prevent over- or under-dosage of drugs [2, 3].

The most common oral dosage form is the polymeric matrix, especially swellable matrices. The swellable matrix is a monolithic system of drug dispersed or dissolved a polymeric bed. It can be manufactured using soluble and swellable polymer which can control the release of the drug due to polymer gelification upon in contact with an aqueous medium.

The gelification of the polymer takes place gradually on time together with the disentanglement of the polymeric chains which elicit matrix erosion. A drug cannot diffuse through the dry (glassy) polymer, but only via the gelled (rubbery) polymer[4].

Figure 1 shows erosion, swelling and diffusion fronts that are encountered in a matrix.

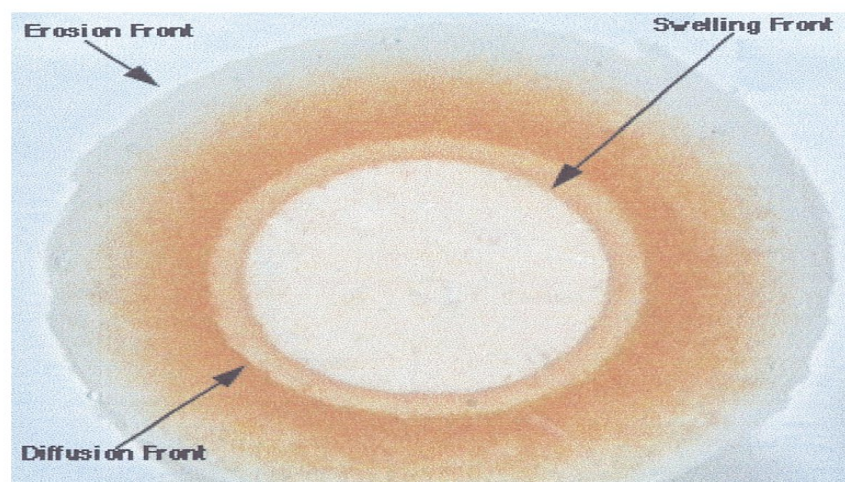


Figure 1. Erosion, diffusion and swelling front

The erosion front defines the limit between the matrix and the dissolution medium. The swelling front indicates the limit between the glassy and the rubbery polymer domains. The diffusion front denotes the limit between solid and un-dissolved drug in core, and the dissolved drug in gel layer. A prolonged release matrix is characterized by a remarkably thick and viscous gel layer that exhibits slow erosion and a diffusion front as close as possible to the swelling front in order to increase the diffusion distance of drug through the gel.

The characteristics of the gel, namely thickness and viscosity, are the key element in drug release control of release and are function of physicochemical properties of the constituent polymers such as weight, type of substituents and their degree of substitution, chain conformation, hydrophilicity, amphiphilicity.

1.1 Kinetics of release

The release kinetics of drug from a swellable and erodible matrix has been described by Ritger and Peppas (1987)[4-7] through the following mathematical model (Eq. 1) :

$$\frac{M_t}{M_\infty} = kt^n \quad (\text{Eq. 1})$$

where M_t = drug released at time t , M_∞ = quantity of drug released at infinite time, k = drug release kinetic constant related to polymer and drug formulation, n = exponent that describes the mechanism of release.

The value of $n = 0.5$ represents Fickian diffusional (Case I) release, $0.5 < n < 1$ represents non-Fickian (Anomalous) release, $n = 1$ indicates Case II (Zero order) release and $n > 1$ indicates Super Case II release. Case II release refers to transport of drug solute via the erosion of polymeric matrix due to relaxation of polymer chains, whereas anomalous release refers to the summation of both drug diffusion and polymer erosion or swelling-controlled drug release. Super Case II release denotes drug dissolution which is controlled by polymer relaxation and is characterized by a sigmoidal release pattern.

1.2 Gastroretentive tablets

Gastroretentive tablets may be useful for the treatment of gastric diseases or for drugs characterized by dissolution and low bioavailability profile. The floating of tablets in the stomach can provide targeted drug dose, increase drug dissolution and absorption. This in turn is envisaged to to a reduction in the required doses[8-10]. In order to realize a gastroretentive tablet, the floating time and the time necessary to achieve the floating are both important. A tablet that floats immediately after reaching the stomach content can prevent its transition into the intestine.

The most common technique to manufacture a gastroretentive dosage form is the use of a low density material or a sponge-like structure (microballons) [11, 12].

1.3 Dome matrix technology

With the advances in pharmacogenomic and pharmacogenetic sciences, individualized therapy is met with challenges of having dosage form that can be mixed and matched to meet the intended drug regimen and pharmacokinetics requirements. In 2006, Colombo et al [13] devised an innovative modular technology platform where the single-unit dosage form design can be constructed through the assembly of the required drug matrix modules into one system for controlled drug release via the oral route.

The Dome Matrix module is constituted of a cylindrically shaped tablet, with one of the base concave and the other convex (Figure 2).

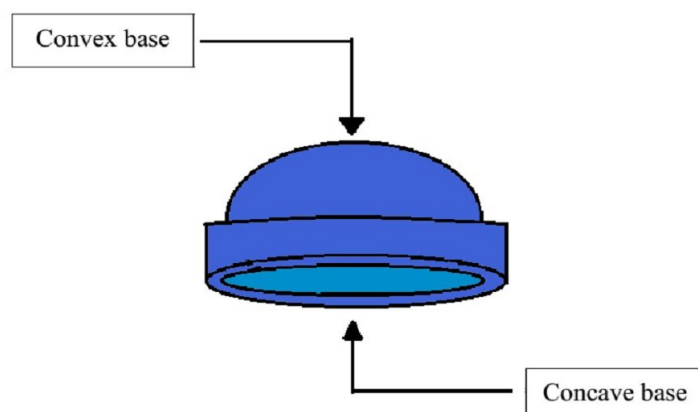


Figure 2. Structure of a Dome Matrix module

Principally, the individual modules are designed to allow the convex base of one

module to be inserted in the concave base of another. The axial section of each module appears as a dome, hence such assembly is named as Dome Matrix. The assembly of two adjacent modules is mediated via frictional interlocking. The basic modules differ in their concave base design[13].

The "male model" is characterized by an annular protrusion on the concave rim base (Figure 3a), whereas the "female model" exhibits a concave base that receives the annular protrusion of the male model (Figure 3b)[13].

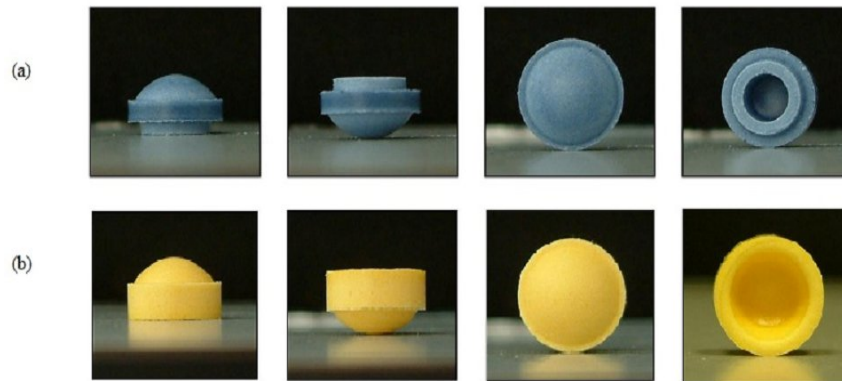


Figure 3. Dome Matrix (a) male and (b) female modules

Both male and female modules can be mixed and matched to produce

1. Piled configuration: Two or more modules are stacked with convex face into concave face (male or female stacked with female module) (Figure 4a).
2. Void configuration: Peculiar assembly through stacking the concave base of one module to the concave base of another module (Figure 4b). This configuration is characterized by an inner empty space that provides buoyancy to the entire assembly of the female with male module. Such construction denotes the potential of matrix acting as a floating drug delivery system and performing gastric-specific drug release and absorption (Figure 4c)[13].

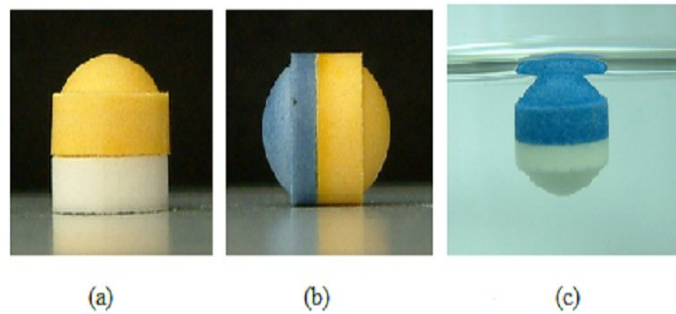


Figure 4. Dome Matrix modules with (a) piled and (b,c) void configuration

Dome matrix shows definite advantages as drug carrier when compared to traditional tablets. Modules of different drug types, doses and release kinetics can be combined in one single unit [14]. The intended therapeutic regimen and drug release kinetics can be tailored in accordance to the state of diseases and convenience of healthcare/patient management simply by changing the number and type of modules that constitute the system [15, 16].

Furthermore, the Dome matrix can be assembled as void configuration to act as a vehicle which releases drug primarily in the gastric region, in addition to piled configuration which is deemed to be able to release drug along the gastrointestinal tract in different dose fractions according to the modular assembly design [17, 18]. The void configuration design is critical for intestinal pH-labile drug, drug exhibiting a poor absorption profile at the intestinal tract, and need of having a direct therapeutic action on the gastric cavity when gastric ulcer, cancer or *Helicobacter pylori* infection are concerned[19].

2 Objective

Dome matrix technology is a module assembly technology for construction of delivery systems for time and space controlled release of drugs.

This technology allows to manufacture floating dosage forms, useful for drugs administration with a very narrow absorption window or not soluble at intestinal pH, or the manufacture of a multi-drugs and multi-kinetics dosage form.

The first part of this thesis will focus on the realization of a multi-kinetics assembled system containing esomeprazole and sucralfate for the delivery of active substances into the stomach for the treatment of gastric ulcer and gastro-esophageal reflux.

The second part will focus on the manufacture of a floating system for the controlled release of norfloxacin using alginate as the polymer of interest for controlled release in order to increase the bioavailability of the drug.

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Chapter 1

1 Introduction

1.1 Structure of the stomach

The stomach is the largest part of digestive tract located between the esophagus and small intestine on the left side of the upper abdomen. The stomach can expand to hold about 2 L of food. Depending on the quantity and contents of the meal, the stomach will digest the food into chyme between forty minutes and few hours.

As shown in Figure 1, it has a characteristic shape which consists of lesser and greater curvatures.

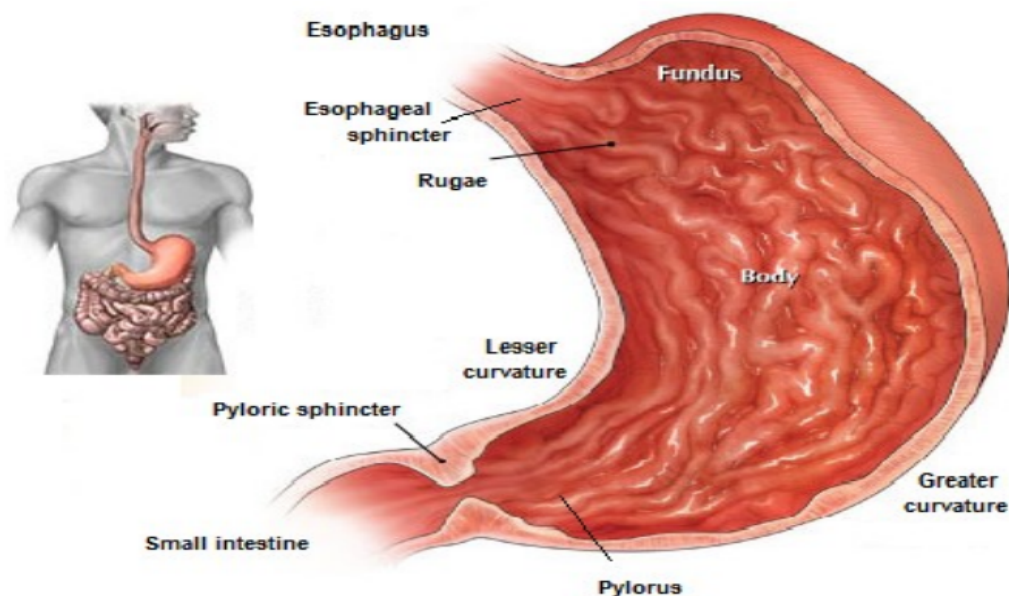


Figure1. Anatomy of The Stomach

Stomach contents are kept in the body of the organ thanks to of two sphincters the esophageal sphincter dividing the tract above, and the pyloric sphincter dividing the stomach from the small intestine. The esophageal sphincter is a collapsible muscular valve. It opens to let the food pass from the esophagus into the stomach as food reaches the end of the esophagus. Moreover, it closes to prevent stomach contents from travelling backward up the esophagus. As for the pyloric sphincter, it opens to allow liquefied food to pass from the stomach to the small intestine. The stomach muscles contract periodically, churning food to enhance digestion. Rugae lines are fold of muscles which aid the digestion of food.

The stomach is divided into four sections, each of which has different cells and functions. The sections are:

- The Cardia: it is the uppermost section of the stomach. As food passes through the esophagus, it reaches through the esophageal sphincter and empties into the cardia. The cardia secretes mainly mucus and HCO_3^- .
- The Fundus: it is located near the greater curvature. It is the part where stomach gases (produced by chemical digestion) accumulate.
- The Corpus: it is the largest and central portion of the stomach. Chief and parietal cells are located in this part. Parietal cells secrete HCl acid and also pepsinogen is produced by chief cells.
- The Pylorus: it is the lower section of the stomach and it contains G cells which secrete gastrin. The passage of gastric contents from the stomach into the duodenum is regulated by the pyloric sphincter.

The stomach wall consists of four main layers. Mucosa is the innermost layer and it releases into stomach acid and digestive juices. Second layer from inside to out is the sub-mucosa. Beside this layer, muscle layers are located. In fact, muscle layers consist of three different layers that moves and mixed the stomach contents. Serosa is the outermost layer of the stomach that wraps the stomach. This kind of structure is visible in Figure 2.

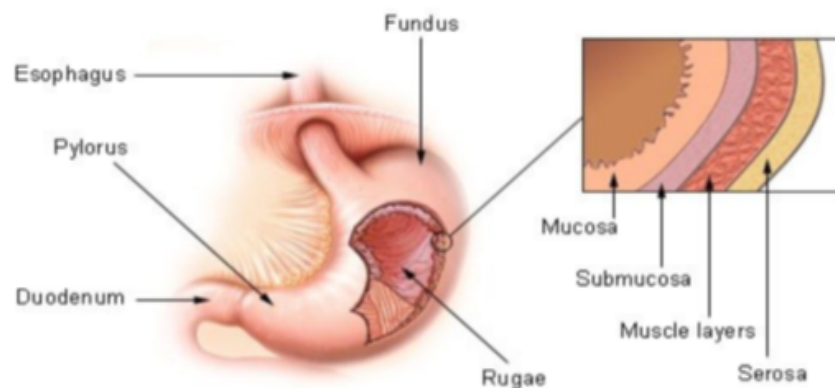


Figure 2. Structure of the stomach wall

The stomach receives chewed food from esophagus and it continues to digest it mechanically and chemically. The stomach has an acidic environment ranging from pH from 1 to 3 due to parietal cells in the wall of the stomach providing hydrochloric

acid (HCl). Hydrochloric acid kills bacteria or other potentially dangerous pathogens and it converts pepsinogen (which is released from chief cells in the stomach wall) into pepsin. On the other hand, mucus cells secrete mucus to protect the stomach from its own acid and enzymes. Gastric acid secretion breaks down food into simpler compounds for further digestion and subsequent absorption in the small intestine. Pepsin begins protein digestion by breaking it down into peptide chains which are made up of amino acids. Chief cells also secrete gastric lipase. Gastrin is the hormone responsible for mobilizing the whole process.[1-3]

The stomach can be the target of different disease:

- Dyspepsia: this most common disease which is known as stomach indigestion. Symptoms are regurgitation, bloating, nausea and vomiting.
- Gastric ulcer: an erosion in the lining of the stomach, often causing pain and/or bleeding. Gastric ulcers are most often caused by non-steroidal anti-inflammatory drugs (NSAIDs) or *Helicobacter pylori* infection.[1, 2, 4]
- Gastric varices: in people with severe liver disease, veins in the stomach may swell and bulge under increased pressure. Called varices, these veins are at high risk for bleeding, although less so than esophageal varices are.
- Gastritis: inflammation of the stomach, often causing nausea, vomiting, loss of appetite and/or abdominal pain. Gastritis can be caused by alcohol, certain medications, *H.pylori* infection or other factors.
- Gastroesophageal reflux: stomach contents, including acid, can travel backward up the esophagus. There may be no symptoms, or reflux may cause heartburn or coughing.
- Gastroesophageal reflux disease (GERD): this chronic disease is abnormal gastric reflux from stomach to esophagus might damage the esophagus. Symptoms are heartburn, difficult swallowing and regurgitation.[5]
- Gastroparesis: in gastroparesis, the stomach motility disappears and food remains stagnant in the stomach. The most common cause of gastroparesis is diabetes. Symptoms of gastroparesis includes abdominal pain, fullness, bloating, nausea, vomiting, loss of appetite.
- *Helicobacter pylori* infection: causes chronic low-level inflammation of the

stomach lining and is known as the leading cause of peptic ulcers, gastritis and stomach cancer.[6-8]

- Peptic ulcer: Peptic ulcer is a sore in the mucous membrane of the stomach although it develops more often in the duodenum.[9, 10]
- Stomach bleeding: Gastritis, ulcers, or gastric cancers may bleed. Seeing blood or black material in vomit or stool is usually a medical emergency.
- Stomach cancer: An uncommon type of cancer. Its symptoms usually include abdominal pain, weight loss, poor digestion, nausea, vomiting, difficult swallowing and black stools.
- Zollinger-Ellison syndrome (ZES): Zollinger-Ellison syndrome is caused by tumors. These tumors produce the hormone gastrin. High levels of gastrin cause production of high level of stomach acid.[1, 2]

1.2 Peptic ulcer

Peptic ulcer disease is an erosion of the lining of the stomach or duodenum caused by gastric acid and the enzyme pepsin. A peptic ulcer of the stomach is called a gastric ulcer while the ones in the duodenum, a duodenal ulcer.

Peptic ulcer develops as a result of an imbalance between irritating factors and the mucosal defence mechanisms that is most common in the first part of the duodenum or lower half of the stomach. When irritating factors become dominant in comparison with defence/protective factor in the stomach or duodenal lining such as increasing acid secretion or reducing mucus production, the mucous layer and mucous-secreting cells are eroded. It is defined as mucosal erosions equal to or greater than 0.5 cm.

Causes of gastric ulcers include the presence of the *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach, NSAIDs (nonsteroidal anti-inflammatory drugs) and cancer (malignancy). Alcohol and smoking are common irritating factors.

The causes of peptic ulcer are:

- *Helicobacter pylori* infection: Peptic ulcers most commonly occur between the ages of 30 and 55. This is a very common disease that produces a characteristic pain and changes of quality of life. *H. pylori* is very common and

it is found in more than 80% of patients with gastric and duodenal ulcers.[2, 6]

- The infection of H.pylori creates an abnormal levels of gastrin, responsible for regulating the amount of gastric acid. The mechanism of how H. pylori causes ulcers is not well explained.[6, 7]
- Non-steroidal anti-inflammatory drugs: NSAIDs such as aspirin, ibuprofen, naproxen and etodolac are medications for arthritis and other painful inflammatory conditions in the body. Prostaglandins preserve the stomach from corrosive acid damage. NSAIDs cause ulcers by repressing prostaglandins that has protective effects in the stomach.[4, 11, 12]
- The other causes: Beverages and foods that contain caffeine can stimulate acid secretion in the stomach. This can aggravate an existing ulcer. Moreover, physical stress such as burn can increase the risk of developing ulcers, especially in the stomach. Emotional stress might increase pain of existing ulcers. Although single cigarette smoking not causes ulcer formation, it increases the risk of ulcer complications such as ulcer bleeding, stomach obstruction and perforation. Also these radiation treatments, bacterial or viral infections and alcohol abuse can be one of the causes of ulcer.[1]

Symptoms of ulcer disease are variable and these vary from patient to patient. The most common symptom of a peptic ulcer is a burning pain in the abdomen between the breastbone and navel. When the stomach is empty after meals in 2 to 5 hours, duodenal ulcer causes symptoms. And it can be relieved by eating. On the other hand, gastric ulcer is made worse by eating. Symptoms are vomiting blood, difficulty swallowing, nausea, black stool, severe pain in abdominal area, weight loss, loss of appetite.[3]

Peptic ulcer can be treated using

- Medications Reducing Acid Secretion
 - H2 Receptor Antagonist: Inhibits gastric acid secretions depend on drug dosage
 - Parasympatholytic Agents

- Proton Pump Inhibitors
- Antacids: Neutralise the gastric acid
- Cytoprotective Agents: Create the sticky barrier with protein and fibrinogen on ulcer area
- Antibacterial Drugs which are combined drugs are used an antibiotic and a proton pump inhibitor

1.3 Esomeprazole

The acid is produced by parietal cells situated at the gastric mucosa. The gastric H^+K^+ ATPase is responsible for gastric acid secretion. H^+K^+ ATPase, located in the membrane between the cytosol and the secretory canal. Hydrogen ions are pumped from the cytosol of the parietal cell to the secretory canal in exchange for potassium ions by an enzyme. The acid is emptied into the lumen of the gland. Inhibition of this H^+K^+ ATPase pump system is the major therapeutic target in treatment of acid-related diseases. The proton pump inhibitors interact with the final step of gastric acid secretion. The proton pump inhibitors diffuse into the parietal cells. The proton pump inhibitors are weak bases. In the acid environment the proton pump inhibitors react to the sulphonamide active group, which covalently binds to sulfhydryl groups of the enzyme and inactivates it. The pharmacological characteristics of proton pump inhibitors are related to their protolytic behavior related to their pKa values.

Esomeprazole belongs to class of proton pump inhibitors (PPIs), the substituted benzimidazoles, known as one of the antiseecretory compounds. It is highly effective inhibitor of gastric acid secretion used in the therapy of peptic ulcer disease, gastroesophageal reflux disease and Zollinger-Ellison syndrome. Furthermore, Esomeprazole is used to decrease the development of ulcers in people who are taking nonsteroidal anti-inflammatory drugs (NSAIDs).

Esomeprazole is the S-enantiomer of Omeprazole which is a racemate. Both of them are proton pump inhibitors, with similar mechanisms of action, with similar drug interactions, and similar adverse effects. However, their difference is the greater bioavailability of Esomeprazole (90%) over Omeprazole (60%). Moreover, according to some studies, Esomeprazole has a better antimicrobial activity in eradication of

H.pylori than the Omeprazole. It might consider that Esomeprazole has higher efficacy than Omeprazole, in terms of stomach acid control.[5, 13-16]

The formulation of esomeprazole is C₃H₁₉N₃O₃S and its molecular mass is 345.417g/mol. The structure of esomeprazole is visible in Figure 3.

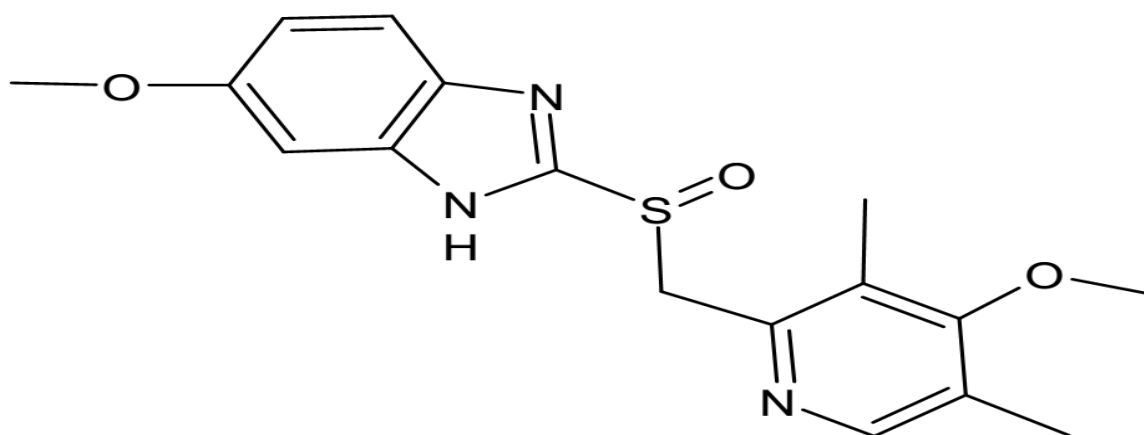


Figure 3. Structure of Esomeprazole

(S)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfonyl]-3H-benzimidazole

Esomeprazole is a weight or slightly coloured and slightly hygroscopic powder that melts with decomposition at 155 °C. Esomeprazole sodium is freely soluble in ethanol (95%) and slightly soluble in water. As the stability of Esomeprazole, although it is rapidly degraded in acid media, it is durable in alkali media owing to its weak basicity. It should be stored in light resistant and airtight container at 2-8° C.

Esomeprazole acts on the hydrogen/potassium adenosine triphosphatase enzyme in the gastric parietal cell, binding to it irreversibly. It suppresses gastric acid secretion by specific inhibition of the H⁺-K⁺ ATPase. By acting specifically on the proton pump, Esomeprazole blocks the final step in acid production, thus causing profound inhibition of acid secretion. In terms of this final step blockade, drugs in this class are significantly more effective than H₂ antagonists and reduce gastric acid secretion by up to 99%.

In the stomach the lack of the acid called hypochlorhydria will aid in the healing of duodenal ulcers, and reduces the pain originating from indigestion. Hydrochloric acid is required for absorption of nutrients, particularly calcium. The proton pump inhibitors acts as a pro-drug thus therefore they are given in an inactive form. The inactive form is neutrally charged (lipophilic and readily crosses cell membranes into intracellular compartments (like the parietal cell canaliculus) that have acidic environments. This inactive form be activated by exposure to acidic pH (< 5). In acidic pH, the inactive drug is protonated and rearranges into its active form. As described before, the activated species irreversibly binds to the H⁺-K⁺ -ATPase enzyme (proton pump) in the parietal cell apical membrane inhibiting its activity. New enzyme has to be synthesized to overcome the inhibition. It effects on gastric acid volume and without effecting gastric motility. Effective in decreasing gastric acidity is more than 95%). This effect is dose-related up to a daily dose of 20 to 40 mg and leads to inhibition of gastric acid secretion.

Single 20-40 mg oral doses of esomeprazole generally achieve peak plasma concentrations of 0.5-1.0 mg/L within 1-4 hours. However after several days of once-daily administration, these levels may increase by about 50%. In addition to this, a 30 minute intravenous infusion of a similar dose usually produces peak plasma levels on the order of 1-3 mg/L. Esomeprazole, like the other proton pump inhibitors, is exposed to low rates of hepatic first pass effect. Esomeprazole is usually administered as a gastro-protected coated dosage forms i.e., tablets or pellets. Upon leaving the stomach, the pellets and tablets are rapidly absorbed rate of 90 % once the preparation enters the small intestine. The absorption of enantiomeric pure esomeprazole is higher than the racemic omeprazole resulting in an higher bioavailability.

After oral administration peak plasma levels (C_{max}) occur at approximately 1.5 hours (T_{max}). This property is another advantage in comparison with Omeprazole which reaches peak plasma levels within 4 hours. The C_{max} increases proportionally when the dose is increased, and there is a three-fold increase in the area under the plasma concentration-time curve (AUC) from 20 to 40 mg. At repeated once- daily dosing with 40 mg, the systemic bioavailability is approximately 90% compared to 64% after

a single dose of 40 mg.

Although, protein binding of all the other proton pump inhibitors are 95%, Esomeprazole is 97% bound to plasma proteins. Plasma protein binding is constant over the concentration range of 2-20 mmol/L. The volume of distribution at steady state in healthy volunteers is approximately 16 L.

The drug is rapidly cleared from the body by way of CYP2C19, largely by urinary excretion of pharmacologically-inactive metabolites such as 5-hydroxymethyleesomeprazole and 5-carboxyesomeprazole.

Esomeprazole, which has elimination half-life of 1.5 hours or less, undergoes elimination in urine and feces. Thus approximately 80% of the administered dose of esomeprazole is excreted as metabolites in urine and the remaining 20% is excreted in feces.[5, 11, 12, 14, 15, 17]

1.4 Sucralfate

Sucralfate is a citoprotective agent and it is used for the treatment of active gastro-duodenal ulcers, gastro-esophageal reflux disease and also stress ulcers.

It is a basic aluminum salt of sulfated sucrose. Sucralfate is a white amorphous solid powder insoluble in cold water, practically insoluble in ethanol, chloroform and soluble in dilute hydrochloric acid and sodium hydroxide solutions. It has to be stored in tightly closed in container a cool, well-ventilated area.[18]

Its formulation is $C_{12}H_{54}Al_{16}O_{75}S_8 \cdot XAl(OH)_3 \cdot yH_2O$ its molecular mass is 2087.75 g/mol, its structure is visible in Figure 4.

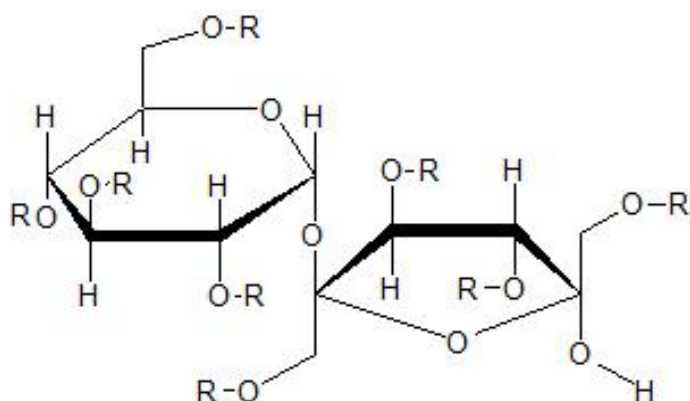


Figure 4. Structure of Sucralfate

Hexadeca-ju-hydroxytetracosahydroxy[ju8-[1,3,4,6-tetra-O-sulfo-PDfructofuranosyl]-a-D-glucopyranosidetetrakis(hydrogensulfato)8-)]hexadecaaluminum

Sucralfate is the hydrous basic aluminium salt of sucrose octasulfate with 8 hydroxi groups that are involved in various extent of dimerixation.

Sucralfate paste is the wet precipitated immediately after production contains about 70% w/w of water on the basis of sucralfate. The water content of sucralfate depends on separation and storage conditions, and it is decreased by drying to reach a minimum value of approximately 5% of dried powder. Therefore, an equilibrium between the water content of sucralfate and the moisture content (humidity) exists in the production/storage environments. Heating to over 200°C the molecule can break the reversibility where the water contents cannot return to their previous values because of the thermal dehydration of aluminum hydroxide moiety, that lead to the conversion from hydroxy cross-linking $[Al-(OH)_2-Al]$ to the oxo crosslinking $[Al-O-Al]$.

Sucralfate is used for the treatment of peptic ulcer disease and to prevent recurrent ulcers after healing of the ulcer. It can also be used to relieve or prevent the ulcers caused by nonsteroidal anti-inflammatory drugs (NSAIDs) although it will be very less effective. The use of sucralfate rather than H₂ antagonists is more effective for stress ulcer prophylaxis.[16, 18, 19]

Sucralfate is a unique oral drug. Unlike the other classes of medications used for treatment of peptic ulcers, sucralfate is a sucrose sulfate-aluminium complex that binds to the mucosa, thus creating a physical barrier that impairs diffusion of hydrochloric acid in the gastrointestinal tract and prevents degradation of mucus by acid.

The mechanism of its action is known as antipepsin and antacid effects. Sucralfate is a locally acting substance that in an acidic environment (pH < 4) reacts with hydrochloric acid in the stomach. Two principal effects of sucralfate are to be forming a highly adhesive gel (cross-linking, viscous, sticky) and in binding with plasma proteins (such as albumin and fibrinogen). These effects serve as protective barriers at the ulcer surface, preventing further damage from acid and pepsin. In addition to these effects, it prevents back diffusion of hydrogen ions. Moreover, according to some recent studies, sucralfate also stimulates the increase of prostaglandin, bicarbonate and gastric mucus (protective agents for gastric mucosa).

Sucralfate has different mechanisms:

- sucralfate binds to the surface of ulcers (attaching to exposed proteins) and coats the ulcer, thus protecting the ulcer surface to some extent from further injury by acid and pepsin;
- sucralfate directly inhibits pepsin (an enzyme that breaks apart proteins) in the presence of stomach acid;
- sucralfate binds bile salts coming from the liver via the bile thus protecting the stomach lining from injury caused by the bile acids;
- sucralfate may increase prostaglandin production, and prostaglandins are known to protect the lining of the stomach. Sucralfate was approved by the FDA in 1981.

The local bioavailability of sucralfate is approximately 3-5%, it has minimally absorption from the gastrointestinal tract. Approximately 95% of a dose remains in the GI tract, with only minute amounts being distributed into liver, kidneys, skeletal muscle, adipose tissue and skin. Excretion of sucralfate is by urine and feces.

The sucralfate used in this research is a special form of the drug named sucralfate gel. It consists in the wet precipitate of sucralfate that possesses rheological and bioadhesion properties similar to the paste obtained by acid interaction. These characteristics are lost in case of drying in normal conditions.[16, 18-21]

2 Objective

The purpose of the work was the production of a double pulse release of esomeprazole in combination with sucralfate by using the Dome Matrix assembling technology

The delivery system should release the first pulse of esomeprazole in the stomach and the second, after 4 to 6 hours, in the upper part of the intestine. We decided not to gastro-protect the first pulse of esomeprazole since the module formulation was designed with alkalinizing agents protecting the drug from the acid environment. The delivery into the stomach of the esomeprazole, despite a partial degradation of the molecule, can drive to a prompt and sufficient amount of drug to be directly absorbed into mucosa where the substance has to act. In addition, the second pulse of esomeprazole is delayed being the drug protected by an impermeable coating during its permanence into the stomach.

For the delivery purpose, this Dome Matrix assembled system consist of five modules (4 female modules and 1 male module), assembled and partially coated. Its structure is depicted in Figure 5.

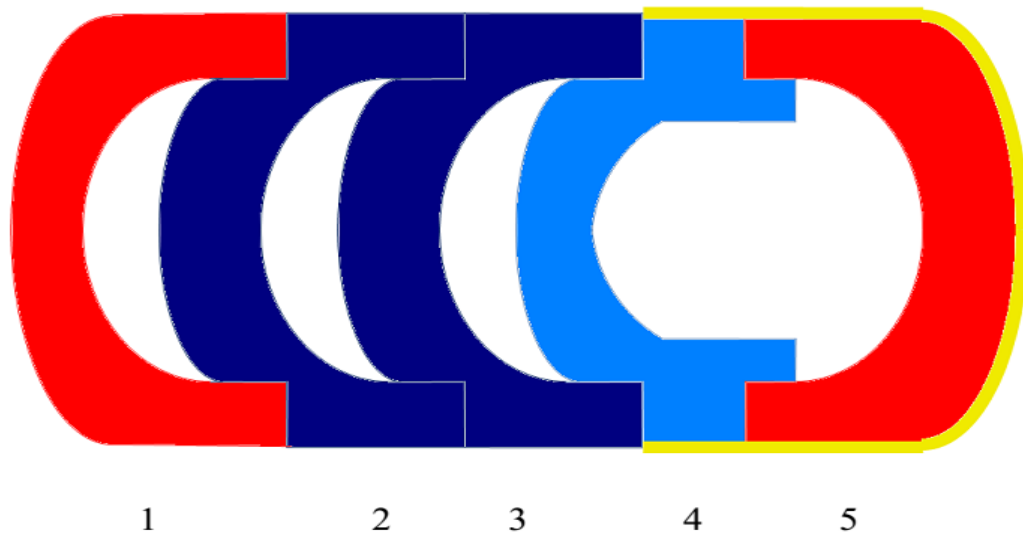


Figure 5. Structure of the assembled system

The two red modules (1 and 5) are esomeprazole immediate release modules while the blue ones (2 and 3) are sucralfate immediate release modules.

The light blue module (4) is a male module for controlled release of sucralfate which, together with an impermeable coating on the esomeprazole module, allows the floating of this assembled system portion controlling the time for the second pulse of esomeprazole.

The coated part of the assembled system should float in the stomach for 2-3 hours and then, it should allow the release of the second pulse after the slow disintegration of the male sucralfate prolonged release module.

An alternative to achieve the delayed release of the second esomeprazole pulse is the production of a partial enteric coated assembled system which should release the second pulse after entering in the intestine thanks to the dissolution of the coating covering the second esomeprazole module.

3 Materials and methods

3.1 Materials

- AcDiSOL type sd-711; FMC; Batch: T0646C
- Avicel PH102; ACEF; Batch: H2692003
- Carbomer 940; BASF; Batch: 42367236
- Cellulosa acetate propionate; ACEF; Batch: H1818001
- Citric acid; Carlo Erba; 403725
- Corn starch; ACEF; Batch: G4283010
- Emcompress; USP/Ph Eur; JRS Pharma; Batch: F17G
- Esomeprazole magnesium dihydrate; Hetero Drugs; Batch: EO0010110
- Ethyl cellulose 22; Lisapharma;
- Eudragit L100-55; Rohm-Pharma; 13-80025
- Eudragit S100; Rohm-Pharma; 05-80028
- HPMC-K15M; Estman Kodek; Batch: 10801
- Kollidon VA 64; BASF; Batch: 55105156P0
- L-HPC; SEPPIC; Batch: 402091
- Magnesium oxide (MgO heavy); ACEF; Batch: H0967001
- Magnesium oxide (MgO light); Giusto Faravelli (Milano), batch 1019131,
- Magnesium stearate; ACEF; Batch: C1402005
- Polyethylene glycol 6000; Fluka; 1308600 43806P01
- 2-Propanol; Merck; Batch: D955007
- Polyox N60K; Estman Kodek; 10801 / Colorcon; DT260727
- Silicon oil; Dow Corning, Batch: XZ050145
- Sodium bicarbonate (NaHCO₃); Riedel de Haen; Batch: 62340
- Sodium carbonate (Na₂CO₃); Riedel de Haen; Batch: 62340
- Sucralfate gel; BK Giulini; Batch: 10-216-30
- Talc; ACEF; Batch: C5239004
- TiO₂; Ph Eur-USP; ACEF; Batch: G5468001
- Triacetin; Eastman; Batch: 850101

- Triethylcitrate; Fluka; Batch: 406296/1-41700

All the solvents were of analytical grade.

3.2 Methods

3.2.1 Preparation of sucralfate granules

The humid sucralfate gel was manually crashed in small pieces and was left in the oven for 2 hours at the temperature of 60 °C. It was then transferred in an oscillating granulator (ERWEKA AR400, Germany) equipped with a 1.8mm opening net.

The granules were dried in oven at 80° C for 1 hour and they were re-granulated using a net with opening of 0.8mm.

Sucralfate granules were finally dried in a Glatt (GmbH, Process technology, Germany) at the temperature of 40° C and pressure of 0.6 bar for 25-30 minutes.

The water content in the sucralfate granules was measured putting the powder under the IR lamp for 1 hour. The amount of water should be between 8% and 12%.

3.2.2 Dry granulation of esomeprazole

Esomeprazole was mixed with the other excipients in a Turbula (WAB, Basilea, CH) for about 20 min.

Powders were compressed using an alternating tableting machine (EK, Korsh, Berlin) equipped with 20 mm punches. Slabs were manually broken in a mortar and they were screened through a net with 1.3 mm openings.

Granules were screened a second time using a net with 0.8mm openings.

3.2.3 Manufacture of tablets

- Dome Matrix modules

Dome Matrix modules were obtained using an alternating tableting machine (KORSCH, Mod. 9341-72, Berlin) equipped with special punches.

The mixture was compressed using Dome Matrix punches 8.5 mm (series 3, Costamac, PsF3, PiT3, PiC3, MN3).

- 8mm cylindrical tablets

Cylindrical tablets (diameter 8mm) were obtained using an alternating tableting machine (KORSCH, Mod. 9341-72, Berlin) equipped with flat punches. The tableting machine was lubricated externally using a mixture of Microcristalline/Talc/Magnesium stearate in a 96:3:1 ratio.

- 2mm cylindrical tablets

Tablets were manufactured using concave punches (diameter 2 mm, concavity range 0.28 mm). The tableting machine was lubricated externally using a mixture of Microcristalline/Talc/Magnesium stearate in a 96:3:1 ratio.

3.2.4 Mechanical characteristics of the tablets

3.2.4.1 Measurement of the hardness of the tablets

The measurement of hardness was carried out according to the procedure in Ph. Eur. 7 ed. Twelve tablets per batch were tested using the Monsanto apparatus.



Figure 6. Monsanto device

The device consists of two opposed clamps, one of which moves to the other. The mobile support is connected to a spring on which it is possible to exert pressure through a rotating screw. The flat surfaces of the clamps are perpendicular to the direction of movement. The module is firmly fixed to the surface of clamps which has to be flat and wider than the area of contact with the module. The modules were placed in two directions as it is shown in Figure 7. The number of the modules per batch which were tested was twelve (six in one position and six in the other). The results, according to Pharmacopoeia, were expressed as mean of minimum and maximum forces measured in kg.

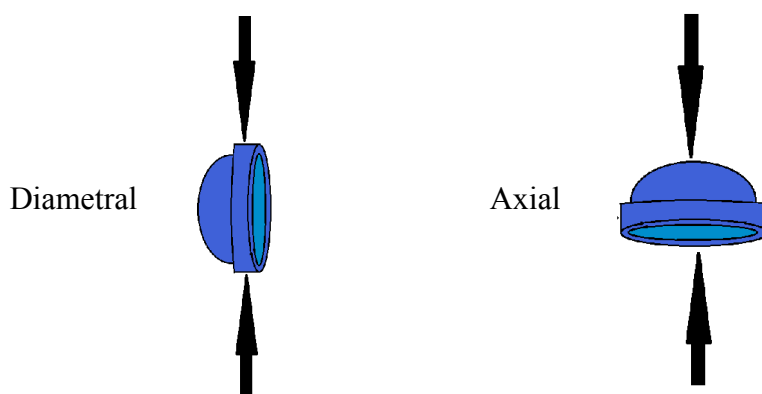


Figure 7. Positions of tablets tested with Monsanto device

3.2.4.2 Friability test

Test was done according to European Pharmacopoeia.

10 tablets were carefully dusted and weighted on an analytical balance.

Tablets were then placed in the drum of the friability test apparatus (ERWEKA, TA3 P) and the drum was rotated 100 times (25 rotation in one minute).

Tablets were dusted again before weighting.

Friability (%) is calculated according to Eq.1.

$$Friability(\%) = \frac{W_1 - W_2}{W_1} * 100 \quad \text{Eq.1}$$

Where W1 is the weight of tablets before testing and W2 is the weight of tablets after testing.

The maximum acceptable weight loss is 1%.

3.2.4.3 Disintegration time

Test was done according to European Pharmacopoeia.

The device is composed of 6 glass cylinders with an openwork bottom and a movable plastic cap. The system is repeatedly dipped in simulated gastric fluid at 37°C and disgregation time is assessed visually.

Maximum time for the test is 15 minutes.

The result is the average value of 6 tablets.

3.2.5 Coating techniques

- Coating of Dome Matrix assembled systems

- Osmotic coating

All the dry components of the solution were weighted on an analytical balance and poured in to a flask. Solvents were then added slowly under stirring.

The solution was left under stirring overnight prior use.

- Enteric coating

All the dry components, beside the polymer, were mixed together in a flask using half of the total volume of the mixture of solvents. The solution was stirred until a dispersion of insoluble components was achieved and the dispersion was homogenized using a Ultraturrex® for 5 minutes.

The polymer was added continuously to the rest of the solvent under stirring and stirred overnight.

The two components were mixed two hours prior the use and kept under stirring during the whole process.

- Coating procedure

An assembled system was dipped in the coating solution in order to coat the convex base and the lateral face of the esomeprazole module. The process can be separated into three steps (Figure 8):

- a) The assembled system was dipped in a crystallizer containing the coating solution.
- b) The assembled system, which was dipped in the coating solution, was rotated in order to facilitate the deposition of polymeric film on the surface of the esomeprazole module.
- c) The assembled system was extracted from the coating solution under continuous rotation, in order to make a thin proper dried film layer, and afterwards was reversed.

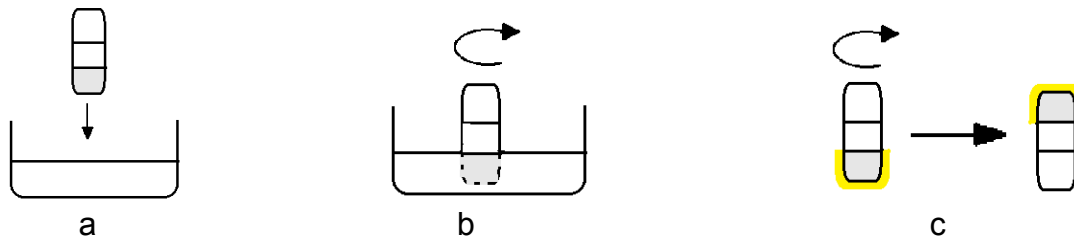


Figure 8. Steps of the coating process.

The procedure was repeated 2/3 times depending on the mechanical characteristics of the dry coating.

The development of the process was determined using the weight increase of the assembled system after drying according to Eq. 2.

$$\text{Weight increase (\%)} = \frac{W_{aft} - W_{bef}}{W_{bef}} * 100 \quad \text{Eq.2}$$

Where W_{bef} was the weight before the coating and W_{aft} the weight after the coating and the drying process.

- Coating of 2mm tablets

Cylindrical (diameter of 2mm) tablets were coated in a coating pan (Erweka AR400) equipped with a 40 cm pan.

Parameters of the process were:

- rotating speed: 24 rpm
- solution flow: 0.8 ml/min
- cool air drying (counter-current).

The end point of the process, expressed as coating weight, was determined according to Eq. 3.

$$\text{Coating weight (\%)} = \frac{A(\text{mm}^2) * L\left(\frac{\text{mg}}{\text{cm}^2}\right)}{W(\text{mg})} \quad \text{Eq.3}$$

Where A is the surface area of the average tablet, W is the weight of the average tablet and L is parameter that depends on the shape of the tablets (ranging from 4 to 6 for cylindrical tablets).

Surface area was calculated using two different methods:

- Tablet was considered as a cylinder which height corresponds to the total height of the tablet;

- 3D model of the tablet realized using SolidEdge software.

The value of coating weight was calculated using the cylindrical model as upper limit, while the real model as lower limit.

Robustness of the coating was assessed visually using 6 tablet in HCl 0.1N (37° C) under stirring for 2 hours.

3.2.6 Esomeprazole HPLC analytical method

The samples are quantified using HPCL (Shimatzu with Waters autosampler) equipped with a UV/vis detector.

The stationary phase was a Supelcosil LC8 column (150 mm; 4.6x5 µm) at 25o C.; the mobile phase was a mixture (7:3 v/v) of potassium dihydrogen phosphate buffer at pH=3 and acetonitrile.

The flow rate was 1 ml/min and sample size was 20 µm.

The wavelength was 280nm.

3.2.7 Esomeprazole degradation study

A weighted amount of esomeprazole was inserted in a darkened volumetric flask and dissolved using a small amount of methanol (2 ml).

The volumetric flask was topped using simulated gastric fluid (37° C) and it was put in an heating bath under continuous stirring.

Samples were collected a different time and analysed via HPLC (described in 3.2.6) without filtration.

Results are expressed as average values of 3 samples.

3.2.8 Drug content analysis of esomeprazole tablets

Ten tablets were crushed in a mortar and 3 different samples of powder were collected. These samples were put in a 200 ml volumetric flask with borate buffer (pH 11) and they were sonicated in an ultrasonic bath for 10 minutes.

Standard solution was prepared in the same way, using borate buffer and a 200 ml volumetric flask. The samples were filtered using cellulose acetate filter (cut-off 0.45µm) and were analysed via HPLC.

Coated tablets were assayed using the same method.

3.2.9 In vitro dissolution test of esomeprazole modules and tablets

- In vitro dissolution test of esomeprazole modules

Dissolution tests were carried out using flow-through apparatus (Sotax CH-4008, Basel, Switzerland) covered by aluminium foil to prevent degradation of esomeprazole because of the light.

A small volume (6.5 ml) cell and simulated gastric fluid at 37° C (flow of 10ml/min) were used to dissolve the tablet. Borate buffer (pH 11) was used to stabilize the sample in a 1:4 ratio, so that the final pH of the solution is close to 10.

Borate buffer (pH 11) was prepared using 9.5 g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ and 1.25 g of EDTA in 2 L of degassed water.

The flowing samples via the device were collected directly into 50ml vials (every of them already containing 30 ml of borate buffer) in a 1 minute interval. After the sample collection step, the vials are filled with borate buffer until the limit of 50 ml.

Every test lasted for 20 minutes and every batch was assayed in triplicate. The first 5 minutes were analysed one by one, while samples from the fifth minute were put together every five minutes (5-10 minutes , 11-15 minutes , 16-20 minutes).

The samples were analysed via HPLC.

The release of the coated tablets was assayed in the same way using intestinal fluid.

The test lasted 30 minutes without stabilization of the sample using borate buffer.

- In vitro dissolution test of 2mm cylindrical tablets

Dissolution tests of 2mm tablets were carried out using the same procedure used for esomeprazole modules described in section 3.2.9.1, but the flow was set at 4ml/min (and the amount of borate was changed accordingly, keeping the ratio constant) to prevent excessive degradation.

3.2.10 Floating of assembled systems

Floating of assembled system was assessed visually using a Type II dissolution test apparatus using 500 ml of simulated gastric fluid (37° C, 50 rpm).

An assembled system was inserted into the vessel and the floating time was assessed visually.

Three samples of every kind of assembled system were examined and the results are the average value of floating times.

3.2.11 In vitro dissolution test of esomeprazole-sucralfate assembled system

Release of esomeprazole was analysed using flow-through apparatus (Sotax CH-4008, Basel, Switzerland).

A small volume (6.5 ml) cell and simulated gastric fluid at 37° C (flow of 10ml/min) were used to dissolve the tablet. High concentration borate buffer (pH 11) was used to stabilize the sample in a 1:1 ratio, so that the final pH of the solution is close to 10. High concentration borate buffer (pH 11) was prepared using 9.5 g of Na₂B₄O₇·10H₂O and 1.25 g of EDTA in 0.5 L of degassed water.

The flowing samples via the device were collected directly into a suitable container together with the flowing buffer. Every time interval was collected in a different container and sample were retrieved, filtered through a cellulose acetate filter (0.45 µm) and analysed via HPLC.

After 4 hours, the simulate gastric fluid was replaced with simulated intestinal fluid and the borate flow was stopped.

Samples were collected, filtered and analysed via HPLC.

3.2.12 Permeation studies

Esomeprazole permeation through a porcine stomach was assessed using vertical Franz type diffusion cells with 2.83 cm² of diffusion area.

Immediate release esomeprazole tablets (8mm flat and 2mm) were used to mimic the release from Dome Matrix esomeprazole module.

Experiments were performed using both simulated gastric fluid and acetate buffer pH 3.5 in order to simulate a non-fasted stomach and to investigate the effect of pH on esomeprazole permeation.

- Cleaning of stomachs

1. Stomachs, belonging to different animals, were washed in water and phosphate buffer saline (PBS).
2. They were cut lengthwise and the the stomach near cardias and pylorus, which has a corrugated surface, was discarded.
3. Muscular layer and mucous layer were separated using a scalpel.
4. Mucous layer was dipped in PBS at 60-65° C for 1 minute .
5. Mucous layer was manually separated from the submucous layer.

6. Mucous layer was cut in 4x4 cm squares and washed with deionized water.
7. Tissues was put in PBS wetted paper, covered in aluminium foil and freezed at -20° C.

- Preparation of stomach

1. Stomachs were defreezed in PBS for 30 minutes.
2. Stomachs, once defreezed, were cut as round disk (diameter of 32mm) and its thickness was measured using a caliper (Mitutoyo, Japan).
3. The stomach was set on a Franz Cell and the donor compartment was filled with PBS to check for leaking through the tissue. Stomachs, from 3 different animals, were used every time together with a fourth one as blank.
4. PBS was then discarded and a cylindrical magnetic stirrer (12.66x3.05) was put in the receiver compartment.
5. Receiver compartment was filled with a precise amount of PBS, while the donor compartment was loaded with simulated gastric fluid and covered with parafilm to prevent evaporation.
6. The system was left in a water bath at 37° C before the test.

The cutting and the final cleaning are visible in Figure 9.



Figure 9. Cleaning of the stomach

- Permeation test

Permeation test were performed at 37° C under stirring (120rpm).

1. Sample was inserted in the donor compartment and parafilm cover was replaced. The whole system was covered in aluminium foil to prevent photodegradation of esomeprazole.
2. Sample (0.5 ml) were collected at 15, 30, 45, 60, 90 and 120 minutes from receiver compartment using a insulin syringe and the volume was replace with fresh PBS.
3. The content of the donor compartment was recovered and stabilised in borate buffer.
4. The stomach was cut in small pieces and dipped in methanol (20ml) for 2 hours in order to recover the esomeprazole trapped inside the tissue.

All experiments were replicated at least three times; results are expressed as the mean \pm standard deviation.

A typical setting for permeation test is visible in Figure 10.



Figure 10. Setting for permeation test

3.2.13 Pharmacokinetics studies of immediate release esomeprazole tablets

Immediate release esomeprazole tablets (both 8mm and 2mm) were employed to perform those experiments.

- In-vivo test settings and blood sampling

Nineteen Male Withstar rats with individual body weight of 405.5 ± 48.6 g (Charles River laboratories, France) were used in this study.

They were kept in an approved area for lab animals (Approval from Parma municipality) on 27/04/1993) under standard environmental condition at an ambient temperature of 25° C and a relative humidity of 65% on a 12 hours light/dark inverted cycle. The rats were given free access to standard pelletized food and deionized water ad libitum.

These rats were subjected to 18 hours of fasting prior to experiment. All experiments were conducted in accordance to the institutional ethics regulations on the conduct of animal experimentation (DL 116/92 and european directive 2010/63).

- Administration of tablets

Tablets (esomeprazole dose: 5mg/kg) were administered orally using a gavage needle loaded with 2 ml of deionized water.

- Blood sampling

Blood sampling were done before administering the tablets (blank) and, after administration, according a different timetable for each formulation:

- Esomeprazole immediate release tablets: 15, 30, 45, 60, 90 and 120 minutes.
- Coated esomeprazole tablets: 30, 60, 90, 120, 150 and 240 minutes.

Blood (0.5 ml) were collected using sublingual veins.

The rat was anaesthetised using diethyl-ether for 2 minutes and it was set in prone position. Its tongue was extended using a Q-tip and one of the sub lingual vein was punctured using a 23G needle. The procedure is depicted in Figure 11.



Figure 11. Blood sampling from the tongue of the rat

The animal was then reversed in supine position, while its neck was gently squeezed to ensure congestion and therefore increasing the blood flow.

The animal, with the tongue sticking out, was put in an inclined position over an EDTA loaded eppendorf vial in order to let the blood drip in to the vials.

Once the proper volume (0.5 ml) was collected, the rat was eased down on the table, the grip on the neck was released to reduce blood flow and the wound was blotted with gauze to stop the bleeding.

The volume of blood collected was replaced with 0.5 ml of warm physiological solution under skin (neck).

- Sample treatment

The blood collected was centrifuged at 5000 rpm for 30 min at 25° C, plasma was separated and collected in an eppendorf vial. Samples were then freezeed at -20° C until testing.

The frozen plasma was thawed at room temperature and 0.5 ml of methanol were added; the sample was then vortexed for 20 minutes to ensure proteins removal and centrifuged at 5000 rpm for 30 min.

The supernatant was subjected to HPLC-MS/MS analysis.

- Analytical method

Esomeprazole in plasma was analysed using HPLC-MS/MS.

Sample preparation

100 µl of plasma were put in a test tube together with:

- 100 µl of Na₂CO₃ solution at pH 9 (1 M);
- 100 µl of methanol;
- 100 µl of NaH₂PO₄ (1 M);
- 500 µl of acetonitrile;
- 4.5 µl of CH₂Cl₂.

Test tubes, covered using parafilm, were vortexed for 2 minutes and then centrifuged at 2200 rpm for 5 minutes. Solvents were then dried out under vacuum and the residue recovered using 100 µl of methanol.

Esomeprazole HPLC-MS/MS analytical method

The samples are quantified using HPLC-MS/MS. (Series 1200, Agilent) equipped

with a QTRAP 4000 (Absciex) detector.

The stationary phase was a Sinergy Fusion C18 column (50x2.1mm, 4 μ m). The mobile phase was a mixture of (A) and (B) at a ratio of 8:2, where

- (A) is a water solution of 1 ml of formic acid and 100 ml of ammonium acetate for a total volume of 1l and pH=3.8.
- (B) is acetonitrile

Flow was set at 0.2 ml/min with a sample size of 5 μ l.

The settings for the equipment were:

- Capillary voltage: 5.5 kV;
- Cone voltage: 50 V ;
- Declustering potential: 50 V
- Extractor potential: 10 V ;
- Source temperature: 450° C.

Spectra were recorded in a 150-300 m/z range using 0.1 step size and a 2 seconds scan time.

4 Results and discussion

4.1 Esomeprazole stability in simulated gastric fluid and intestinal fluid

Esomeprazole stability was assayed in simulated gastric fluid at 37°C.

Esomeprazole was inserted in a volumetric flask containing simulated gastric fluid and placed in a water bath

The results, reported in Figure 12, showed a marked and fast degradation of the drug in the simulated gastric fluid during dissolution test.

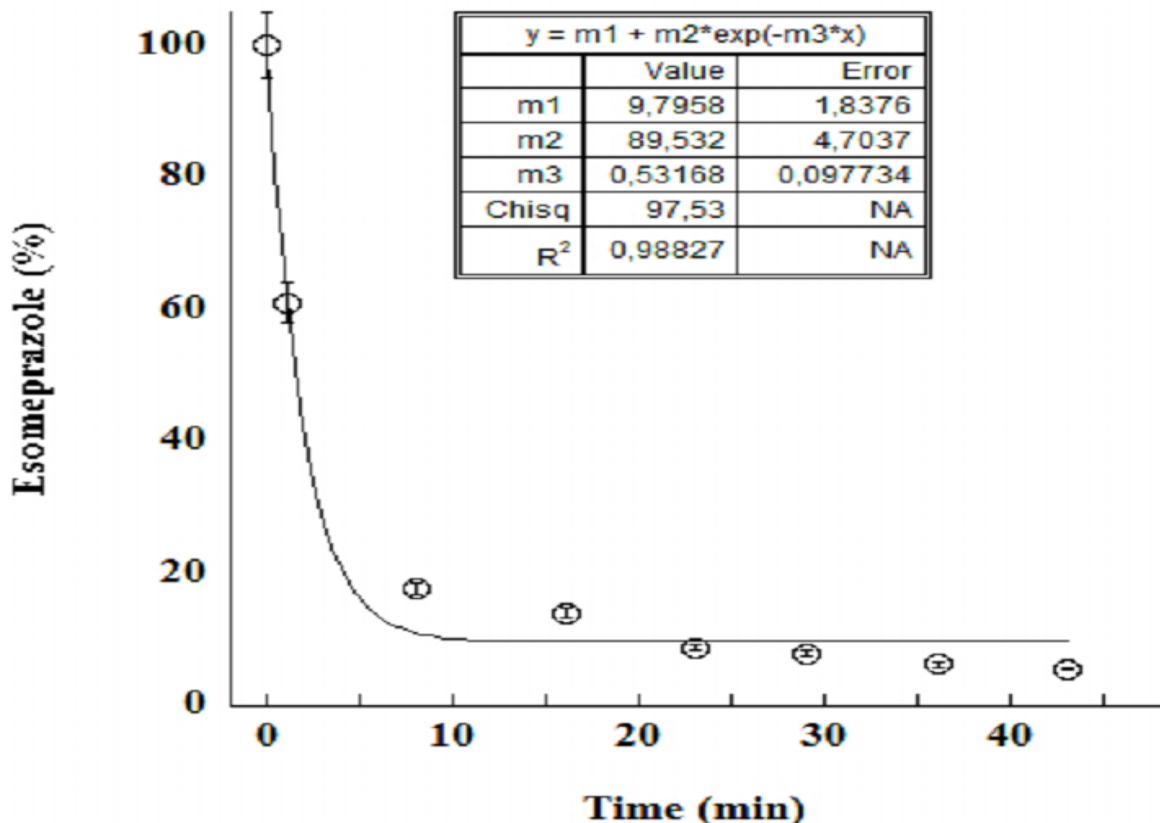


Figure 12. Degradation of esomeprazole in simulated gastric fluid

Stability was also assayed in intestinal fluid using the same method adopted for simulated intestinal fluid over a 2 hours period (Figure 13).

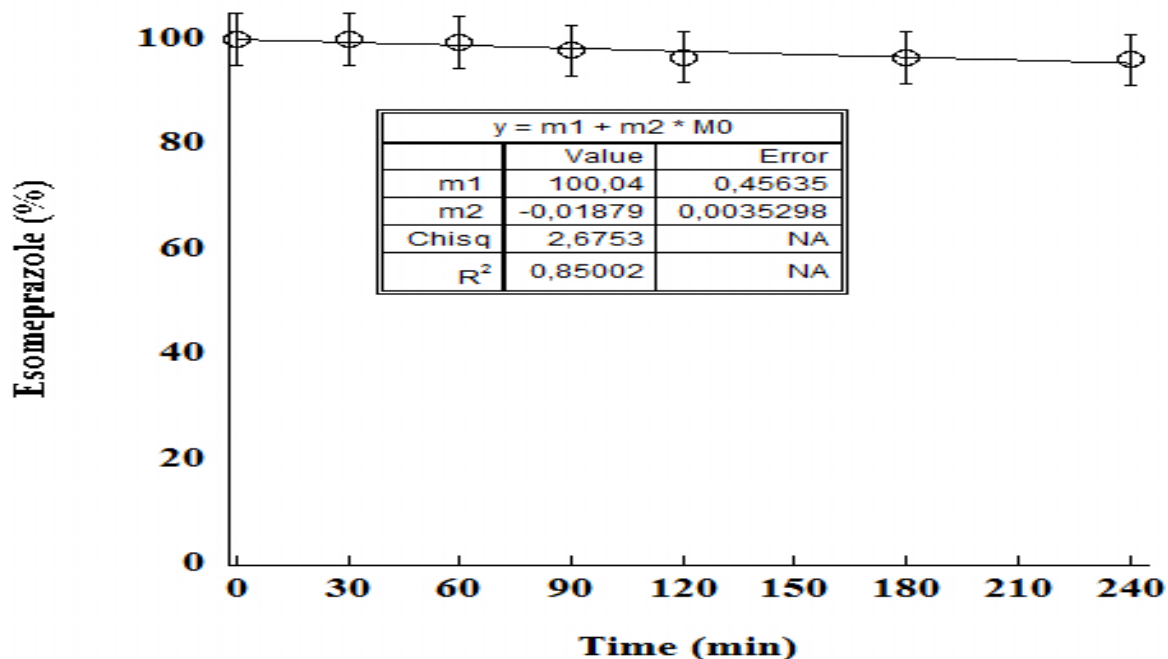


Figure 13. Degradation of esomeprazole in simulated intestinal fluid

4.2 Sucralfate immediate release module

Modules were manufactured in order to contain the maximum amount possible of sucralfate and present appropriate mechanical and release properties.

Sucralfate gel was granulated according to the procedure described in 3.2.1 to manufacture granules having an average content of water between 8 and 12%.

Granules were mixed with magnesium stearate (Turbula mixer, 10 minutes) to improve the flowability, which was a drawback observed during preliminary test. Lubricated sucralfate granules were then mixed with other excipients in the same Turbula mixer for 10 minutes.

Modules were manufactured according to the procedure described in 3.2.3.

4.2.1 Formulation SUC IR #1SIR

The formulation, described in Table 1, contains starch to improve disgregation time. Emcompress (calcium phosphate monohydrate) and Avicel (microcrystalline cellulose) are both necessary to achieve a proper compression of the module. Corn starch and AcDiSol (sodium croscarmellose) were used as disintegrating agents and magnesium stearate as a lubricant.

Table 1. Formulation SUC IR #1SIR

	mg	%
Sucralfate granules	130.43*	60.26
Starch	20	10.04
Avicel PH102	30	15.06
Emcompress	20	10.04
AcDiSol	8	4.02
Magnesium stearate	1	0.5
Purple lake	0.15	0.08
	209.58	

* 120 mg of dry sucralfate

The characteristics of the cylindrical modules are summarized in table 2.

Table 2. Characteristics of SUC IR #1SIR

Friability (%)	6.58
Disgregation time(sec)	74 ± 12
Hardness (kg, axial)	2.3 ± 0.8
Hardness (kg, diametral)	1.4 ± 0.5

Diametral hardness is lower than axial because the side of the module are thinner and less compressed than the dome, because of the shape of the module.

Friability is too high and over the limit, so PEG6000 as hydrophilic binder was added. This excipient should strengthen the module and increase water uptake, lowering the disintegration time.[22]

Sodium bicarbonate replaced starch as disintegrating agent, due to the bubbles formation and their effect on the module integrity.

Talc, as glidant was also used to improve mixing.

4.2.2 Formulation SUC IR #2SIR

The formulation, described in Table 3, contain a larger amount of sucralfate compared to formulation #1 (150 mg of dry sucralfate instead of 120 mg).

Emcompress was eliminated to accommodate more sucralfate.

Table 3. Formulation SUC IR #2SIR

	mg	%
Sucralfate granules	162.16*	63.25
PEG 6000	20	8.43
Avicel PH102	30	12.65
NaHCO ₃	20	8.43
AcDiSol	10	4.22
Talc	5	2.11
Magnesium stearate	2	0.84
Purple lake	0.15	0.06
	249.83	

* 150 mg of dry sucralfate

The characteristics of the modules are summarized in table 4.

Table 4. Characteristics of SUC IR #2SIR

Friability (%)	3.26
Disgregation time(sec)	64 ± 13
Hardness (kg, axial)	2.6 ± 0.5
Hardness (kg, diametral)	1.7 ± 0.5

Although friability values was improved, it was still over the limit.

4.2.3 Formulation SUC IR #3SIR

The formulation, described in Table 5, contain an even bigger amount of sucralfate compared to formulation #2 (190 mg of dry sucralfate instead of 150 mg). PEG6000 was discarded to accommodate HPMC K15M.

HPMC is a cellulose based polymer (hydroxypropyl methyl ether cellulose) usually used for controlled release, but also for improving mechanical hardness.

Table 5. Formulation SUC IR #3SIR

	mg	%
Sucralfate granules	206.07*	43.6
HPMC K15M	20	7.75
Avicel PH102	20	7.75
NaHCO ₃	10	3.87
AcDiSol	10	3.87
Talc	6	2.32
Magnesium stearate	2	0.77
Purple lake	0.15	0.06
	274.22	

* 190 mg of dry sucralfate

The characteristics of the module are summarized in table 6.

Table 6. Characteristics of SUC IR #3SIR

Friability (%)	0.21
Disgregation time(sec)	36 ± 6
Hardness (kg, axial)	5.5 ± 1.2
Hardness (kg, diametral)	3.8 ± 0.2

This formulation forms a buoyant layer of sucralfate particles on the surface of the medium used for release test, as shown in Figure 14.



Figure 14. Floating layer of sucralfate

This formulation was selected as final for the assembled system.

4.3 Esomeprazole immediate release module

Esomeprazole modules (two in every assembled system: one immediate release and one delayed release) contain 20mg of drug as 22 mg of esomeprazole magnesium dihydrate.

Esomeprazole is not stable in the acid environment of the stomach, therefore alkalisating agents were included in the formulation. Three of them and their combination were tested:

- d) Magnesium oxide (heavy and light): this excipient has the higher alkalisating capacity, but tablets show mechanical weakness. [23]
- e) Sodium carbonate: it has lower alkalisating capacity compared to magnesium oxide, but tablets are very resilient.[24, 25]
- f) Sucralfate: the choice of this substance presents a double advantage, because it's a buffer and its inclusion can increase the loading of sucralfate in the assembled system.

Esomeprazole as powder showed compaction problem, therefore dry granulation was necessary.

Granules were mixed with excipients (apart magnesium stearate) using a Turbula mixer for 10 minutes. Magnesium stearate was then added to the mixture and the mixture was remixed for 5 minutes.

Modules fulfilled friability test and disgregation time tests.

Release of drug was assessed according to the method described in 3.2.8 and 3.2.9: tests were done as triplicate and results are expressed as esomeprazole release (%) on the assayed drug content of the modules.

4.3.1 Formulation ESO light magnesium #1ESO

Granules in Table 7 contain pregelatinized starch as a binder[23], because previous formulations base on light magnesium oxide showed lamination during compaction.

Table 7. Granules in ESO light magnesium #1ESO

	mg
Esomeprazole magnesium di-hydrate	22*
Light Magnesium oxide	160
Pregelatinized starch	35
	217

* corresponding to 20 mg of esomeprazole

Modules formulation is described in Table 8.

Glyceryl palmitostearate (Precirol ATO 5) was the only excipients that allowed compression of the modules using granules listed in Table 7. Magnesium stearate didn't prove necessary because Precirol ATO 5 can also act as a lubricant.[26, 27]

Table 8. Formulation ESO light magnesium #1ESO

	mg	%
Esomeprazole granules	217*	86.8
Precirol ATO 5	23	9.2
AcDiSol	10	4
	250	

* corresponding to 20 mg of esomeprazole

Modules disintegrated in 29 ± 8 seconds and the friability value was 0.08%.

The release of esomeprazole in gastric fluid was $73.24 \pm 4.76\%$ and the profile of release is depicted in Figure 15.

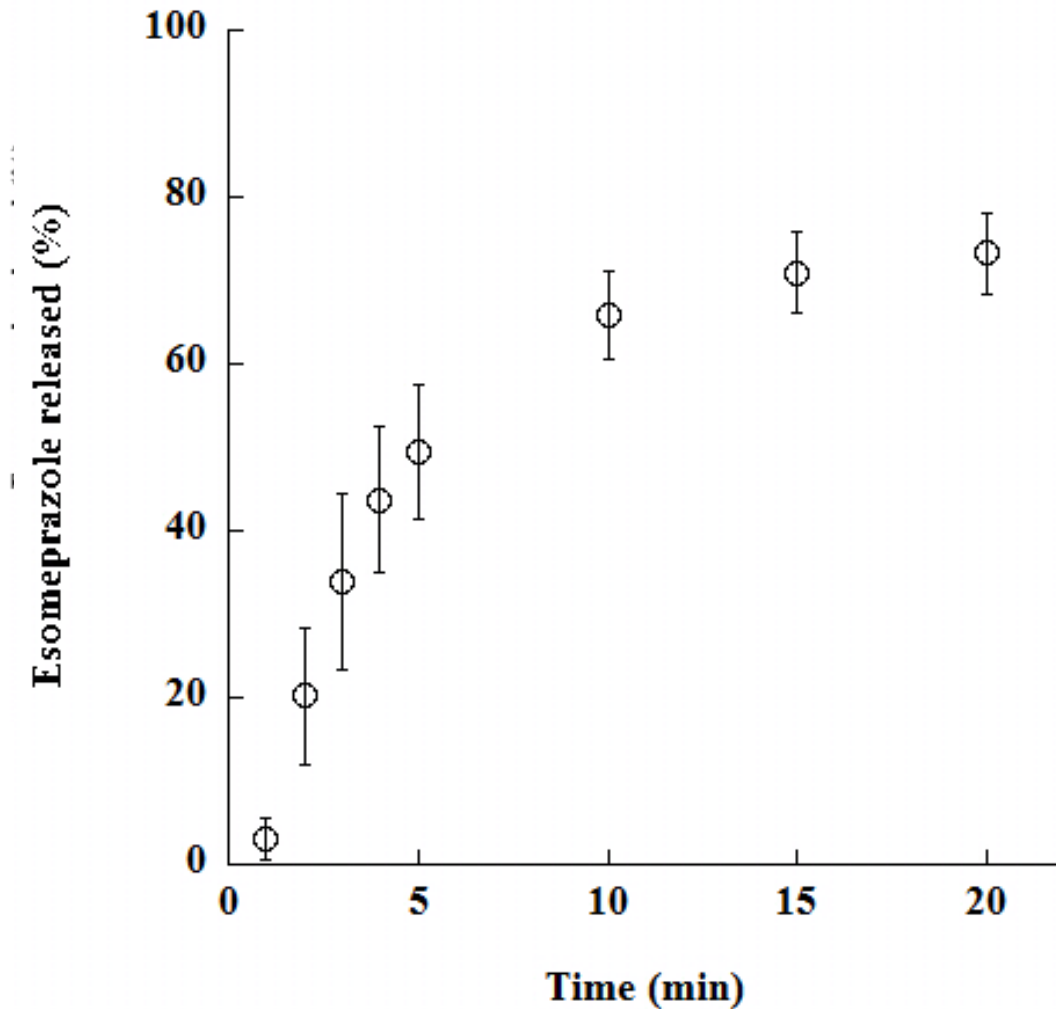


Figure 15. Esomeprazole release in gastric fluid of ESO light magnesium #!ESO

The remaining esomeprazole degraded because of the gastric fluid: the effectiveness of the formulation depends on the amount of non degraded esomeprazole at the end of the analysis.

4.3.2 Formulation ESO heavy magnesium #2ESO

Granules in Table 9 contain starch to improve flowability which is a main issue during mixing and tableting. [23, 28, 29] The starch in the granules also improves disintegration time.

Table 9. Granules in ESO heavy magnesium #2ESO

	mg
Esomeprazole magnesium dihydrate	22*
Heavy Magnesium oxide	160
Corn starch	35
	217

* corresponding to 20 mg of esomeprazole

Modules formulation is described in Table 10.

Avicel was used as direct compression material to ensure compactability.

Table 10. Formulation ESO heavy magnesium #2ESO

	mg	%
Esomeprazole granules	217*	83.46
Avicel PH102	30	11.54
AcDiSol	11	4.23
Magnesium stearate	2	0.77
	260	

* corresponding to 20 mg of esomeprazole

Modules disintegrated in 9 ± 2 seconds and the friability value was 0.08%.

The release of esomeprazole in gastric fluid is showed in Figure 16.

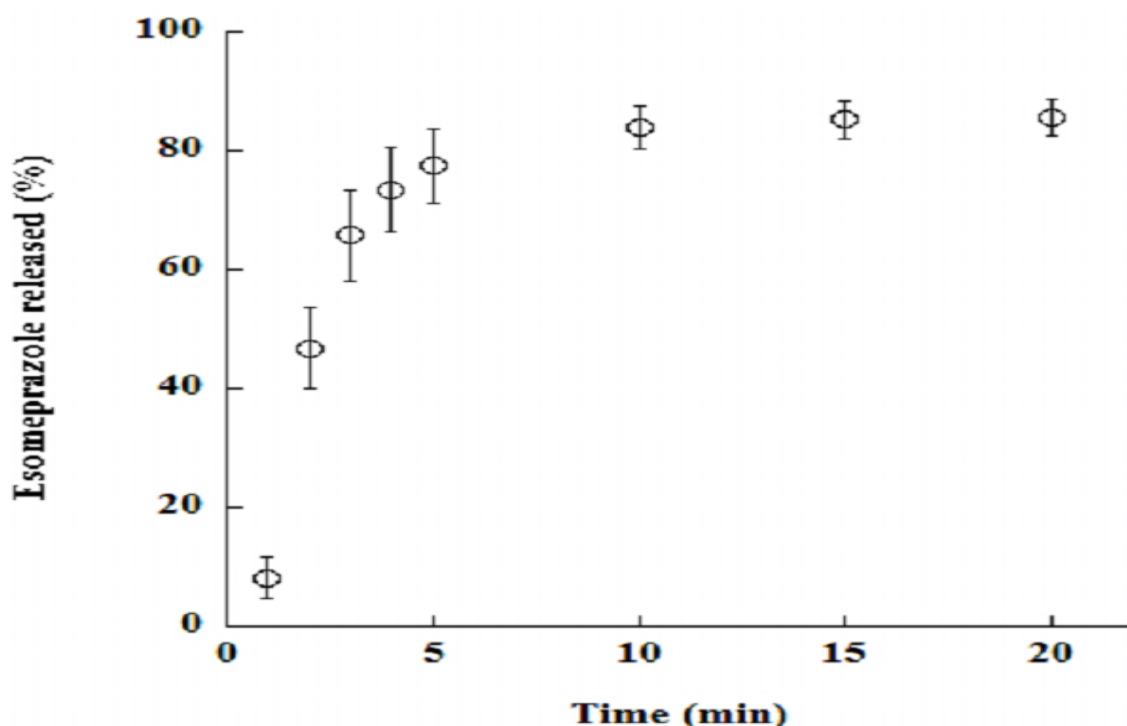


Figure 16. Esomeprazole release in gastric fluid of ESO heavy magnesium #5

4.3.3 Formulation ESO magnesium and sucralfate #3ESO

Granules in Table 11 contain lower amount of heavy magnesium oxide compared to granules in Table 9 in order to accommodate sucralfate in the formulation. Heavy magnesium oxide was selected as alkalisng agent because of it better release of drug (Figure 15 and 16).

Table 11. Granules in ESO magnesium and sucralfate #3ESO

	mg
Esomeprazole magnesium di-hydrate	22*
Heavy Magnesium oxide	80
Pregelatinized starch	10
	112

* corresponding to 20 mg of esomeprazole

Modules formulation is described in Table 12.

Granules, both sucralfate and esomeprazole were mixed with excipients (beside magnesium stearate) and mixed in a Turbula mixer for 10 minutes. Magnesium

stearate was added and the mixture was mixed again for 5 minutes.

Sucralfate was added separately because of the water content of sucralfate gel (raw material) which made impossible to granulate the two substances together.

Table 12. ESO magnesium and sucralfate #3ESO

	mg	%
Esomeprazole granules	112*	53.79
Sucralfate	65**	31.32
Avicel PH102	20	9.61
AcDiSol	10	4.8
Magnesium stearate	1	0.48
	208	

* corresponding to 20 mg of esomeprazole

** corresponding to 60 mg of dry sucralfate

The release of esomeprazole in gastric fluid was $67.92 \pm 1.78\%$ and the profile of release is depicted in Figure 17.

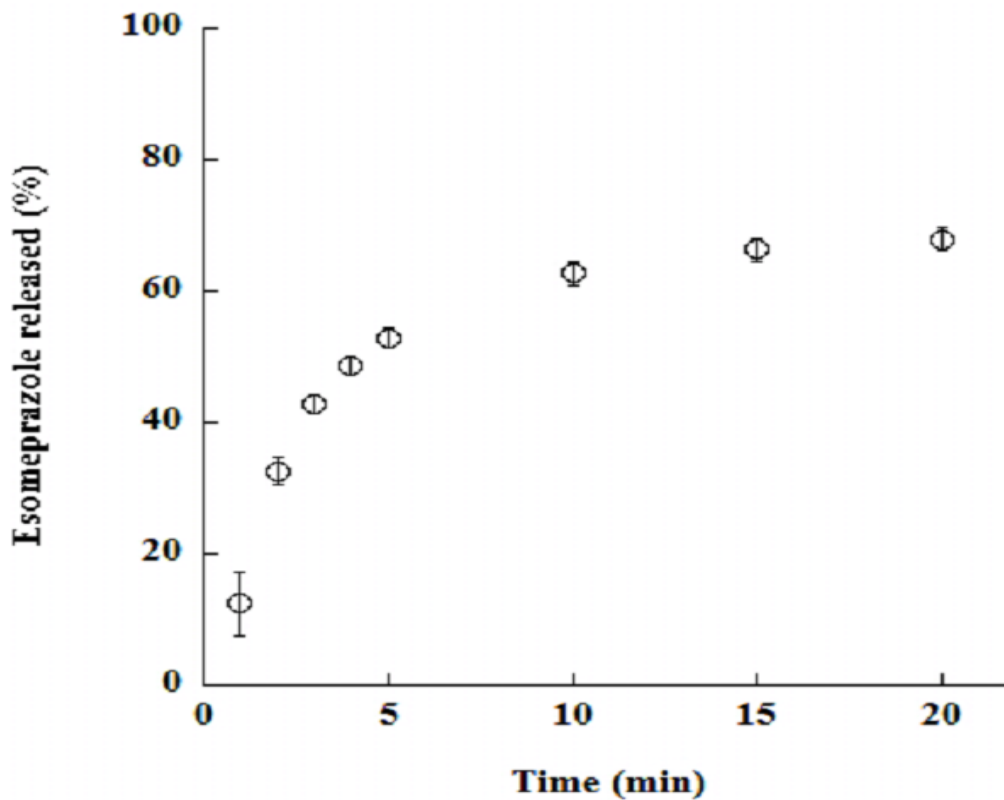


Figure 17. Esomeprazole release in gastric fluid of ESO magnesium and sucralfate

4.3.4 Formulation ESO carbonate #4ESO

Sodium carbonate was used in place of magnesium oxide. This material has a lower buffering capacity, but the best mechanical properties.[23, 30]

Granulation wasn't used because the mixture was directly compressed.

Two disintegrating agents, AcDiSol and cross-linked polyvinylpyrrolidone (PVP CL) were used at once to improve disgregation process; Avicel was also used for the same purpose via its swelling capability and its insolubility in water.

Components, magnesium stearate apart, were mixed in Turbula for 10 minutes. Magnesium stearate was added and the mixture was remixed for 5 minutes.

Table 13. ESO carbonate #4ESO

	mg	%
Esomeprazole magnesium di-hydrate	22*	9.82
Sodium carbonate	160	71.43
Avicel PH102	20	8.93
AcDiSol	8	3.64
PVP CL	8	3.64
Magnesium stearate	2	0.91
	220	

* corresponding to 20 mg of esomeprazole

Modules disintegrated in 4 minutes \pm 39 seconds and the friability value was 0.02%.

The release of esomeprazole was $48.11 \pm 3.82\%$ and the profile of release is depicted in in Figure 18.

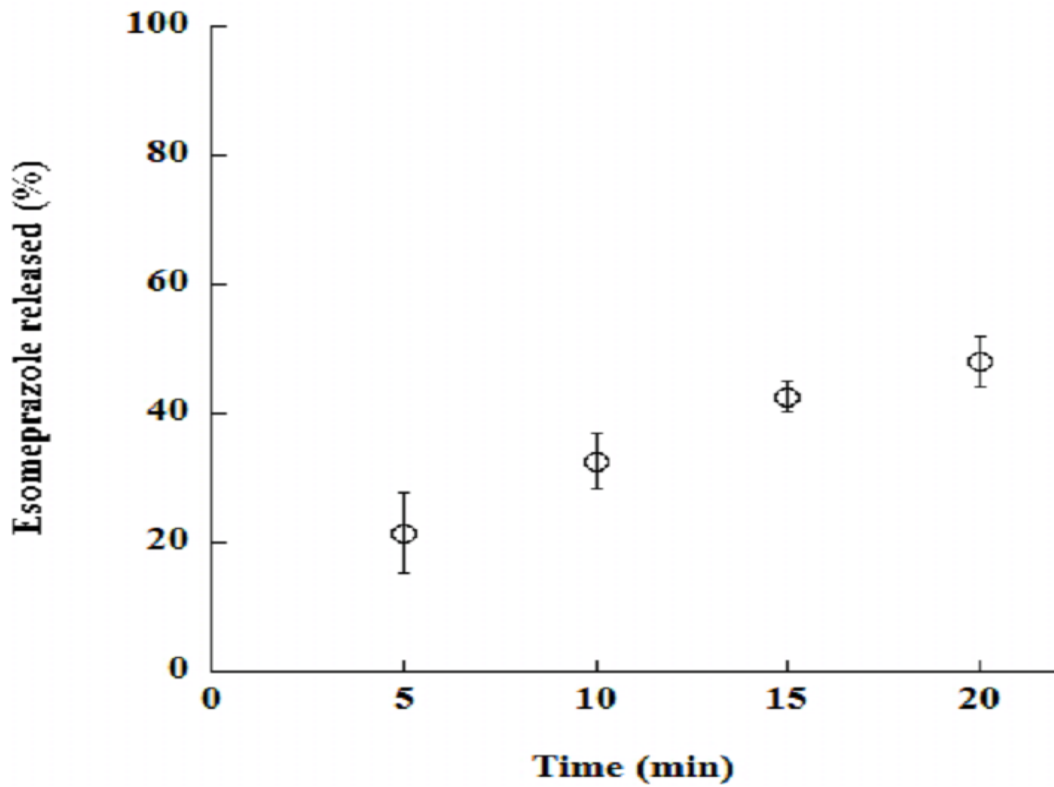


Figure 18. Esomeprazole release of ESO carbonate #7

The tablets showed a long disintegration time due to the limited flow in the cell of the dissolution test apparatus.

The release of the ESO formulations in gastric fluid is compared in Figure 19.

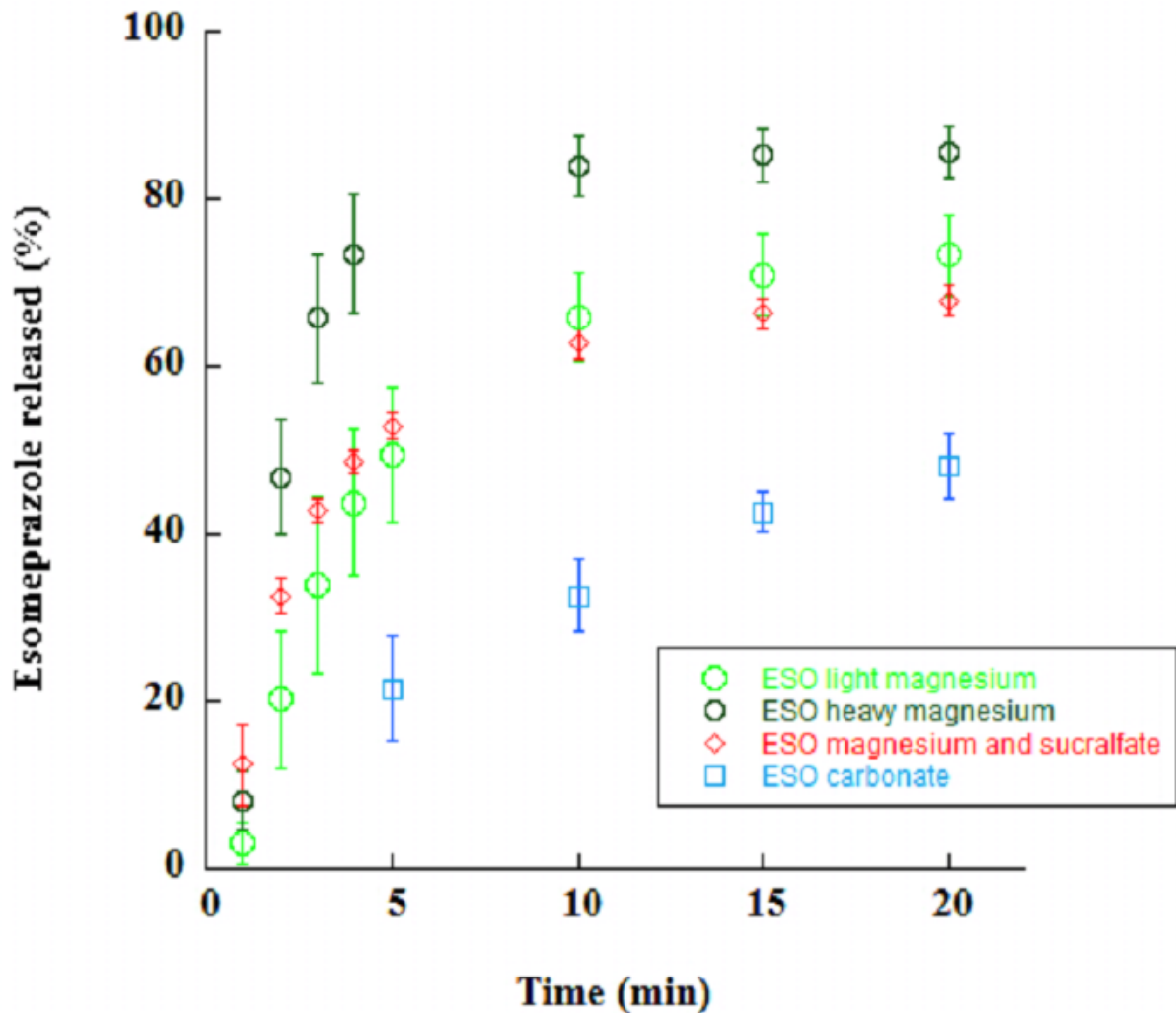


Figure 19. Comparison between ESO formulations in gastric fluid

Heavy magnesium oxide formulation (ESO#2ESO) showed the highest release of esomeprazole thanks to its higher alkalisng capacity.

The lower release of the light magnesium oxide formulation (ESO#1ESO) depends on the longer disintegration time of the module which leave the esomeprazole exposed to the simulated gastric fluid for a long time and therefore leads to high degradation of the esomeprazole.

The use of sucralfate (ESO#3ESO) as alkalisng agent proved unsuccessful: the module release can be compared to the light magnesium formulation while being more complex to manufacture.

The carbonate formulation (ESO#4ESO) showed the lowest release, both for the low alkalisng capacity and the long disgregation time.

Therefore, heavy magnesium oxide formulation (ESO#2ESO) was selected for the assembled system.

4.4 Coating of Dome Matrix assembled systems

The module assembly was partially coated in correspondence of the void assembly portion in order to protect the second dose or pulse of esomeprazole..

Coating prevented disintegration of the esomeprazole module assembled in void configuration with the male module of sucralfate, thus allowing the system to stay afloat on the gastric content until the intestinal transport. Since the coating also cover part of the sucralfate male module, it physically reduces its swelling and the erosion.

Two different coating were selected: osmotic and enteric.

- Osmotic coating allows water entrance in the module regardless of the environment pH: it means that the coating will withstand gastric and intestinal fluid. The release of the second pulse will depend only on the disintegration of the male module of sucralfate.
- Enteric coating solubilizes at intestinal pH, allowing a space controlled release of the second esomeprazole pulse. The sucralfate male module will determine the floating time preventing the assembled system to sink. The dissolution of the coating will also increase the area of the module exposed to the intestinal fluid, thus allows the release of the second pulse of esomeprazole.

The drawback of enteric coating is the necessity of a sub-coating to protect the acid sensitive esomeprazole from the acid groups of the polymer employed for enteric coating.

The assembled system were tested for the film resistance using placebo male module. Modules were also analysed for drug content before and after coating

Experiments were performed on modules belonging to the formulation ESO heavy magnesium #2ESO.

4.4.1 Osmotic coating #1Coat

Film formulation (Table 14) is based on cellulose acetate propionate. The polymer was dispersed in 2-propanol together with other solid excipients, and successively the acetone was added.

Table 14. Osmotic coating #1Coat

	mg	Function
Cellulose acetate propionate	8	Polymer
Triethylcitrate	2.9	Plasticizer
Silicon oil	20	Glidant/Anti-foaming agent
TiO ₂	0.3	Opacifier
2-propanol	30	Solvent
Acetone	40	Solvent
	83.7	

Assembled systems were dipped twice in the coating solution so that the weight increase for the assembled system was $10.86 \pm 2.14\%$.

HPLC analysis showed that the coating didn't affect the drug content of the modules. Release of esomeprazole was assayed in intestinal fluid and the results are in Figure 20.

Esomeprazole release was $51.24 \pm 11.1\%$, while esomeprazole recovered at the end of the test was $36.58 \pm 10.41\%$

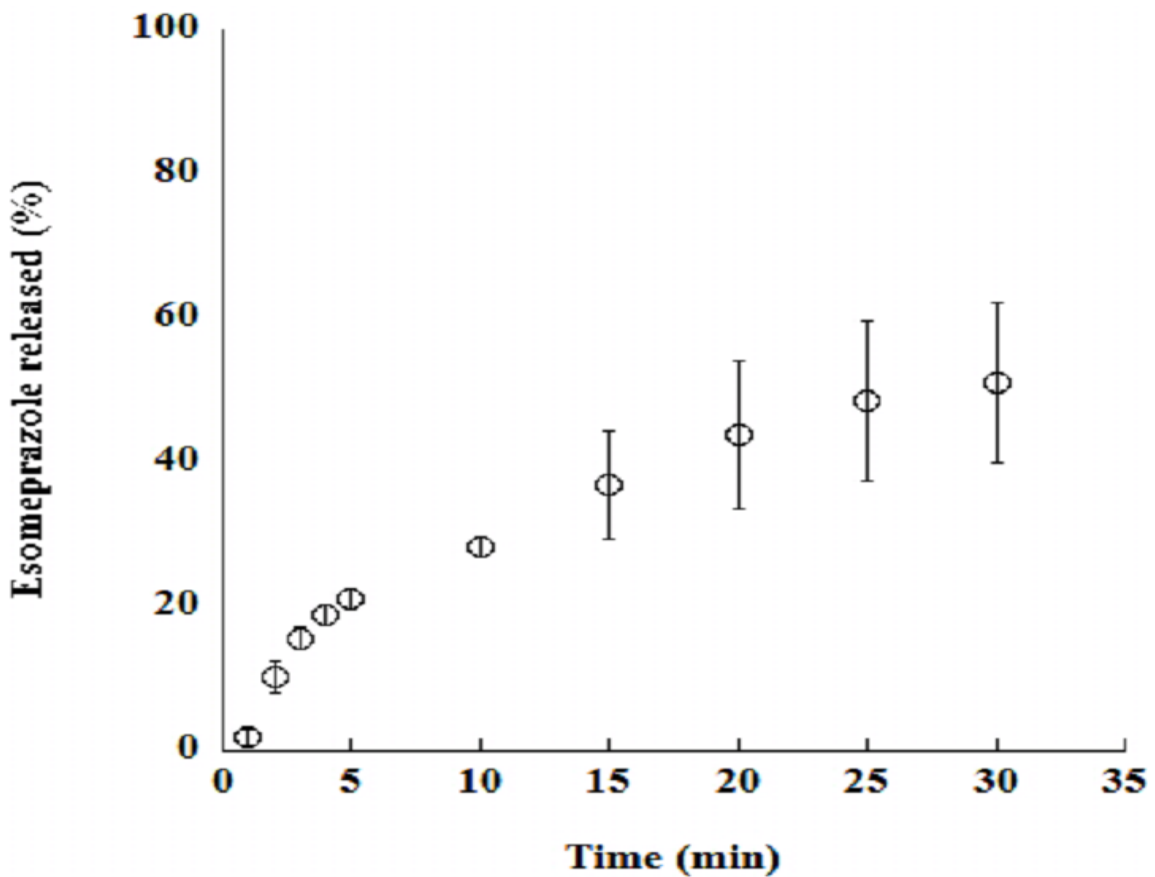


Figure 20. Esomeprazole released from coated system in intestinal fluid

The release is lower than expected despite the increased stability in intestinal fluid, because part of the drug remain trapped in the coated module.

The starch forms a sort of cap that cover the exit of the drug from uncoated face of the module.

4.4.2 Enteric coating #2Coat

Enteric coating was prepared according to section 3.2.5.

Enteric coating was a double layer coating: an inner protective coating and an outer enteric coating. Both layers are based on Eudragit: the inner layer is made of neutralized Eudragit in order not to react with esomeprazole, while showing a fast dissolution in intestinal fluid.[31, 32]

Sub-coating formulation is in Table 15, while enteric coating is described in Table 16.

Table 15. Subcoating formulation

	mg	Function
Eudragit S100	10	Polymer
Triethylcitrate	3.5	Plasticizer
NaOH 1M	1	Alkalisising agent
PEG3000	0.5	Viscosity modifier
Citric acid	0.2	Alkalisising agent
2-propanol	30	Solvent
Acetone	40	Solvent
	100.2	

The sub-coating was repeatedly applied until weight increase was 2.05 ± 0.35 .

Table 16. Enteric coating formulation

	mg	Function
Eudragit L100-55	20.6	Polymer
Silicon oil	0.5	Plasticizer
2-propanol	38.5	Solvent
Acetone	48.7	Solvent
	108.3	

The enteric coating was repeatedly applied until weight increase of the assembled system was 8.36 ± 0.72 .

HPLC analysis didn't show any degradation of the drug.

The profile of release is visible in Figure 21.

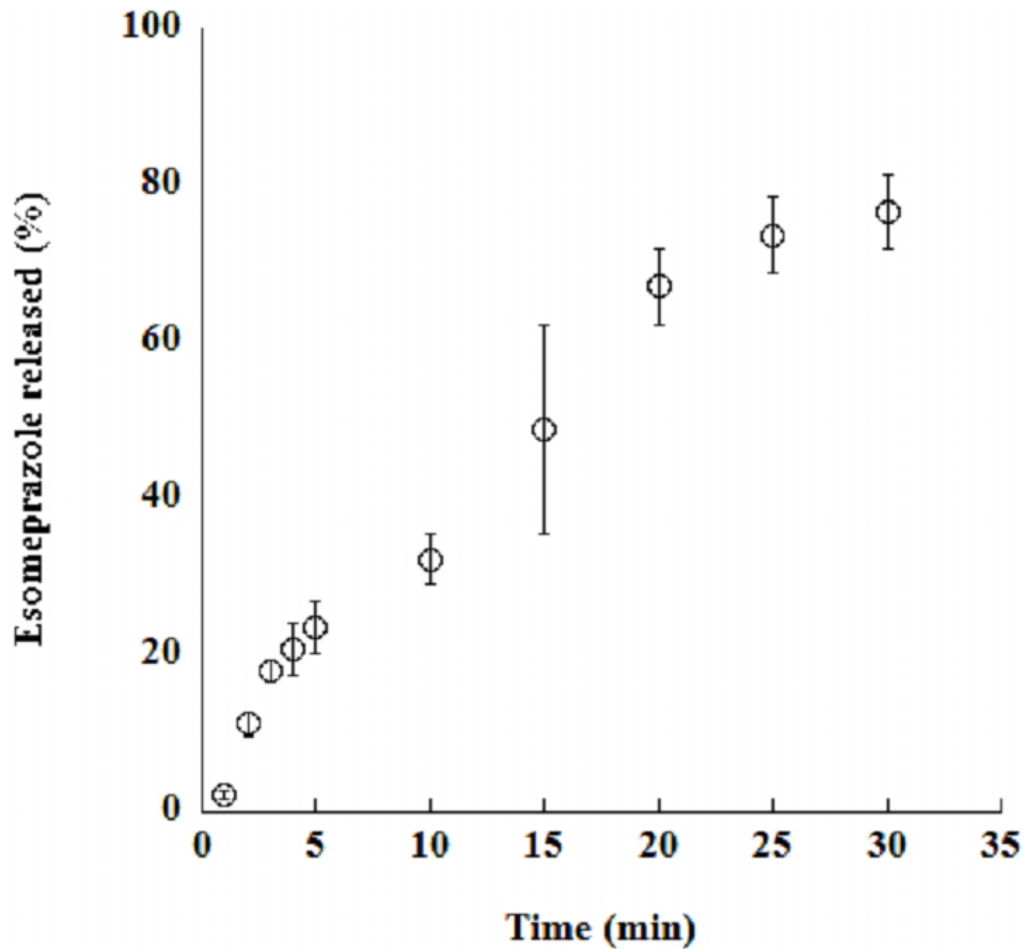


Figure 21. Esomeprazole released from enteric coated module in intestinal fluid

Esomeprazole release after 30 minutes was $76.6 \pm 4.73\%$, while esomeprazole recovered at the end of the test was $31.28 \pm 6.9\%$.

Enteric coating proved better suited for an immediate release thanks to its solubility at intestinal pH.

4.5 Sucralfate controlled release male modules

The purpose of the male module in the void part of assembled system is to achieve the floating of the void portion (from 4 up to 6 hours) thanks to the coating that prevent disgregation of the esomeprazole module. The fully assembled system did not float but after the disintegration of the immediate release part of it, the void portion starts floating on the gastric fluid.

The male module of sucralfate is the key element to regulate the time of the second pulse of esomeprazole.

Sucralfate granules were mixed with excipients, apart magnesium stearate, using a Turbula mixer for 10 minutes). After adding magnesium stearate the mixture was remixed for 5 minutes. Lubricated sucralfate granules were then mixed with other excipients using Turbula mixer for 10 minutes.

The assembled full system were tested for their floating using the following composition for modules and coating (Figure 5):

- SUC IR #3SIR
- ESO heavy magnesium #2ESO
- Enteric coating #2Coat

In order to find the optimal formulation for the male module, different formulations were tested. Male modules were manufactured using polyethylene oxide (Polyox) as polymer for controlled release, because HPMC showed compactability issues, like lamination or breaking during extraction.

4.5.1 Formulation SUC CR #1SUCR

Male module formulation SUC CR #10, (Table 17), used NaHCO₃ as gas forming agent to improve floating and PEG 6000 as promoter for water uptake.

Table 17. Formulation of SUC CR #1SUCR

	mg	%
Sucralfate granules	87*	43.01
Polyox N60K	52	27.96
PEG 6000	8	4.3
NaHCO ₃	40	21.51
Talc	4	2.15
Magnesium stearate	2	1.08
	192.96	

* 80 mg of dry sucralfate

The assembled systems including this male module floated after 15 minutes, but lasted more than 6 hours.

4.5.2 Formulation SUC CR #2SUCR

Male module formulation SUC CR #11 described in Table 18, replaced part of the sucralfate with Emcompress in order to improve tableting process and increase erosion via its low solubility in water.

Table 18. Formulation of SUC CR #2SUCR

	mg	%
Sucralfate granules	64*	32.17
Polyox N60K	40	21.45
Emcompress	20	10.72
NaHCO ₃	60	32.17
Talc	5	2.68
Magnesium stearate	1.5	0.8
	190.5	

* 60 mg of dry sucralfate

Assembled systems floated after 10 minutes and they sank before 6 hours.

4.5.3 Formulation SUC CR #3SUCR

Formulation SUC CR #12 describe in Table 19, uses a mixture of Polyox and an acrylic acid polymer (Carbopol).

Table 19 Formulation of SUC CR #3SUCR

	mg	%
Sucralfate granules	64*	31.07
Polyox N60K	60	29.13
Carbopol 940	20	9.71
Emcompress	20	9.71
NaHCO ₃	40	19.42
Magnesium stearate	2	0.97
	206	

* 60 mg of dry sucralfate

Assembled systems floated immediately sinking at 195 ± 7 seconds.

Formulation of male module SUC CR #3SUCR was selected for the assembly of the Dome Matrix assembled system.

4.6 Permeation test

In order to assess esomeprazole in vitro absorption in the stomach tissue, which is the target of the first pulse of esomeprazole, permeation test was performed.

Flat tablets (diameter: 8 mm) were employed instead of Dome Matrix modules due to the low amount of simulated gastric fluid in the donor compartment of the Franz cells used for experiments. Those tablets release esomeprazole immediately upon contact with medium.

4.6.1 Permeation study in simulated gastric fluid

Three different alkalisng agents were employed in the tablet used for the test: heavy magnesium oxide, sodium carbonate and lactose as a filler to investigate the permeation of the drug without any stabilising agent.

Their formulation are described in Table 20, Table 21 and Table 22. All tests are conducted in triplicate.

Table 20. Formulation of ESO 8mm MgO #ESOMgO

	mg
Esomeprazole magnesium di-hydrate	11*
Heavy magnesium oxide	80
AcDiSol	5
	96

*10 mg of esomeprazole

Table 21. Formulation of ESO 8mm Na₂CO₃ #ESONa

	mg
Esomeprazole magnesium di-hydrate	11*
Sodium carbonate	80
Microcrystalline cellulose	40
AcDiSol	5
	136

*10 mg of esomeprazole

The use of microcrystalline cellulose (Avicel PH102) was necessary to ensure the disgregation of the tablet in a short time and with a small amount of fluid.

Table 22. Formulation of ESO 8mm Lactose #ESOlac

	mg
Esomeprazole magnesium di-hydrate	11*
Lactose	80
AcDiSol	5
	96

*10 mg of esomeprazole

The permeation profiles of esomeprazole from the immediate release tablets through porcine gastric mucosa are shown in Figures 22-24.

Tablets, containing Na₂CO₃ as alkalizing agent (Table 21) showed a slow initial permeation in the first hour, followed by increased flux in the second hour. At a volume of 1 ml of simulated gastric fluid in the donor compartment, a flux of 0.11 ±

0.04 mg cm⁻²h⁻¹ was reached between 60 and 120 min and the amount of esomeprazole permeated in 2 h through the porcine gastric mucosa was 0.23 ± 0.06 mg/cm². Moreover, the amount of drug accumulated in the tissue was 1.00 ± 0.06 mg. The mass balance of the experiment allowed to recover 97.43 ± 3.51 %. At the end of the experiment, the pH of the solution in the donor compartment had a value of 11.0. In the case of 3 ml volume in the donor compartment, the flux was increased (0.16 ± 0.03 mg/cm²h) in the 90 – 120 min range, while the drug amount permeated in 2 h (0.24 ± 0.06 mg/cm²) was not significantly different from the value observed at a volume of 1 ml in the donor compartment. The amount of drug accumulated in the tissue was 0.72 ± 0.16 mg, the mass balance corresponded to 88.43 ± 2.21 % and the pH in the donor compartment was 10.0. At a volume of 5 ml in the donor compartment, the values of flux (0.12 ± 0.05 mg/cm²h in the 60 – 120 min range) and of drug amount permeated in 2h (0.21 ± 0.06 mg/cm²) were not significantly different to those observed with the volume of 1 ml in the donor compartment. The amount of drug accumulated in the tissue was 0.85 ± 0.25 mg, the mass balance resulted 87.23 ± 3.15 % and the pH in the donor compartment was 10.0.

In summary, the cumulative transport of esomeprazole through the mucosa after 2 h was not influenced by the volume in the donor compartment.

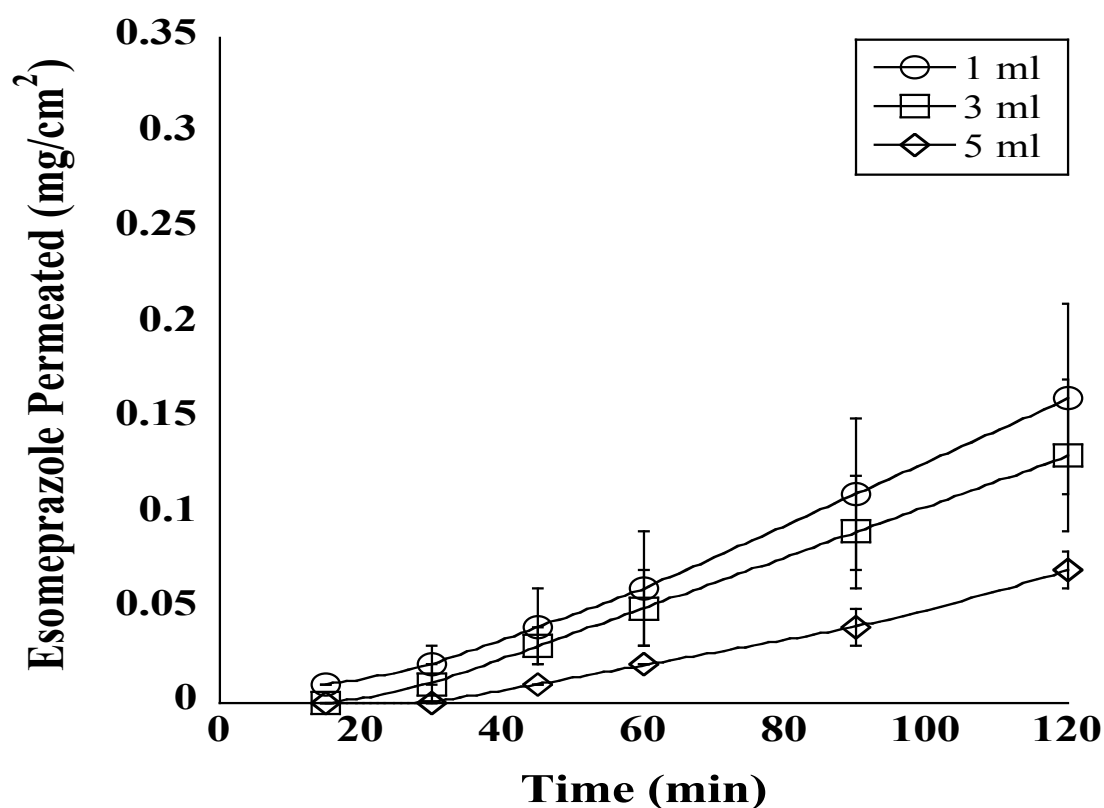


Figure 22. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets containing Na_2CO_3 as alkalizing agent ($n = 3$), from different simulated gastric fluid volumes in the donor compartment: 1 ml (\circ), 3 ml (\square) and 5 ml (\diamond).

In presence of MgO as alkalizing agent in the tablet, the profiles versus time are in Figure 23. At a volume of 1 ml of simulated gastric fluid in the donor compartment, an average flux of $0.10 \pm 0.03 \text{ mg/cm}^2\text{h}$ was reached between 45 and 120 min and the amount of esomeprazole permeated in 2 h through the porcine gastric mucosa was $0.16 \pm 0.05 \text{ mg/cm}^2$. The amount of drug accumulated in the tissue was $0.66 \pm 0.19 \text{ mg}$ and the mass balance was $90.58 \pm 0.38 \%$. At the end of the experiment, a pH of 10.0 in the donor compartment was measured. In the case of 3 ml volume in the donor compartment, the values of flux ($0.09 \pm 0.03 \text{ mg/cm}^2\text{h}$) and drug amount permeated ($0.13 \pm 0.04 \text{ mg/cm}^2$) were comparable with those measured previously. On the other hand, the amount of drug accumulated in the tissue was increased to $1.12 \pm 0.35 \text{ mg}$ and the mass balance resulted to be $100.71 \pm 1.81 \%$. The pH in the donor compartment was 7.0.

At a volume of 5 ml in the donor compartment, different behavior was observed. The lag time was greater with respect to the other permeation profiles. Moreover, the values of average flux ($0.05 \pm 0.01 \text{ mg/cm}^2\text{h}$) and drug amount permeated ($0.07 \pm 0.01 \text{ mg/cm}^2$) were significantly lower with those measured at volumes of 1 ml and 3 ml in the donor compartment. The amount of drug accumulated in the tissue was negligible ($0.03 \pm 0.06 \text{ mg}$) and the mass balance resulted to be $74.63 \pm 1.45 \%$. By increasing the volume in the donor compartment, the buffering capacity of the MgO decreases. In fact, the reduced mass balance could be due to the greater esomeprazole degradation in the donor compartment where the pH measured at the end of the experiment was 7.0.

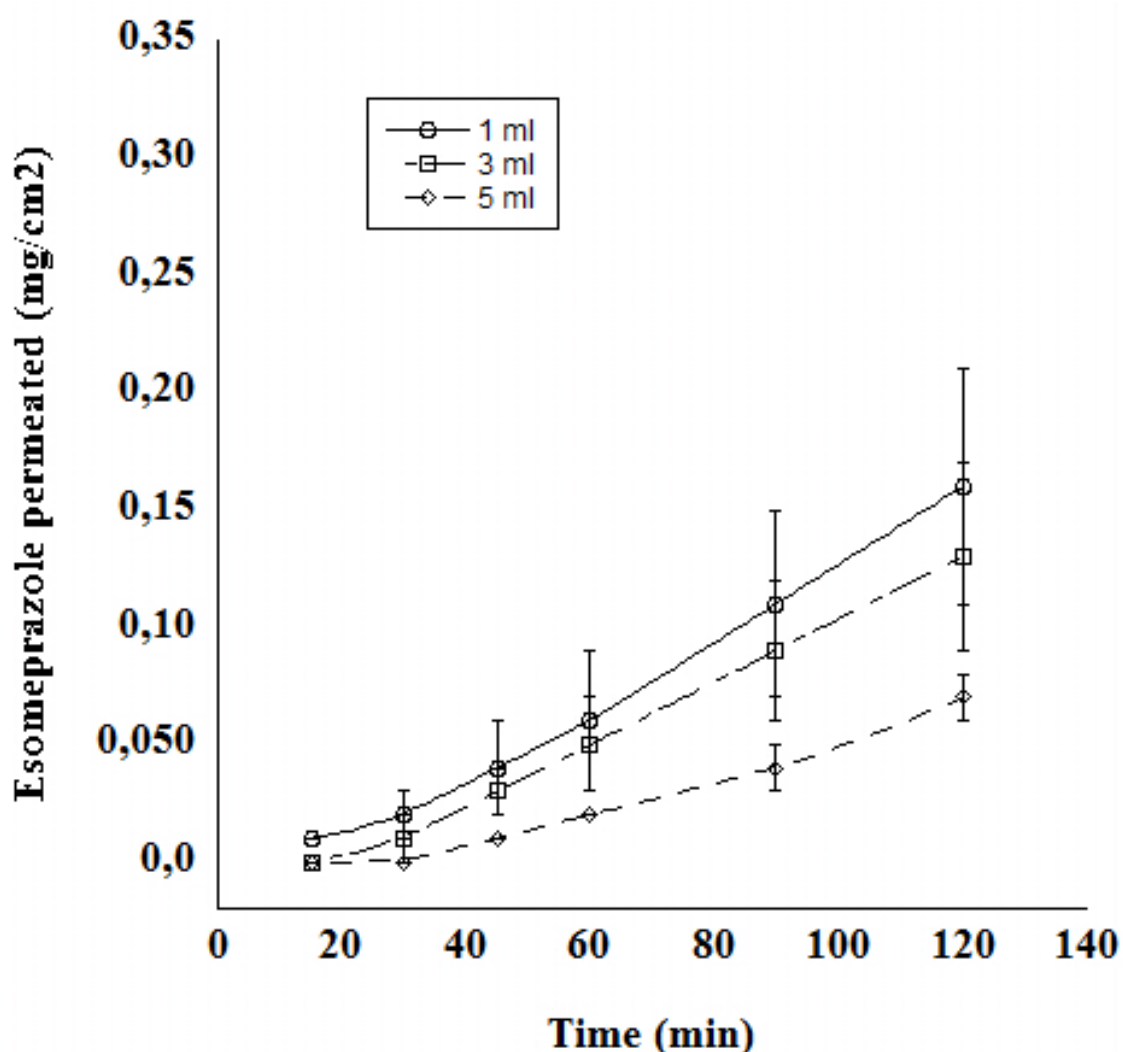


Figure 23. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets containing MgO as alkalizing agent ($n = 3$), from different simulated gastric fluid volumes in the donor compartment: 1 ml (\circ), 3 ml (\square) and 5 ml (\diamond).

In summary, Na_2CO_3 is able to increase the permeation of the drug through the porcine gastric mucosa and resulted more efficient than MgO in protecting esomeprazole from degradation.

However, the capacity of alkalizing agent to protect the esomeprazole from degradation in acid environment was compared with the permeation behavior of esomeprazole from the tablets containing lactose. In the absence of alkalizing agent, at volumes of 1 ml and 3 ml in the donor compartment the transport of esomeprazole was low ($0.04 \pm 0.01 \text{ mg/cm}^2$); the amount of drug accumulated in the tissue was less than $0.1 \pm 0.06 \text{ mg}$ and the average flux was $0.02 \pm 0.01 \text{ mg/cm}^2\text{h}$. Moreover, the mass balance was very low ($13.33 \pm 3.37 \%$) due to the degradation of the drug as the pH in the donor compartment was 4.0. At volume of 5 ml in the donor compartment, the permeation of esomeprazole was negligible.

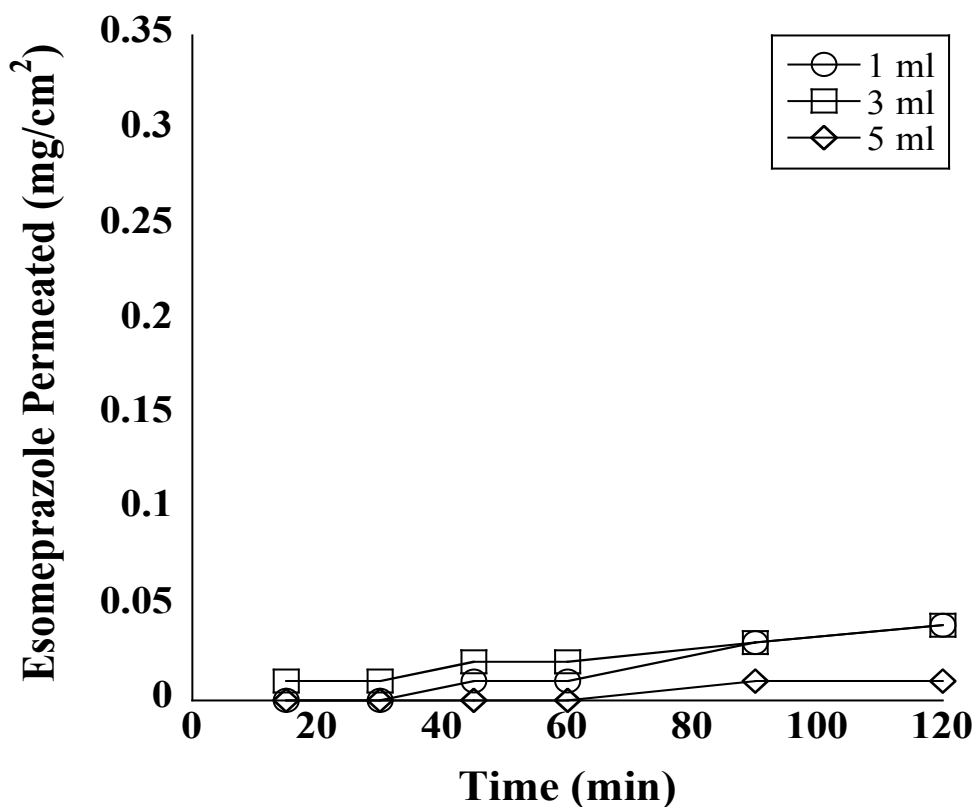


Figure 24. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets without alkalizing agent ($n = 3$), from different simulated gastric fluid volumes in the donor compartment: 1 ml (\circ), 3 ml (\square) and 5 ml (\diamond).

Figure 25 show the average value of esomeprazole permeated for each formulation and volume of donor employed.

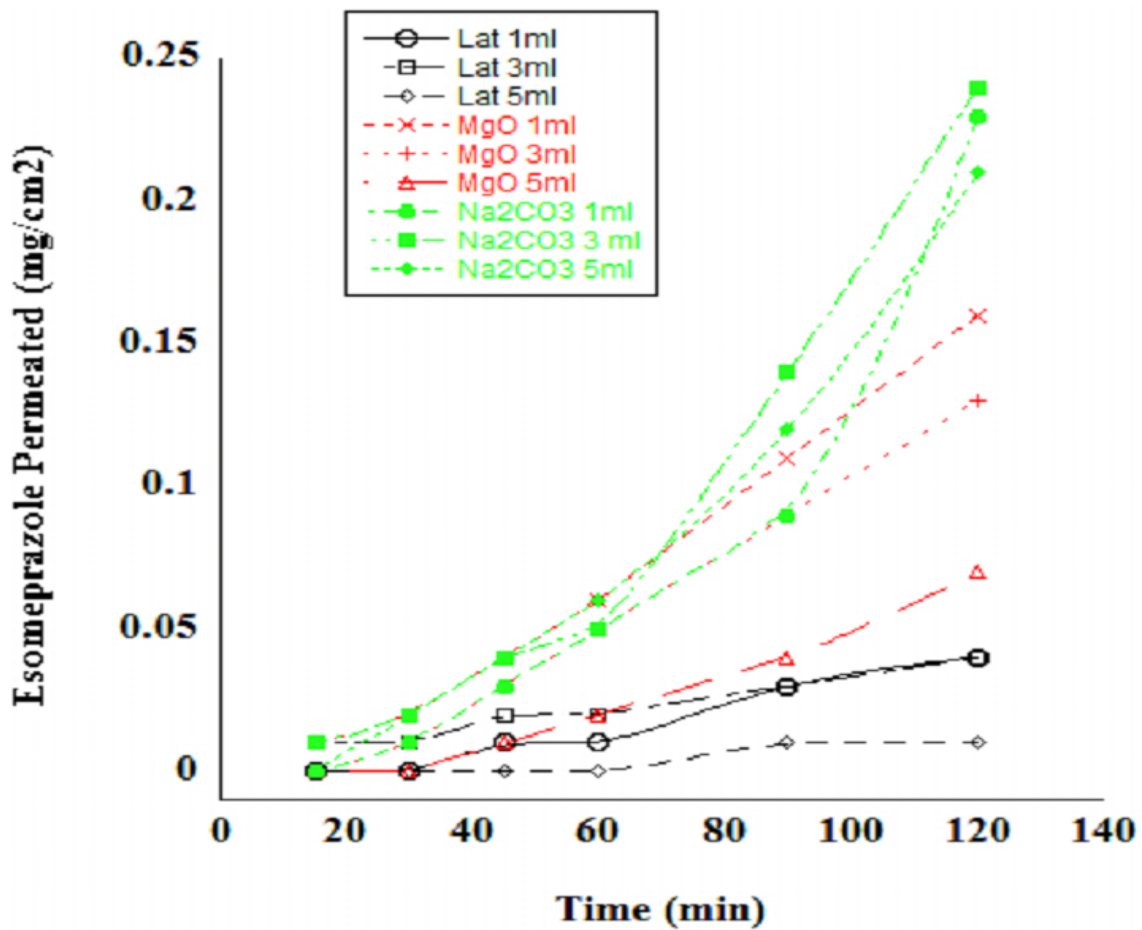


Figure 25. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets (n = 3), from different formulation and simulated gastric fluid volumes.

Anova test on the data states that all the results are statistically different ($p < 0.001$) from each other.

4.6.2 Permeation study at pH 3.5

The permeation study with acetate buffer pH 3.5 in the donor compartment were performed on the same tablets described before at pH 1.2.

In presence of Na_2CO_3 , the amount of esomeprazole permeated at pH 3.5 was $0.20 \pm 0.09 \text{ mg/cm}^2$ whit 1 ml volume in the donor compartment. Moreover, the drug accumulated in the tissue and the mass balance were $0.95 \pm 0.11 \text{ mg}$ and $97.86 \pm 5.8 \%$, respectively. These values were similar to those observed at pH 1.2, in fact

the permeation profiles were almost superimposable. The situation changed with increasing volume of acetate buffer pH 3.5 in the donor compartment. The permeation profiles evidenced a decrease of esomeprazole permeated both at 3 ml volume ($0.06 \pm 0.04 \text{ mg/cm}^2$) and 5 ml volume ($0.06 \pm 0.01 \text{ mg/cm}^2$), significantly different with respect to pH 1.2 (Anova test showed a $p < 0.01$ and $p < 0.05$, respectively). Besides, an increased drug degradation was observed. The permeation profiles of esomeprazole from the immediate release tablets through porcine gastric mucosa are shown in Figure 26.

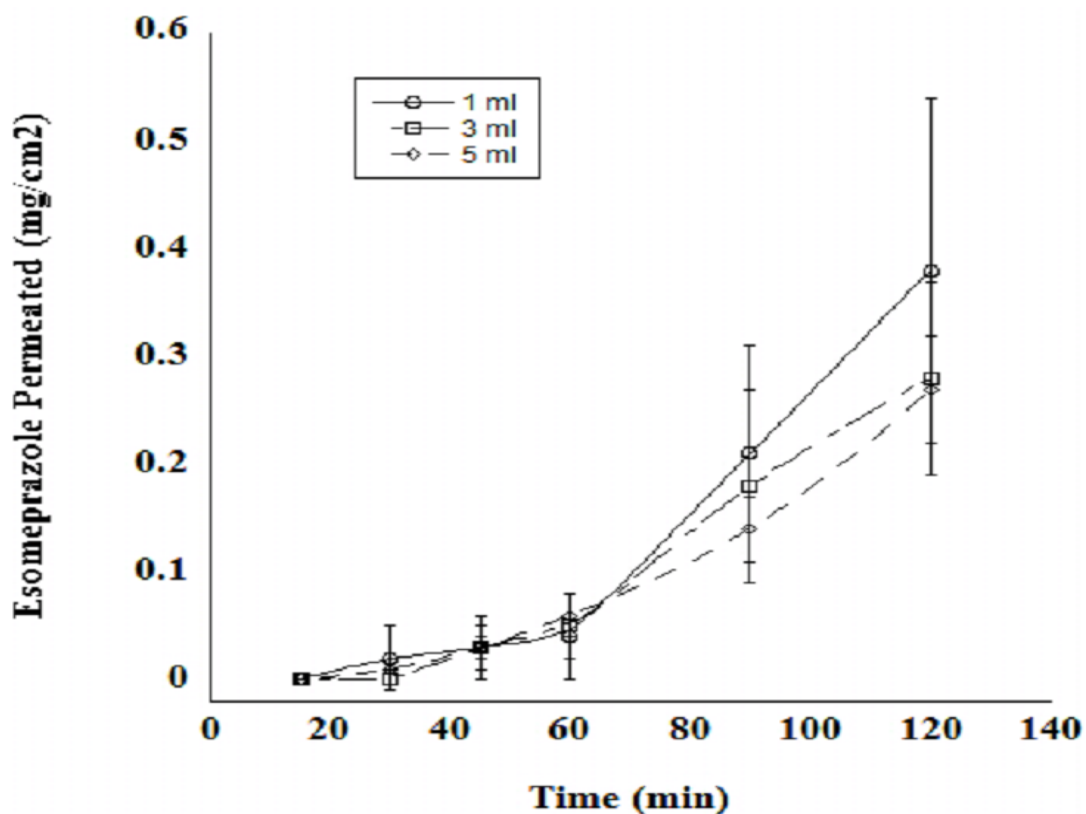


Figure 26. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets with sodium carbonate ($n = 3$), from different simulated gastric fluid volumes at $\text{pH} = 3.5$.

The permeation profiles of esomeprazole with MgO through porcine gastric mucosa are shown in Figure 27.

In the case of MgO, as alkalinizing agent, the permeation of esomeprazole through the porcine gastric mucosa was not significantly different from that observed at pH 1.2 when the volumes in the donor compartment were 1 ml or 3 ml: the amount of

esomeprazole permeated was $0.12 \pm 0.02 \text{ mg/cm}^2$ and $0.12 \pm 0.02 \text{ mg/cm}^2$, respectively, and the drug accumulated in the tissue was $0.22 \pm 0.16 \text{ mg}$ and $0.25 \pm 0.01 \text{ mg}$. Nevertheless, with respect to the behavior observed at pH 1.2, the drug degradation was greater and the drug accumulated in the mucosa was lower in both cases.

Otherwise, at higher volume in the donor compartment (5 ml), the transport of esomeprazole through the porcine gastric mucosa was increased ($0.19 \pm 0.05 \text{ mg/cm}^2$, Anova $p < 0.01$), even if a degradation of the drug of about 30% was observed.

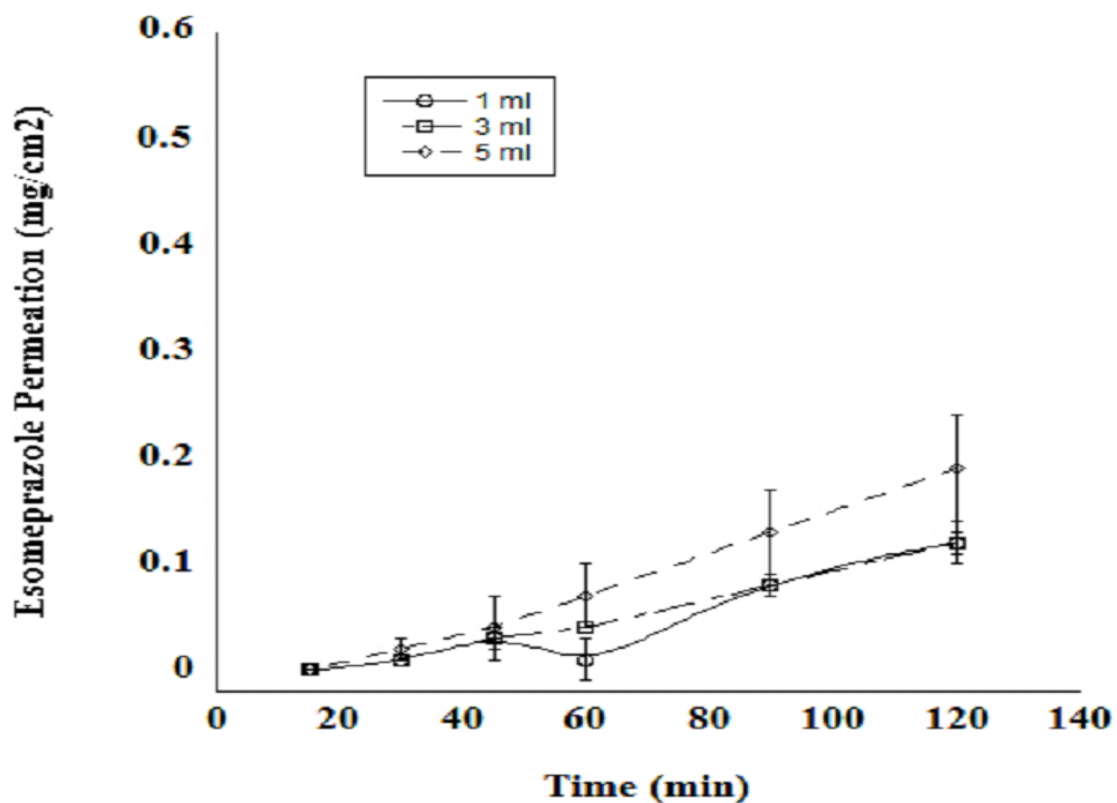


Figure 27. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets with magnesium oxide ($n = 3$), from different simulated gastric fluid volumes at pH=3.5.

Lactose loaded tablets didn't show any significant difference from the transport at pH1.2 and the permeation profiles of esomeprazole from the immediate release tablets through porcine gastric mucosa are shown in Figure 28.

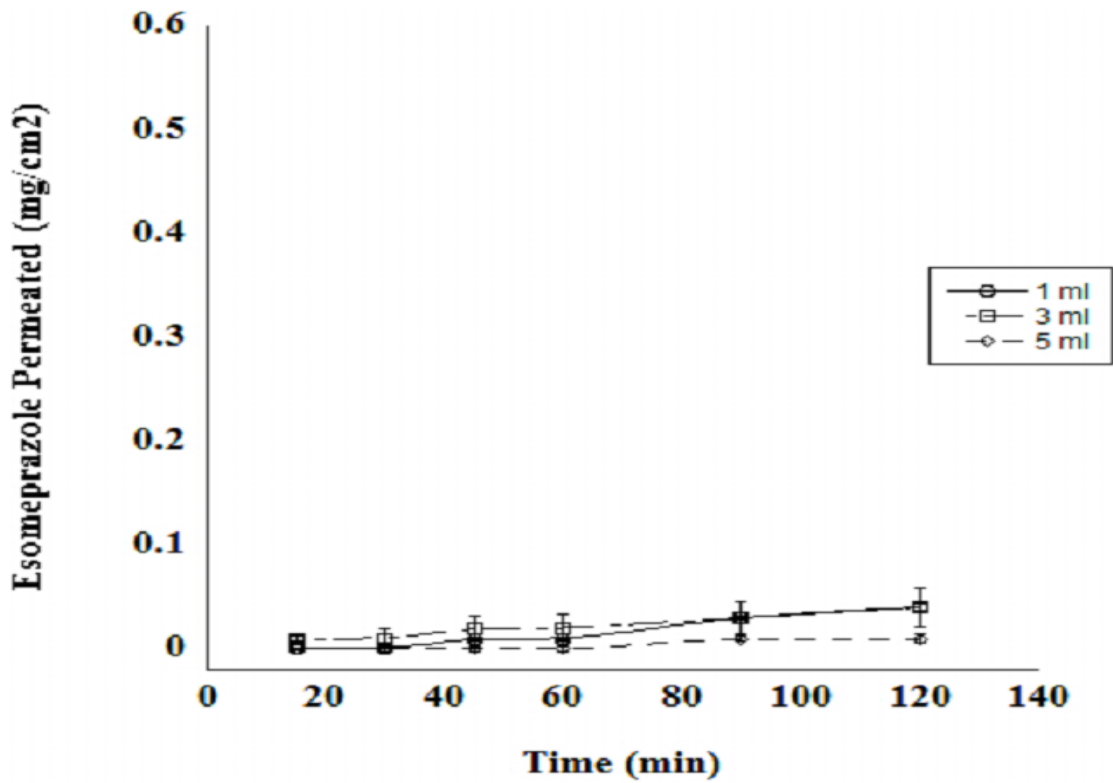


Figure 28. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets with lactose (n = 3), from different simulated gastric fluid volumes at pH=3.5.

Figure 29 show the average value of esomeprazole permeated for each formulation and volume of donor employed.

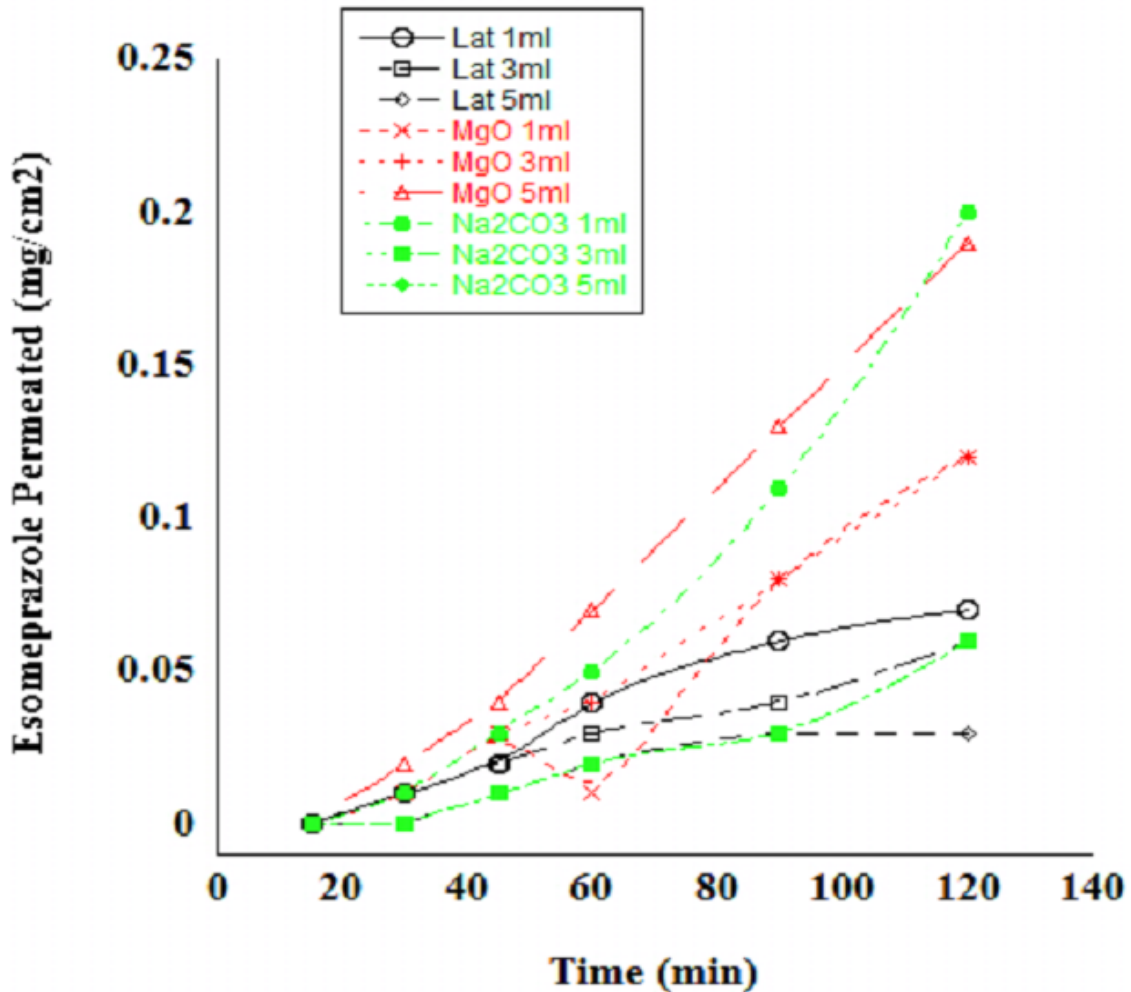


Figure 29. Esomeprazole permeation profile through pig gastric mucosa from 8 mm tablets (n = 3), from different formulation and simulated gastric fluid volumes at pH 3.5.

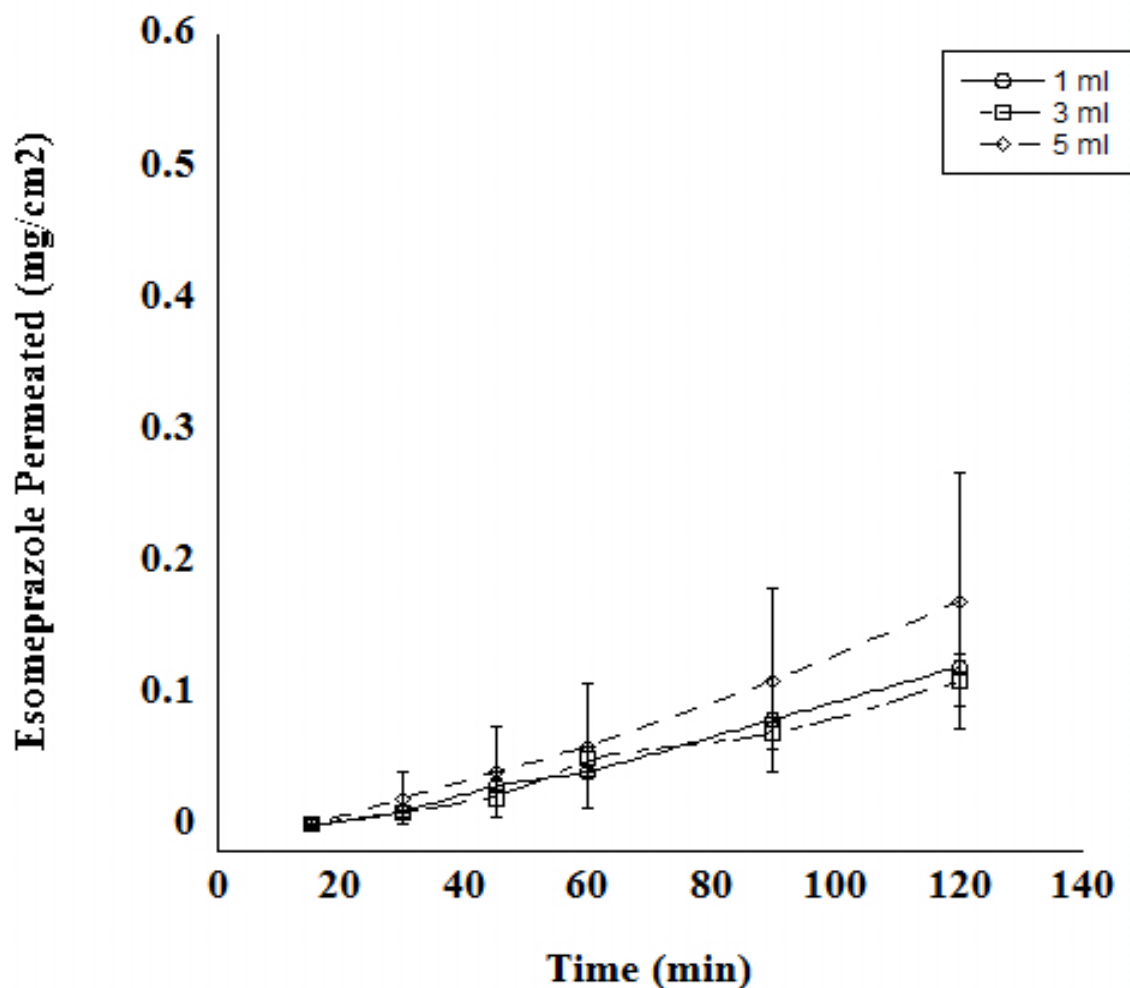
Anova test on the data states that all the results are statistically different ($p < 0.001$) from each other.

Those results are also statistically different from the results of permeation in simulated gastric fluid of Figure 25. The probability is below 0.0001.

4.6.3 Permeation study of 2 mm tablets in simulated gastric fluid.

Since to perform *in vivo* release test on rats, 2 mm cylindrical tablets were manufactured using the same formulation used for the 8 mm tablets.

The *in vitro* permeation study with simulated gastric fluid in the donor compartment were performed on 2 mm cylindrical tablet manufactured using the same



formulations.

Up to ten 2 mm tablets (depending on the drug content of the tablets) were used in order to reach the same amount of esomeprazole in a 8 mm tablet.

The results are depicted in Figure 30, Figure 31 and Figure 32. Those results were compared with those obtained at pH 1.2 using 8 mm tablets..

It was also assessed if the size and shape of the tablets affected the esomeprazole permeation.

The esomeprazole permeation from Esomeprazole-magnesium oxide tablets is slightly different compared to 8 mm tablets: in 120 minutes 0.17 mg/cm² instead of 0.04 mg/cm² for 5 ml were transported.

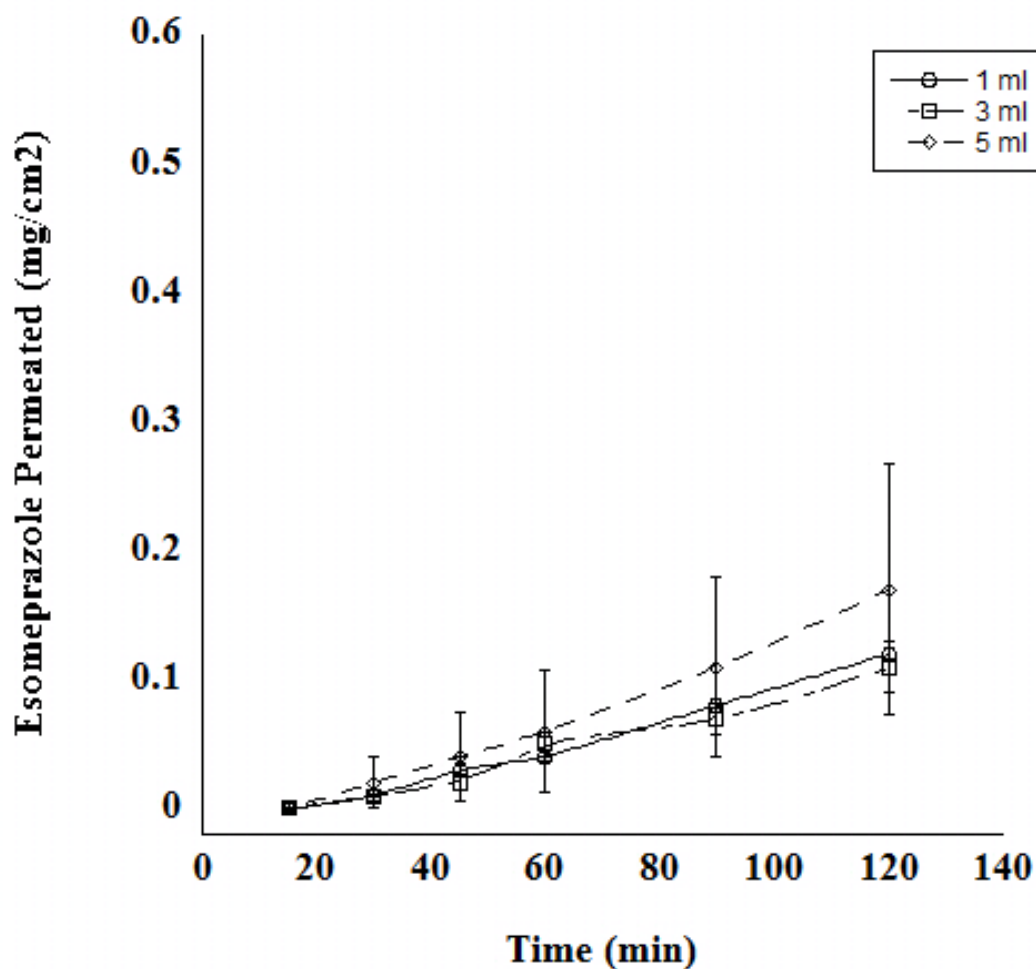


Figure 30. Esomeprazole permeation profile through pig gastric mucosa from 2 mm tablets containing MgO as alkalizing agent (n = 3), from different simulated gastric fluid volumes in the donor compartment: 1 ml (○), 3 ml (□) and 5 ml (◇).

The esomeprazole permeation is not statistically different from 8 mm tablets ($p > 0.05$).

The esomeprazole permeation in case of tablets containing Na₂CO₃ was slightly different compared to 8 mm tablets: 0.38 mg/cm² instead of 0.23 mg/cm² for 1 ml, 0.28 mg/cm² instead of 0.24 mg/cm² for 3 ml and 0.27 mg/cm² instead of 0.21 mg/cm² for 5 ml.

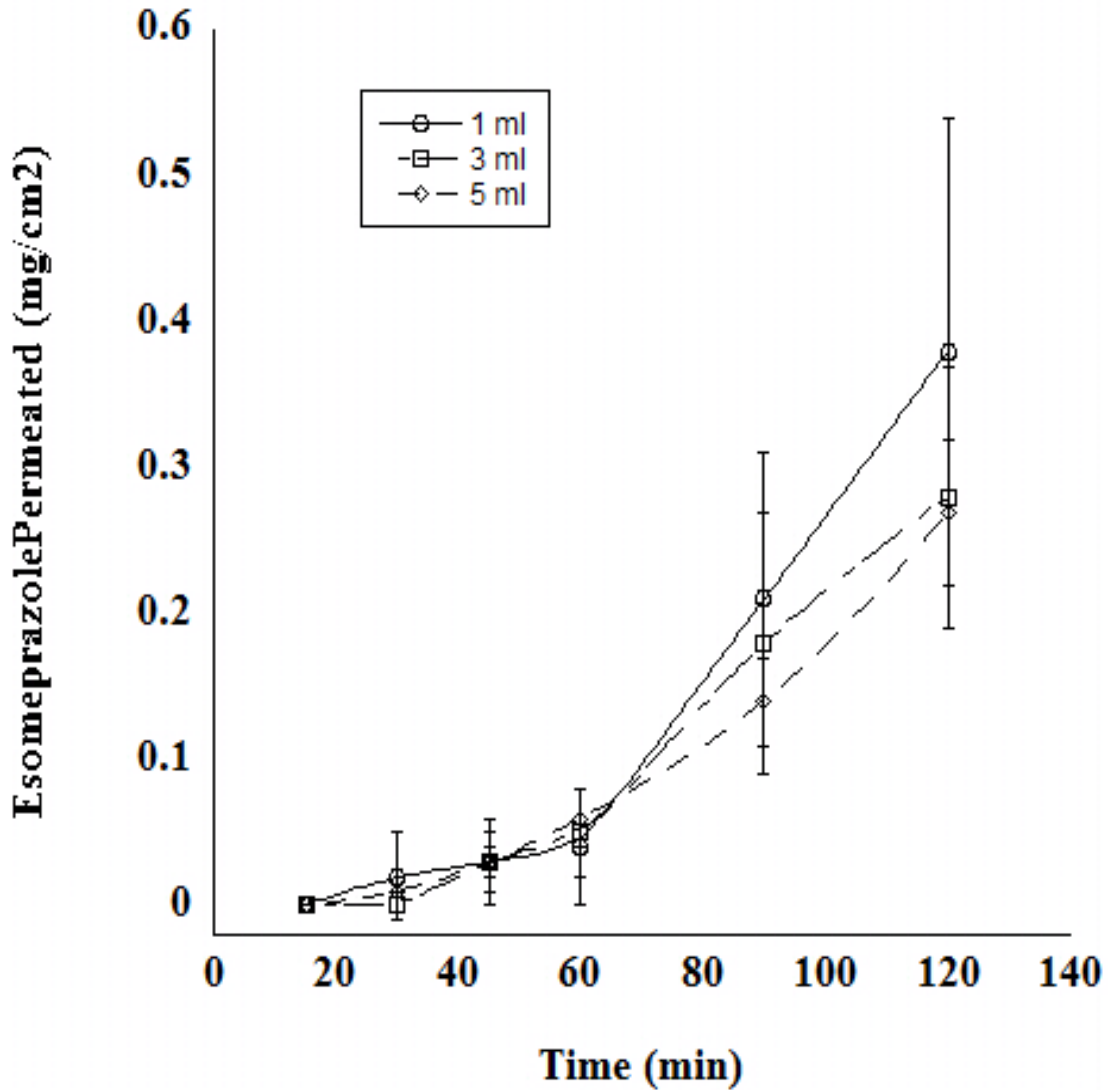


Figure 31. Esomeprazole permeation profile through pig gastric mucosa from 2 mm tablets containing Na_2CO_3 as alkalizing agent ($n = 3$), from different simulated gastric fluid volumes in the donor compartment: 1 ml (\circ), 3 ml (\square) and 5 ml (\diamond).

There were no statistical differences between the values of esomeprazole permeation belonging to 8mm and 2mm tablets because Anova test states a $P > 0.40$ and all the Turkey pairwise comparisons result negative.

The 2 mm tablet containing lactose could not be manufactured using formulation #ESOlac (Table 22) without the use of a small amount of microcrystalline cellulose (Avicel PH102) to ensure proper compaction.

Table 23. Formulation of ESO 2mm Lactose #ESOlacModified

	mg
Esomeprazole magnesium di-hydrate	1.13*
Lactose	6.18
Microcristalline cellulose	1.03
AcDiSol	0.41
	8.75

*10 mg of esomeprazole

Esomeprazole permeation of 2 mm tablets (Figure 32) containing lactose in 1 ml of simulated gastric fluid is statistically significant compared to the other values and esomeprazole permeation of 8 mm tablets too (ANOVA $p=0.0048$).

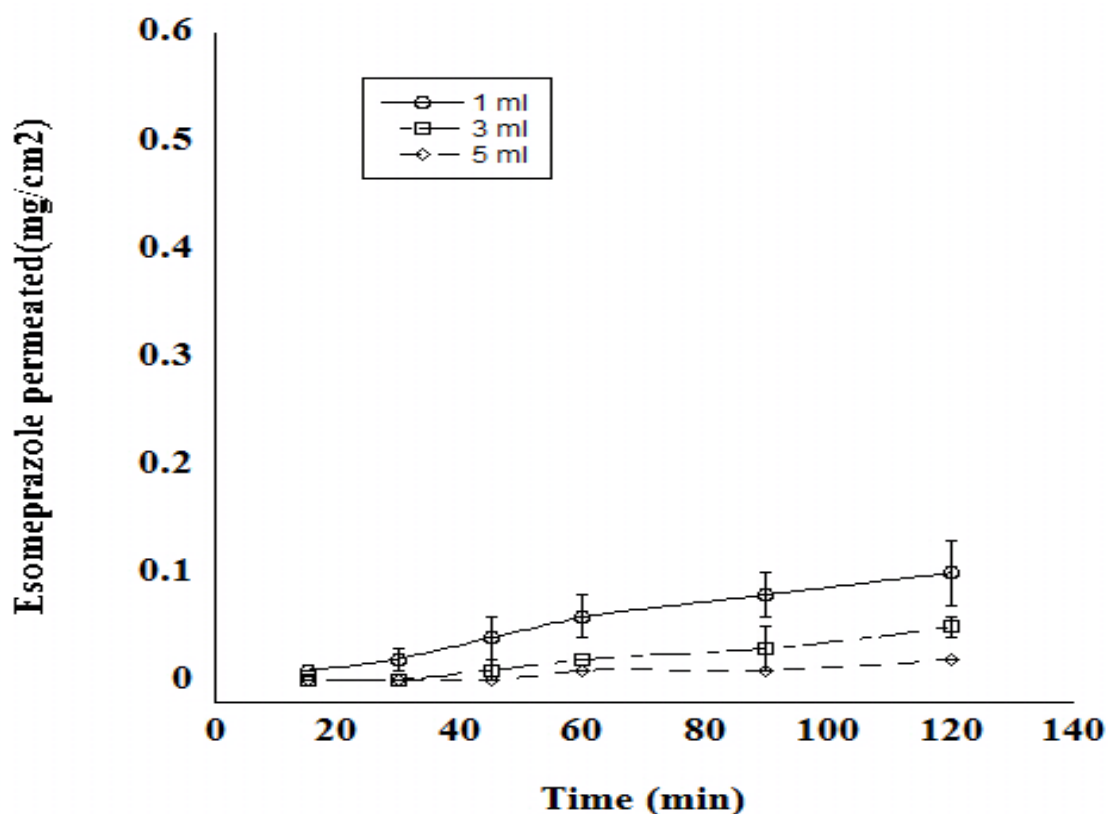


Figure 32. Esomeprazole permeation profile through pig gastric mucosa from 2 mm tablets containing lactose ($n = 3$), from different simulated gastric fluid volumes in the donor compartment: 1 ml (\circ), 3 ml (\square) and 5 ml (\diamond).

Figure 33 show the average value of esomeprazole permeation (mg/cm^2) from 2mm tablets in simulated gastric fluid.

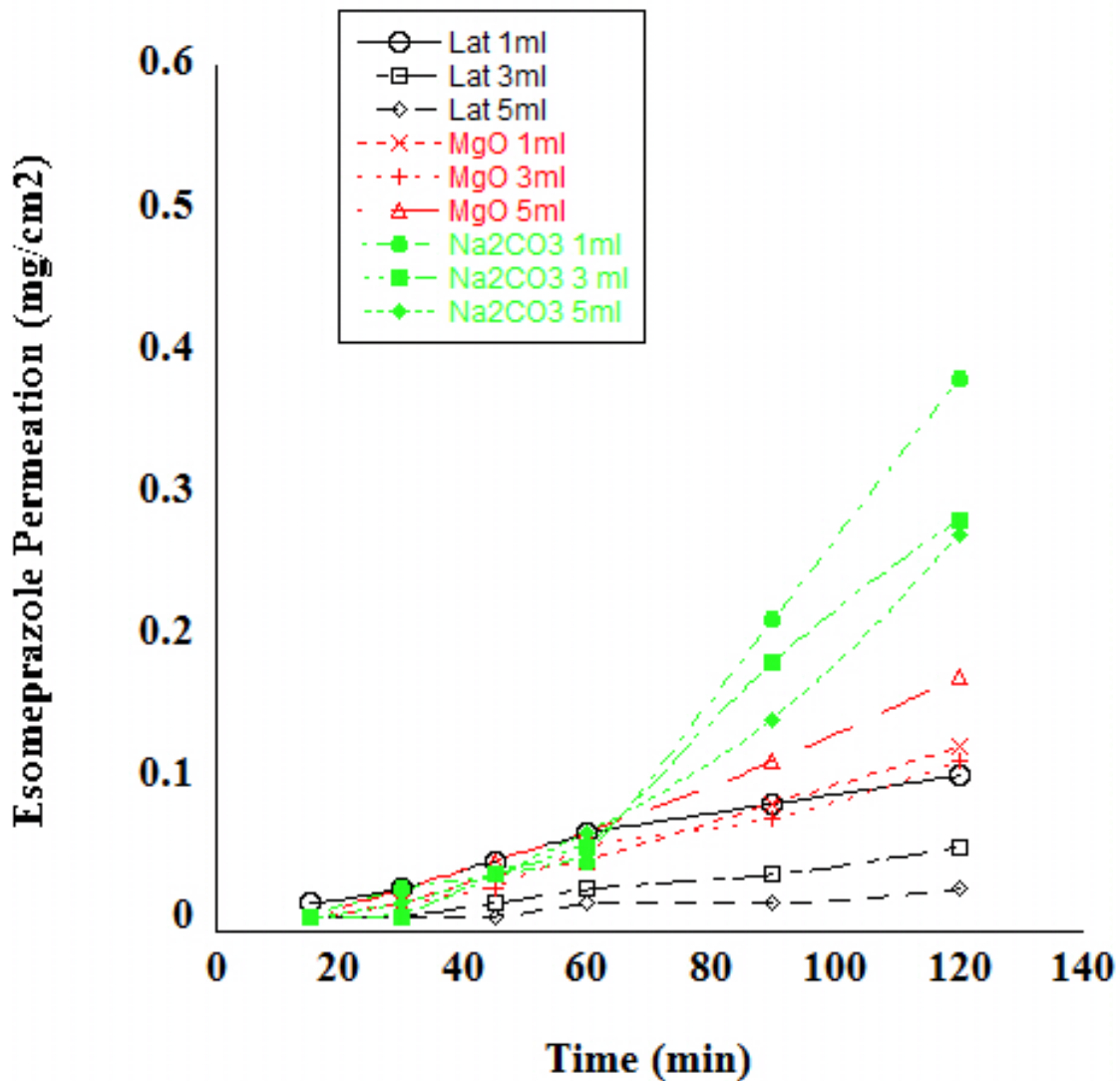


Figure 33. Esomeprazole permeation profile through pig gastric mucosa from 2 mm tablets ($n=3$) from different formulation and simulated gastric fluid volumes.

Because of the results in Figure 25, Figure 29 and Figure 33, sodium carbonate improves esomeprazole permeation through pig gastric mucosa despite its lower alkalisng capacity emerged from the in-vitro dissolution test of esomeprazole modules described in 4.4. Two Way Anova confirmed that the shape of the tablets has no effect on the permeation of the esomeprazole ($p>0.10$).

Therefore sodium carbonate was selected as alkalisng agent for Dome Matrix modules and for 2mm tablets for in-vivo pharmacokinetics study.

4.7 Pharmacokinetics study

Pharmacokinetics studies of esomeprazole immediate release tablets and comparison with esomeprazole gastro-protected tablets and esomeprazole IV.

The purpose of this study was to demonstrate the effectiveness of the release of esomeprazole in the stomach when protected by alkalizing opposed to the more traditional intestine release via gastro-protected tablets.

Esomeprazole as IV injection was used to calculate the bioavailability. Esomeprazole magnesium dihydrate (2.5 mg/ml in saline solution) was dissolved just before the experiment in and it was injected in tail vein to 18 h fasted rats. Intravenous injection dose volumes were determined on the basis of individual body weights.

Esomeprazole 2 mm tablets were manufactured as a direct compression mixture based on formulation ESO carbonate #4ESO adding the 0.9% of blue lake dye which has the function to allow an easier visual survey of the coating process.

The average weight of the tablets was 14.80 ± 1.40 mg; the average weight of the tablets was 2.49 ± 0.03 mm.

The average content of esomeprazole was 0.93 ± 0.01 mg.

4.7.1 Coating

Coating of esomeprazole tablets was done accordingly to the following procedure.

Tablet were initially coated with a cellulose solution (Opadry OY white dispersion) (6% w/v) in a mixture of 2-propanol/water (8:2 ratio) to prevent degradation of esomeprazole during enteric coating application.

The end-point of the process was calculated as an increase of 1% on the weight of the batch of tablets. The Opadry coating of the 2mm tablets used for in vitro permeation studies was 1.39%.

The enteric coating formulation is described in Table 24

Table 24. Enteric coating formulation #3Coat

	mg	% v/v	
Eudragit L100-55	5	5.7	Polymer
Triethylcitrate	0.5	0.6	Plasticizer
Talc	1	1.1	Glidant
Silicon oil	0.5	0.6	Antifoaming agent
Viola lake	0.05	0.1	Coloring agent
2-propanol	39.5	44.7	Solvent
Acetone	39.5	44.7	Solvent
water	2.4	2.7	Solvent

The surface area of the tablets calculated was between 13.62 and 17.58 mm² (lower/higher value). Therefore the lower limit of weight increase(%), calculated accordingly to size and shape of the tablets, was 12% while the upper limit was 15.5%.

The value of weight increase of the batch was 15.1%.

Figure 34 shows the tablets along the coating process from the uncoated to the fully coated tablets.

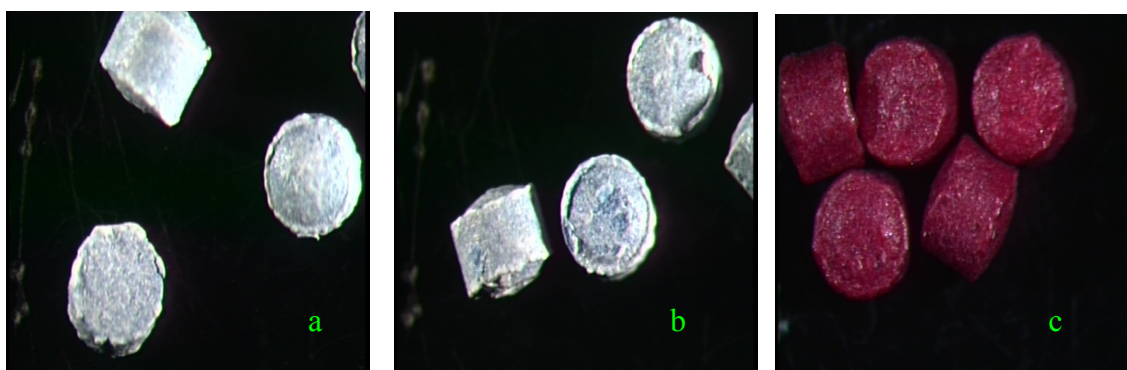


Figure 34. Uncoated tablets(a), tablets coated with Opadry(b), enteric coated tablets(c)

The coating robustness was tested in 0.01N HCl at 37° C for two hours.

The average content of esomeprazole in one tablet was 0.93 ± 0.01 mg.

Esomeprazole release from coated tablets was assessed for in-vitro dissolution. The results in Figure 35 are the average value and standard deviation of 3 tablets. The esomeprazole release was $79.94 \pm 1.63\%$ after 150 minutes: during the first hour (in simulated gastric fluid or SGF) esomeprazole wasn't released due to the enteric coating.

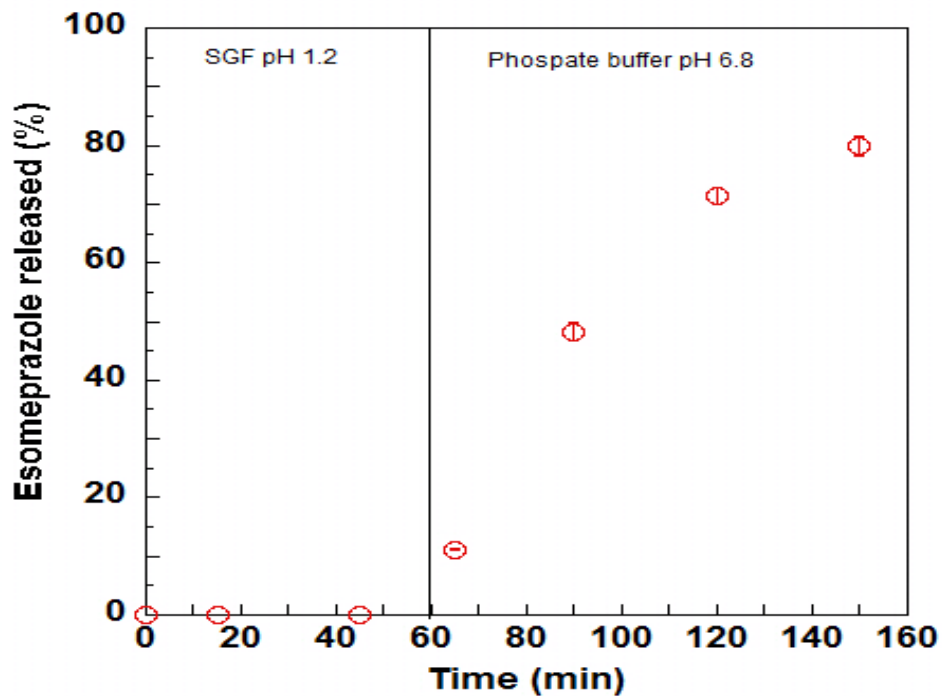


Figure 35. Esomeprazole release from coated tablets of ESONa (Table 22) coated with coating solution #3Coat (Table 24)

4.7.2 Results

Pharmacokinetic of esomeprazole was assayed determining the drug concentration in plasma of the rats receiving the drug. Esomeprazole as IV injection was compared to uncoated tablets of esomeprazole with sodium carbonate and coated tablets.

C_{max} , T_{max} and dose normalized AUC were also calculated. AUC calculations were limited to 120 minutes because the main focus of this work was absorption of the drug in the stomach.

All the results are presented as average value and standard error.

Esomeprazole IV (n=9) showed a C_{max} of $8.80 \pm 0.96 \mu\text{g/ml}$ after 1 minute. The profile is visible in Figure 36.

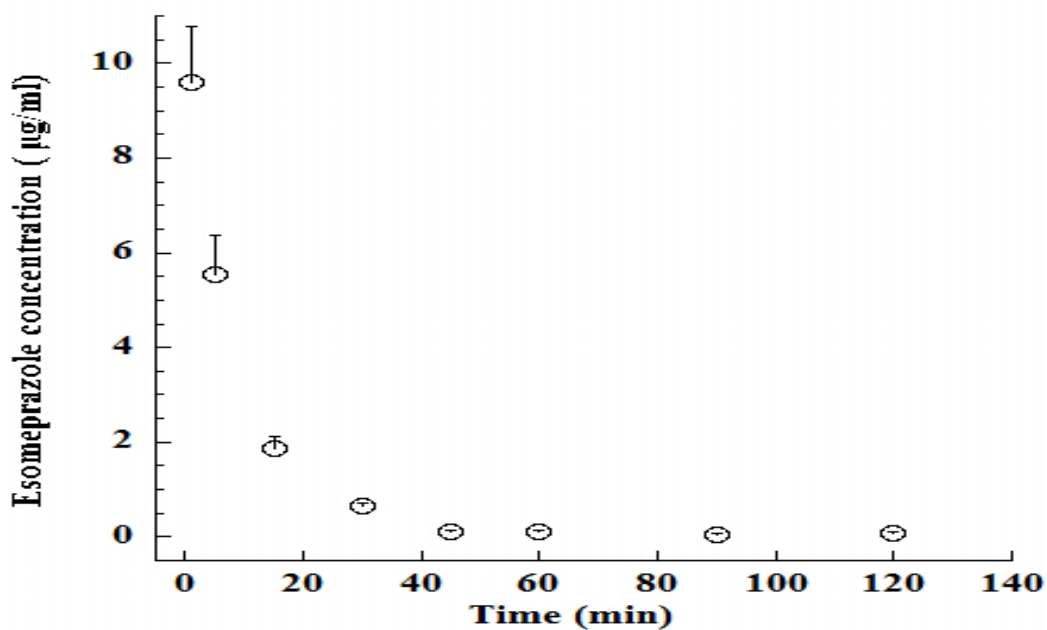


Figure 36. Esomeprazole concentration in plasma after IV injection

Esomeprazole immediate release tablets (n=10) showed a C_{max} of 0.42 ± 0.10 µg/ml after 56 ± 18 minutes. The profile is visible in Figure 37.

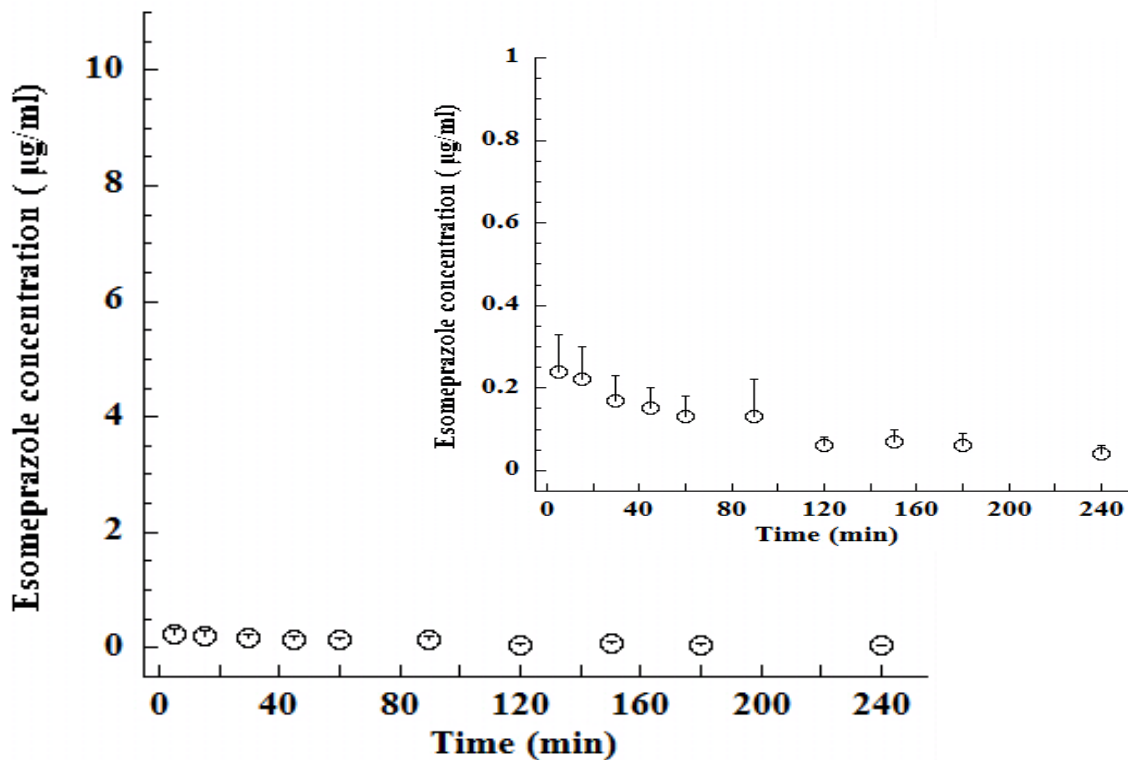


Figure 37. Esomeprazole concentration in plasma for immediate release tablet

Esomeprazole enteric coated tablets (n=10) showed a C_{max} of 0.41 ± 0.15 µg/ml after 140 ± 11 minutes. The profile is visible in Figure 38.

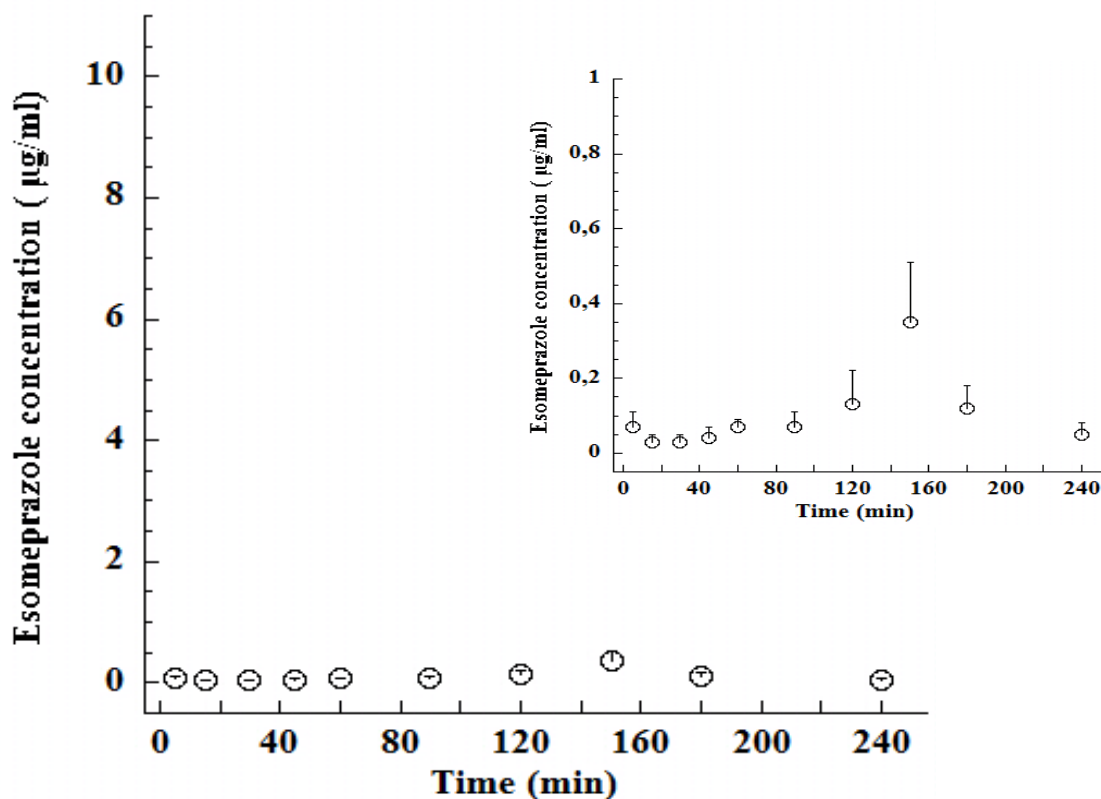


Figure 38. Esomeprazole concentration in plasma for enteric coated tablet

Dose normalized AUC, calculated between 0 and 120 minutes, was 92.65 ± 10.73 for esomeprazole IV, 21.99 ± 6.77 for esomeprazole Na₂CO₃ immediate-release tablets and 9.90 ± 4.58 for esomeprazole enteric coated tablets.

Availability was calculated using esomeprazole IV as a comparison via Eq.4.

$$Bioavailability(\%) = \frac{AUC_X}{AUC_{ESO IV}} * 100 \quad \text{Eq.4}$$

Where X is the dose normalized AUC of the selected dosage form.

Bioavailability(%) was 23.74 for immediate-release tablets and 10.68 for enteric coated tablets. It means that immediate-release tablets can be absorbed twice more than enteric coated tablets in a 2 hours window.

4.8 Dissolution assembled system

Dissolution of the whole assembled system in order to assay esomeprazole release was conducted according to the procedure in 3.2.11.

The experiment were done in triplicate and the results (Figure 39) are expressed as percentage of esomeprazole released calculated on the drug content.

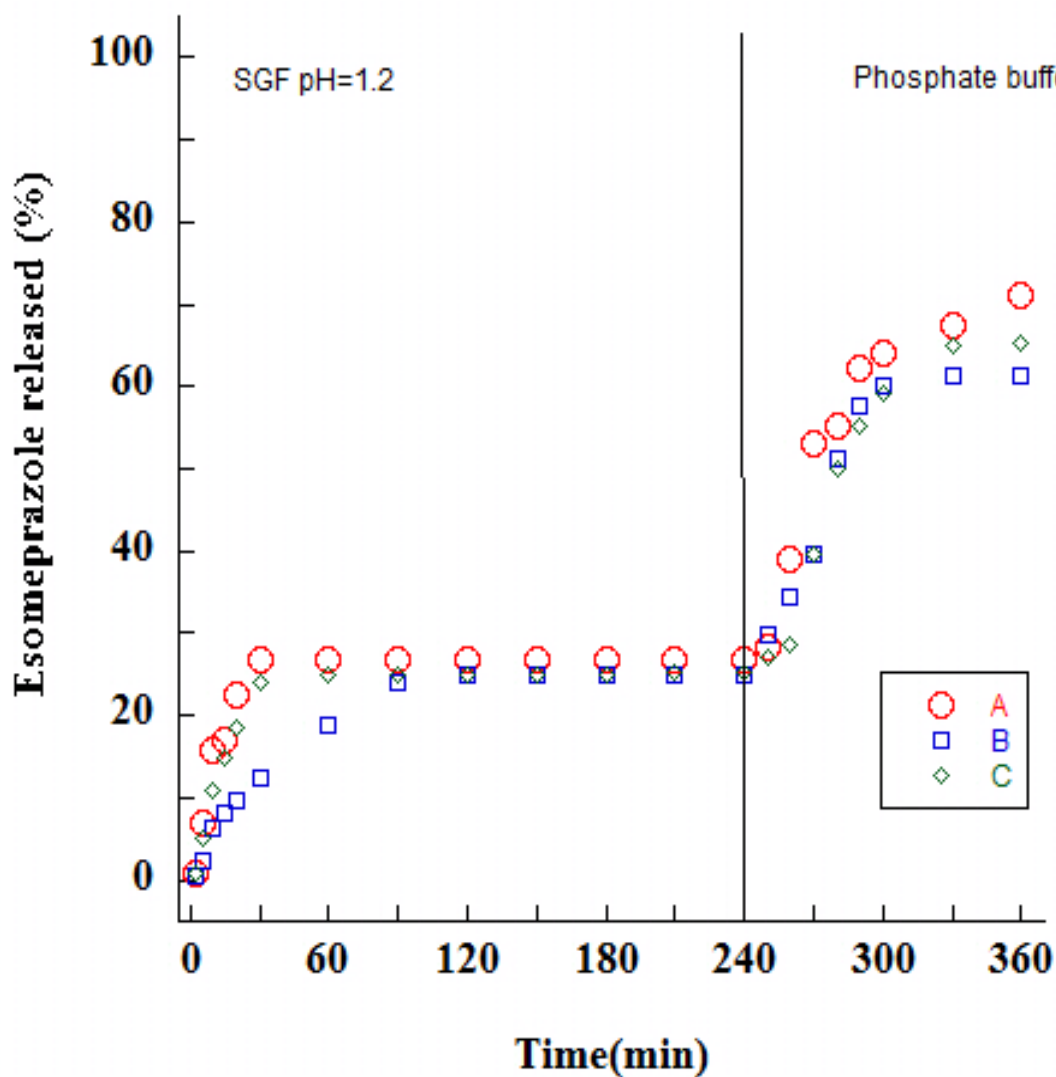


Figure 39. Esomeprazole dissolution of Dome Matrix assembled systems

Esomeprazole release at 360 minutes was the $65.92 \pm 4.98\%$, while the esomeprazole recovered in the cell of the dissolution test apparatus was $2.75 \pm 2.40\%$. Thus the total amount of esomeprazole recovered at the end of the system was the 68.67 ± 2.60 .

The release of the first pulse of esomeprazole is slower and smaller compared to the single module analysis of ESOcarb#4ESO (Table 13). That depends on the presence of the the assembled system which reduces the surface area exposed of the module, thus slowing down the disgregation process which is a key element in the release of the drug.

Besides the floating layer of sucralfate acts as an additional filter prior the recovery and analysis of the sample, increasing the time needed for the sample to come trough the cell.

The amount of esomeprazole recovered is lower because the additional time leads to an higher extent of disgregation.

Between 60 and 240 minutes, esomeprazole wasn't detected: it means disgregation of the second esomeprazole module (assembled in void configuration e coated) didn't took place until the pH value increased leading to the coating disintegration.

The time of the second pulse depend on the dissolution of the coating and the disgregation of the male module: the speed of the release can be compared to the first pulse, but with an higher extent thanks to the stability of the drug in simulated intestinal fluid compared to the simulated gastric fluid. Likewise the first pulse, the second release is affected by the floating layer of sucralfate that delay the sample.

5. Conclusions

In this PhD thesis the main focuses were the applications of the Dome Matrix technology for the controlled release of drug and the advantage of an immediate release formulation for the esomeprazole.

Dome matrix technology proved to be successful in achieving the floating of the void configuration section of the assembled system, despite the disadvantage of the coating, which reduce the floating capability of the dosage form. The inner space of the void system was therefore necessary in achieving the proper kinetics of release.

The permeation test developed allowed an additional screening of the available formulations: this test on an animal tissue revealed the behaviour of the formulation *in-vivo*, which differed from the results of the in-vitro dissolution test.

The results of those tests were confirmed via *in-vivo* test on rats: this proved the robustness and reliability of the animal model utilized.

The release of the esomeprazole in the stomach, which was made possible via its stabilization, led to an innovative dosage form showing also an improved bioavailability of the drug compared to the conventional dosage form.

Appendix I: Analytical methods validations

1. Esomeprazole HPLC method for *in-vitro* tests validation

Esomeprazole was quantified via HPLC method described in 3.2.6. A calibration curve was constructed using 8 concentrations of esomeprazole dissolved in simulated gastric fluid/borate buffer (1:4 ratio) as reported in the Figure 1. Each sample was injected three times.

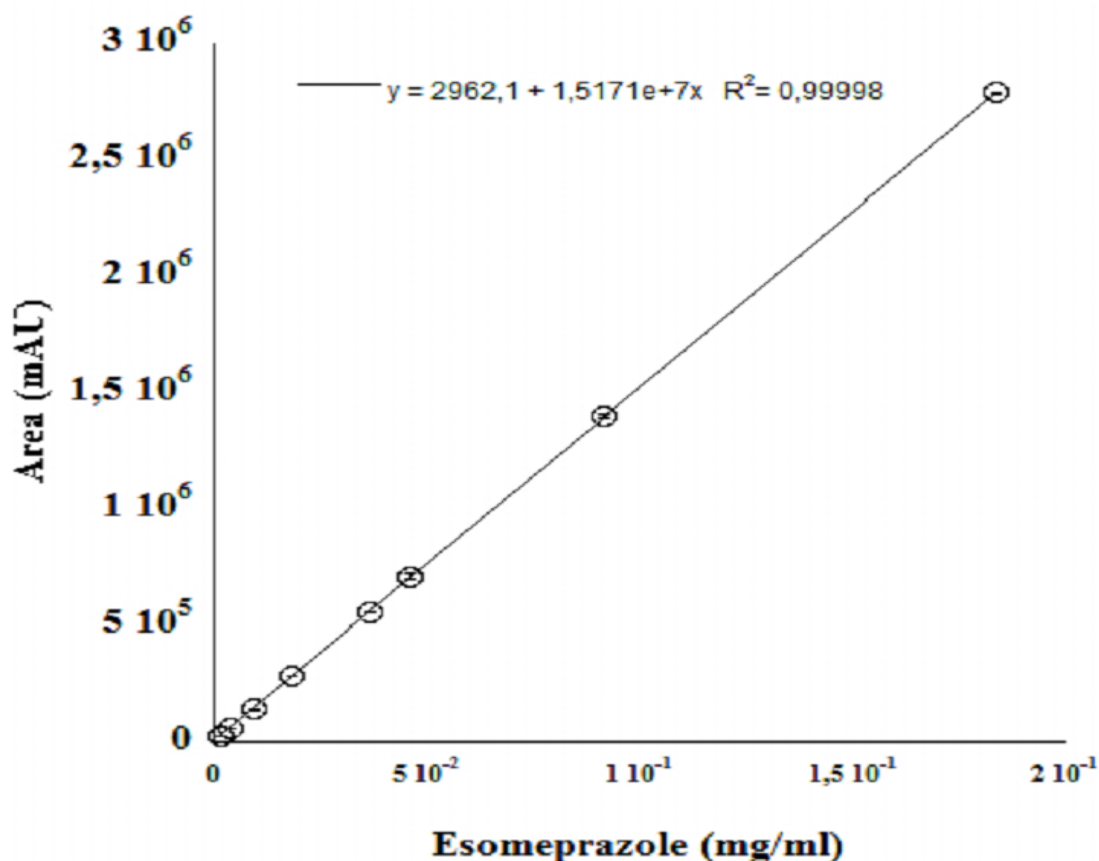


Figure 1. Area versus concentration of esomeprazole in borate buffer/simulated gastric fluid

The correlation coefficient (R^2) for the calibration curve was 0,99998 in the range of 0.002-0.18 mg/ml.

2. HPLC-MS/MS method validation

The analytical method described in 3.12.13.2 was validated in a concentration range of 5-100 ng/ml.

The equation of the regression line was $y = 42181(\pm 1922)x$ with a $R^2=0.98$.

LOD and LOQ were 10 ng/ml and 20 ng/ml respectively, while precision (indicated as %RSD) ranged between 0.4% and 5.6%.

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Chapter II

1. Introduction

1.1 Norfloxacin

Norfloxacin is a synthetic broad-spectrum antibacterial drug firstly selected for the treatment of diseases caused by *Campylobacter*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio Cholera*. [1, 2]

It is also used for the treatment of urinary tract infections.

Norfloxacin is very slightly soluble in water. It is an amphoteric drug (Figure 1) and its solubility increases sharply at pH below 4.0 and above 10.0. [3]

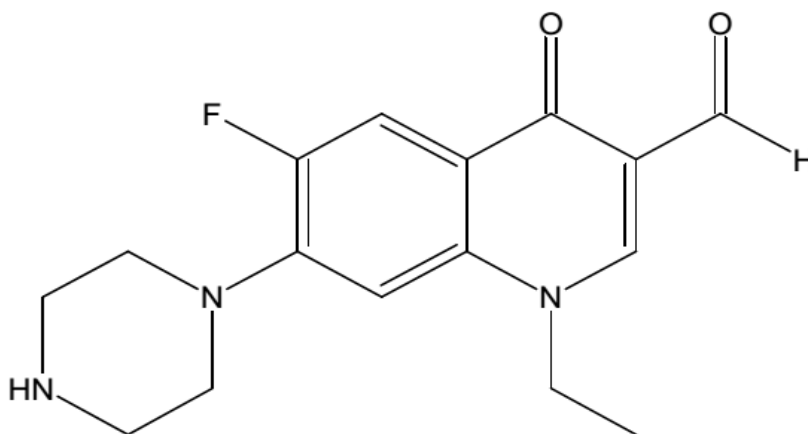


Figure 1. Chemical structure of norfloxacin.

The recommended dosage is 400 mg twice daily for urinary tract infections. The half-life of norfloxacin in serum is 3 to 4 hours. Only approximately 30% to 40% of an oral dose is absorbed and the fecal recovery accounts for 30% of the administered dose.[4]

Norfloxacin is more soluble in acidic media. A prolonged gastric residence of dosage form is expected to lead to an increased in vivo dissolution rate. Dome Matrix modules assembled in void configuration can float for up to 4 h on gastric content. Formulation of norfloxacin into gastro-retentive site-specific drug delivery system is expected to improve the drug bioavailability. [3, 5, 6]

1.2 Calcium alginate

Alginate is a water-soluble linear polysaccharide composed of binary blocks of D-mannuronate (M) and L-guluronate (G) linked by $\beta(1-4)$ and $\alpha(1-4)$ glycosidic bonds, respectively, in varying proportions and sequences, interspersed with alternating MG blocks.

The crosslinkage of alginate with calcium ions leads to its transition from the soluble sodium salt to insoluble calcium alginate. [7]

The concept of calcium alginate crosslinkage has been used for the development of multi-particulate sustained release dosage form.

The use of calcium alginate based dosage form as sustained release vehicle is met with challenges where $\text{Ca}^{2+}/\text{H}^+$, $\text{Ca}^{2+}/\text{Na}^+$ and or $\text{Ca}^{2+}/\text{K}^+$ exchanges in gastrointestinal tract can rapidly induce loss of insoluble crosslinkage which renders matrix erosion and fast drug release.

1.3 Melt agglomeration

Melt agglomeration refers to the process of converting pharmaceutical powder into granules or pellets. It involves agitation of powder in presence of a molten binding liquid or a solid binder which melts due to functional heat of powder agitation. [8-10]

Conventional wet agglomeration of alginate and calcium salt into multi-particulate system results in a porous pre-crosslinked structure that is characterized by fast drug release ref.

Using melt agglomeration, both alginate and calcium salts or the similar will not react during the agglomeration process. These excipients however will be solvated in the gastrointestinal milieu and form crosslinkages thereby resulting in drug release retardation.[10, 11]

2. Objective

The present study aims to design a floating dosage form using calcium alginate as controlled release vehicle.

Dome Matrix assembled in void configuration will be instrumental in achieving the floating of the dosage form.

Norfloxacin was selected as the model drug as it exhibited a high solubility in gastric environment and a low bioavailability at higher pH values.

Effect of polymer-polymer coacervation and cross-linkage of the alginate were also investigated together with physiochemical interaction of excipients in the matrix of release.

The floating of dosage form is envisaged to improve the bioavailability of norfloxacin.

3. Materials and methods

3.1 Materials

- Barium sulphate, Bendonesn, C1363-7727437
- Calcium Acetate, Merck, K41024725
- Calcium Carbonate, Merck, A827266-806 (CaCO₃)
- Calcium Lactate, Aldrich, BCBK1569V
- Chitosan (food grade), Zhejiang Aoxin, NW ID GW13
- Eudragit E PO, Evonik, G110931200
- Norfloxacin, Zhejiang Aoxin, 120401
- Polyethylene glycol 3000 (PEG3000), Merck, S37368-412
- Potassium chloride, Merck, K41042236-026
- Potassium phosphate dibasic (KH₂PO₄), Ajax finechem Pty Ltd, 391-500G
- Potassium phosphate monobasic (K₂HPO₄), Merck A531473-432
- Sodium Alginate (E401) , ISP (UK) limited , 11310736269
- Stearic acid, VWR, 54056899

3.2 Methods

3.2.1 Melt granulation

- Melt granules for placebo modules

The required excipients were mixed in a mortar and they were poured in a glass beaker (75ml capacity). The mixture was heated at 60°C for 60 minutes and cooled under continuous stirring. The molten mass was granulated through a 1mm sieve.

- Melt granules for norfloxacin loaded modules

The required excipients were mixed in a mortar and they were poured in a glass beaker (75 ml capacity). The mixture was heated at 60°C for 45 minutes, the norfloxacin was added under continuous stirring and the mass was mixed using a spoon for 2 minutes. The mixture was subjected to further heating for 15 minutes at

60°C, cooled under continuous stirring and granulated through a 1mm sieve.

- Melt granules for barium sulphate modules

All the excipients were mixed in a mortar and they were poured in a glass beaker (75ml capacity). The mixture was heated at 60°C for 60 minutes, with barium sulphate added and mixed prior cooling to room temperature and granulated through a 1mm sieve.

3.2.2 Manufacture of Dome Matrix modules

- Diameter 7.5 mm

The Dome Matrix modules were manufactured using a single punch tableting machine (TDP15, Shanghai Tiafeng Pharmaceutical Equipment) equipped with 7.5 mm punches.

Each placebo module (male and female) was made with a weighted amount of granules equal to 126.3 mg. Each norfloxacin-loaded module (male and female) was made with a weighted amount of granules equal to 176.3 mg.

Dome Matrix modules were assembled through pushing their respective concave faces together until interlocking via friction was achieved.

- Diameter 3 mm

The Dome Matrix modules were manufactured using a single punch tableting machine (TDP15, Shanghai Tiafeng Pharmaceutical Equipment) equipped with 3mm Dome Matrix punches.

The modules manufactured were female modules and the assembled systems were realized sticking 2 modules together along the concave base using a 30% w/v solution of polyvinylpyrrolidone K90 in ethanol.

3.2.3 Floating test

The floating test was run in 500 ml of USP pH 2.2 buffer to simulate gastric condition. The simulated gastric fluid was placed in a glass bottle and heated at 37°C in a horizontal shaker bath (Mettler, GmbH Company, Germany).

Tested Dome Matrix was first weighted and had its physical dimensions characterized. It was then placed in the dissolution medium and subjected to shaking 50 rpm for 480 minutes.

The matrices were retrieved at 0, 2, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 minutes and had its size examined.

Through the experiment, time to float and the floating duration of Dome Matrix were recorded.

At least triplicates were conducted and results averaged.

3.2.4 Weight/Volume ratio

Weight/Volume ratio (W/V) represents the capability of the dosage form to float because of its porous nature.

This parameter is the up-thrust force resulting from the difference between the upward force due to the mass of water occupied by the module and the weight of the module itself. If the up-thrust force is bigger than the downward force of the weight, the dosage form will float in the medium.

Volume was calculated using a 3D model of the tablet made using SolidEDGE software using the real dimensions of the assembled system: length, width and depth were determined via photo-analysis of the sample.

The W/V ratio is calculated according to Eq.1.

$$W/V \text{ ratio} = \frac{\text{Weight (mg)}}{\text{Volume (mm}^3\text{)}} \quad \text{Eq.1}$$

If the W/V ratio has a value below 1, the dosage form should float immediately.

3.2.5 Module Porosity

The porosity of the module was determined by a mercury porosimeter (AutoPore IV, Micromeritics Instruments Corporation, Norcross, Georgia, USA). The porosimeter characterizes a material's porosity by applying various levels of pressure to a sample immersed in mercury. The pressure required to intrude mercury into the sample's pores is inversely proportional to the size of the pores.

Each sample was analysed in a calibrated pen stem of 3 cm³ (Micromeritics, Pen Stem, Powder 3cc, IV- .39cc).

The test consisted of two different analysis on the same sample:

1. A low pressure analysis, during which a vacuum was created inside the penetrometer followed by mercury flow filling the outer layer of the sample.
2. A high pressure analysis, during which the mercury was pushed into the inner

core of the sample using a compressed air.

At least triplicates were conducted and results averaged.

3.2.6 Swelling of assembled systems

Swelling refers to growth in size of the module due to absorption of water and gelification of the polymer.

The swelling test was run in 500 ml of USP pH 2.2 buffer. The simulated gastric fluid was placed in a glass bottle and heated at 37°C in a horizontal shaker (Memmert, GmbH Company, Germany).

The tested Dome matrix was first subjected to length and width characterization by ImageJ software. It was then placed in the dissolution medium and subjected to shaking at 50 rpm for 480 minutes.

The physical changes of Dome Matrix were photographed at 0, 10, 30, 60, 120, 240, 360 and 480 minutes.

Photographs were analysed using ImageJ software to determine the exact length and width of the system. Swelling is calculated according to eq. 2, using dimensions at t=0 as initial size.

$$Swelling (\%) = \left(\frac{Length_{t=xmin} - Length_{t=0min}}{Length_{t=0min}} + \frac{Width_{t=xmin} - Width_{t=0min}}{Width_{t=0min}} \right) * \frac{100}{2} \text{ Eq.2}$$

The swelling for a module at each sampling time was the average value of horizontal and vertical dimension changes.

3.2.7 Water uptake and erosion of assembled system

Water uptake is the amount of water absorbed into the gelled module at the end of a dissolution test.

Tested Dome Matrix was first weighed and it was then placed in the dissolution medium and subjected to shaking 50 rpm for 480 minutes.

Assembled systems were the recovered at the end of the floating test and the weight of wet matrix was determined after removing its surface moisture by blotting the matrix gently over a dry petri dish.

The assembled systems were then dried in an oven at 40°C for 48 hours and equilibrated to a constant weight by storing in a desiccator at 25°C.

Water uptake was defined in Eq.3.

$$Water\ Uptake\ (\%) = \frac{W_{480min} - W_{dry}}{W_{dry}} * 100 \quad Eq.3$$

where W_{480min} was the weight of the wet matrix after 480 minutes of dissolution while W_{dry} was the weight of the matrix after oven and desiccator drying.

Erosion is the disentanglement and solubilization of the polymer at the end of a dissolution test. It is described in Eq.4.

$$Erosion\ (\%) = \frac{W_{0min} - W_{dry}}{W_{0min}} * 100 \quad Eq.4$$

where W_{0min} was the weight of the matrix at 0 min, while W_{dry} is the weight of the system after oven and desiccator drying to constant weight.

3.2.8 Module wettability

To assess the wettability of a module, a modified water uptake test of section 3.2.8 was developed.

A module was inserted upward in a saddle made of iron coil as shown in Figure 2.



Figure 2. Apparatus for wettability test.

The module was first weighed on an analytical balance. Ten ml of USP pH 2.2 buffer (previously heated at 37°C) were poured on the module using a graduated pipette. After two minutes, the lower part of system was blotted on a paper tissue to remove the excess water.

The system was then dried in an oven at 40°C for 48 hours and equilibrated to a constant weight by storing in a desiccator at 25°C.

The wettability parameter was calculated according to Eq.5.

$$Wettability(\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} * 100 \quad \text{Eq.5}$$

where W_{wet} is the weight of the wet system while W_{dry} is the weight of the system after drying.

3.2.9 High Pressure Liquid Chromatography analytical method

Norfloxacin was analysed using a high pressure liquid chromatography HPLC equipped with a UV-Vis detector (Agilent1200 series, Agilent technology, USA).

The stationary phase was a C18 column (250 mm x 4.6, 5 μ m packing) while the mobile phase was a mixture (7:3 v/v) of potassium dihydrogen phosphate buffer pH 3 and acetonitrile.

The flow rate of mobile phase was 1 ml/min. The temperature of the column was set at 40°C. Sample size was 20 μ l. The detector was operated at a wavelength of 260nm.

3.2.10 Dissolution test

The dissolution test was run in 500 ml of USP pH 2.2 buffer.

The simulated gastric fluid was placed in a glass bottle and heated at 37°C.

Dome matrix assembled systems were first weighed and then placed in the glass bottles. Bottles were placed in a horizontal shaker bath (Mettler, GmbH Company, Germany).

Aliquots were withdrawn at 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes.

Aliquots were filtered through cellulose acetate membrane filter (0.45 μ m) and analysed using HPLC.

Triplicates were conducted and results averaged.

3.2.11 Drug content of modules

Assembled systems at the end of the dissolution test were collected and placed in a 500 ml volumetric flask which was filled with USP buffer pH2.2.

The assembled system was subject to magnetic stirring overnight.

Aliquots were filtered through cellulose acetate membrane filter and analysed using

HPLC.

The total amount of drug was the sum of the amount of drug found in the assembled system after the dissolution test and the amount of the drug released during the dissolution test.

Triplicates were conducted and results averaged.

3.2.12 Similarity factor calculation

The drug dissolution data were statistically analysed and compared using Fit Factor described by Moore and Flanner. Fit Factor is a model independent method that directly compares the difference between percent drug released per unit time for a test and a reference product. The similarity factor (f₂) can be calculated comparing different pairs of dissolution profiles using Eq. 6.

$$f_2 = 50 * \log \sqrt{1 + \frac{1}{n} \sum (R_t - T_t)^2} * 100 \quad \text{Eq.6}$$

where n is the number of dissolution sample times, R_t and T_t represent the mean percent drug released at each time point (t) for the reference and the test products, respectively.

The similarity factor (f₂) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percentage released between curves. In simple terms, f₂ values should be close to 100 (between 50 and 100). When f₂ < 50, two release profiles are considered different.

3.2.13 Molecular interaction

Molecular interaction between excipients and drug was assessed using differential scanning calorimetry and Fourier transform infrared spectroscopy techniques.

Both starting materials and Dome Matrix modules (before and after dissolution test) were analysed.

3.2.13.1 Fourier transform infra-red spectroscopy (FTIR)

Two mg of sample were mixed with 98 mg of dry potassium bromide. The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm⁻¹ over a wavenumber region of 400 to 4000 cm⁻¹ using a FTIR spectrophotometer (Spectrum RX1 FTIR system, Perkin Elmer, USA).

Triplicates were conducted and results averaged.

3.2.13.2 Differential scanning calorimetry (DSC)

DSC thermograms were obtained using a differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA).

Two mg of sample were closed in a standard aluminium pan and heated from 30 to 380°C at a heating rate of 10°C/min under a constant purging of nitrogen at 40 ml/min.

The characteristic peak temperature and enthalpy values of the endotherms or exotherms of samples were recorded. Triplicates were conducted and results averaged.

3.2.14 Scanning Electron Microscopy analysis

The morphological features of the matrices, before and after dissolution test, were examined by means of scanning electron microscopy (SEM) technique (JSM-6360LA, JEOL, Japan). Representative sections were photographed using different magnifications.

The samples were fixed onto an aluminium stub with carbon based glue before being sputter coated with platinum.

The amount of calcium element on the surfaces of samples was examined using the SEM technique with a built-in Energy Dispersive X-ray (EDX) system (JED-2300 series, JEOL, Japan). The prepared studs were viewed using the backscatter electron imaging mode at an accelerating voltage of 10 kV followed by image analysis by means of EDX system.

3.2.15 In vivo floating

In vivo experiments were conducted according to the ethic application presented to the Committee on Animal Research and Ethics.

The barium sulphate loaded assembled systems were used to assess the floating characteristics of the assembled systems in vivo in Sprague-Dowley rats (male, body weight = 383.6 to 536.4 g, n = 18).

One Dome Matrix assembled system was administered to each rat using a modified gavage needle.

The rats were anaesthetized using diethyl ether and X ray images were taken every hour using a Kodak multimodal ImageStation (Field of view=140mm, exposure time= 10 seconds and 2x2 binning) to trace the location of the assembled system in to the gastrointestinal tract with time.

Triplicates were conducted and results averaged.

Image analysis was performed using ImageJ software to remove the background noise and enhance resolution.

3.2.16 Pharmacokinetics

In vivo experiments were conducted according to the ethic application presented to the Committee on Animal Research and Ethics. Pharmacokinetics studies were performed in Sprague-Dowley rats to assess the bioavailability of norfloxacin using Dome Matrix technology compared to conventional dosage form and pure drug.

Without fluid replacement, the maximum blood volume which can be safely removed for a one time sample is 10% of the total blood volume or 5.5-7 ml/kg. For a 350 g rat, this is equivalent to 1.9-2.5 ml. With fluid replacement, up to 15% of the total blood volume or approximately 8.3-10.5 ml/kg can be removed. For a 350 g rat, this is equivalent to 2.5-3.2 ml. Therefore up to 3.5 ml (7 sampling points of 0.5 ml each) of blood can be collected during a pharmacokinetic study using a 400 g rat.

Three different formulations were tested:

- Norfloxacin solution
- Non-gastroretentive dosage form
- Dome Matrix assembled system

Two separated dome matrix modules (administered at the same time) were used as a non-gastroretentive dosage form.

Rats (n=6 per group) were fasted for 12 hours before the test.

The animal was restrained using a plastic restrainer in order to administer the norfloxacin solution using a gavage needle, while solid matrices by means of a modified version of a gavage needle equipped with a plastic sample holder.

To collect blood samples, rats were anaesthetized using diethyl ether. They were then put in a prone position with its tongue extended using a Q tip and a plastic

pincer.

The sublingual veins were visible on both sides of the median line. The blood vessel was punctured using a 25 gauge needle.

The rat was reversed in supine position and inclined over an eppendorf vial. At the same time, the skin of the neck was gently grasped to ensure congestion and speed up the blood collecting process. The bleeding was stopped via tapping the tongue with gauze and releasing the skin of the neck.

Both sides of the tongue were used alternatively moving toward the basal part of the tongue, in order not to use the same position several times which can lead to edema. The volume of blood collected was replaced with 0.5ml of warm physiological solution under skin.

The blood samples were withdrawn at different intervals according to the formulation:

- Pure drug: 30 minutes, 1, 2, 3, 4 and 6 hours.
- Non gastroretentive dosage form: 1, 2, 4, 6, 8 and 10 hours.
- Dome Matrix system: 1, 2, 4, 6, 8, 10 and 12 hours.

Blood samples (0.5 ml), collected in an EDTA-coated eppendorf, were centrifuged at 5000 rpm for 30 min.

The plasma was kept in a freezer (-20°C) until analysis. Prior to the analysis, the samples were thawed and an aliquot of methanol (0.5 ml) was added.

The mixture was then vortexed for 20 min and centrifuged at 5000 rpm for 30 min. A volume of 0.5 ml of the supernatant was diluted using the same amount of mobile phase before subjecting to HPLC analysis for norfloxacin content.

- HPLC analytical method for in-vivo pharmacokinetics studies

Norfloxacin in plasma was analysed using a HPLC equipped with a UV-Vis detector (Agilent, 1200 series).

The stationary phase was a C₁₈ column (250mm x 4.6, 5µm) while the mobile phase was a mixture (7.5:2.5 v/v) of acetonitrile and phosphate buffer (0.05M) adjusted to pH 3.

The flow rate of mobile phase was 1.2 ml/min and sample size was 20 µm.

The detector was operated at a wavelength of 279 nm.[12]

4. Results and discussion

The initial part of the work focused on the manufacture of the placebo tablets for use as drug carrier.

Such formulation should float immediately and it should also stay afloat for 8 hours.

In order to achieve this goal, the matrix should present:

- high swelling and low water uptake at the same time in order to keep density of the system as low as possible, thus improving floating capabilities;
- low erosion, in order to effectively control the release of the drug.

The Dome Matrix systems were manufactured according to the procedure described in 3.2.1. using granules prepared in accordance to the procedure described in 3.2.1.1.

The floating, swelling, erosion and water uptake profiles of matrices were tested according to the procedure described in 3.2.3; Swelling, water uptake and erosion test were performed as described in 3.2.6 and 3.2.7.

4.1 Preliminary formulations

The first formulation was based on previous works involving calcium alginate as polymer for controlled release in multi-particulate dosage forms.[10]

Sodium alginate is the water-soluble polymer, PEG3000 is the meltable binder while calcium carbonate (CaCO_3) has a double roles: it serves as a source of calcium ions for matrix crosslinking and slowing drug release, it is also a gas forming agent which may increase porosity of the matrix, aid floating but induce drug release.

Dome matrix assembled systems were tested because single dome matrix modules cannot achieve floating.

In order to underline the role of the calcium carbonate, different formulations containing lower and higher amounts of calcium carbonate were tested. Three formulations were produced and examined for their floating property (Table 1).

The weight was kept constant for all the modules, therefore the amount of sodium alginate was reduced in order to accommodate the carbonate salt.

Table 1. Formulations of calcium carbonate loaded assembled systems.

	A (mg)	B(mg)	C(mg)
PEG3000	32	32	32
Calcium carbonate	25.4	12.7	6.4
Sodium alginate	62.6	75.3	81.6
Floating time (min)	25 ± 23	7 ± 3	0 ± 0
Time afloat (min)	180 ± 210	443 ± 51	169 ± 51

Formulation B shows a longer floating time, so the formulation was kept as basis for the subsequent formulation development.

4.2 Role of calcium carbonate

To point out the need for the calcium carbonate, 2 different formulations were tested: one containing only alginate and PEG3000, while the second one was introduced with calcium carbonate. Those formulations are shown in Table 2.

Because of manufacturing issues due to the small amount of powder in the dye of the tableting machine, especially in relation to the shape of the Dome Matrix punches, the amount of sodium alginate was increased to improve the filling of the dye during the compression process.

Therefore the weight of the tablet was increased from 120mg to 126.3 mg as stated in section 3.2.2.

Table 2. Formulations of Dome matrix with and without calcium carbonate.

	D (mg)	E (mg)
PEG3000	32	32
CaCO ₃	0	12.7
Sodium alginate	94.3	81.6

Table 3 shows the swelling, erosion, water uptake and floating profiles of alginate dome matrix with and without calcium carbonate.

Table 3. Swelling, water uptake and erosion test of alginate Dome matrix with and without calcium carbonate.

	Floating time (min)	Time afloat (min)	Swelling (%)	Water uptake(%)	Erosion (%)
D	27.33 ± 29.69	192.67 ± 63.57	188.09 ± 29.87	2226.70 ± 359.07	61.56 ± 11.44
E	2.33 ± 2.52	177.67 ± 2.52	173.95 ± 31.97	2131.25 ± 432.55	42.50 ± 11.10

Crosslinkage of alginate by Ca^{2+} of calcium carbonate reduced swelling, erosion and water uptake extent of Dome matrix (Table 3 and Figure 3).

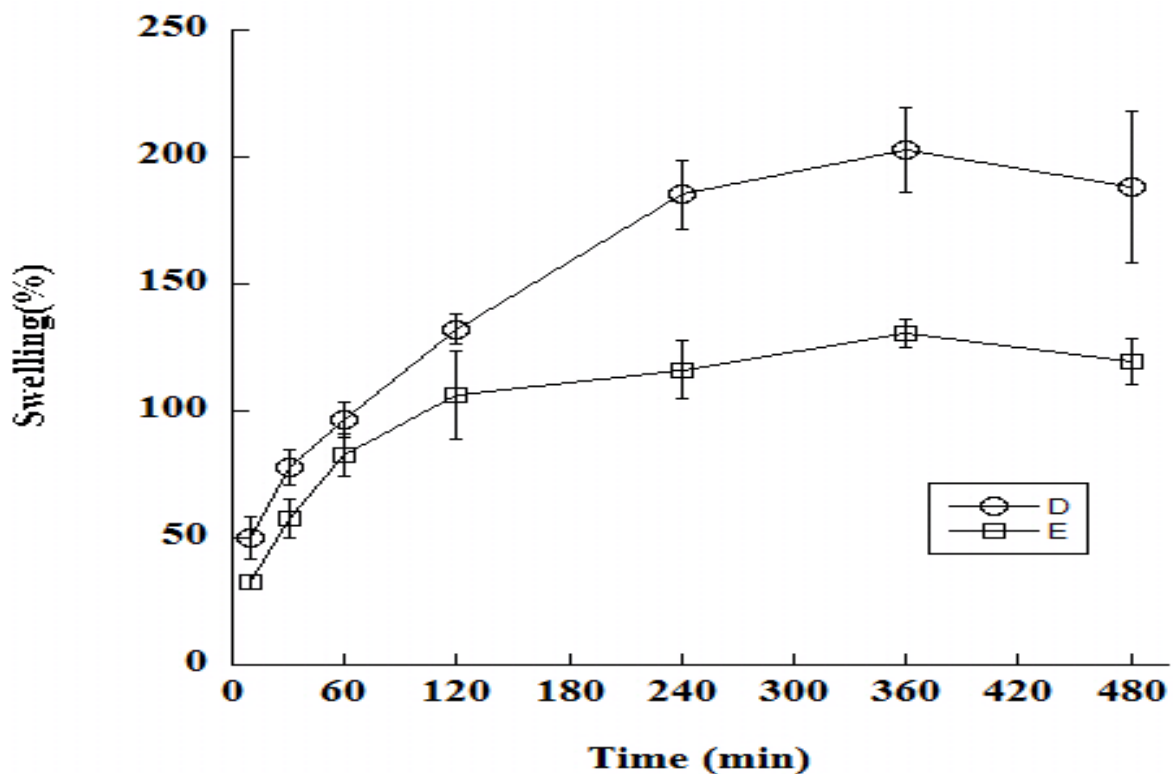


Figure 3. Swelling profiles of D and E.

Time for matrix to float was greatly reduced when calcium carbonate was loaded into the matrix and a more consistent floating duration was obtained. This can be possibly attributed to matrix porosification by calcium carbonate that released carbon dioxide as porogen in simulated gastric medium, in addition to reduced erosion and water uptake.

4.3 Coacervation

Coacervation of alginate with polyelectrolyte polymer was tested in order to increase the robustness of the modules and therefore improving floating capabilities and controlled drug release.

Two different polymers were selected: chitosan (Chit) and Eudragit E PO (EU).

Both polymers are soluble when in contact with gastric fluid and they presents protonated amino groups that may react with the negatives charges of the alginate.

Different ratios of chitosan/alginate and eudragit/alginate were tested (Table 4).

Table 4. Formulations used in alginate-chitosan and alginate-eudragit coacervate matrix.

	LowChit (mg)	HighChit (mg)	VeryHighChit (mg)	EU (mg)	HighEU (mg)
PEG3000	32	32	32	32	32
CaCO ₃	12.7	12.7	12.7	12.7	12.7
Sodium alginate	72.8	66.5	55.5	66.5	55.5
Chitosan	8.8	15.1	26.1	0	0
Eudragit E PO	0	0	0	15.1	26.1

Chitosan is a well known disintegrating agent in multi-particulate system.[13, 14]

Formulation using an excessive amount of chitosan (VeryHighChit formulation) disaggregated after 20 minutes and it was therefore discarded in subsequent physiochemical testing.

Floating, swelling, erosion and water uptake profiles of alginate-chitosan and alginate-eudragit Dome matrices are shown in Table 5.

Table 5. Floating, swelling, erosion and water uptake profiles of alginate-chitosan and alginate-eudragit Dome matrices.

	Floating time (min)	Time afloat (min)	Swelling(%)	Water uptake(%)	Erosion (%)
LowChit	20.0 ± 17.3	140 ± 34.6	209.6 ± 26.1	2137.1 ± 121.9	41.5 ± 11.1
HighChit	0	177.7 ± 2.5	187.9 ± 15.3	1791.6 ± 20.4	40.9 ± 9.7
EU	0	213.3 ± 23.1	101.0 ± 25.9	611.0 ± 84.3	38.7 ± 5.9
HighEU	0	181.7 ± 4.1	89.8 ± 21.7	584.4 ± 54.0	39.0 ± 5.2

The alginate-eudragit coacervated Dome matrices exhibited reduced swelling, erosion and water uptake, increased duration of floating when compared to alginate-chitosan coacervate Dome matrices (Table 5, Figure 4)

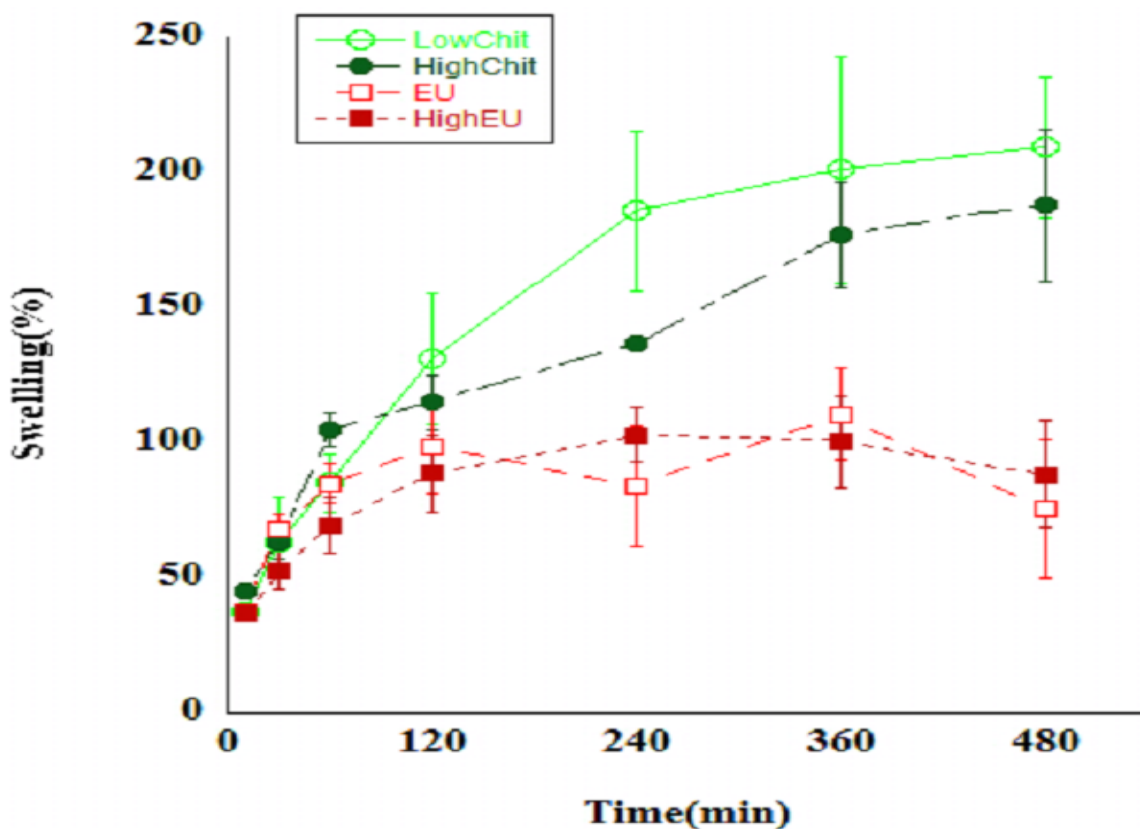


Figure 4. Swelling profiles of alginate-chitosan and alginate-eudragit coacervated Dome matrices.

One possible reason was that eudragit was less hydrophobic than chitosan. Through

reduced erosion and specifically reduced water uptake, the floating behaviour of alginate-eudragit Dome matrices was improved.

4.4 Cross-linkage

Dome matrix strengthening was further examined through using calcium salts of higher aqueous solubility than carbonate salt.

The salts selected were calcium acetate (CA) and calcium lactate (CL) (Table 6). Calcium acetate has a solubility of 34.7 mg/ml in water while calcium lactate has a solubility of 7.9 mg/ml.[15]

Both salts show higher acid solubility compared to calcium carbonate, with calcium acetate has a higher solubility compared to calcium lactate. They were employed in association with the calcium carbonate without any modification to its amount.

Table 6. Calcium acetate/lactate loaded formulations

	LowCL(mg)	HighCL(mg)	CA(mg)
PEG3000	32	32	32
CaCO ₃	12.7	12.7	12.7
Sodium alginate	75.2	62.5	75.2
Calcium lactate	6.4	19.1	0
Calcium acetate	0	0	6.4

The formulation with calcium acetate disintegrated in less than 20 minutes. The same happened with HighCL formulation. This was attributed to high water solubility of calcium acetate and lactate, leading to matrix disintegration in short times.

Table 7. Floating, swelling, erosion and water uptake profiles of LowCL assembled system.

	Floating time (min)	Time afloat (min)	Swelling (%)	Water uptake(%)	Erosion (%)
LowCL	5.67 ± 4.04	226 ± 136.72	57.37 ± 7.95	591.84 ± 11.82	62.69 ± 3.57

LowCL formulation was deemed unsuccessful because it showed higher erosion and high variability in the time afloat.

For those reasons, eudragit coacervated formulation(EU) was selected as basis for norfloxacin loaded system development.

4.5 Floating time

All the assembled systems in Table 4 and 6 showed a W/V ratio, calculated according to the method described in 3.2.5, equal to 0.737 ± 0.042 .

Those systems were supposed to float immediately thanks to the low density feature of Dome Matrix, but it didn't happen. A porous system might not float as the weight of the system could have overcome its floating capability. A matrix system, showing low pores size and low metastability, might float successfully because of low wettability that prevented water entry into the pores. This was characterized by reduced W/V ratio during floating.

Formulations, that were able to withstand after 480 minutes of dissolution, were selected. The selected formulations are reported in Table 8.

Table 8. Formulations selected for wettability and porosity studies

	LowChit (mg)	HighChit (mg)	EU (mg)	HighEU (mg)	LowCL (mg)
PEG3000	32	32	32	32	32
CaCO ₃	12.7	12.7	12.7	12.7	12.7
Sodium alginate	72.8	66.5	66.5	55.5	75.2
Chitosan	8.8	15.1	0	0	0
Eudragit E PO	0	0	15.1	26.1	0
Calcium lactate	0	0	0	0	6.4

4.5.1 Wettability test

In order to assess wettability, the method described in 3.2.9 was developed.

The aim of the method was to assess the wettability on the entire module without cutting or crushing it as it would have been necessary for the contact angle test.

The module wettability results are shown in Table 9.

Table 9. Wettability of modules.

Formulation	Wettability (%)
LowChit	31.02 ± 10.32
HighChit	32.55 ± 11.24
EU	15.66 ± 9.87
HighEU	31.02 ± 2.19
LowCL	31.02 ± 2.48

The wettability was successfully correlated to the components of the formulation as shown in Table 10, where the WettabilityEQ indicated the wettability value reconstructed using the mathematical model indicated in Table 10.

Table 10. Multivariate regression of wettability parameter of the formulations reported in Table 8

Wettability	R2=	0,9814			
	R2adj=	0,97753			
	F=	253,29			
	p=	0,000000			
	Coeff.	Std.err.	t	p	R²
Constant	-57,281	21,833	-2,6235	0,014889	0
Chit	0,26965	0,049679	5,4278	1,4127E-05	0,24587
CaCO3	-0,94785	0,10171	-9,3192	1,9127E-09	0,33999
PEG	2,255	0,33114	6,8098	4,8218E-07	0,01619
EU	-0,7721	0,041026	-18,82	7,0869E-16	0,75191
Lactate	-0,8975	0,14432	-6,2188	1,9943E-06	0,066736
	Wettability	Wettability EQ	Residual		
LowChit	67,20	66,53	0,67		
HighChit	75,19	74,62	0,56		
EU	36,27	40,78	-4,51		
LowCL	60,08	55,63	4,45		
HighHE	28,09	25,49	2,60		
			3,77		

Wettability value reconstructed from the model (wettabilityEQ) was compared to the measured wettability. Residual value indicates the goodness of fitting.

Therefore the wettability was related to the components of the formulation

4.5.2 Mercury porosimetry

Porosity and pore size of the modules were assayed according to the procedure described in 3.2.6 (Table 11).

Table 11. Results of mercury porosimetry

Formulation	Porosity (%)	Average pore diameter (μm)
LowChit	22.5 \pm 5.6	26.48 \pm 3.08
HighChit	30.2 \pm 4.3	28.53 \pm 3.15
EU	31.2 \pm 6.9	54.07 \pm 11.30
HighEU	30.2 \pm 7.1	50.45 \pm 1.53
LowCL	17.4 \pm 2.1	48.48 \pm 5.58

Wettability, porosity and pore size can be correlated to floating time using multivariate regression. The results are visible in Table 12, where FloatingEQ (table 12) is the floating time value reconstructed from the model.

Table 12. Multivariate regression of floating time versus wettability, porosity and pore size of formulations reported in Table 8

Wettability	R2=	0,9814			
	R2adj=	0,97753			
	F=	253,29			
	p=	0,000000			
	Coeff.	Std.err.	t	p	R²
Constant	-57,281	21,833	-2,6235	0,014889	0
Chit	0,26965	0,049679	5,4278	1,4127E-05	0,24587
CaCO3	-0,94785	0,10171	-9,3192	1,9127E-09	0,33999
PEG	2,255	0,33114	6,8098	4,8218E-07	0,01619
EU	-0,7721	0,041026	-18,82	7,0869E-16	0,75191
Lactate	-0,8975	0,14432	-6,2188	1,9943E-06	0,066736
	Wettability	Wettability EQ	Residual		
LowChit	67,20	66,53	0,67		
HighChit	75,19	74,62	0,56		
EU	36,27	40,78	-4,51		
LowCL	60,08	55,63	4,45		
HighHE	28,09	25,49	2,60		
			3,77		

The R^2 of the model is low compared to the previous one: it means that the model doesn't fit properly to the existing data.

Using existing parameters, like swelling at the time of the floating of the assembled system, did not improve the model as shown in Table 13.

Table 13. Multivariate regression of floating time versus to wettability, porosity, pore size and swelling after 10 minutes of formulations reported in Table 8

Floating	R ² =	0.641
	R ² adj=	0.530
	F=	5.801
	p=	0.0066

	Coeff.	Std.err.	t	p	R ²
Constant	85.28	36.39	2.34	0.03564	0.000
Pore Size	-0.12	0.51	-0.24	0.81631	0.260
Porosity	-1.63	0.51	-3.19	0.00709	0.239
Wettability	0.24	1.00	0.24	0.81234	0.173
Swelling 10min	-0.89	0.57	-1.56	0.14334	0.002

Floating Time	FloatingEQ	Residual
20.00	19.60	0.40
0.00	0.24	-0.24
0.00	-1.41	1.41
0.00	0.72	-0.72
5.67	5.80	-0.13
		0.72

The R^2 of the model is low and it doesn't fit properly to the existing data.

4.6 Preliminary results on placebo tablets

The work on placebo modules led to several conclusions:

- Use of chitosan was characterized by a high water uptake and negatively affected the time afloat.
- Calcium lactate, despite improving the floating, makes results unreliable. This could be due to erosion/disintegration prone of such formulation.
- Eudragit was the best polymer of choice.

The behaviour of the formulation reported in Table 8 can be correlated to the components of the modules after normalization as shown in Table 14,15 and 16).

Table 14. Multivariate regression of swelling versus components of formulations in Table 8.

Swelling (%)	R2=	0.91179			
	R2adj=	0.86367			
	F=	18.95			
	p=	0.00311			
	Coeff.	Std.err.	t	p	R2
Constant	19,703	292,18	0,067435	0,94745	0
Sodium alginate	-18356	7147,8	-2,5681	0,02614	0,024011
Chitosan	-1101,7	432,65	-2,5464	0,027169	0,69134
CaCO3	6472,8	5152,4	1,2563	0,23504	0,2232
PEG3000	16636	9829,8	1,6924	0,11866	0,22146
Eudragit	-3222,4	1241,3	-2,596	0,024869	0,24051
Calcium lactate	-325,97	120,11	-2,7139	0,020151	0,24528

Swelling after 480 minutes of dissolution depends mostly on PEG3000 and Eudragit: the first increasing the swelling, probably thanks to his hydrophilic, while Eudragit affect swelling negatively, maybe because of its effect on the strength of the matrix.

Table 15. Multivariate regression of water uptake versus components of formulations in Table 8.

Water Uptake(%)	R2=	0.96399			
	R2adj=	0.94435			
	F=	49.077			
	p=	2.63E-007			
	Coeff.	Std.err.	t	p	R2
Constant	-861.29	2097.7	-0.41058	0.68926	0
Sodium alginate	-2,486E05	51317	-4,8443	0,00051545	0,12613
Chitosan	-14927	3106,2	-4,8054	0,0005486	0,77927
CaCO3	44229	36992	1,1956	0,25697	0,18994
PEG3000	2,6915E05	70573	3,8138	0,0028739	0,18599
Eudragit	-43623	8911,7	-4,895	0,00047536	0,44991
Calcium lactate	-4329,3	862,34	-5,0204	0,00038978	0,093144

Water uptake depends largely on Chitosan and Eudragit: chitosan seems to reduce the water uptake value mostly because of its disintegrating effect.

Table 16. Multivariate regression of erosion versus components of formulations in

Table 8 .

Erosion (%)	R2=	0.86745
	R2adj=	0.79515
	F=	11.998
	p=	2.86E-004

	Coeff.	Std.err.	t	p	R2
Constant	170.05	61.534	2.7635	0.01844	0
Sodium alginate	1490,1	1505,3	0,98991	0,34349	0,44732
Chitosan	91,132	91,118	1,0002	0,33873	0,016752
CaCO3	-2581,9	1085,1	-2,3794	0,036543	0,0028714
PEG3000	584,38	2070,2	0,28228	0,78297	0,004012
Eudragit	259,63	261,42	0,99315	0,34198	0,26332
Calcium lactate	29,543	25,296	1,1679	0,26754	0,7546

Erosion depends on PEG3000 and Eudragit: Eudragit reduce the erosion while PEG3000 increase it. PEG3000 is a poring agent and pores makes the matrix more susceptible to water uptake and therefore erosion.

While swelling, water uptake and erosion can be described using the formulation components, floating time cannot.

Therefore floating time was deemed to be dependent on mechanical characteristics of the module, like porosity, short time swelling and probably short time water uptake and the gelification speed of the polymer itself.

4.7 Manufacture of Norfloxacin-loaded modules

The EU formulation described in Table 4 was loaded with 50 mg of norfloxacin (Table 17). Since an assembled system is composed of two modules, the total amount of norfloxacin is 100mg. Its comparison with the placebo system (Table 4) is shown in Table 17.

Table 17. Norfloxacin loaded EU module.

	mg
Norfloxacin	50
PEG3000	32
CaCO3	12.7
Sodium alginate	66.5
Eudragit E PO	15.1

Table 18. Floating, swelling, water uptake and erosion of placebo and norfloxacin loaded Dome Matrices.

	Floating time (min)	Time afloat (min)	Swelling(%)*	Water uptake(%)*	Erosion (%)*
EUplacebo	0	213 ± 23	101.0 ± 25.9	611.0 ± 84.3	38.7 ± 5.9
Norfloxacin-loaded EU	-	0 ± 0	92.9 ± 7.6	678.8 ± 86.4	38.9 ± 6.5

*The measurement was made based on the major pieces after 360 min.

The drug loaded formulation didn't float and partially broke down after 360 minutes of dissolution test (Table 18).

Swelling and erosion were similar for the formulations in Table 18. That could mean that the presence of norfloxacin doesn't affect the strength of the matrix formed by the alginate.

4.8 Use of stearic acid as binder

To ensure norfloxacin loaded Dome matrix systems float with minimum disintegration, a hydrophobic low density binder was proposed. Stearic acid as lipophilic binder was used (Table 18) to improve the floating of the system, replacing the PEG 3000 used previously (Table 16). The low density attribute of stearic acid (compared to the PEG 3000) can also help the system to stay afloat. The amount of sodium alginate was kept constant and a higher amount of stearic acid was matched with a reduction in Eudragit content in order to keep the weight constant.

The formulations are shown in Table 19 and the comparison between them is in Table 20.

Table 19. Norfloxacin loaded EU module using stearic acid as binder

	Hstea(mg)	LowEU(mg)	NoEU(mg)
Norfloxacin	50	50	50
Stearic acid	32	38.3	47.1
CaCO ₃	12.7	12.7	12.7
Sodium alginate	66.5	66.5	66.5
Eudragit E PO	15.1	8.8	0

Table 20. Floating, swelling, water uptake and erosion profiles of formulations using stearic acid as binder.

	Floating time (min)	Time afloat (min)	Swelling(%)	Water uptake(%)	Erosion (%)
Hstea	0 ± 0	240 ± 60	84.74 ± 3.07	405.32 ± 26.45	8.33 ± 1.06
LowEU	0 ± 0	340 ± 35	68.60 ± 7.52	290.29 ± 12.88	12.01 ± 1.03
NoEU	0 ± 0	420 ± 104	101.62 ± 3.67	421.47 ± 61.00	20.45 ± 7.86

All the formulations floated instantaneously. NoEU formulation, despite having the higher time afloat, presented the same problem as calcium lactate formulation, ie low float reproducibility even in different batches (any 2 over 3 tablets managed to float up to 480minutes (Table 20).

A low content of stearic acid can lead to a low matrix floating and erosion because it reduced the matrix wettability, but an increase in stearic acid content may induce erosion because the matrix tended to erode as separation between the hydrophilic domain of alginate and the lipophilic domain of stearic acid took place.[16-19]

4.9 Norfloxacin release

The norfloxacin release profile of Hstea and LowEU were similar with a F2 value of 85.7 (Figure 5).

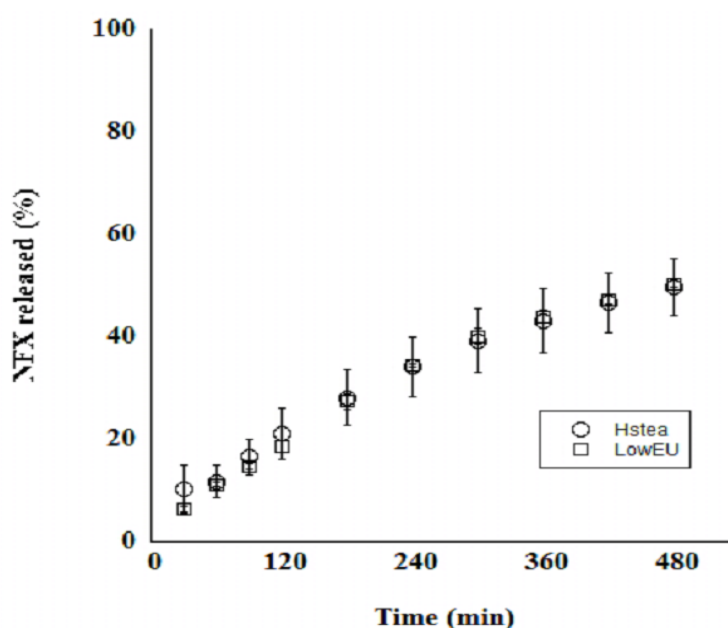


Figure 5. Norfloxacin release profiles of Hstea and LowEU assembled systems.

Figure 6 shows the thermogram of the raw materials used during modules manufacture, while Figure 7 shows the infrared spectra of the raw materials.

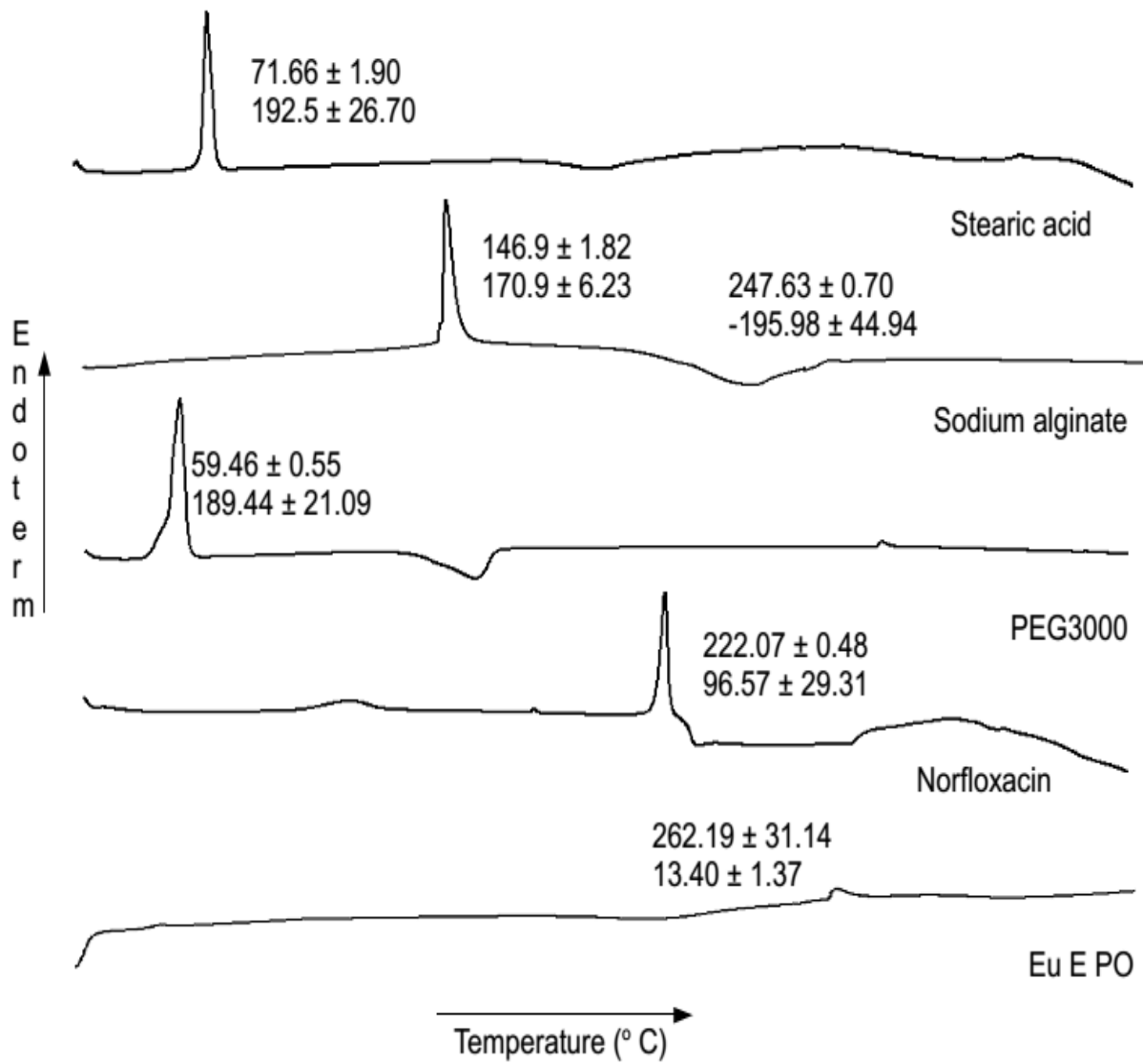


Figure 6. Thermograms of raw materials.

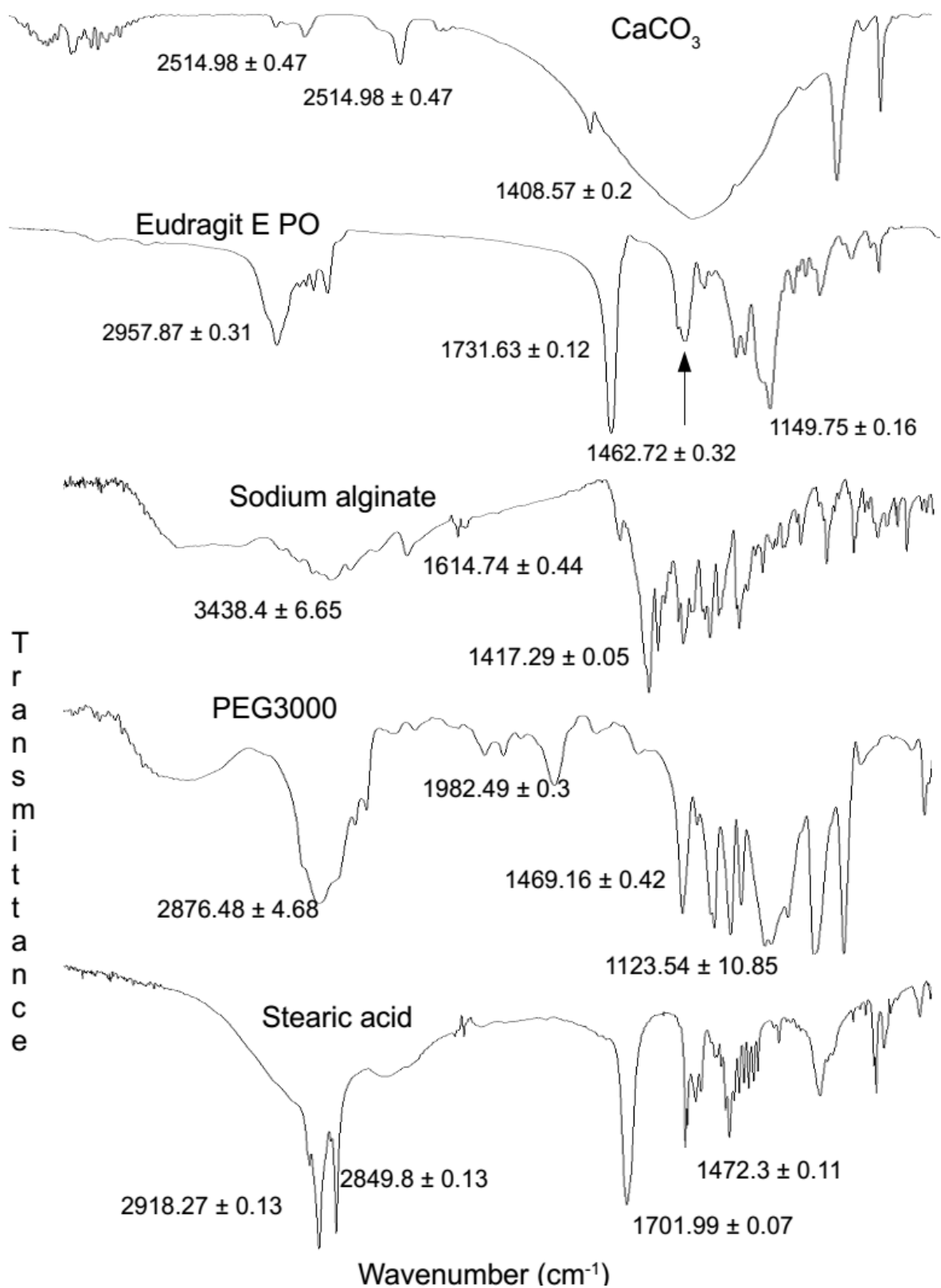


Figure 7. IR spectra of raw materials.

Thermograms in Figure 8 presented:

- An intermediate endothermic peak temperature of about 180°C denoting polymer-drug interaction in fresh matrices.
- reduction of endothermic peak temperature to lower than 180°C after dissolution test for both samples analysed. This indicated polymer-drug dissociation during the drug release process.

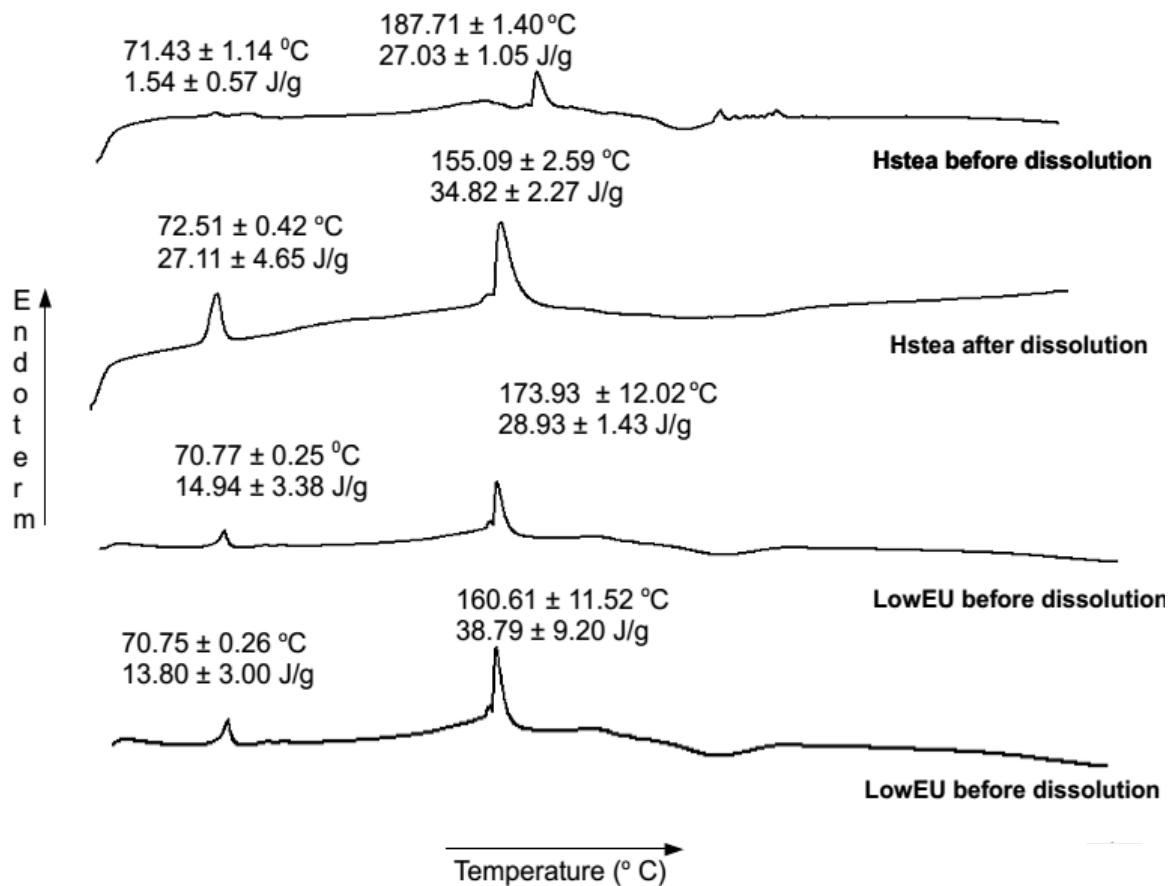


Figure 8. DSC thermogram of Hstea and LowEU assembled systems, before and after 480 minutes of dissolution.

The reduction of melting temperature of the endothermic peak at about 180°C indicated that the matrix after dissolution was weaker compared to the fresh sample.

Infrared spectra of Hstea and LowEU assembled systems, both before and after dissolution, showed that there were no significant differences between the spectra which meant that there was no chemical interaction between chemicals (Figure 9).

The peak at 1730 cm^{-1} (C=O stretch) ascribing to the norfloxacin and it's almost

undetectable in the second spectra (after dissolution).

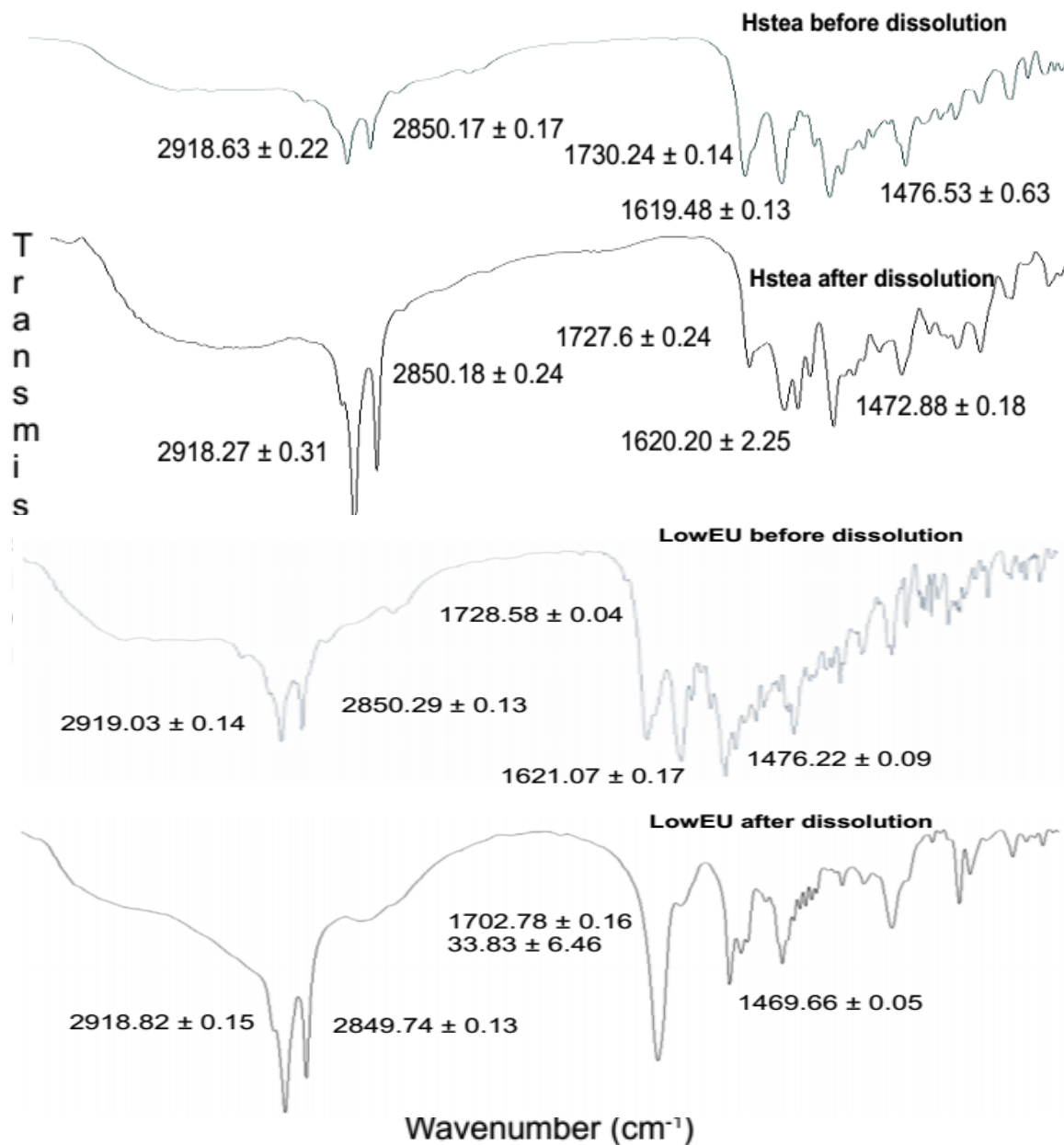


Figure 9. FTIR spectra of Hstea and LowEU assembled system before and after 480minutes of dissolution.

Both DSC and FTIR findings indicated that stearic acid was largely remained in the matrices following the dissolution process. This was essential to prevent early drug release and induce floating.

The extent of crosslinkage was also investigated via SEM-EDX to determine the amount of calcium on assembled systems after dissolution test.

Table 21. Calcium content of Hstea and LowEU assembled systems after 480 minutes of dissolution

	Ca ²⁺ ions content (%)
Hstea	3.31 ± 1.59
LowEU	5.23 ± 0.46

The correlation between calcium alginate cross-link and release doesn't exist. A higher cross-link degree should lead to a stronger matrix and therefore to a lower release (and erosion), but according to the data, LowEU exhibited a similarly high drug release as Hstea even with a higher degree of cross-link.

Taking in account that some soluble compounds like NFX, PEG, Eudragit and CaCO₃ itself are not present anymore in the samples at the time of analysis as inferred from DSC/FTIR/drug dissolution analysis, calculation of cross-link degree should be modified and related to the amount of the insoluble compound as shown in Table 22 and Eq 7.

Table 22. Calcium content calculation correction.

	Hstea mg
NFX	50
Stearic acid	32
CaCO ₃	12,7
NaAlg	66,5
PEG3000	
Eudragit E PO	15,1
	176,3
Soluble elements	77,8
insoluble elements	98,5
Ca ⁺⁺ content (%)	3,31
>>Ca Crosslink	3,26

where

$$Ca\ Crosslink = \frac{Insoluble\ elements \cdot Ca\ content\ (\%)}{100} \quad Eq. 7$$

Using the second method, the value of cross-link changed as visible in Table 23.

Table 23. Corrected cross-link values of Hstea and LowEU.

	Cross-link value(%)	
	Old	New
Hstea	3.31	3.26
LowEU	5.23	5.48

SEM-EDX analysis of Hstea and LowEU Dome matrices indicated that LowEU matrix contained a higher Ca^{2+} load than Hstea matrix (Table 223). Nevertheless, it appeared that alginate- Ca^{2+} crosslinkage did not make drug release slower in LowEU matrix beyond that of Hstea matrix.

Overall, less than 60% of norfloxacin were released within 8 hours of dissolution. DSC and FTIR analysis of the matrices indicated the stearic acid was available in the Dome matrix after a prolonged period of time (Figure 8 and 9). This aptly explained that the sustained drug release behaviour of the matrices and their ability to stay afloat for six hours in simulated gastric fluid.

4.10 Optimization

Among all formulations, LowEU formulation exhibits the highest floating capability though it was still far from being satisfactory.

The selected formulation was thus modified through adding PEG3000 and reducing stearic acid content (Table 24). PEG3000 was introduced to promote complete drug release within 8 hours of dissolution and it was envisaged that PEG3000 fast dissolution may induce porous structure that aid matrix floating.

Table 24. SteaPegMod formulation.

	SteaPegMod(mg)
Norfloxacin	50
Stearic acid	29.5
CaCO ₃	12.7
Sodium Alginate	66.5
Eudragit E PO	8.8
PEG3000	8.8

The assembled systems floated up to 480 minutes and it was characterized by higher swelling and erosion (Table 25, Figure 10) compared to LowEU formulation (Tables 18 and 19).

Table 25. Floating, swelling, water uptake and erosion profiles of SteaPegMod and LowEU.

	Floating time (min)	Time afloat (min)	Swelling (%)	Water Uptake (%)	Erosion (%)
LowEU	0 ± 0	340 ± 35	68.60 ± 7.52	290.29 ± 12.88	11.46 ± 1.09
SteaPegMod	4 ± 2	476 ± 2	147.75 ± 39.18	489.73 ± 50.47	24.39 ± 4.25

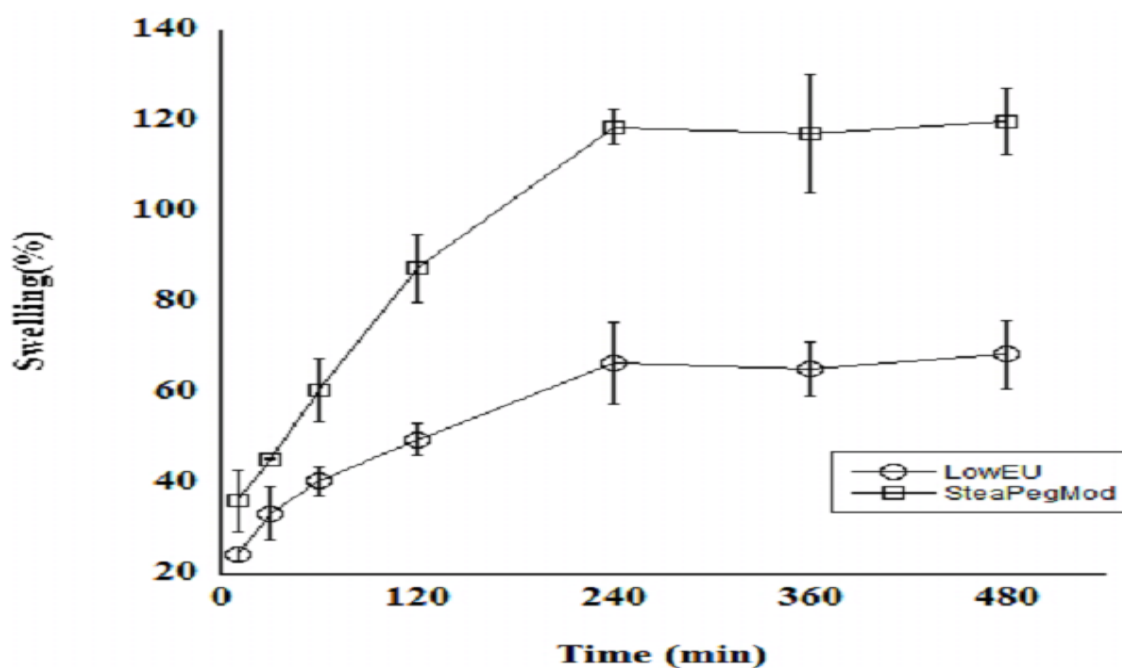


Figure 10. Swelling profiles of LowEU and SteaPegMod assembled systems

This formulation behaved accordingly to the mathematical models developed in section 4.6 (Table 14-16) and the comparison between experimental data and real data is in Table 26.

Table 26. Comparison between real and theoretical behaviour of SteaPegMod.

	Swelling (%)	Water Uptake (%)	Erosion (%)
Theoretical value	84.92	455.03	17.65
SteaPegMod	147.75 ± 39.18	489.73 ± 50.47	24.39 ± 4.25

Figure 11 and 12 show matrix morphology of LowEU and SteapegMod assembled systems after undergoing dissolution. SteapegMod, formulation, containing PEG3000, was characterized by pore formation following PEG3000 leaching.

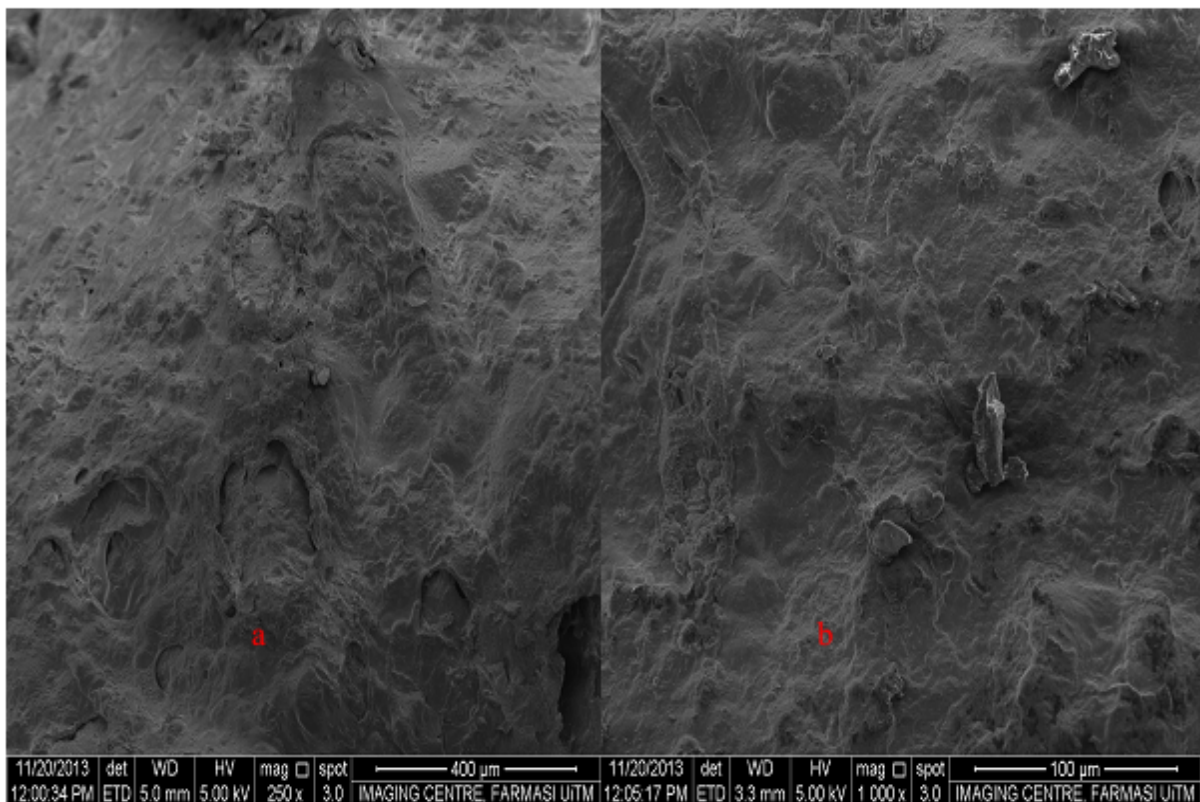


Figure 11. LowEU module surface: 200x(a) and 1000x(b) magnification power.

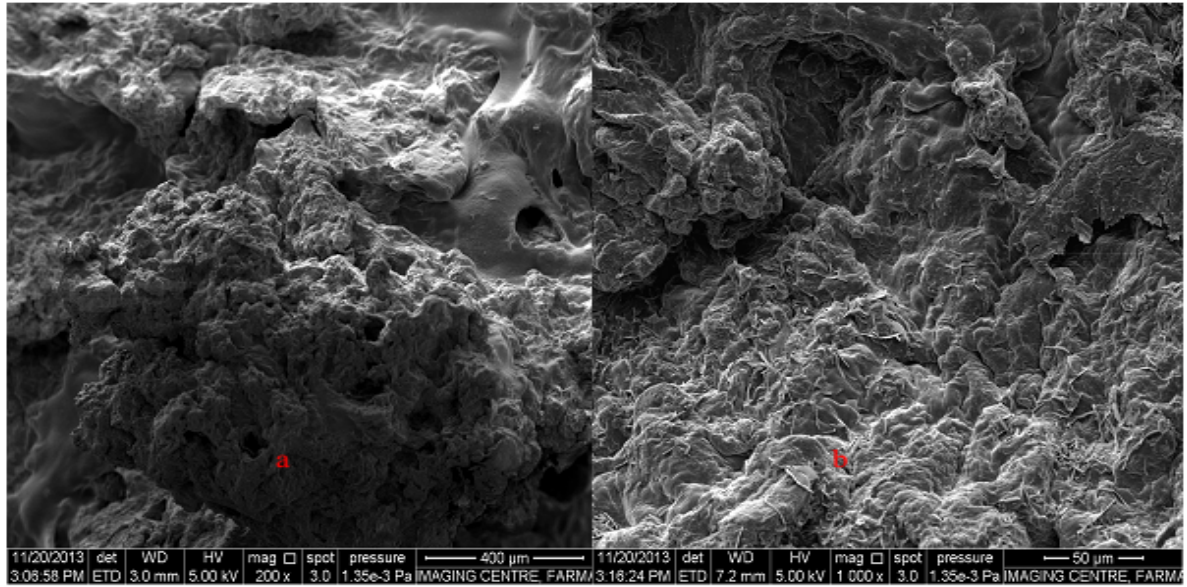


Figure 12. SteaPegMod module surface: 200x(a) and 1000x(b) magnification power.

Drug release profile of the Dome matrix assembled system is shown in Figure 13.

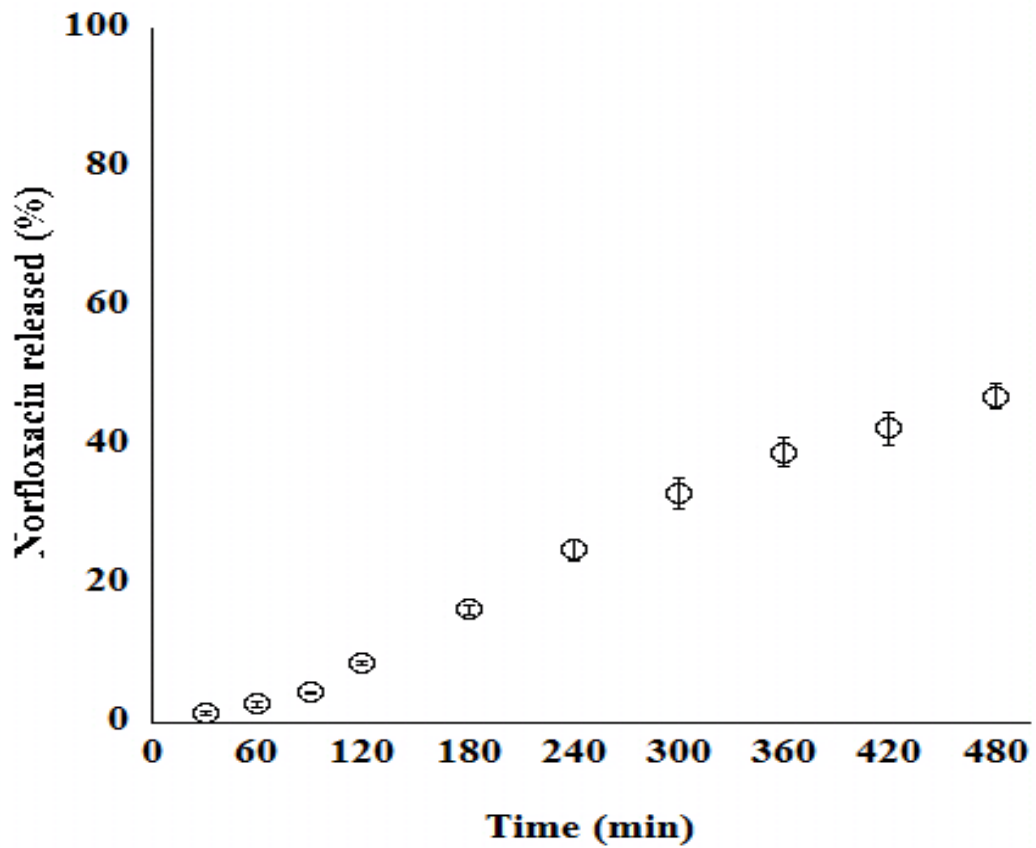


Figure 13. Profile of drug release from SteaPegMod formulation

The release of norfloxacin from SteaPegMod at 8 hours of dissolution was $47.02 \pm 1.82\%$.

F2 value was over 54.78 for LowEU and 52.60 for Hstea, therefore there was no difference between drug release profiles of Hstea, LowEU and SteaPegMod.

DSC thermogram showed that SteapegMod matrix was weaker than Hstea and LowEU as marked by lower endothermic temperatures in general (Figure 14).

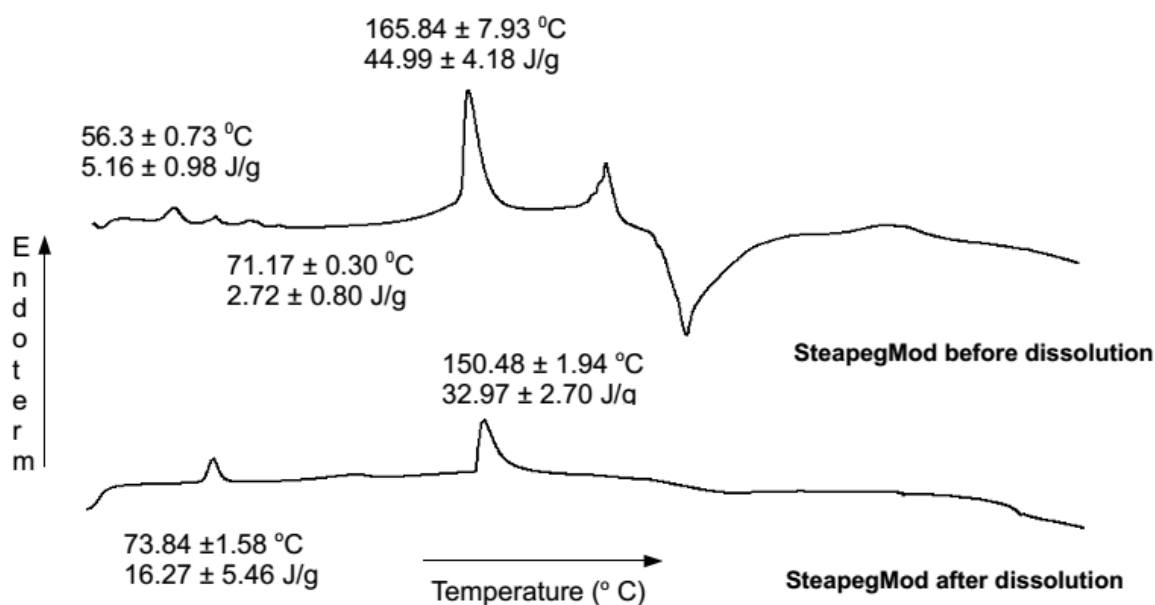


Figure 14. DSC thermogram of SteaPegMod.

The thermogram also shows that the peaks of PEG3000 disappeared in the thermogram of samples undergoing dissolution. This was due to PEG 3000, being water-soluble, can be readily dissolved in the aqueous medium. Nonetheless, the use of PEG 3000 did not appear to aid drug release till completion within 8 hours of dissolution. The stearic acid, being a hydrophobic low density excipient, remained in the matrix throughout the dissolution as reflected by DSC thermograms and FTIR spectra (Figure 14 and 15). Coupled with porous structure induced by PEG 3000 leaching, the floating ability of the said matrix was thus sustained.

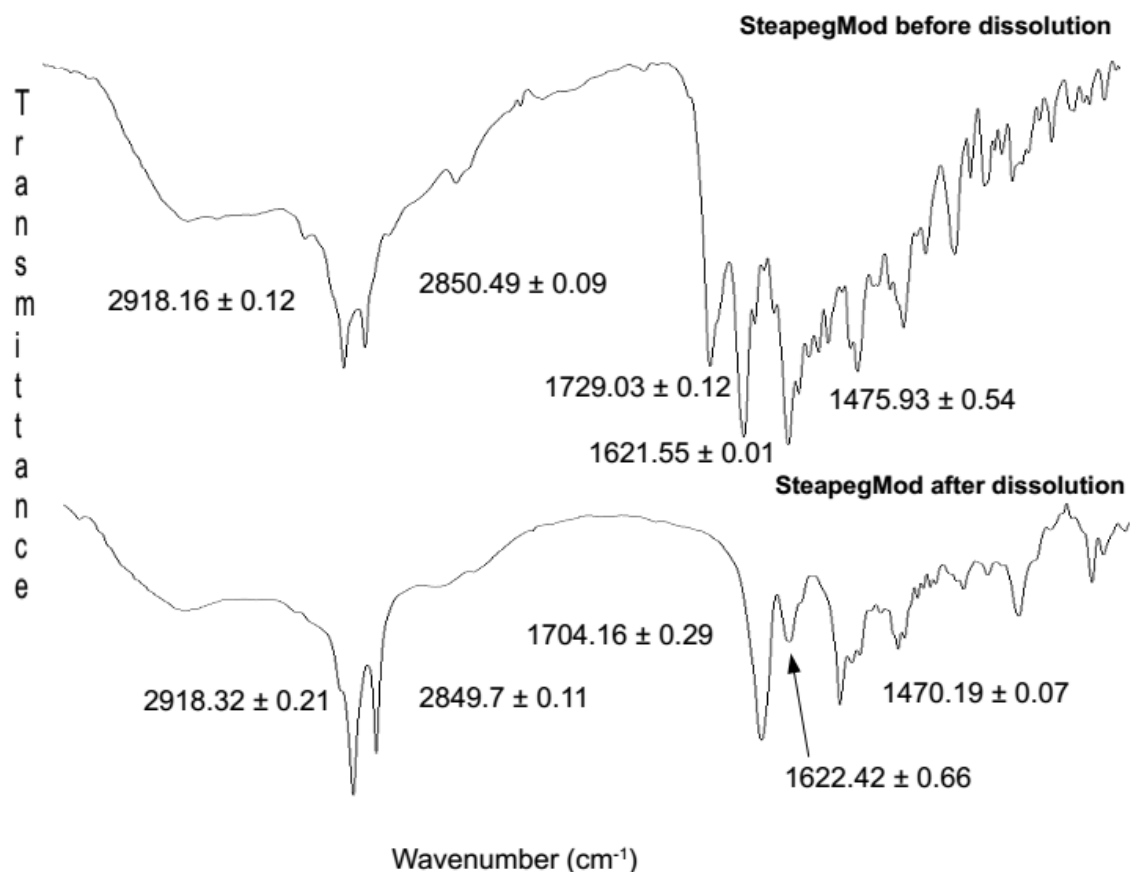


Figure 15. FTIR spectra of SteaPegMod.

SEM-EDX indicated that SteaPegMod exhibited a low level of crosslinkage (Table 27). The crosslinkage did not seem to govern the drug release profiles of SteaPegMod, Hstea and LowEU.

Table 27. Cross-link values of formulations in Table 19 and 24

	Cross-link value
Hstea	3.26 ± 1.57
LowEU	5.48 ± 0.49
SteaPegMod	3.05 ± 0.85

4.10.1 PEG 3000 content

An increase in PEG3000 was hypothesised to be able to further increase matrix porosity, floating and drug release properties.

Stearic acid was reduced in order to keep the weight constant in relation to an

increase in PEG3000 (Table 28).

Table 28. Modified PEG3000 content formulations

	PegM (mg)	PegMbis (mg)
Norfloxacin	50	50
Stearic Acid	20.7	25.1
CaCO ₃	12.7	12.7
Sodium Alginate	66.5	66.5
PEG 3000	17.6	13.2
Eudragit E PO	8.8	8.8

Table 29. Floating, swelling, water uptake and erosion profiles of PegM and PegMbis

	PegM	PegMbis
Floating time (min)	23 ± 36	17 ± 6
Time afloat (min)	207 ± 23	403 ± 107
Swelling(%)	129.0 ± 16.61	124.65 ± 10.46
Water uptake(%)	442.84 ± 140.47	674.45 ± 145.08
Erosion (%)	20.08 ± 2.48	19.56 ± 3.58

The floating capability of SteapegMod Dome matrix was not improved by varying PEG3000 content (Table 28).

4.10.2 Eudragit E PO

The use of PEG 3000 as stearic acid substitute was replaced by less water soluble Eudragit E PO (Table 30). Owing to its lower solubility attributes compared to PEG3000, the floating behaviour was envisaged to be improved with a lower drug release retardation effect than that of stearic acid.

Table 30. Modified Eudragit content formulations

	EuM (mg)	EuMbis (mg)
Norfloxacin	50	50
Stearic Acid	20.7	25.1
CaCO ₃	12.7	12.7
Sodium Alginate	66.5	66.5
PEG 3000	8.8	8.8
Eudragit E PO	17.6	13.2

Table 31. Floating, swelling, water uptake and erosion profiles of EuM and EuMbis

	EuM (mg)	EuMbis (mg)
Floating time (min)	17 ± 6	2 ± 3
Time afloat (min)	173 ± 45	268 ± 32
Swelling(%)	76.74 ± 9.32	77.78 ± 12.06
Water uptake(%)	293.06 ± 50.31	334.54 ± 16.94
Erosion (%)	13.05 ± 0.71	12.99 ± 1.25

Floating time, swelling, water uptake and erosion are statistically lower than PEG3000 formulations in Table 29. Neither EuM and EuM Dome matrix assembled systems floated up to 8 hours.

4.10.3 Stearic acid content

Stearic acid, though hydrophobic, was expected to induce hydrophilic-hydrophobic region detachment and possibly led to pore formation that promoted floating and drug release when higher contents were used.

Stearic acid content was increased with the weight of the modules increased and without changing the content of other components (Table 32).

Table 32. Stearic acid based formulations

	Spm (mg)	SpmB (mg)	SpmC(mg)
Norfloxacin	50	50	50
Stearic acid	38.3	44.6	50.9
CaCO ₃	12.7	12.7	12.7
Sodium alginate	66.5	66.5	66.5
Eudragit E PO	8.8	8.8	8.8
PEG3000	8.8	8.8	8.8
Total	185.1	191.4	197.7

The hydrophilic-hydrophobic region detachment induced by stearic acid could have been accompanied by matrix erosion and disintegration (Table 33). The latter was negated by PEG3000, a more tacky and viscous binder than stearic acid.

Table 33. Floating, swelling, water uptake and erosion profiles of Spm, SpmB and SpmC

	Spm	SpmB	SpmC
Floating time (min)	12 ± 3	17 ± 6	4 ± 2
Time afloat (min)	468 ± 3	463 ± 6	476 ± 2
Swelling(%)	135.35 ± 28.5	84.11 ± 7.67	96.27 ± 10.90
Water uptake(%)	402.42 ± 18.22	317.57 ± 12.59	353.02 ± 15.95
Erosion (%)	20.87 ± 0.55	19.03 ± 0.90	21.72 ± 1.11
Norfloxacin release at 8 hours (%)	60.20 ± 2.88	63.42 ± 2.49	52.89 ± 6.84

Profiles of drug release for stearic based formulations are shown in Figure 16.

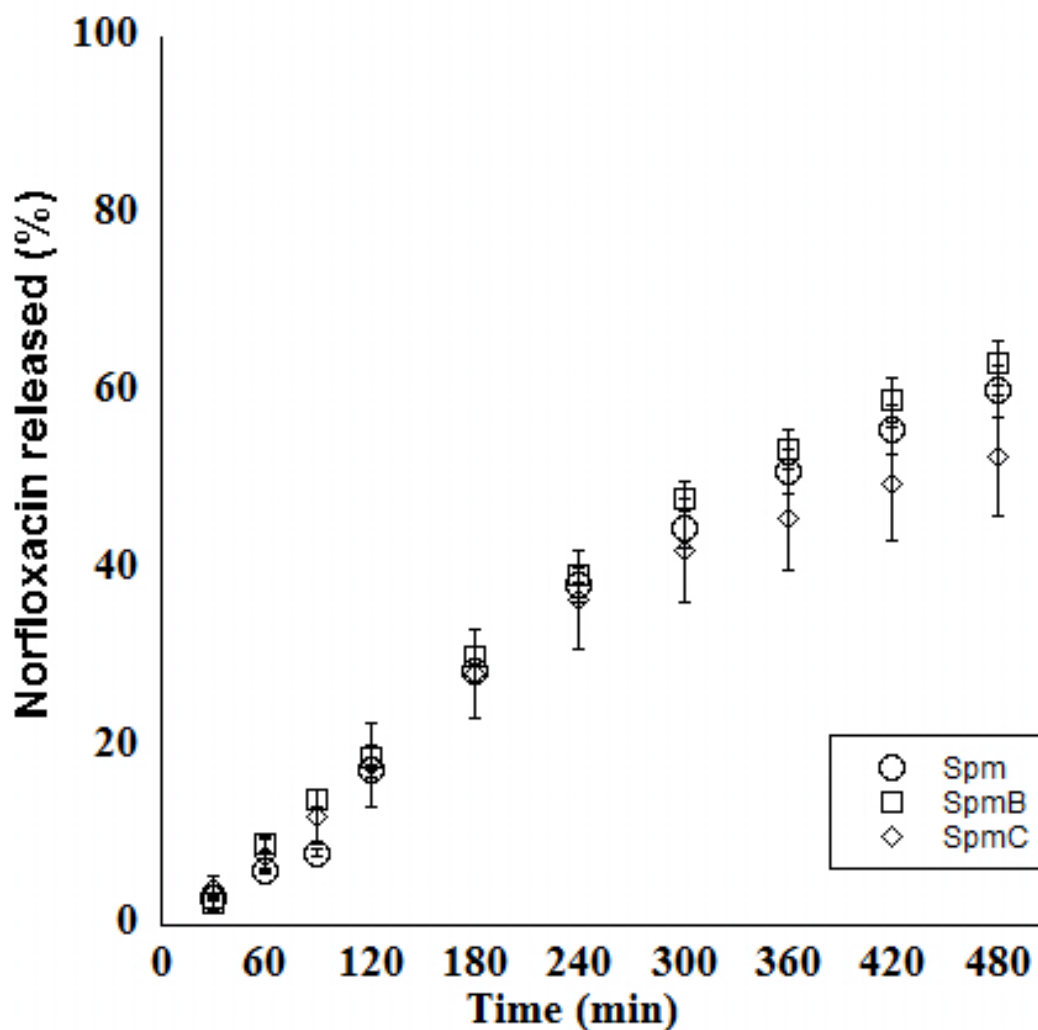


Figure 16. Profile of drug release from stearic acid based formulations

Further addition of stearic acid led to increased drug release and minimal changes to the floating behaviour of SteapegMod Dome matrix (Table 25 and 26, Figure 13). The drug release was promoted by hydrophobic stearic acid addition in spite of its presence reduced the water uptake and swelling extent of matrix substantially.

Drug release profiles had a f2 factor over 60 using Spm (Table 34) as comparison and Anova test give a $p=0.0017$.

Table 34. Similarity factor of stearic acid based formulations (Table 32)

	SpmB	SpmC
Spm	74.58	70.12
SpmB		62.12

SpmB was the formulation showing the highest release and it was therefore selected for the in-vivo experiments.

Figure 17 shows the DSC endothermic and exothermic peaks of all stearic acid based formulations.

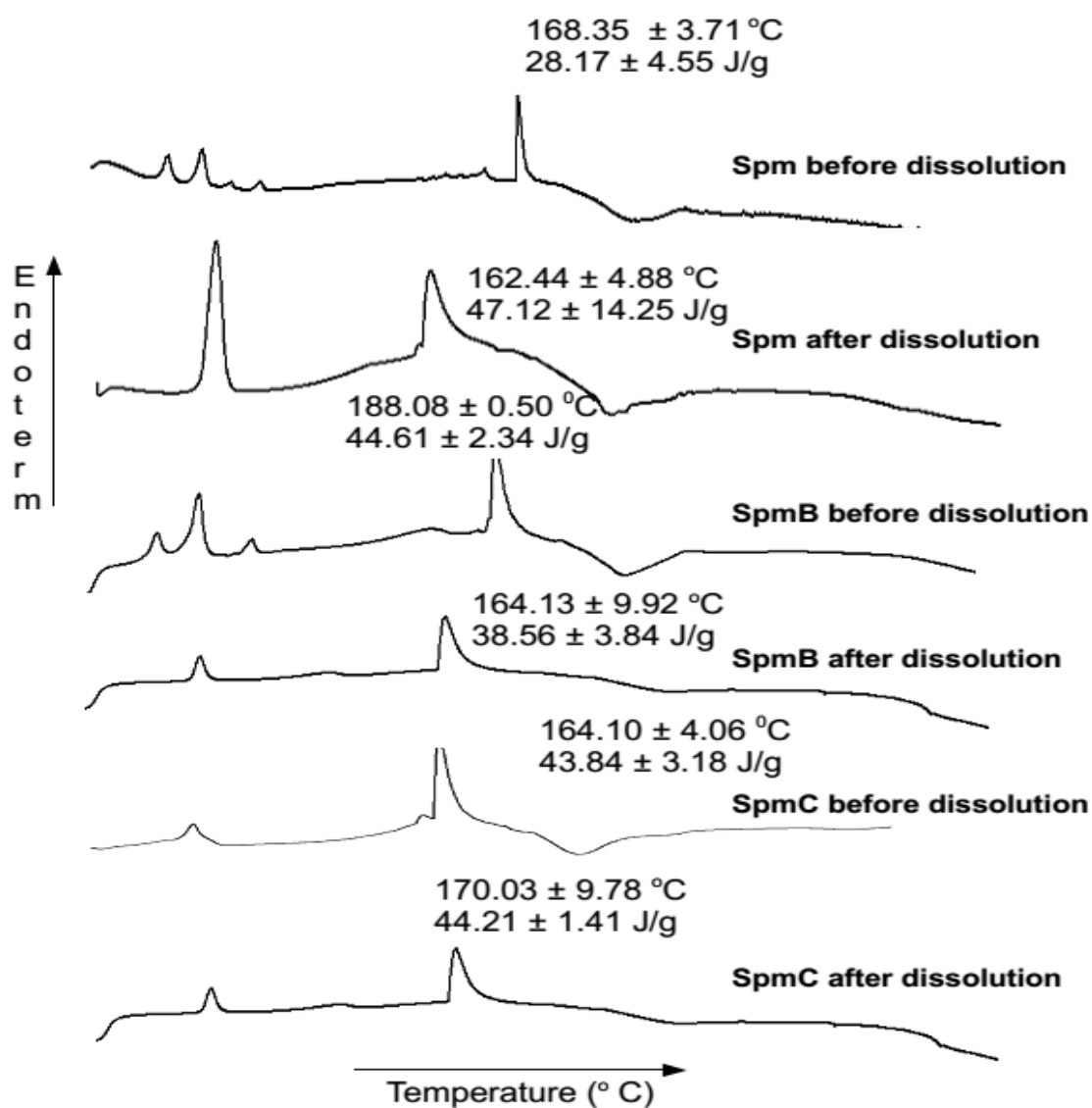


Figure 17. Thermograms of stearic acid based formulations

The DSC thermograms of stearic acid based formulations exhibited a similar thermal profile as matrices made of stearic acid and/or PEG 3000 (Figure 17). Using excessive stearic acid as in the case of SpmC, the tendency of matrix weakening following dissolution was lower. This could be associated with reduced drug release due to excessive hydrophobic effect and a lesser extent of dissociation of polymer-drug interaction.

FTIR spectra of stearic acid based formulations did not present any significant difference between batches (Figure 18). The spectra characteristics of all formulations were similar after dissolution. It was assumed that all formulations had similar chemical environments.

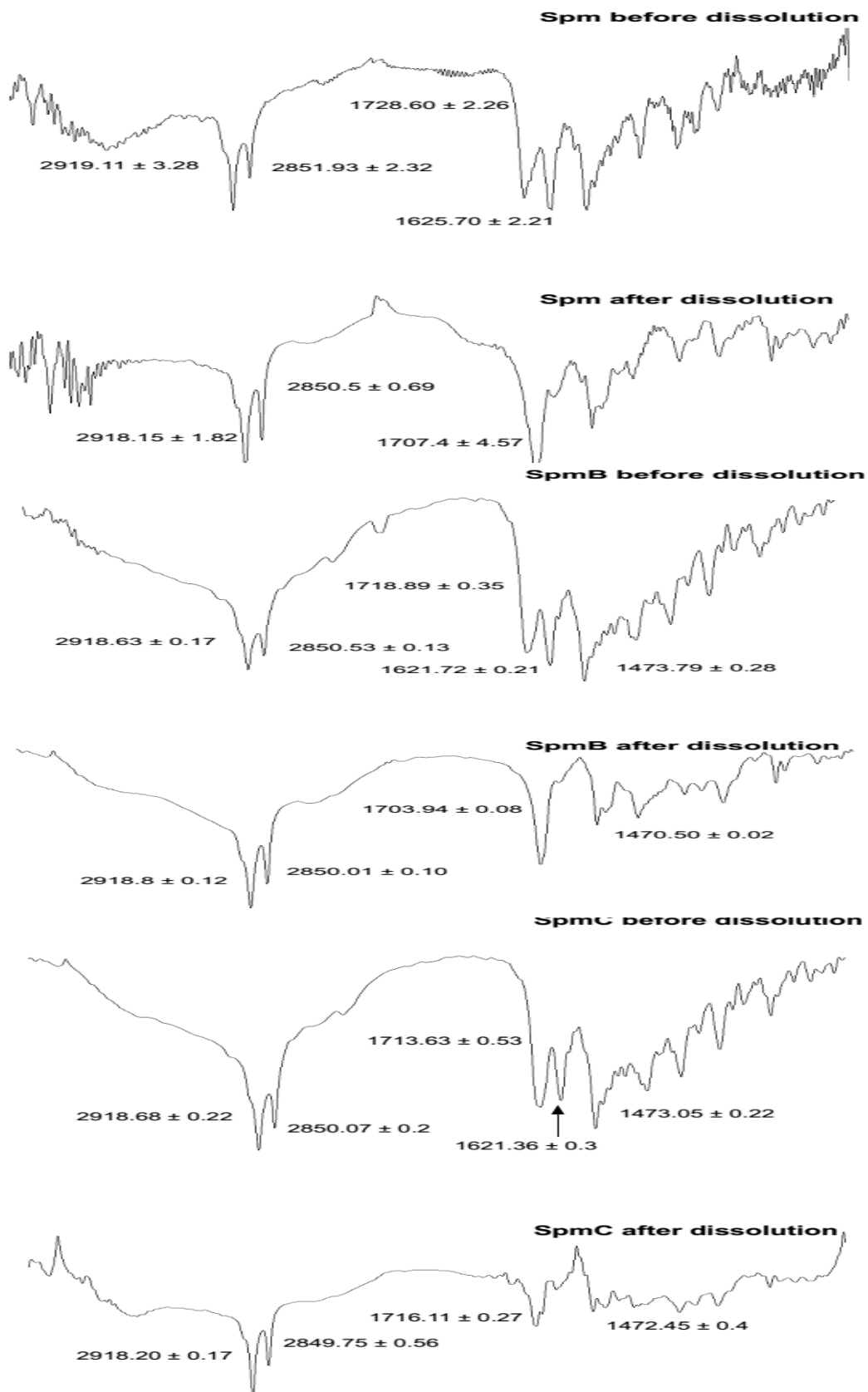


Figure 18. Infrared spectra of stearic acid based formulations

Table 35. Summary of cross-link values of formulations described in Table 19, Table 24 and Table 32

	Cross-link value
Hstea	3.26 ± 1.57
LowEU	5.48 ± 0.49
SteaPegMod	3.05 ± 0.85
Spm	5.65 ± 0.87
SpmB	4.49 ± 1.60
SpmC	3.28 ± 1.02

A higher cross-link value led to a higher drug release (Table 35, Figure 19).

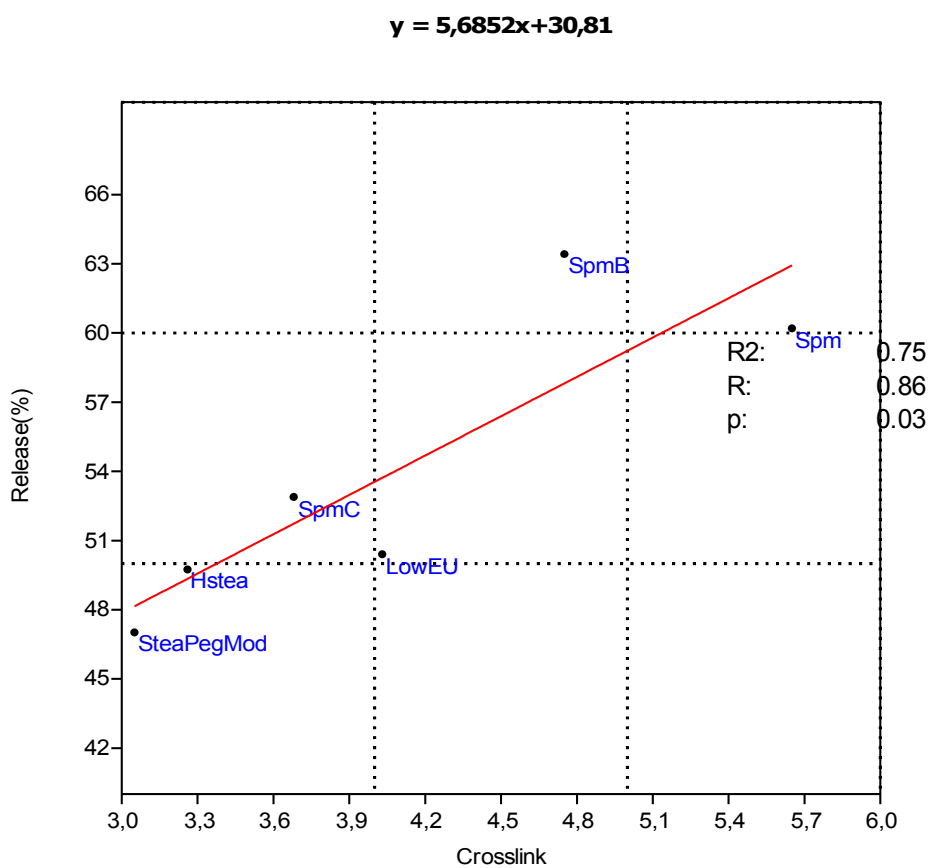


Figure 19. Correlation between cross-link and norfloxacin release

The opposite would have been expected, but the cross-link between calcium and

alginate is very loose and it may lead to the formations of pore because there is no entangling of polymeric chains.

4.11 *In-vivo* floating

In-vivo floating of 3mm Dome Matrix assembled system was assembled using two female modules glued together via PVP K90 (30% w/v) in ethanol.

Barium sulphate is commonly used as a contrasting agent for X-ray at the gastrointestinal tract thanks to its insolubility and low toxicity. The barium can be loaded in a Dome matrix module replacing the norfloxacin.

The loading of barium sulphate depends on the characteristics of the matrix itself: the 3mm Dome Matrix assembled systems were tested *in vitro* before the analysis in order to ensure that the behaviour of the tablets was the same of the full size assembled systems.

Two different formulations (Table 36) were tested: one based on Formulation A (Table 1) as a reference and one based on SpmB (Table 32). Formulation SpmB was the successful formulation from the previous section of the project, while FormA (using PEG 3000 as binder) was one of the first formulations employed.

Table 36. Barium sulphate loaded tablets

	FormA (mg)	SpmB (mg)
Barium sulphate	6.85	4.35
Stearic acid	0	7.77
CaCO ₃	6.96	2.21
Sodium Alginate	17.16	11.58
PEG3000	8.77	1.53
Eudragit E PO	0	1.53
Average weight (mg)	39.74	28.97

FormA floated for 4.3 ± 0.6 hours, while SpmB floated for 7 ± 0 hours *in vivo*.

Figure 20 shows the outcome derived from the *in vivo* X-ray study: the green circle indicated the position of the stomach, while the red arrow marked the position of the 3mm Dome Matrix assembled system.

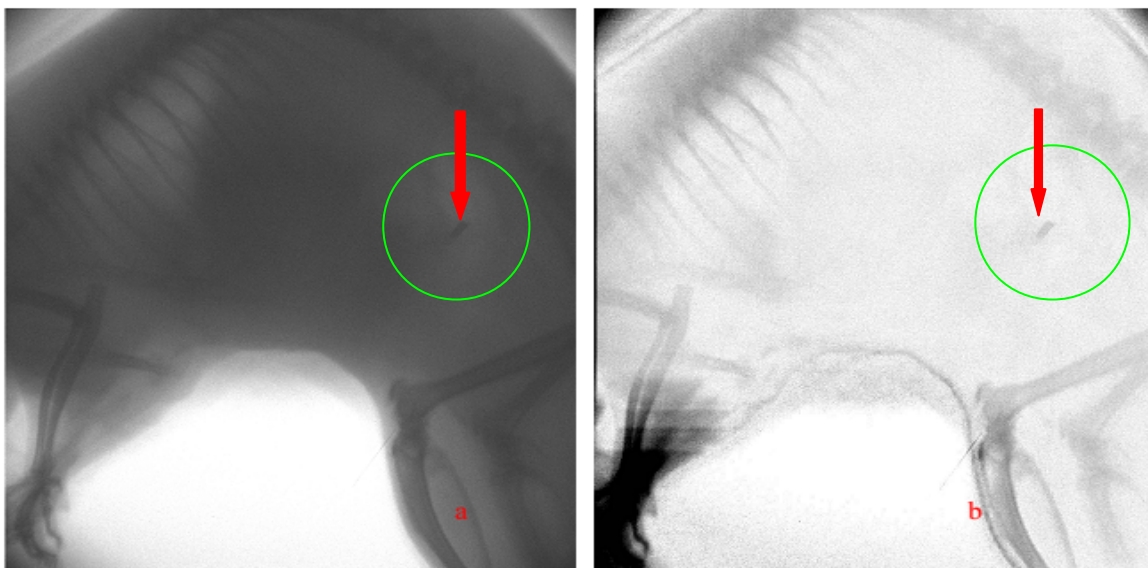


Figure 20. X ray photographs indicating the matrix position in rat stomach (a) before and (b) after background noise reduction.

4.12 Pharmacokinetic

The pharmacokinetic study demonstrated that the bioavailability of norfloxacin in the form of Dome Matrix was improved.

Formulation SpmB (Table 32) was compressed as 3mm Dome Matrix modules, which had an average weight of 29.55 ± 1.54 mg and an average norfloxacin content of 8.04 ± 0.37 mg.

Dome Matrix assembled systems were compared to non-gastroretentive dosage form (unassembled Dome matrix modules) and norfloxacin solution.

Each assembled system was composed of 2 modules, therefore the amount of norfloxacin administered to each rat was 16.08 mg. With reference to non-gastroretentive dosage form, 2 Dome matrix modules were administered.

Pharmacokinetic profile of norfloxacin is shown in Figure 21, while pharmacokinetic parameters (C_{max} , T_{max} , normalized AUC and Half life) are listed in Table 37.

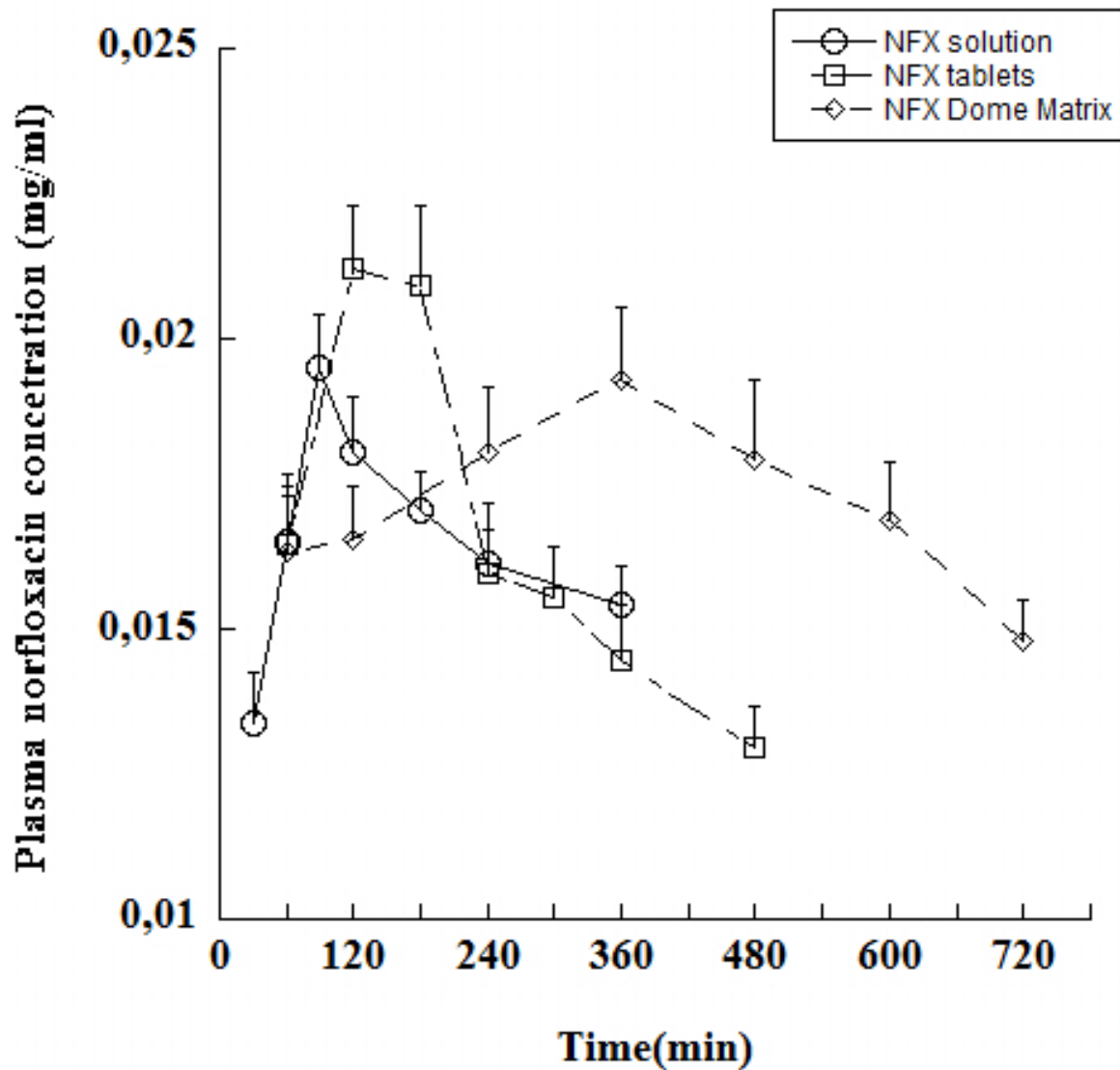


Figure 21. Plasma norfloxacin concentration-time plot

Table 37. Pharmacokinetics parameter of norfloxacin solution and norfloxacin modules (both as assembled system and as non-gastroretentive dosage form)

	Cmax (mg/ml)	Tmax	AUC normalized	Half life (min)
Norfloxacin solution	0.0202 ± 0.0021	95 ± 23	0.3924 ± 0.0251	66 ± 29
Norfloxacin non gastroretentive dosage form	0.0223 ± 0.002	160 ± 31	0.4801 ± 0.0384	80 ± 41
Norfloxacin Dome Matrix	0.0205 ± 0.0035	330 ± 141	0.757 ± 0.1140	185 ± 108

Anova test was performed on each single parameter:

- No significant difference in C_{max} between formulations.
- T_{max} was statistically different only for Dome Matrix systems ($p < 0.01$), while norfloxacin solution and norfloxacin tablet had comparable T_{max} ($p > 0.4$).
- AUC normalized was statistically different between formulations.
- Half-life was statistically higher with Dome Matrix system ($p < 0.05$).

5. Conclusions

Dome Matrix technology is a new platform for oral controlled drug release (rate and site control) characterized by two modules (male and female) having one concave and one convex base assembled in void configuration.

This technology proved effective in the manufacture of a floating dosage form while the calcium alginate was able to modulate the release of the drug for the entire test duration without disaggregation, through judicious choice of additives.

The integrity of the assembled system proved also effective in achieving a floating in vivo and therefore improving the bioavailability of the drug.

There are some points which may need additional research:

- Achieving a floating Dome Matrix system with faster and more complete drug release attributes.
- Identifying the speed of polymer's gelification which may affect erosion and release of the drug. X ray may be employed as a non-destructive analytical method to monitor the process even without using barium loaded modules.
- Clinical trials on human volunteers to verify the validity of animal pharmacokinetics research.

Appendix I: Validation of analytical methods

1) HPLC method for *in vitro* dissolution

The norfloxacin was quantified by HPLC equipped with an UV-Vis detector.

A calibration curve (Figure 1) was constructed by dissolving the drug in buffer at pH=2.2 following the method reported in 3.2.10.

Each sample was injected three times, the linearity and precision (expressed as relative standard deviation, RDS%) of the assay were determined.

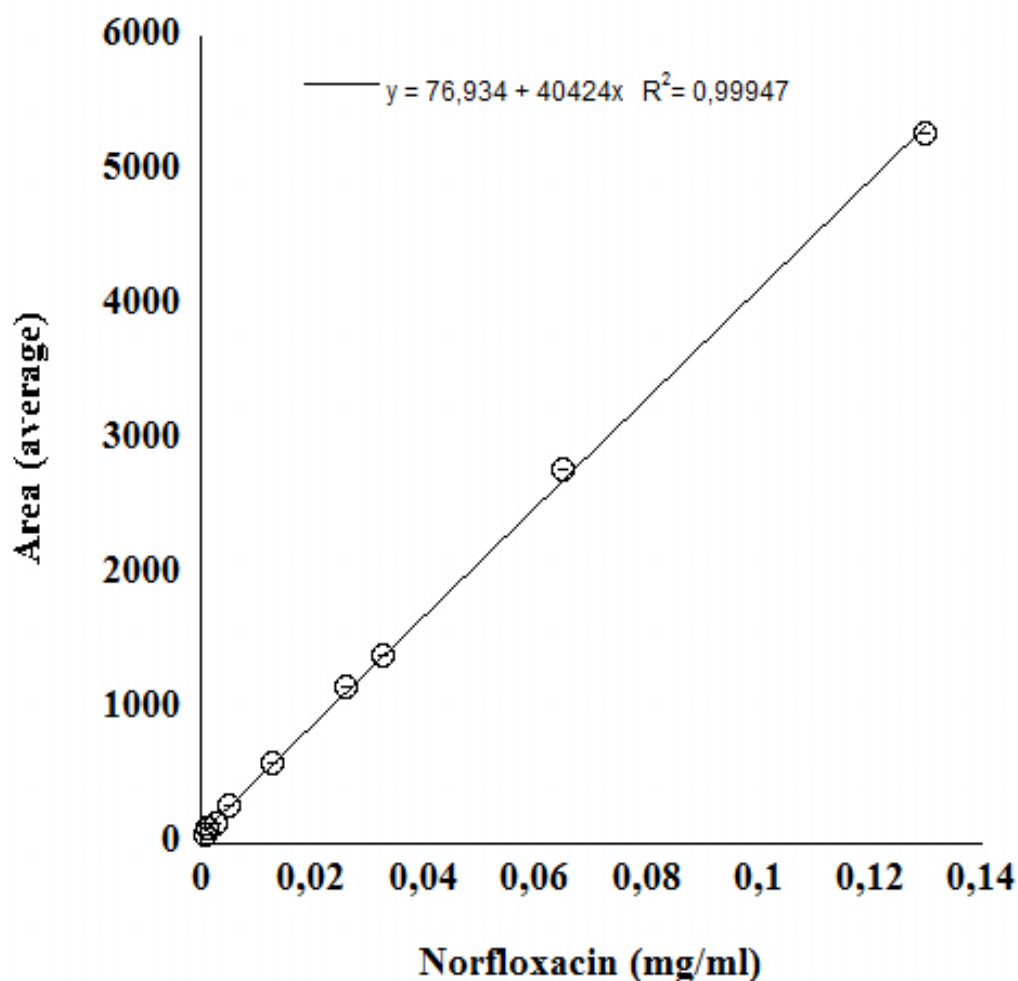


Figure 1. Area vs Norfloxacin concentration in buffer pH 2.2

In the drug concentration range of 6.5 $\mu\text{g/ml}$ – 0.13 mg/ml, the correlation coefficient (R^2) for the calibration curve was 0.99947, indicating good linearity. The reproducibility of the method was considered acceptable as the RSD% values obtained were for each standard <2%.

Limit of detection was 2.04 $\mu\text{g/ml}$, while limit of quantification was 3.78 $\mu\text{g/ml}$.

Variability intraday and extraday were also assessed: 0.22 the first and 1.33 the latter.

2) HPLC method for *in vivo* pharmacokinetic studies

The norfloxacin was quantified by HPLC equipped with an UV-Vis detector.

A calibration curve (Figure 2) was constructed by dissolving the drug in blank plasma following the method reported in 3.2.17.

Each sample was injected three times, the linearity and precision (expressed as relative standard deviation, RSD%) of the assay were determined.

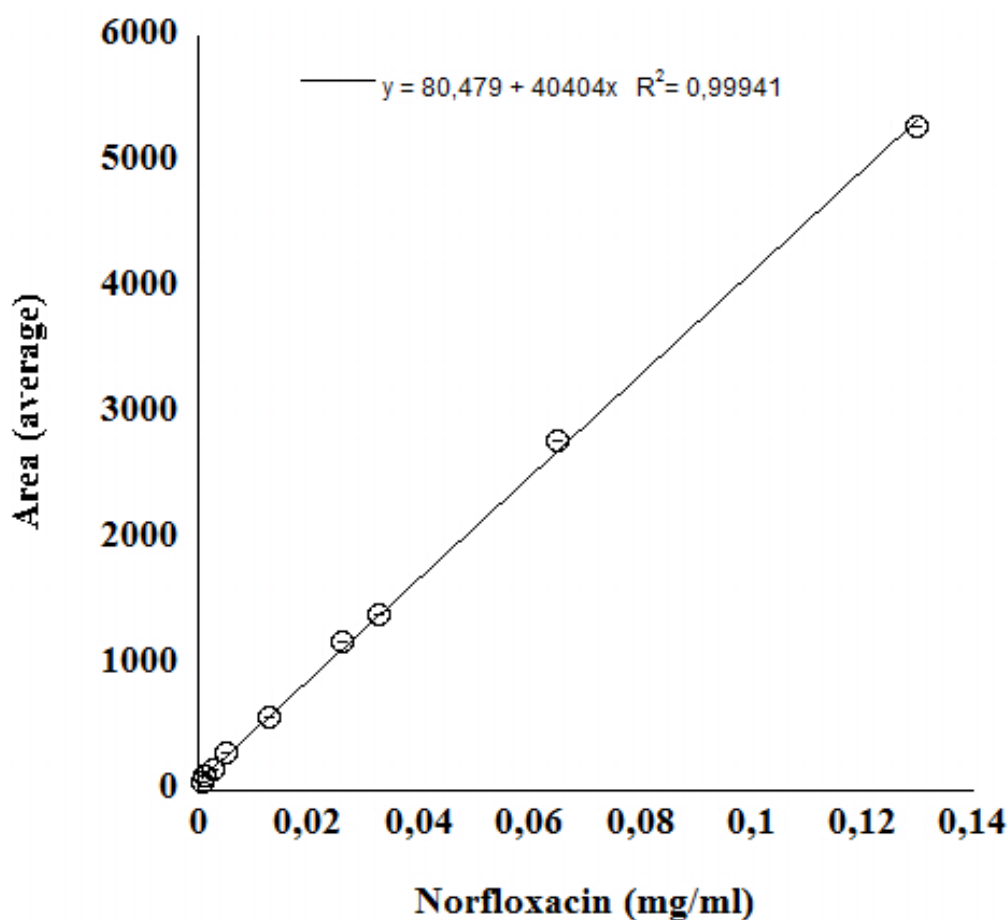


Figure 2. Area vs norfloxacin concentration in blank plasma

In the drug concentration range of 6.5 $\mu\text{g/ml}$ – 0.13 mg/ml, the correlation coefficient (R^2) for the calibration curve was 0.99941. The reproducibility of the method was considered acceptable as the RSD% values obtained were for each standard <2%.

Limit of detection was 2.56 $\mu\text{g/ml}$, while limit of quantification was 4.40 $\mu\text{g/ml}$.

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Conclusions

Dome Matrix technology is a platform for the controlled release of drug. This technology is based on the assembling of modules used as elements for controlling the drug release.

The research project of the doctorate thesis was devoted to the application of Dome Matrix technology for the preparation of gastro-retentive dosage forms thanks to the module assembly in void configuration. According to the co-tutorship agreement, the research was carried out both at University of Parma and Universiti Teknologi MARA, Kuala Lumpur.

During the first part of the project, carried out at University of Parma, a modular assembled system for a double pulse release (immediate and delayed release) of esomeprazole in combination with sucralfate was studied. The assembled system was build up by assembling 5 modules, three sucralfate modules and two esomeprazole modules, in "mixed" configuration (void and stacked configurations) with three different release kinetics. The alkalising agent in the esomeprazole immediate release module prevented the degradation of the drug in acid environment *in vitro*. The role of alkalising agent in preventing the degradation of the drug in acid environment was further investigated via permeation studies and *in-vivo* pharmacokinetics studies on rats.

The second pulse was obtained via the partial coating of one esomeprazole module and its assembly in void configuration with a sucralfate controlled release module.

During the second part of the project, carried out at UiTM (Kuala Lumpur), a floating dosage form for the controlled release of norfloxacin was developed. The control of drug release was determined by the *in situ* cross-linkage of alginate with calcium ions when in contact with gastric fluid. The floating of the dosage form was confirmed *in vivo* using Dome Matrix assembled system loaded with barium sulphate. The floating of the Dome Matrix assembled system *in-vivo* led to an increased bioavailability of the drug compared to the conventional non-gastroretentive tablets that was confirmed via pharmacokinetic studies on rats.