ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΤΜΗΜΑ ΦΑΡΜΑΚΕΥΤΙΚΗΣ
ΤΟΜΕΑΣ ΦΑΡΜΑΚΕΥΤΙΚΗΣ ΤΕΧΝΟΛΟΓΙΑΣ

Αναπτυξη ενός ευέλικτου συναρμολογούμενου φαρμακευτικού προϊόντος με τη χρήση της τεχνολογίας Dome Matrix® στοχεύοντας στη θεραπεία του H. Pylori

ΜΕΤΑΠΤΥΧΙΑΚΟ ΔΙΠΛΩΜΑ ΕΙΔΙΚΕΥΣΗΣ
ΣΤΗ ΒΙΟΜΗΧΑΝΙΚΗ ΦΑΡΜΑΚΕΥΤΙΚΗ

ΤΣΕΛΙΟΥ ΑΙΚΑΤΕΡΙΝΗ
ΦΑΡΜΑΚΟΠΟΙΟΣ

ΑΘΗΝΑ 2017

Τριμελής Εξεταστική Επιτροπή

Δημήτριος Μ. Ρέκκας	Αναπ. Καθηγητής ΕΚΠΑ (Επιβλέπων)
Γεωργία Βαλσαμή	Αναπ. Καθηγήτρια ΕΚΠΑ
Παρασκευάς Δάλλας	Επικ. Καθηγητής ΕΚΠΑ

Στην οικογένειά μου

Το παρόν μεταπτυχιακό δίπλωμα ειδίκευσης είναι το αποτέλεσμα μίας επιτυχούς συνεργασίας του Εργαστηρίου Φαρμακευτικής Τεχνολογίας του Τμήματος Φαρμακευτικής του Εθνικού και Καποδιστριακού Πανεπιστημίου Αθηνών με το Εργαστήριο Φαρμακευτικής Τεχνολογίας του Τμηματος Φαρμακευτικής του Πανεπιστημίου της Πάρμας κατά τα έτη 2016-2017.

Οφείλω να ευχαριστήσω θερμά τον επιβλέποντα καθηγητή μου, τον Αναπληρωτή Καθηγητή κ. Δημήτριο Μ. Ρέκκα, ο οποίος με την καθοδήγησή του, την πολύτιμη βοήθειά του, τις εύστοχες παρατηρήσεις και συμβουλές, το συνεχές ενδιαφέρον και την επίβλεψή του σε όλη τη διάρκεια της μελέτης, συνέβαλε στο μέγιστο στην επιτυχή εκπόνηση της εργασίας. Η άψογη συνεργασία μας μου έδωσε την ευκαιρία να έρθω σε επαφή και να ανακαλύψω τη γοητεία του αντικειμένου.

Ένα μεγάλο ευχαριστώ στην Αναπ. Καθηγήτρια κα Βαλσαμή Γεωργία για τις πολύτιμες συμβουλές και υποδείξεις της και το χρόνο που αφιέρωσε για την παρούσα εργασία, και στον Επικ. Καθηγητή κ. Δάλλα Παρασκευά για τις χρήσιμες υποδείξεις παρατηρήσεις και συμβουλές του στην εργασία.

Επίσης θα ήθελα να ευχαριστήσω θερμά τον Καθηγητή Paolo Colombo του Πανεπιστημίου της Πάρμας, για την πολύτιμη συμβολή του σε όλα τα στάδια της εργασίας κατά την παραμονή μου στο εργαστήριο. Ένα μεγάλο ευχαριστώ στην Καθηγήτρια Alessandra Rossi για τις χρήσιμες παρατηρήσεις της και την συμβολή της στην ομαλή συνεργασία μας κατά την παραμονή μου στην Πάρμα. Ένα ευχαριστώ στους υποψήφιους διδάκτορες Greta Adorni, Ayca Altay και Davide Frascio για την βοήθεια τους για εξοικείωση με την αρχη λειτουργίας των μηχανημάτων, συσκευών και λογισμικών και την άψογη συμπεριφορά τους με σκοπό τη γρήγορή ένταξή μου στο περιβάλλον.

Τέλος, θα ήθελα να εκφράσω τη βαθιά μου ευγνωμοσύνη στην οικογένεια μου που με στηρίζει σε όλες τις επιλογές μου καθ’ όλη τη διάρκεια των σχολικών και ακαδημαϊκών μου σπουδών με αστείρευτη συμπαράσταση και υπομονή.

Τσέλιου Αικατερίνη

Αθήνα, Ιούλιος 2017

Index

Abstract

Purpose

The purpose of this project was the treatment of Helicobacter Pylori by the use of Dome Matrix technology, which allow us to manufacture floating dosage forms that keep the concentration of the antibiotics above the respective minimum inhibitory concentration (MIC) in the stomach for 4–5 hours. In this way, the drug doses and the number of dosage forms administered daily could be reduced, for better patient compliance.

Methods

The substances used are tetracycline hydrochloride, metronidazole, sucralfate and mastic of Chios. Male Dome Matrix modules of tetracycline and female modules of metronidazole combined in a single dosage form designed to float in the gastric content and to sustain the intra-gastric concentrations of these two antibiotics used for the eradication of Helicobacter pylori. The modules having a disc shape with curved bases were formulated as hydrophilic matrices, containing 20 % of HPMCK15M. One module of tetracycline and one of metronidazole were assembled by sticking the concave base of one to the concave base of the other, creating an internal void chamber that showed immediate floatation and gastro-retention. Moreover, immediate release modules of sucralfate and mastic of Chios were manufactured as a bilayer tablet, in order to enhance the efficacy of antibiotics against H. pylori. A 00 type hard capsule was used to include two assembled systems of the antibiotics and one of the bilayer tablet as the final configuration. A USP dissolution apparatus II was used for the in vitro drug release of individual modules and assembled system with dissolution medium pH 1.2 and pH 3.5. In the end, were studied the dissolution profiles of the various individual modules and the assembled system, all substantially hydrophilic matrices, in order to analyze the drug release rate and kinetics.

Results

In both dissolution medium pH 1.2 and 3.5 the void assembly system displayed an immediate floating lasting more than 5 h. Tetracycline from the assembly system was 65% released within 5 hours at pH 1.2, while when tested at pH 3.5, where it is less soluble, the module showed a lower drug release (40% in 8 h). Metronidazole from assembly system dissolved at about 90% and 70% in pH 1.2 and 3.5 respectively at 5 hours. The release kinetics of the various individual modules and the assembled system has been described by Ritgel and Peppas equation, in which the value of exponent (n) gives indication on the mechanism of drug release. The n value is between 0.45 and 0.89, so mainly diffusion through the gel layer (but also relaxation) is the mechanism that controls the release of both antibiotics. The only case where $n < 0.45$ ($n=0.3967$) is the individual male-middle module of tetracycline, because of the geometry effect of the system.

1. Introduction

1.1. Structure of the stomach

The stomach is the largest part of digestive track 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 chime between forty minutes and few hours.

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

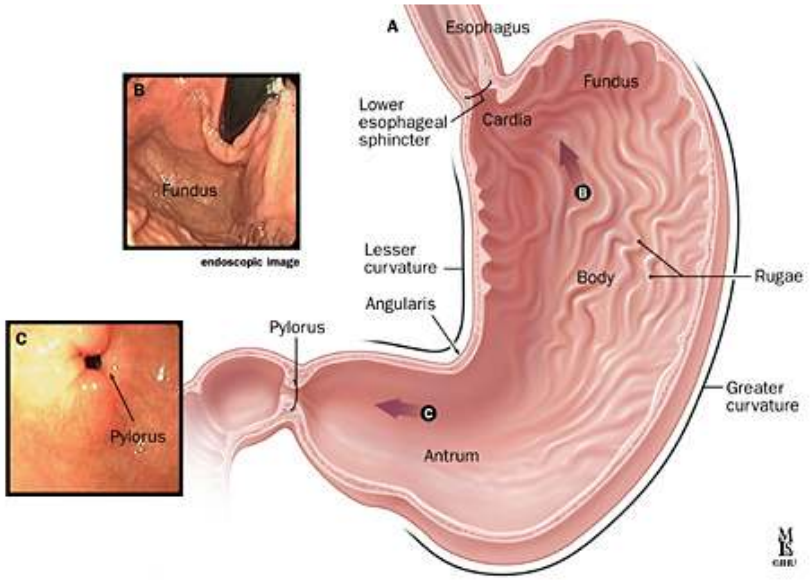


Figure 1. Anatomy of the stomach

Stomach contents are kept in the body of the organ thanks to the 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 move and mix the stomach contents. Serosa is the outermost layer of the stomach that wraps the stomach. This kind of structure is visible in Figure 2.

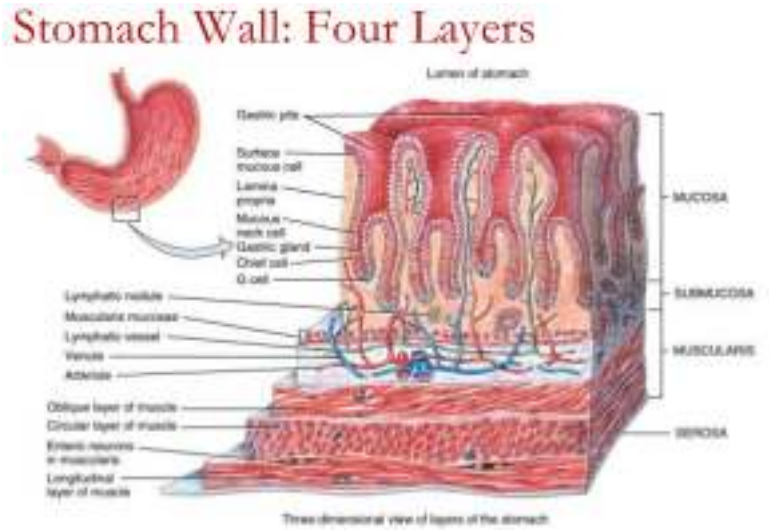


Figure 2: The main layers of the stomach

The stomach receives chewed food from esophagus and it continues to digest it mechanically and chemically. The stomach has an acidic environment ranging 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 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 aminoacids. 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 diseases:

- Dyspepsia: this is the 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 caused heartburn or coughing.
- Gastroesophageal reflux disease (GERD): this chronic disease is abnormal gastric reflux from the 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 this disease includes abdominal pain, fullness, bloating, nausea, vomiting, loose of appetite.
- Peptin ulcer: Peptin ulcer is a sore in the mucus membrane of the stomach although it is developed more often in the duodenum.
- Stomach bleeding: Gastritis, ulcers, or gastric cancers may bleed. Seeing blood or black material in vomit or stool is usually a medicine emergency.
- Stomach cancer: an uncommon type of cancer. Its sypmtoms usually include abdominal pain, weight loss, poor digestion, nausea, vomiting,difficult swallowing and black stools.
- Zollinger-Ellison syndrome (ZES): It is usually caused by tumors. These tumors produce the hormone gastrin. High levels of gastrin cause high levels of gastric acid[1,2].
- 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].

1.2. Helicobacter Pylori

Helicobacter pylori (Figure 3) is a Gram-negative, helix-shaped bacterium that is about 3 micrometers long with a diameter of 0.5 micrometers. *H. pylori* is a microaerophilic bacterium which means that it requires oxygen to function. However, *H. pylori* requires much lower concentrations of oxygen than those found in our atmosphere. This bacterium contains a hydrogenase which it can use to obtain energy by oxidizing molecular hydrogen (in the form of H_2) produced by intestinal bacteria. *H. pylori* also produces oxidase, catalase, and urease. It has an outer-membrane consisting of phospholipids and lipopolysaccharide which are characteristic of typical Gram-negative bacteria. *H. pylori* bacteria are known to inhabit various areas of the stomach and duodenum. Infections caused by this bacteria lead to chronic inflammation in the stomach lining (Gastritis). *H. pylori* infections are also strongly associated to the development of gastric ulcers and even stomach cancer. Although *H. pylori* is known to cause several problems, over 80% of individuals infected with this bacterium show no symptoms. Over half of all people in the world are thought to harbor this bacterium in their upper gastrointestinal tract[9].

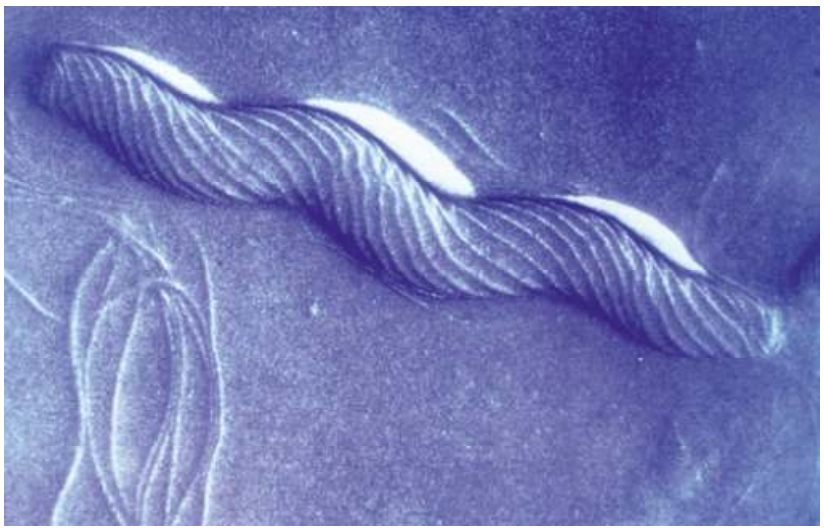


Figure 3: Picture of the spiral form of the H. pylori bacterium, which also shows the whips

1.2.1. H. pylori from ancient times to the final discovery

Infection with H. pylori has co-evolved with mankind since the Paleolithic era. Thus, both humans and H. pylori migrated from East Africa around 58 000 years ago [10, 11]. The spiral-shaped microorganism later identified as H. pylori was noticed in the human stomach by the clinical researcher Jaworski at Krakow University, Poland in 1899 [12] after Bizzozero had found spiral organisms in dogs in 1893 [13]. The main crucial events of our knowledge of H. pylori came in 1979-82 by the groundbreaking experiments of the two Australian scientists, the pathologist Robin Warren who identified the bacterium underneath the protecting lining mucus coat in the stomach, and Barry Marshall that finally, albeit accidentally, successfully cultured the bacterium [14, 15]. In 1985, Marshall reported H. pylori to be the cause of gastritis [16]. Finally, Borody in 1987 was the first to document that H. pylori caused peptic ulcer disease by developing the triple therapy (bismuth, metronidazole and tetracycline) used in the eradication of H. pylori [17, 18]. This has later been reported as “Marshall found the bug and Borody the drug”.

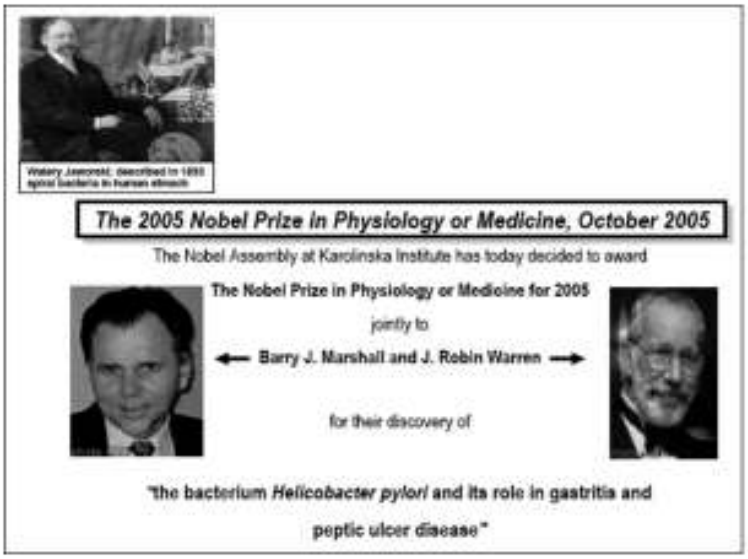


Figure 4: From Presentation on theme: "Update on Helicobacter pylori: Molecular Diagnosis of Helicobacter pylori Prof. Fatma A.

Amer Microbiology & Immunology Department, Faculty of Medicine, Zagazig University, Egypt"

1.2.2. Helicobacter Pylori – Pathogenesis

There is no single pathway of transmission for *Helicobacter pylori* that has been clearly identified. Scientists have had difficulty locating the bacterium anywhere outside of gastric tissue. Because of this, it has been difficult for scientists to locate the portal by which the bacterium is spread. However, scientists do believe the bacterium is passed from person to person and most transmissions occur during early life. It has been suggested that transmission may occur in faecal-oral and oral-oral pathways. Other pathways have not been ruled out [19]. However the bacterium enters the body it eventually makes its way to the gastrointestinal tract. *H. pylori* makes its way into the stomach to begin colonization. In order to colonize the stomach *H. pylori* must survive the acidic pH of its environment. It burrows into the mucous lining that coats the stomach. An *H. pylori* bacterium has flagella and uses them to move through the stomach lumen and drill into the mucous lining of the stomach[20]. *H. pylori* must insert itself near the stomach's epithelial cell layer which is this bacterium's niche. *H. pylori* can be found deep in the mucous lining of the stomach. Mucus is continuously secreted by mucous cells and removed on the side of the lumen.

To avoid being carried into the lumen by the newly formed mucus, *H. pylori* senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface[21]. *H. pylori* can also be present on the inner surface of the epithelial cells of the stomach. Sometimes the bacterium is even found inside the epithelial cells[22]. *H. pylori* produces adhesins that bind to membrane-associated lipids and carbohydrates which helps it to adhere to the epithelial cells[23]. The bacterium also produces great quantities of urease, and enzyme located inside and outside of the cell. This enzyme is capable of breaking down urea that is secreted into the stomach. It breaks down the urea into carbon dioxide and ammonia (Figure 5). The ammonia is converted into

an ammonium ion by accepting a hydrogen ion from self-ionized water. The left-over hydroxyl ions then react with the carbon dioxide to produce bicarbonate which neutralizes gastric acid.

Therefore, *H. pylori* is dependent upon urease for its survival in the acidic environment found in the stomach. Without the urease enzyme the bacterium would almost inevitably die. The ammonia that is a byproduct of the urease reaction is toxic to epithelial cells of the stomach. *H. pylori* also produces protease (an enzyme that breaks down proteins), vacuolating cytotoxin A (VacA), and phospholipases (enzymes that hydrolyze phospholipids into fatty acids and other lipophilic substances). All of these products are damaging to the epithelial cells of the stomach[24]**.**

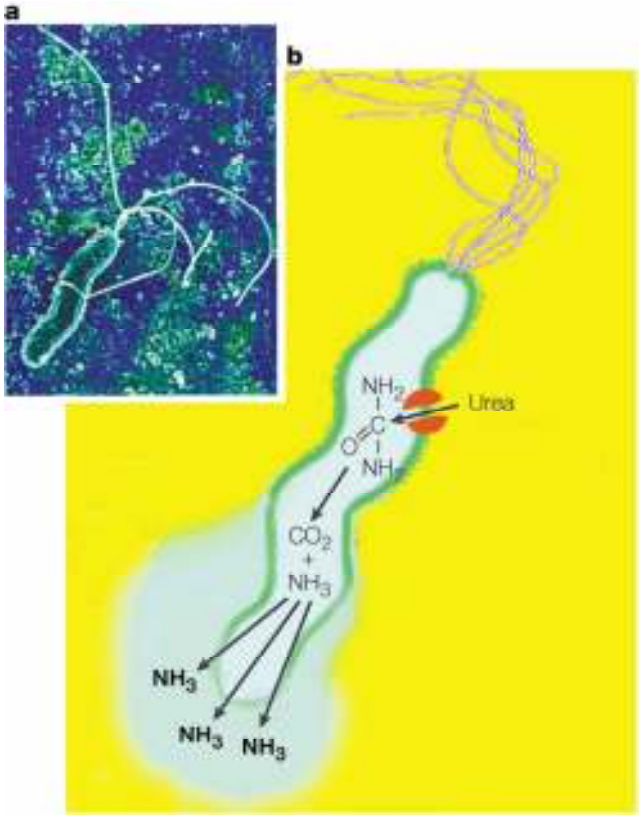


Figure 5: **a.** Image from an electron microscope, **b.** A schematic representation of the shape, whips, urease, the channel that allows the

entry of urea and the production of ammonia that neutralizes the acidic environment (yellow color) inside and outside the bacterium [25].

When *H. pylori* colonizes the stomach it often results in chronic gastritis, an inflammation of the stomach lining. Stomach and duodenal ulcers occur when the inflammation allows the acid and pepsin in the stomach lumen to overpower the mechanisms that protect the stomach and duodenal mucosa from these substances. The location of the chronic gastritis (which occurs at the site of *H. pylori* colonization) dictates the type of ulcer that subsequently develops[25]. The amount of acid within the stomach lumen has an effect on the colonization patterns of the bacterium *H. pylori*. It will therefore, in the end, establish the location at which the gastric or duodenal ulcer will form. For example, in people that produce large amounts of acid, *H. pylori* will colonize the pyloric antrum of the stomach to avoid the parietal cells in the corpus of the stomach that secrete acid[26].

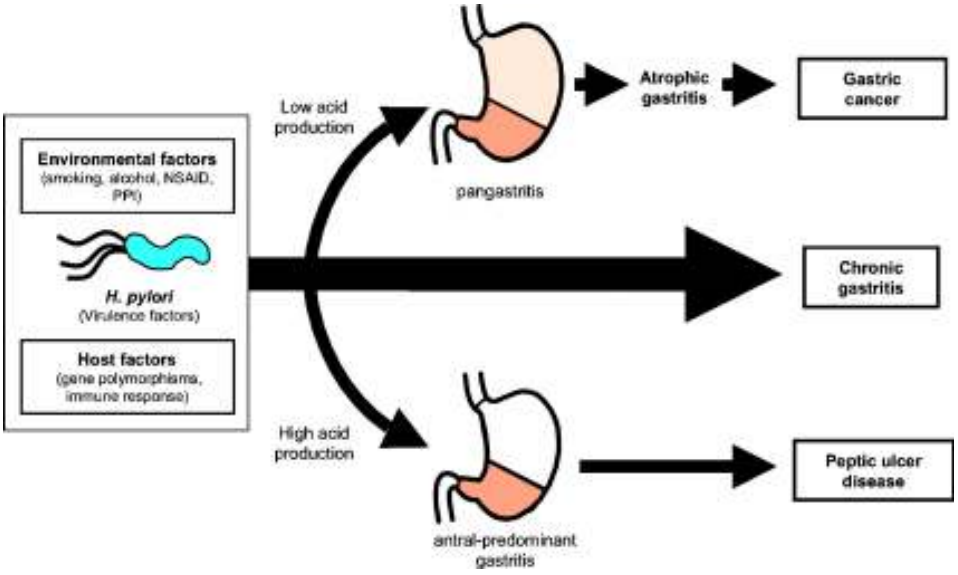


Figure 6: Pathogenesis of H. Pylori

The inflammatory response to the bacteria induces G cells in the antrum to secrete the hormone gastrin. Gastrin travels through the bloodstream

to the corpus of the stomach[27]. Gastrin excites the parietal cells in the corpus to release even more acid into the stomach lumen. Persistently increased gastrin levels eventually cause the number of parietal cells to also increase, further increasing the amount of acid secreted[28].

The increased amount of acid damages the duodenum and may consequently result in the formation of duodenal ulcers. Gastric ulcers on the other hand are often linked with reduced or normal levels of acid production. This suggests that the mechanisms that protect the gastric mucosa are faulty in that individual[28]. *H. pylori* capitalizes on this defect and begins to colonize the corpus of the stomach where the parietal cells are located. The chronic inflammation caused by the colonization of this bacterium causes further reduction in the stomach's acid production, causing atrophy of the stomach lining. The deterioration of the stomach's lining may lead to future gastric ulceration and even an increased risk for stomach cancer[29].There are some strains of *H. pylori* that are capable of triggering a greater inflammatory response in the stomach of its host. This strain carries the cag pathogenicity island (cag PAI). Over half of the *H. pylori* strains in Western countries are thought to carry the cag PAI[30].

Not only do these strains create a stronger inflammatory response in the stomach but they also create a greater risk for developing ulcers or cancer than the strains lacking the cag PAI[26]. The cag PAI expresses a type IV secretion system after the attachment of the bacterium to the epithelial cells of the stomach. This system inserts peptidoglycan from the bacterial cell wall into the epithelial cells. This peptidoglycan acts as an inflammatory inducing agent within epithelial cells. It is recognized by the cytoplasmic immune sensor Nod1 that stimulates the expression of cytokines which promote inflammation[31].

The type IV secretion system also injects the cag PAI-encoded protein CagA into the stomach's epithelial cells, where it disrupts the cytoskeleton, adherence to adjacent cells, intracellular signaling, cell polarity and other cellular activities[32]. Once it reaches the inside of the cell the CagA protein is phosphorylated on tyrosine residues by a host cell membrane-associated tyrosine kinase. Pathogenic strains of *H. pylori* have been shown to activate the epidermal growth factor receptor

(EGFR), a membrane protein with a tyrosine kinase domain. Activation of the EGFR by *H. pylori* is linked with altered signal transduction and gene expression in host epithelial cells that may contribute to pathogenesis.

It has also been suggested that a c-terminal region of the CagA protein (amino acids 873–1002) is able to regulate host cell gene transcription independent of protein tyrosine phosphorylation[33,34]. There is a large amount of diversity between different strains of *H. pylori*, and often times the strain with which a person is infected is predictive of the outcome of the infection.

There are two possible mechanisms currently being investigated by which *H. pylori* could cause cancer. One of these mechanisms involves the enhanced production of free radicals near *H. pylori* as well as an increased rate of host cell mutation. The other mechanism under investigation is called the "perigenetic pathway" [35] and involves enhancement of the transformed host cell phenotype by altering cell proteins such as adhesion proteins. It is thought that *H. pylori* causes inflammation and locally high levels of TNF-α and/or interleukin 6. According to the perigenetic mechanism, inflammation-associated signaling molecules such as TNF-α can alter gastric epithelial cell adhesion and cause the dispersal and relocation of mutated epithelial cells without the need for additional mutations in tumor suppressor genes such as genes that code for cell adhesion proteins [36].

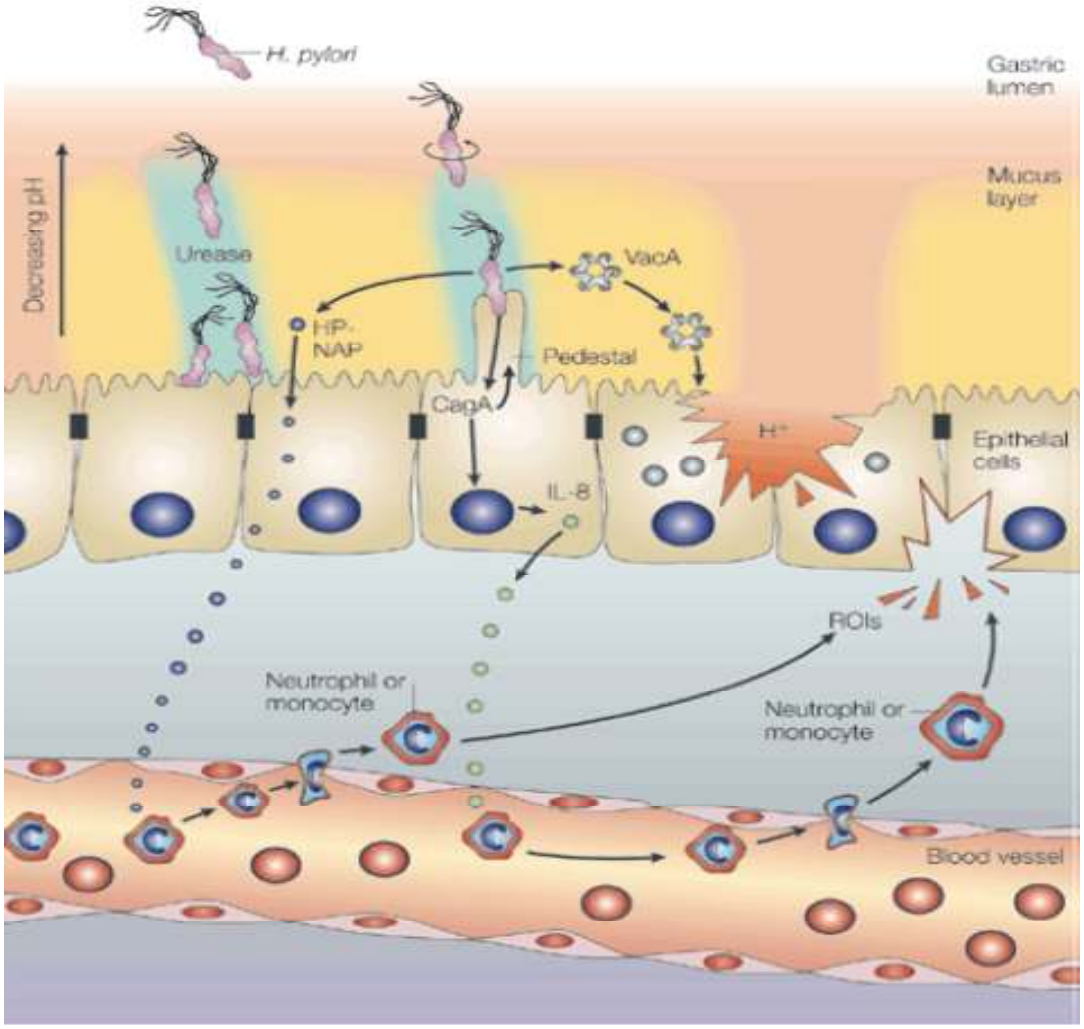


Figure 7: Schematic representation of the gastric mucosa during colonization of H. pylori, where are presented all the toxic agents that take part to the colonization process and the disease. In the early stages of infection, the bacterium enters the gastric fluid where the urease it carries helps it to survive in the acidic environment (red color indicates strongly acid pH, yellow color suggests weak acidic pH) by producing the ammonia that regulates the cytoplasmic and periplasmic pH as well as pH at bacteria's periphery (the blue color indicates neutral pH). The whips help the bacterium to move into the mucus layer until it reaches its upper surface epithelial cells. There, the plethora of adhesive proteins it produces, allow the bacterium to bind strongly to the epithelial cells. It then injects the cagA protein into the cells via a type IV secretion mechanism, while secreting other toxic factors, such as the neutrophil-activating HPNAP protein and VacA. VacA induces relaxation in tight joints between epithelial cells while inducing the formation of vacuoles in host cells.

HPVAR penetrates the epithelial layer and recruits neutrophils and monocytes, which are extravasated and cause tissue damage by producing Reactive Oxygen Intermediates (ROI). The CagA protein causes changes in the cytoskeleton while some of the cagPAI proteins activate the production of pre-inflammatory cytokines that intensify it inflammatory response by recruiting lymphocytes. The combined effect of ROI and VacA are increased tissue damage [36].

1.2.3 Helicobacter Pylori – Therapeutic Approaches

The treatment of H. pylori infection aims, on the one hand, in the eradication of the pathogenic microorganism and, on the other hand, in the suppression of inflammatory chronic gastritis. Because of changing resistance patterns, the recommended treatment for H. pylori consisted of at least 3 drugs, which generally included 2 antibiotics (Table 1) [37]. The American College of Gastroenterology published updated treatment guidelines for the management of H. pylori infections in 2007. The recommended primary therapy (Table 2) included a proton pump inhibitor, clarithromycin and amoxicillin, or metronidazole (clarithromycin-based triple therapy) for 14 days or a proton pump inhibitor or H2-receptor antagonist, bismuth, metronidazole, and tetracycline (bismuth quadruple therapy) for 10 to 14 days[38]. Modern patterns used by the gastrointestinal community are currently categorized as single, double, triple and multiple, depending on the number of drugs used during the regimen. Single treatments with bismuth salicylate or other antacid agent alone have been abandoned as ineffective (eradication rates below 40%) as opposed to bismuth triplets, double and multiple patterns all applied to clinical practice.

Common Drugs Used in the Treatment of *H. pylori*

Drug Class	Examples of Drugs Within the Class
Antibiotics	Amoxicillin, clarithromycin, metronidazole, tetracycline
H_2-blockers	Cimetidine, famotidine, nizatidine, ranitidine
Proton pump inhibitors	Esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole
Cytoprotective agents	Bismuth subsalicylate, bismuth subcitrate potassium

Table 1: Common Drugs used in the treatment of H. Pylori

I. Triple therapy based on bismuth compounds

One of the commonly used forms is the classic treatment with bismuth colloidal subcitrate (or bismuth subsalicylate), metronidazole and tetracycline or amoxicillin. With one week of total treatment, gastric and duodenal ulcers are healed even with no suppression of gastric secretion with H2 histamine reuptake antagonists or proton pump inhibitors [Thijs et al., 1996]

II. Double Therapy

Double therapy consists of a combination of a. a gastric secretion-suppressing compound (H2 antagonists-ranitidine or famotidine or proton pump inhibitors (a class of drugs that include omeprazole, lansoprazole, rabeprazole and pantoprazole); and b. an antibiotic (usually amoxicillin or clarithromycin). The strong inhibition of gastric secretion results in pH rise to 5 or higher and acts synergistically with antimicrobial therapy. This treatment achieves eradication rates of between 20% and 90%. The most frequent dose of clarithromycin (500mg three times a day achieves even more effective eradication rates between 63% and 81%) (Harris et al., 1995).

III. Triple Therapy

However, as is evident from modern bibliography, the most popular treatment regimen seems to be the triple combination therapy with an antisecretory agent and dual antimicrobial treatment. The MACH study 1 has shown that the use of omeprazole in combination with various antimicrobials such as amoxicillin, tetracycline or metronidazole achieves effective eradication of H. pylori in one week (Lind et al., 1996). And in this case the addition of clarithromycin achieves more permanent and optimal results. It has also been demonstrated that the replacement of the prazole with a new formulation (ranitidine with bismuth citrate) achieves the same eradication rates by combining the anti-secretory properties of ranitidine with the mucoprotective and directly toxic for H. pylori properties of bismuth (Savarino et al. 1997).

IV. Quadruple treatment

The quadruple therapy combines a suppressive secretion factor with triple antimicrobial therapy. Typical quadruple therapy is the combination of omeprazole, tetracycline, metronidazole and bismuth salts or sucralfate [37]. At present, quadruple treatment is maintained as a second-line treatment for the failure of the above-described regimens, and is experimentally tested to reduce the duration of treatment.

First-Line Treatment Regimens for *H. pylori* Infections Recommended by the American College of Gastroenterology

Regimen	Duration (days)	Eradication Rates (%)	Comments
Standard-dose proton pump inhibitor twice daily (esomeprazole is once daily), clarithromycin, 500 mg twice daily, amoxicillin, 1000 mg twice daily	10-14	70-85	Consider in nonpenicillin allergic patients who have not previously received a macrolide
Standard-dose proton pump inhibitor twice daily, clarithromycin, 500 mg twice daily, metronidazole, 500 mg twice daily	10-14	70-85	Consider in penicillin allergic patients who have not previously received a macrolide or are unable to tolerate bismuth quadruple therapy
Bismuth subsalicylate, 525 mg orally 4 times daily, metronidazole, 250 mg 4 times daily, tetracycline, 500 mg 4 times daily, ranitidine, 150 mg twice daily, or standard-dose proton pump inhibitor once to twice daily	10-14	75-90	Consider in patients allergic to penicillin
Proton pump inhibitor plus amoxicillin, 1 g twice daily for 5 days, followed by proton pump inhibitor plus clarithromycin, 500 mg and tinidazole, 500 mg twice daily for 5 days	10	> 90	Requires validation in North America

Reprinted with permission from Chey WC et al.[3]

Table 2: Therapeutic regimens for treating H. pylori infection by the American College of Gastroenterology

The widespread use of antimicrobial agents over the last decade has led to high levels of microbial resistance and growth of H. pylori strains resistant to combined treatments. Moreover, the complexity and duration of treatment are associated with the low level of compliance with therapy. Metronidazole tolerance varies between 10% and 90% between countries, due to the mutation of enzymes in bacterial strains that reduce the antibiotic to nitroimidazole (Ling et al., 1996).

The resistance to clarithromycin increases rapidly with rates of up to 15% due to its common use in respiratory infection (Tompkins et al., 1997). Even the best therapies fail at a rate of 10% with re-infection rates remaining high (Sung et al., 1998). These data, combined with the growing literature on this issue, impose a skepticism and perhaps a redefinition of future therapeutic strategies.

For this reason, new innovative pharmaceutical technologies as well as alternative active substances, such as the Mastic of Chios, are under investigation for use in the eradication of Helicobacter.

1.2.4. Product availability on the market

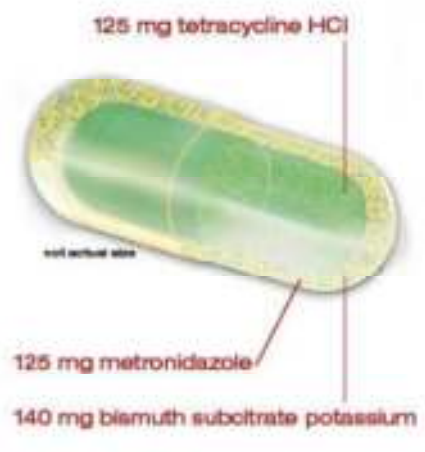


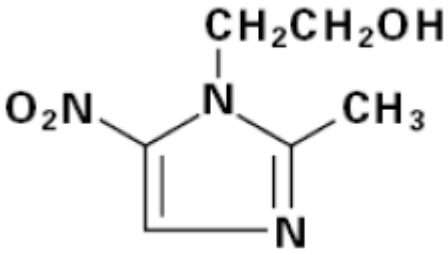
Table 1: Daily Dosing Schedule for PYLERA

Time of dose	Number of capsules of PYLERA
After morning meal	3
After lunch	3
After evening meal	3
At bedtime	3

The Pylera formulation is designed to decrease the pill burden associated with H. pylori therapy and improve patient adherence with the prescribed dosing regimen. Pylera contains bismuth subcitrate potassium, metronidazole, and tetracycline in a specially designed capsule. The outer part of the capsule contains a blend of 140 mg of bismuth subcitrate potassium and 125 mg of metronidazole. The inner part of the capsule contains 125 mg of tetracycline HCl [39]. The use of this capsule formulation does not impact the bioavailability of any of the product components (Table 3). The treatment regimen for H. pylori infection with this product is 3 capsules 4 times daily after meals and at bedtime in conjunction with omeprazole 20 mg at breakfast and dinner for 10 days. Administration with food decreases the relative bioavailability of each drug, but the change in the bioavailability should be insufficient to influence the efficacy of the drug regimen[39,40].

1.2.5. Helicobacter Pylori – Pharmacology of the treatment

- **METRONIDAZOLE**



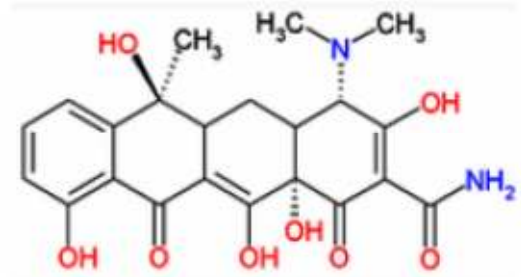
Metronidazole is a bactericidal antibiotic against anaerobic bacteria. It works with two possible mechanisms of action:

a. Works as an electron reservoir. Operating as an electron acceptor deprives the bacterial cell of its reductive power. In addition, electron uptake by the drug results in its conversion to an intermediate derivative (nitroimidazole), which is toxic to the DNA, leading to rupture.

b. The second mode of action relates to the properties of metronidazole to bind to DNA and to break the chains that prevent it from functioning properly as a template for DNA polymerase resulting in inhibition of DNA duplication by DNA polymerase.

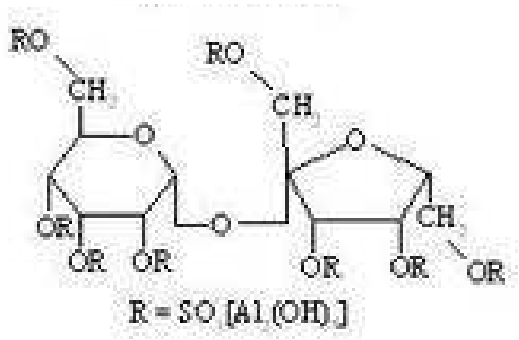
The antimicrobial mechanism of action of metronidazole is dependent upon the reduction of its nitro moiety by nitroreductase and other reductases to the nitro anion radical and other moieties (eg, nitroso, hydroxylamine). These reduced moieties then produce a damaging effect on the DNA of the bacteria. In situations where there may be some metronidazole resistance, the resistance may be overcome with higher doses of metronidazole and the involvement of alternative nitroreductase pathways [41].

- **TETRACYCLINE**



The antimicrobial mechanism of action of tetracycline is dependent on the drug binding to the 30S ribosomal subunit, which then prevents the binding of the tRNA to the mRNA ribosome complex and interfering with the protein synthesis of the bacteria [42].

- **SUCRALFATE**



Hexadeca-μ-hydroxytetracosahydroxy[μ8-[1,3,4,6-tetra-O-sulfo-β-Dfructofuranosyl-α-D-glucopyranoside tetrakis(hydrogen sulfato)8-)]]hexadecaaluminum

Sucralfate is a cytoprotective agent and it is used for the treatment of active gastro-duodenal ulcers, gastro-esophageal reflux and also stress ulcers. It was approved by the FDA in 1981. It is a basic aluminium 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 hydroxide solutions. It has to be stored in tightly closed containers, in a well-ventilated area [43].

Sucralfate is a unique oral drug. It is a sucrose sulfate-aluminium complex that brings to the mucosa, thus creating a physical barrier that impairs diffusion of hydrochloric acid in the gastrointestinal track 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 the 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:

1. 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.
2. Inhibits directly pepsin (an enzyme that breaks apart proteins) in the presence of stomach acid
3. Binds bile salts coming from the liver via the bile thus protecting the stomach lining from injury caused by the bile acids
4. May increase prostaglandin production, and prostaglandins are known to protect the lining of the stomach.

- **MASTIC OF CHIOS**

a.  b. 

Figure 8: a. Mastic of Chios as a resin **b.** The mastic tree

The mastic of Chios is produced by the mastic tree, which belongs to the Pistacia lentiscus L. specie of the Anacardiaceae family. The mastic tree is cultivated or found naturally in the south of the island of Chios, which is the only area that can thrive. Mastic is a characteristic product of Chios since it is not produced in any country other than Greece, and it has the privilege of being a unique product at the level of the European Union and perhaps even globally. Mastic is excreted, in the form of resin (figure 8a) from "wounds" (figure 8b) that are created in the tree trunk with a pointed tool (embroidery), in its natural state.

The biological activities of Chios Mastic can be attributed to various compounds. The components which might contribute to its therapeutic effects belong to the class of mono- and sesquiterpenoids (Barra et al, 2007) and triterpenoids (f.i. masticadienonic acid) . Apart from the above, approximately 25% of its total weight is a polymer, which in an acid environment becomes a runny resin that could have cytoprotectant effects in patients [44]. More than 20% of its total weight is the neutral fraction. In total, 70 components have been isolated from pure Chios Mastic [45]. The high concentrated compounds found in the mastic essential oil and the mastic itself are: α-pinene (63% and 40% respectively), β-pinene (3.3% and 1.5% respectively), β-mycene (25% % respectively), limonene (1.5% and 1%, respectively) and β-caryophyllene (1% and 5%, respectively) [46].

Chios Mastiha has reportedly been used in traditional Greek medicine for the relief of diverse gastrointestinal disorders, such as abdominal pain, dyspepsia, gastritis and peptic ulcer for more than 2.500 years. Studies have been done for the investigation of therapeutic properties of Mastic in the gastrointestinal tract. Al-Habbal et. Al., administered crude mastic (1g daily for 2 weeks) and lactose (1g daily for 2 weeks - placebo) in duodenal ulcer patients [47]. Symptoms were reduced in 80% of patients, while 70% of patients were treated with ulcer (rates are 50% and 22% respectively for placebo). Subsequent studies have shown that crude mastic has a potential cytoprotective effect and action on stomach and duodenal ulcer [48].

Apart from the gastroprotective protective effect, mastic has also been found to have bactericidal properties. It has activity against H. pylori in vitro as well as against strains isolated from biopsies [47, 48]. Also, the essential oils of mastic as well as the resin have antibacterial activity against the bacteria Escherichia coli, Staphylococcus aureus and Bacillus subtilis [49]. Of course, there are studies with conflicting results, supporting that mastic cannot eliminate the bacterium from the stomach, nor reduce its number [50].

1.3. Modified release solid oral dosage forms

Among all drug delivery systems, oral delivery is the most convenient and commonly preferred route for administration of several drugs. It has some specific advantages, such as ease of administration (can be self-administered), pain free, flexibility in the design and low cost.

In the last few decades, pharmaceutical research has focused its attention to the necessity to modify drug release. Modified or controlled released oral drug delivery systems have been shown to offer advantages over conventional system [51]. Some problems correlated with the conventional drug delivery systems are:

- Poor patient compliance, increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary (especially for chronic disease).
- Fluctuations of drug concentration levels that may lead to under or over medication.
- Difficult on the attainment of steady-state condition.

Controlled release can overcome various issues tied to the conventional release, changing the kinetics of drug release, offering prolonged delivery of drugs and maintenance of plasma levels within the therapeutic range. Oral modified release dosage forms are developed by altering the drug release to achieve predetermined clinical objectives. Possible therapeutic benefits of a modified release product include improved efficacy, reduced side effects, increased patient compliance, optimized performance and reduced dosing frequency. Several formulations capable to control both the rate and the site of drug release have been studied.

Oral modified release dosage forms can be divided in three different categories:

- Delayed release : the drug is released after a certain interval of time after administration
- Extended release : the drug is released slowly over time
- Pulsatile release: the drug is released in sequential way and repeated in the time.

In this way it is possible to obtain constant drug release rate reducing drug level fluctuations in blood and maintain a steady state concentration over a prolonged period of time.

Furthermore, drug plasma levels are kept within a narrow window with no sharp peaks, with a subsequent reduction of adverse side effects, of the dose frequency and an improvement of patient compliance. Thus, it is possible to target the drug delivery to its site of absorption or action, reducing unwanted side effects [52].

1.4. Hydrophilic Matrix Systems

Among the various modified-release pharmaceutical forms designed for the oral route, the most widespread is the one constituted by swellable matrix systems. The active ingredient is dissolved or dispersed in a swellable hydrophilic polymer able to swell and dissolve in contact with an aqueous solvent[53-56]. These systems are commonly manufactured by direct compression, wet or dry granulation and hot melt extrusion. The choice of the type of polymer to be used determines the control of release. The polymer acts as a control element of the issue, and must meet certain requirements such as biocompatibility, mechanical strength and permeability to the drug [57]. The polymers more frequently used are some cellulose esters (hypromellose, hydroxypropyl cellulose and sodium carboxymethylcellulose), polyethylene oxide, carbomers, sodium alginate and xantham gum [58]. Drug release from these delivery systems is basically regulated by solvent-polymer interaction and solvent-drug interaction. The polymer primarily used for the production of swellable matrix is hydroxypropylmethylcellulose (HPMC, Figure 9) due to its technological characteristics, availability, handling and use, reduced toxicity and cost [59].

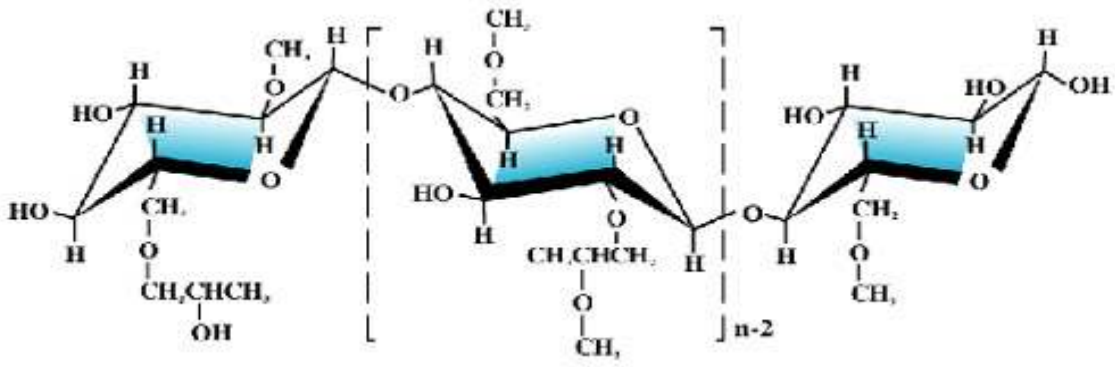


Figure 9: Structure of hydroxypropylmethylcellulose.

The HPMC is a hydrophilic polymer capable of absorbing large quantities of water. The characteristics of the polymer, in particular the degree of viscosity, the amount, the size of the particles and the solubility, affect the release of the drug from the matrix [60]. The different viscosity of HPMC depents on the degree of substitution by methyl and hydroxypropyl groups that affect the interaction of the chains in the polymer with the water, and then the hydration of the matrix. If the polymer has a high viscosity it has more rapid hydration and forms quickly a layer of dense and thin gel at the beginning, which decreases the entry of water and then the release of the drug [58,59]. When the content of HPMC in the matrix reaches high percentages (30-40%), the degree of substitution becomes less influential on the release kinetics of the drug. High polymer content causes the formation of a gel layer that slowly erodes by controlling the release of the drug; a small amount of polymer allows a greater penetration of water into the matrix and a consequent increase in rate of drug release.

Also the polymer particles size, affects the speed of hydration of the polymer itself modifying the release of the drug. Small polymer particles lead to the formation of dense matrix because of the bonds between the particles while large particles lead to a high porosity and therefore to a fast release of the drug due to a slow hydration of the particles and to

the formation of an uneven gel which causes the disintegration of the matrix.

The swelling of the matrix occurs when the system is placed in contact with an aqueous solvent, such as a biological fluid, and is influenced by the penetration of solvent into the polymer that allows the transition from the glassy state to the rubbery state as a result of the relaxation of the polymer chains [61]. The transition from glassy to rubbery state is accompanied, as well as by swelling , also by the formation of the layer of gel on, the outer surface of the matrix which hinders simultaneously the water uptake and the release of drug.

The swelling of these matrixes can be described by the position of the fronts, inside the matrix, due to the changes of the physical state and the polymer and/or the drug (Figure 10) [62].

Usually starting from the center of the matrix, three fronts can be observed:

- Swelling front: boundary between the dry core and the swollen matrix
- Diffusion front: boundary between the undissolved solid drug and the drug in solution within the gel layer. It is more common to observe the diffusion front if the drug has limited water solubility
- Erosion front: boundary between the matrix and the dissolution medium [63]

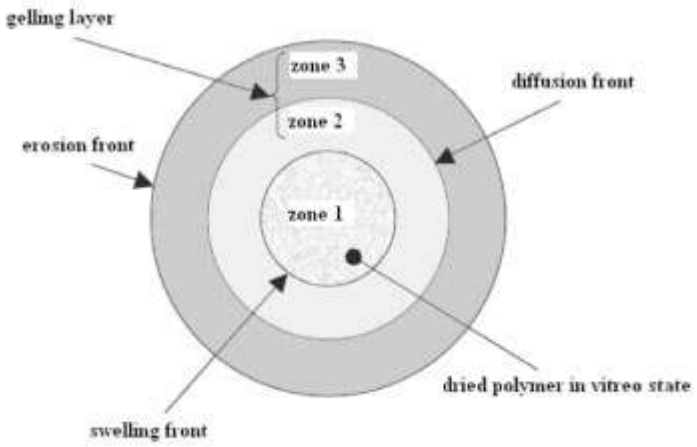


Figure 10: Illustration of fronts (swelling, diffusion and erosion) during drug delivery by matrix swelling process [64].

The relative movement of erosion and swelling fronts determines the thickness of the gel layer and drug release rate and kinetics, since drug release occurs by diffusion through the gel layer. The fronts are continuously in motion and this is associated to physical phenomena that follow one another within the matrix:

- The movement of the swelling front depends on the speed with which the water enters into the matrix;
- The diffusion front changes position based on the rate of drug dissolution;
- The erosion front moves following the dissolution of the polymer, when the polymer chains are distant from each other and surrounded by the solvent.

At the beginning, the erosion front moves outward to the solvent, while the swelling front moves inwards as a result of water penetration. Thus, at the early stage of the dissolution process, the gel thickness increases. As the hydration process continues, the erosion front moves inwards, in the same direction of the swelling front, because of the progressive dissolution of polymer chains in the medium. If the rate of movement of both fronts is the same, the gel thickness is constant. In this phase, the synchronization of the two fronts produces a constant drug release.

Finally, when the entire polymer is swollen, only erosion occurs and the gel thickness decreases until the matrix is completely dissolved [65]. The presence of a visible diffusion front depends on the drug solubility. If the drug is highly soluble, it easily dissolves in the small volume of water present in the gel layer. Therefore, undissolved drug is present only in the dry core of the matrix and the diffusion front coincides with the swelling front. Otherwise, for poorly soluble drugs, the diffusion front is observed with the swollen gel layer. The movement of this front depends on the drug solubility: it tends to be faster when the drug solubility is higher, providing also higher release rate. In addition drug solubility affects also the thickness of the diffusion front and its rate of change. In systems where a diffusion front is present, the polymer relaxation near the swelling front may be reduced by the undissolved drug. Here, the drug dissolution and its subsequent diffusion through the gel layer is more important in controlling drug release than the gel thickness [63].

It is observable that the performance of a matrix system, in terms of drug release rate and release kinetics, is highly influenced by the nature of the drug and the polymer used in the formulation. In addition to the characteristics of the matrix include the solubility of the excipients, the size and shape of the matrix, the relationship between surface area and volume of the matrix.

The release of the drug from the matrix is controlled by the interaction between water, polymer and drug [66]. The polymer is mainly responsible for the release kinetics of the drug, because its characteristics affect the speed of the matrix swelling and the viscocity of the gel layer that the drug has to cross. The characteristics of the drug that influence the release are the solubility and the particle size. More the drug is soluble, faster will be its release.

1.5. Release Kinetics

The release kinetics of drug from a swellable and erodible matrix has been described by RItgel and Peppas (1987) [67-69] through the following mathematical model: (eq.1)

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

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 is used to characterize different release for cylindrical shaped matrices and it characterizes the release mechanism of drug. The value of n = 0.5 represents system where the Fickian diffusion is the main mechanism of release (Case I), 0.5<n<1 describes anomalous-Fickian release where both diffusion and relaxation mechanism contribute to drug release, n = 1 indicates Zero order release (Case II) if the drug is released upon relaxation of the hydrated polymeric chains 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 polymeric chains, whereas anomalous release to the summation of both drug diffusion and polymer erosion or swelling-controlled drug release. The extreme values of 0.5 and 1 are valid only for matrices with the geometry of a thin film. For cylindrical matrices n takes the value of 0.45 for diffusion-controlled release and 0.89 for relaxation-controlled release [70-71.]

The mathematical description of the entire process of drug release from swellable matrix remains rather difficult, because of the number of physical characteristics that must be take into consideration, such as water penetration into the matrix, polymer swelling and dissolution, erosion process, drug dissolution and diffusion, radial and axial transport

in 3-dimensional system, moving fronts and changes in matrix dimension, porosity and compositions.

1.6. Dome Matrix technology

With the advances in pharmacogenomics and pharmacogenetics 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 [72]. From the therapeutic point of view, the personalization of therapy addresses the specific health needs and personal of the individual patient.

In 2006, Colombo et al [73] developed 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 a single unit for controlled drug release via the oral route.

The dome matrix module is constituted of a cylindrically shaped tablet or matrix, with two bases, one concave and the other convex (Figure 11). The axial section of the module appears as a dome, hence the name “Dome Matrix”.

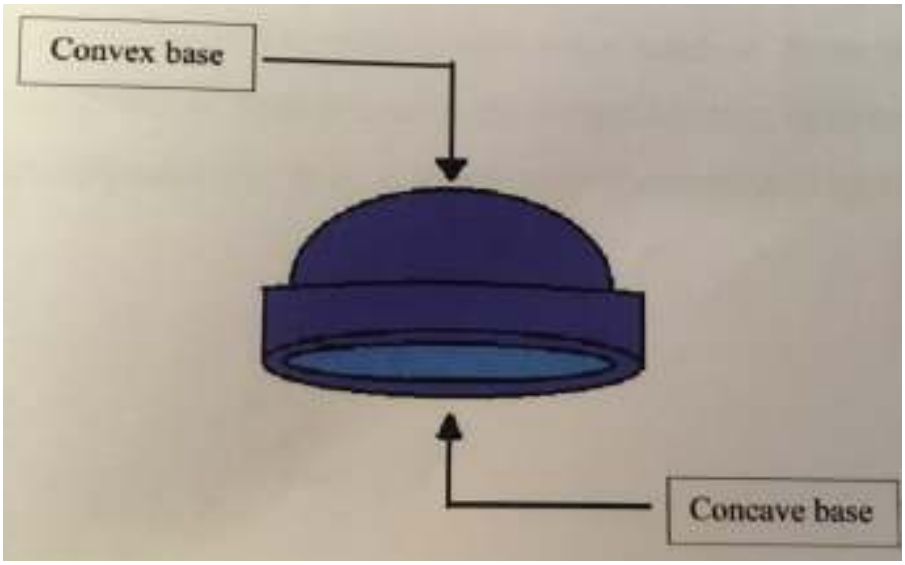


Figure 11: Structure of Dome Matrix module

Principally, the individual modules are designed to allow the convex base of one module to be inserted in the concave base of another. There are two different types of modules, identified as male and female module, differing from each other from the concave base design.

1.6.1. Female Module

Two different female modules can be obtained. The first one, terminal module allows only the interlocking of a male or female module into its concave base. In contrast, the second type besides being able to accommodate a module into the base concave, can be stacked on its convex base thanks to the particular geometry of its face, into the concave base of another module female [74] (Figure 12).

Figure 12: Dome Matrix female modules

1.6.2. Male Module

The male modules (Figures 13) are characterized by the presence of an annular protrusion on the concave rim base, which allows the interlocking with the concave face of female modules.

Figure 13: Dome Matrix male modules

1.6.3. Dome Matrix assembling configurations

The modules assembling may take place in different ways, it depends by the features of the two modules: stacked configuration, void configuration and mixed configuration.

1.6.3.1. Stacked Configuration

This type of configuration is obtained putting the convex base of a module into the concave base of another (male or female stacked with female module) (Figure 16a)

In this way it is possible the assemblage of more modules to control the drug release rate thank to the relationship volume/surface exposed to the dissolution medium.

1.6.3.2. Void Configuration

The Void Configuration is obtained by interlocking the annular protrusion of a male module with the concave base of a female module (Figure 16b)

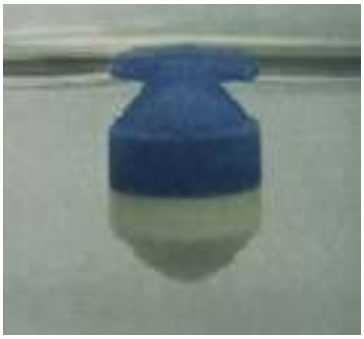
a.  b.  c. 

Figure 16: Dome Matrix modules with a. stacked configuration b,c. void configuration

This configuration is characterized by an inner empty space that provides buoyancy of the assembled system, obtaining delivery systems able to float on the gastric-content that perform drug release in the stomach (Figure 16c) [75,76]

A configuration of this kind can be useful for the drugs with an absorption window and site of action into the stomach. Obviously, for these gastroretentive drug forms is necessary to keep in consideration the emptying of the stomach. The factors that influence the gastric emptying (hence the retention time) of oral dosage forms are the density, the shape and the dimensions the simultaneous ingestion of food or drug like anticholinergic and biological factors as age, sex, race, body mass index and presence of pathology [77].

1.6.3.3. Mixed Configuration

Using both configurations, void and mixed, it is possible to obtain a delivery system in which the modules are assembled in mixed configuration (Figure 17).

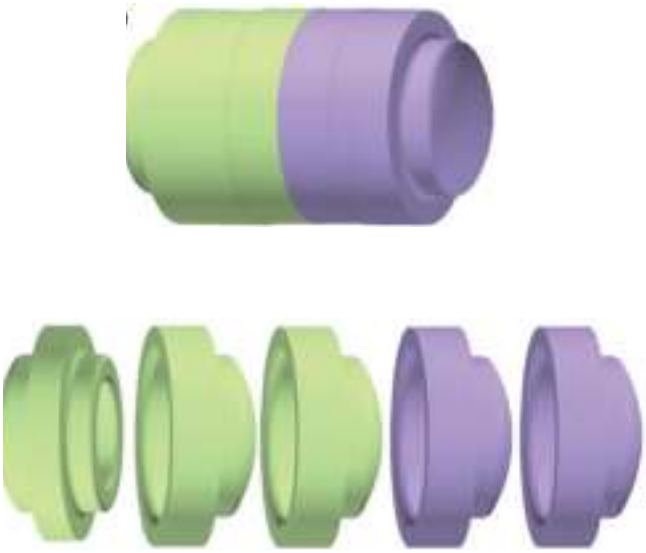


Figure 17: Dome Matrix modules with mixed configuration

Dome Matrix shows some definite advantages as drug carrier when compared to traditional tablets. For example, in the case of chronic diseases, which require the administration of several medicinal products, the system of issuing Dome Matrix allows to reduce the number of dosage units and to customize therapy, increasing patient compliance.

In dependence on the module assemblage way, different system configurations can be obtained. The modular approach makes possible to administer different drugs at the same time at different rate and in selected site. Modules of different drug types, doses and release kinetics can be combined in one single unit. 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. Modular technology solves the eventual problem of incompatibility between the actives, because each module is individually manufactured by compression and controlled for weight and release.

2. Aim of the work

The purpose of this master thesis was the treatment of Helicobacter Pylori by the use of Dome Matrix technology which allow us to manufacture floating dosage forms. The substances used are tetracycline hydrochloride, metronidazole, sucralfate and mastic of Chios. Modules of tetracycline and metronidazole combined in a single dosage form designed to float in the gastric content and to sustain the intra gastric concentrations of these two antibiotics. By maintaining a prolonged direct contact between H. pylori and the antibiotic molecules, the assembled system has the potential to reduce the drug doses and the number of dosage forms administered daily. Moreover, immediate release modules of sucralfate and mastic of Chios were combined in different configurations, in order to enhance the efficacies of antibiotics against H. pylori. A hard capsule of size 00 were used to include these assembled systems, for better patient compliance.

3. Materials and Methods

3.1. Materials

- AcDiSOL type sd-711; FMC; Batch: T0646C
- Avicel PH102; ACEF; Batch: H2692003
- Corn Starch; ACEF; Batch: G4283010
- HPMC-K15M; Estman Kodek; Batch: 10801
- Kollidon VA 64; BASF; Batch: 55105156P0
- Magnesium Stearate; ACEF; Batch: C1402005
- Mastic of Chios, Natural resin; Chios Gum Mastic Growers Association
- Metronidazole; Special Product's Lines (Rome, Italy)
- Sodium Bicarbonate (NaHCO3); Riedel de Haen; Batch: 62340
- Sucralfate gel; BK Giulini; Batch: 10-216-30
- Talc; ACEF; Batch: C5239004
- Tetracycline Hydrochloride; Lisapharma S.p.A. (Erba, Italy)

3.2 Methods

3.2.1 Development of an HPLC analytical method for Tetracycline HCL and Metronidazole

Tetracycline HCL and Metronidazole were analyzed using a High Pressure Liquid Chromatography (HPLC) equipped with a UV-VIS detector (Shimatzu with Waters autosampler). The stationary phase was a Supercosil C18 column (250 mm, 4.6x5 µm) at ambient temperature and the mobile phase was a mixture (65-35) of potassium dihydrogen phosphate buffer 0.05 M at pH 1.5 and methanol. The flow rate was 0.7 ml/min; the injection volume 10 µl

and the wavelength 280 nm. For the validation of the method, the linearity was examined for both antibacterials.

3.2.2 Preparation of mastic powder

Pieces of mastic resin (Figure 1a.) were grounded by a mill for 15 seconds to produce a fine powder (Figure 1b.)

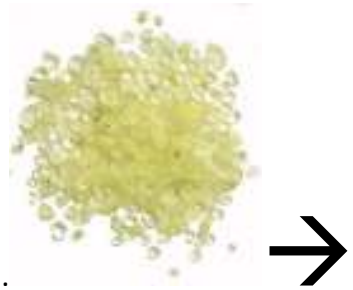
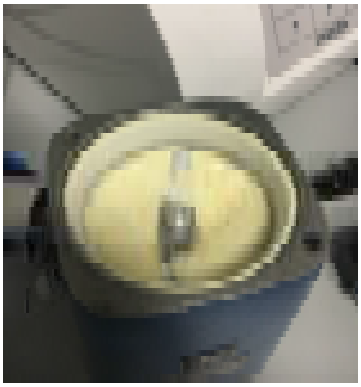
a.  b. 

Figure 1: a. Mastic as a resin b. Powder of Mastic

3.2.3 Preparation of sucralfate granules

The humid sucralfate gel was manually crashed in small pieces and left in the oven for 4 hours at the temperature of 60°C. It was then transferred in an oscillating granulator (Figure 2a) equipped with a 1.2 mm opening net. The granules (Figure 2b) were dried in oven (Glatt, Germany) at 60°C for 2 hours. The water content in the sucralfate granules was measured by thermogravimetric analysis (TGA). The amount of water was 18.6%.

a.  

Figure 2: a. Wet granulator (ERWEKA AR400, Germany) b. Granules of sucralfate

3.2.4 Manufacture of tablets

3.2.4.1. Dome Matrix modules

(process for Tetracycline and Metronidazole)

Dome Matrix modules were obtained using an alternative tableting machine (KORSCH, Mod. 9341-72, Berlin) equipped with special punches (figure 3b). The mixture was compressed using Dome Matrix punches 7.5 mm, female and male.

a.  b. 

Figure 3: a. KORSCH tableting machine, Mod. 9341-72, Berlin. b. Special punches of Dome Matrix modules

3.2.4.2. 7 mm cylindrical tablets

(process for bilayer tablet of sucralfate-mastic of Chios)

Cylindrical bilayer tablets (diameter 7 mm, Figure 4b) were obtained using the Steel'One Tableting Machine (Figure 4a) equipped with flat punches.

a.  b. 

Figure 4: a. Sty'one Tableting machine, Medelpharm S.A.S Beynost, France

b. Cylindrical flat punches diameter of 7 mm

3.2.4.3. Assembly of Dome Matrix modules

The assembled systems were obtained by ''clicking'' together the modules by hand in the following way: one female module of Metronidazole (Figure 5a) and one male module of Tetracycline (Figure 5b) were interlocked concave to concave face. This assembly, named void configuration, ensured the floatation of the release system to be

exploited in vivo for localizing and maintaining the drug release in the stomach.

Figure 5: **a.** Male module of Tetracycline, **b.** Female module of Metronidazole, **c.** Assembly system of the two antibacterials (void configuration)

3.2.5 Mechanical characteristics of the tablets

3.2.5.1 Measurement of the hardness of the tablets

The measurement of hardness was carried out according to the procedure

in Ph. Eur. 7. 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

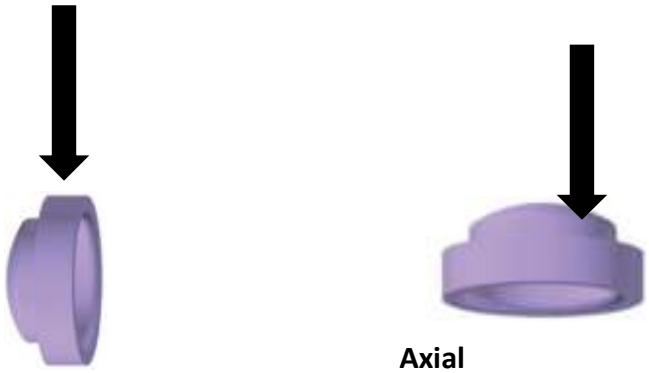
other. The mobile support is connected to a spring on which it is possible

to exert pressure through a rotating screw. The flat surfaces of the clamps

are perpendicular to the direction of movement. The module is firmly

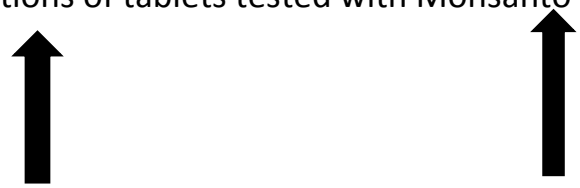
fixed to the surface of clamps which has to be flat and wider than the area

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). Theresults, according to Pharmacopoieia, were expressed as mean of minimum and maximum forces measured in kg.



Diametral **Axial**

Figure 7: Positions of tablets tested with Monsanto device



3.2.5.2 Friability Test

Test was according to 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,TA3P, Figure 8) and the

drum was rotated 100 minutes (25 rotation in one minute). Tablets were dusted again before weighting.

Figure 8: Friability Test Apparatus

Friability (%) is calculated according to the following equation

$$F(\%) = \frac{(W_{initial}) - (W_{final})}{(W_{initial})} X\,100$$

where Winitial is the weight of tablets before testing and Wfinal is the weight of tablets after testing.

The maximum acceptable weght loss is 1%.

3.2.5.3 Disintegration time

Test was done according to European Pharmacopoieia. The device (Figure 9) 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 oC and disintegration time is assesed visually.

Maximum time for test is 15 minutes. The result is the average value of 6 tablets.

Figure 9: Disintegration Test Apparatus

3.2.6 In Vitro dissolution test of Metronidazole and Tetracycline HCL (as individual modules and as assembly system)

The USP dissolution apparatus II (Sotax CH-4008, Basel, Switzerland) with

addle rotating at 75 rpm was employed. The modules were tested in two different media; in the European Pharmacopeia pH 1.2 simulated gastric fluid without enzymes and in a Phosphate buffer pH 3.5 at 37 oC, and 900mL were used in each vessel for dissolving the individual modules and the assembled systems. Samples collected at fixed time points (5, 10, 15, 20, 30, 45, 60, 120, 180, 240, 300) were filtered through a 0.45 mm membrane (CA 0.45 mm, LabService Analytica, Bologna, Italy) and analyzed using the HPLC assay method. Triplicates were conducted for the average result.

3.2.7 Floating of assembly system

The floatation of the assemly system was assesed visually using a Type II dissolution test apparatus with 900 ml of dissolution medium. The trial was done for both of the medium that were used (pH 1.2 and 3.5). An assemled system of Metronidazole- Tetracycline HCL was inserted into the vessel and the floating time was assesed visually.

a.  b.  c. 

Figure 10: Visually assesment of floatation of the assemled system at 2 sec (a), after 4 minutes (b), after 6 hours (c).

4. RESULTS AND DISCUSSION

4.1. Validation of the HPLC method for the Tetracycline-Metronidazole assay

Tetracycline HCL and Metronidazole were quantified via HPLC method descibed in 3.2.1. A calibration curve was constructed using 7 concentrations of TTC-MND dissolved in simulated gastric fluid as reported in Figure 1 and 2 . Each sample was injected three times.

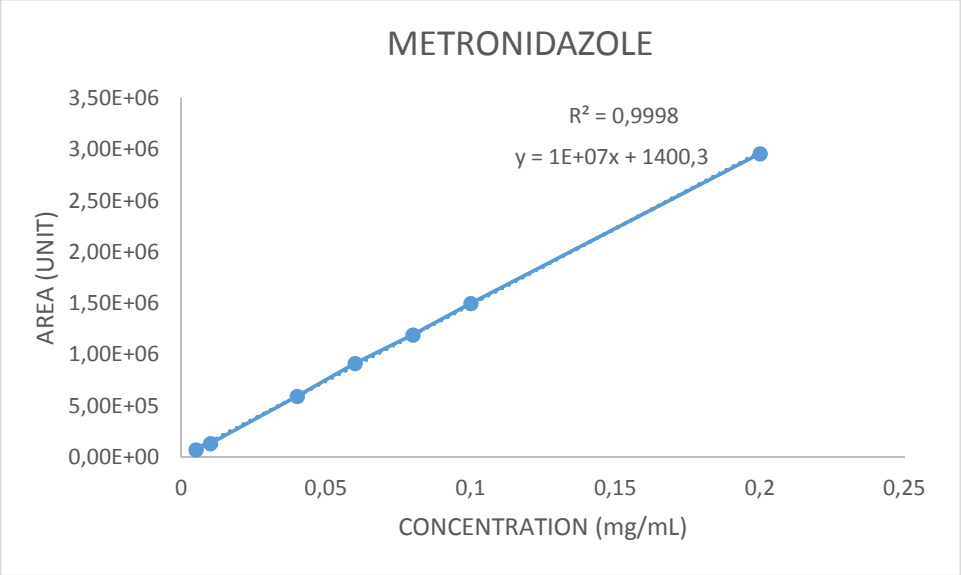


Figure 1. Area versus concentration of Metronidazole in simulated gastric fluid pH 1.2

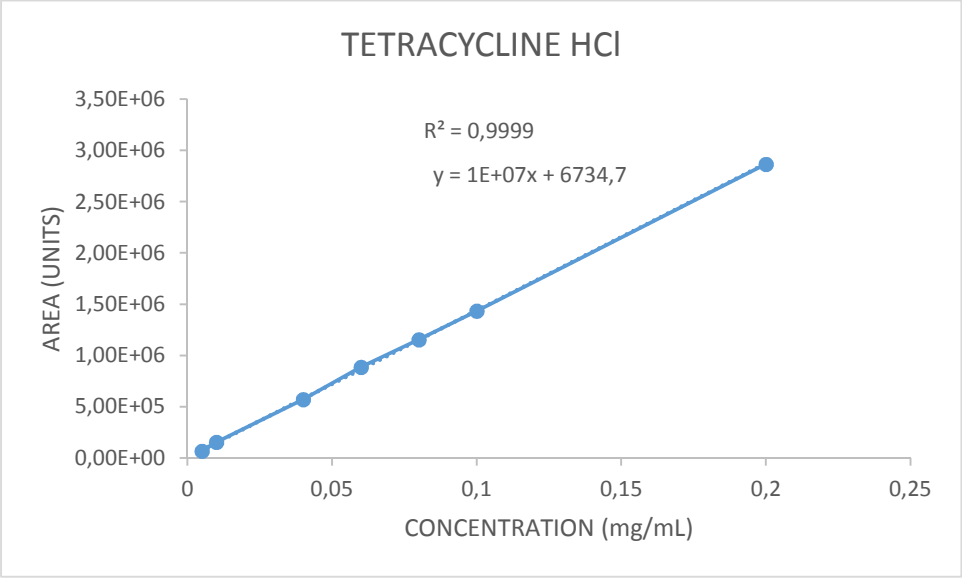


Figure 2. Area versus concentration of Tetracycline HCl in simulated gastric fluid pH 1

4.2. Stability of the antibiotics in simulated gastric fluid pH 1.2 and pH 3.5

The stability of the antibiotics was assayed in the European Pharmacopeia pH 1.2 simulated gastric fluid without enzymes and in a Phosphate buffer pH 3.5 at 37 ^{o}C. Tetracycline HCL and Metronidazole were inserted together in a volumetric flask containing the buffer, placed in a water bath and HPLC analysis of the samples was done in frequent time points (0, 3, 4, 5, 6, 24 hours).

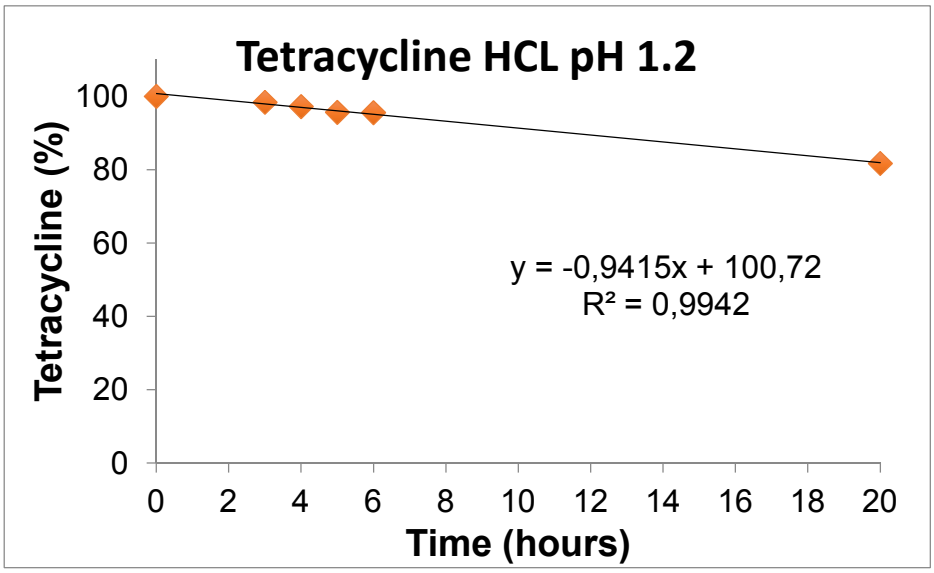


Figure 3. Stability of Tetracycline HCL in European Pharmacopeia pH 1.2 simulated gastric fluid without enzymes

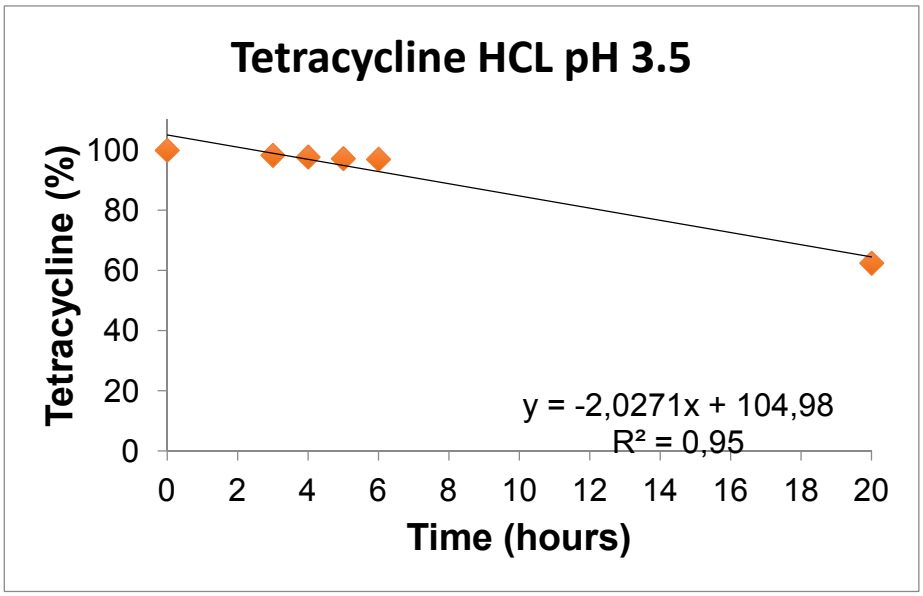


Figure 4. Stability of Tetracycline HCL in Phosphate buffer pH 3.5

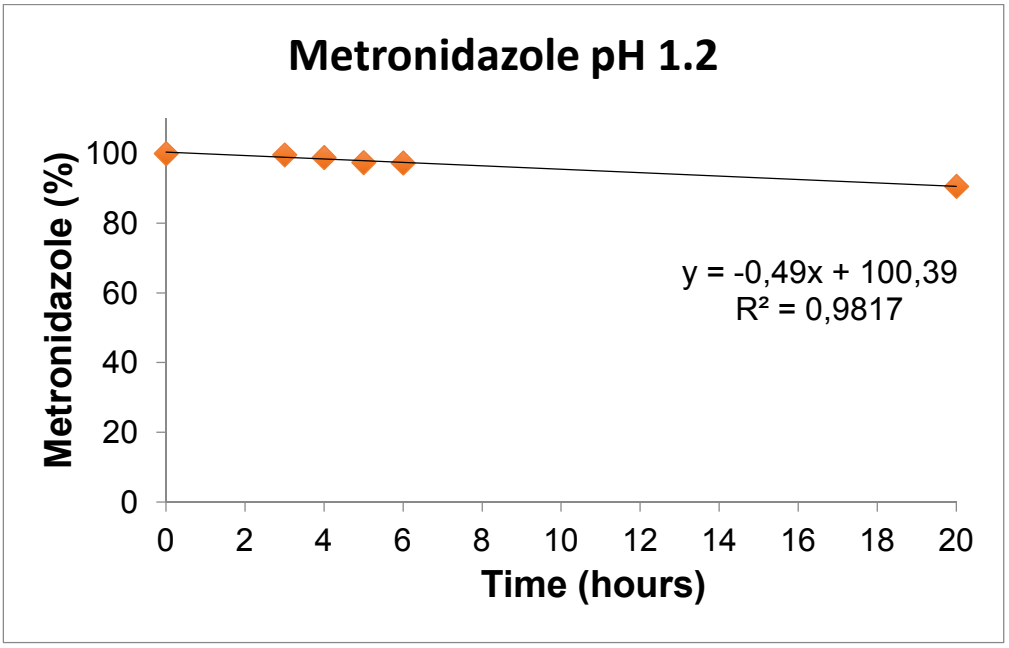


Figure 5. Stability of Metronidazole in European Pharmacopeia pH 1.2 simulated gastric fluid without enzymes

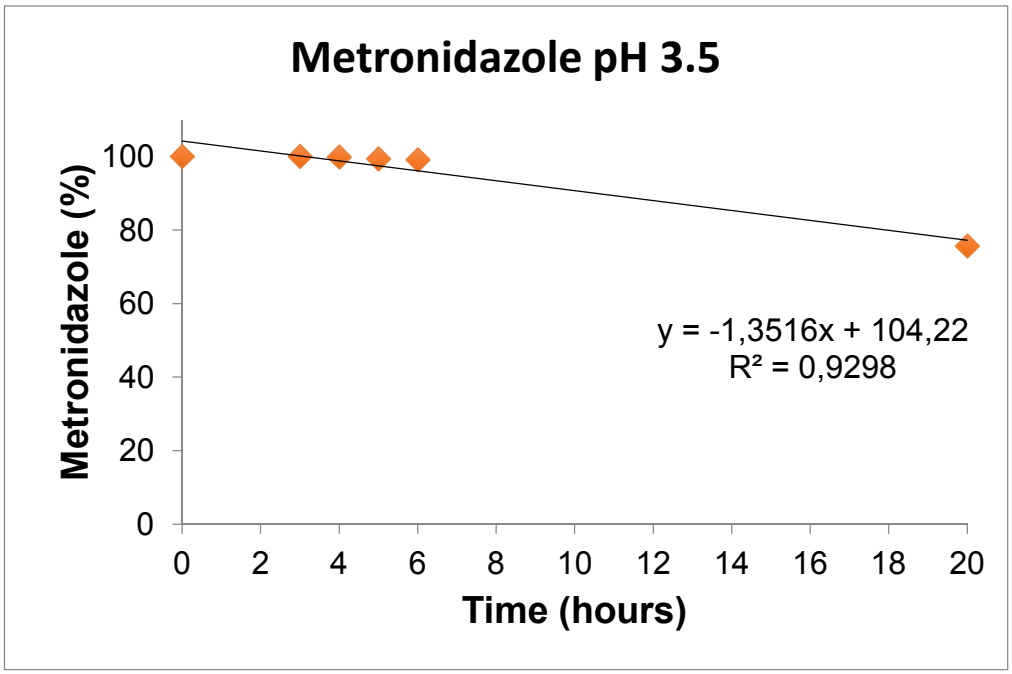


Figure 6. Stability of Metronidazole in Eu.Ph Phosphate buffer pH 3.

4.3. Formulation of antibiotics

Clarithromycin and amoxicillin modules were formulated taking into account the biopharmaceutical characteristics of the active substances and the therapeutic program for H. pylori eradication. The high doses of these antibiotics, required in therapy, restricted the formulation design space, but at the same time simplified the module composition. Tetracycline and Metronidazole oral recommended doses for Helicobacter eradication are 375 mg each antibiotic , four times a day for 10-14 days of treatment. Given such high-drug amounts and the need to construct a dosage form in a size convenient for swallowing, the modules used in this study had a diameter of 7.5 mm and a thickness between 4.4 and 5.8 mm. With this size, each module could accommodate at about 150 mg of powder formulation and, for administration purposes, two assembled modules could be introduced into a size 00 hard zelatin capsule. The modules were prepared as hydrophilic matrices for prolonged release.

Concerning the formulation of sucralfate and Mastic of Chios, which is described in Table 3 and 4, contains Starch, Sodium bicarbonate and AcDiSol as disintegration agents. Avicel (microcrystalline cellulose) is necessary to achieve a proper compression of the module, Magnesium Stearate as a lubricant and Talc as glidant to improve mixing.

4.3.1. Manufacturing of Tetracycline HCL controlled release modules

Modules of Tetracycline HCL were manufactured in order to contain 62.5 mg each and present appropriate mechanical and release properties.

Tetracycline HCL was mixed with HPMC K15M, Avicel PH102 and PEG6000 in a Turbula blender (WAB, Basel, Switzerland) for 25 minutes, then Talc and Magnesium Stearate were added and mixed for another 10 minutes. The percentage of each substance is reported on Table 1.

Male Dome Matrix modules of Tetracycline were manufactured by direct compression, according to the procedure described in 3.2.4. With this formulation it is feasible to obtain both male and female modules of Tetracycline.

	mg per module	%
Tetracycline HCL	62.5	41.66 %
HPMC K15M	30	20 %
Avicel PH102	30	20 %
PEG 6000	20	13.33 %
Talc	4.5	3 %
Mg Stearate	1.5	1 %
	148.5	

Table 1. Formulation of Tetracycline HCL contolled release

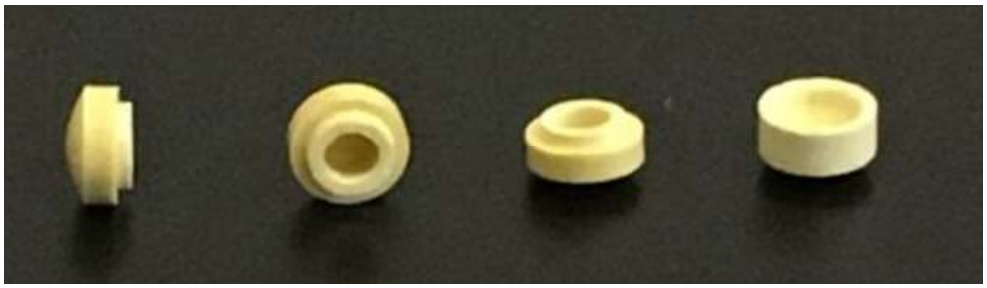


Figure 7 : Male and female modules of Tetracycline

4.3.2. Manufacturing of Metronidazole controlled release modules

Modules of Metronidazole were manufactured in order to contain 62.5 mg each.

Metronidazole was mixed with HPMC K15M, Avicel PH102 and PEG6000 in a Turbula blender (WAB, Basel, Switzerland) for 25 minutes, then Talc and Magnesium Stearate were added and mixed for another 10 minutes. The percentage of each substance is reported on Table 2.

Female Dome Matrix modules of Metronidazole were manufactured by direct compression, according to the procedure described in 3.2.4. With this formulation, only the female module of Metronidazole it is feasible to obtain.

	mg per module	**%**
Metronidazole	62.5	41.66 %
HPMC K15M	30	20 %
Avicel PH102	30	20 %
PEG 6000	20	13.33 %
Talc	4.5	3 %
Mg Stearate	1.5	1 %
Total	**148.5**	

Table 2. Formulation of Metronidazole contolled release

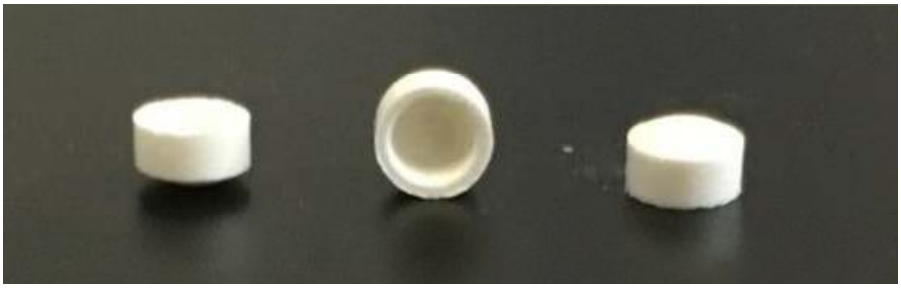


Figure 8: Female module of Metronidazole

4.3.3. Manufacturing of Sucralfate immediate release tablets

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

Sucralfate gel were was granulated according to the procedure described in 3.2.3 to manufacture granules having an average content of water

between 15 and 20%. Granules of sucralfate were mixed with the apropriate excipients in order to produce immediate release tablets.

4.3.4. Manufacturing of Mastic immediate release tablets

Mastic resin was processed according to the procedure described in 3.2.2, in order to produce the Mastic powder.

Tablets of Chios Mastic were manufactured after mixing the powder with the apropriate excipients for immediate release action.

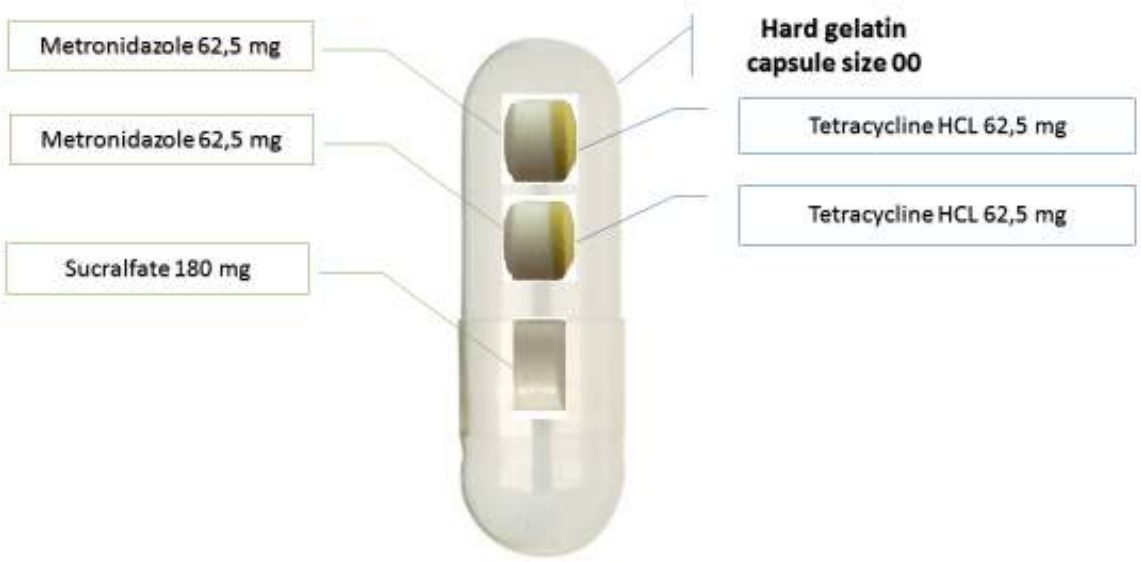
Cylindrical bilayer tablets of Sucralfate- Mastic were manufactured by direct compression, according to the procedure described in 3.2.4.

4.4. Configuration of the final formulation

The two antibacterials with sucralfate and mastic are all included in a hard gelatin capsule size 00, for better patient compiance.

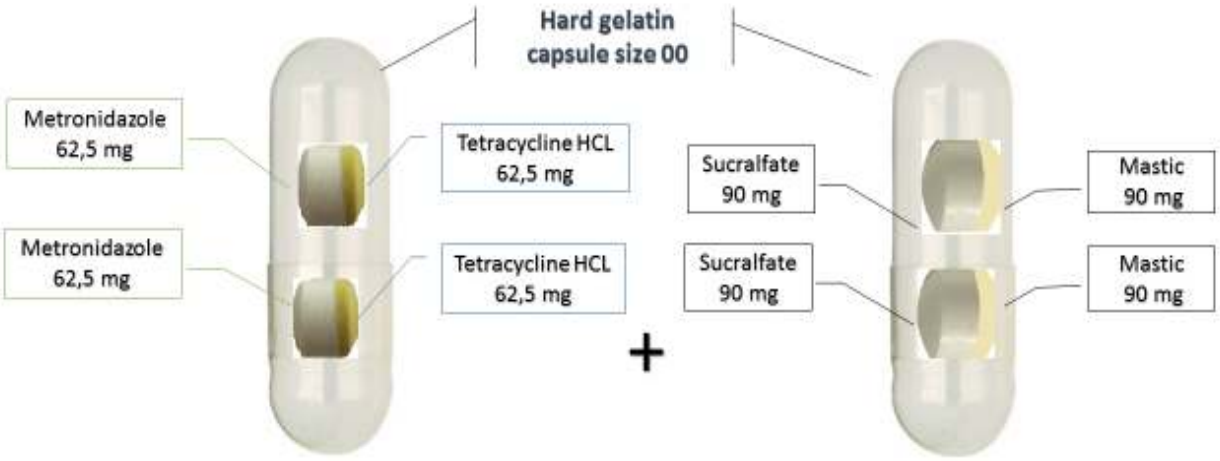
Concerning the final configuration, there are 3 different options:

- Option 1 (respectively with Pylera capsule)



This configuration is designed respectively with the Pylera capsule. In a hard gelatin capsule (type 00) was inserted two assemly Dome Matrix modules of Tetracycline-Metronidazole (62.5 mg each module), and a conventional tablet of Sucralfate 180 mg (instead of bismuth of Pylera).

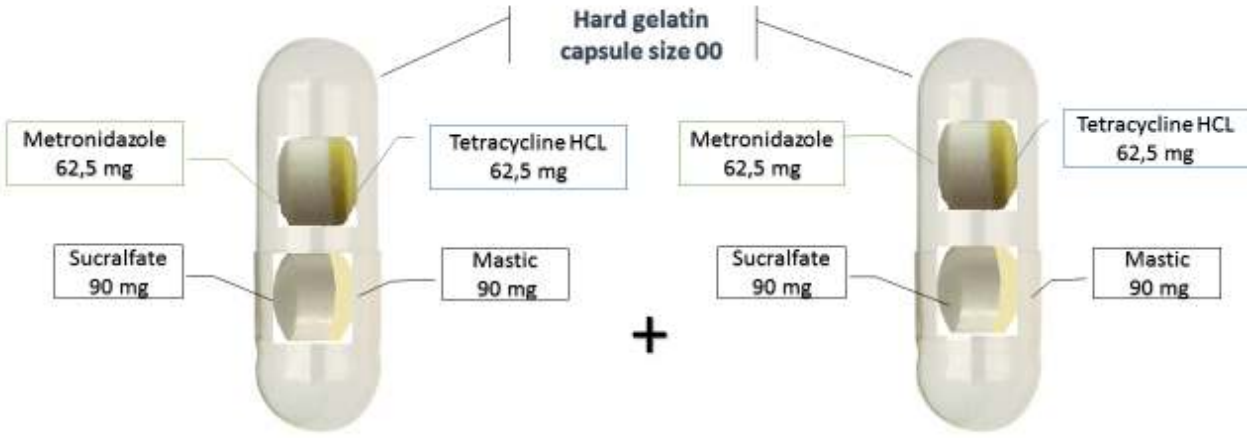
- Option 2



Option 2 suggests the administration of two separate capsules, one with two Dome Matrix assembly systems of antibiotics (62.5 mg each module) and one other with two bilayer tablets of Mastic-Sucralfate (180 mg each bilayer).

One advantage of this option is that there is enough space left in each capsule for possible increase of the modules according to doctor's requition. Moreover, we could use different colors in each capsule for identification of their content and better patient compliance.

- Option 3



Option 3 suggests the administration of two separate capsules but identical concerning the composition. So the patient will take two same capsules each one consisted of one Dome Matrix assembly system with the antibiotics (62.5 mg each module) and one bilayer tablet of Mastic-Sucralfate (180 mg each bilayer).

The advantage of this option is that there is enough space left in each capsule for possible increase of the modules according to doctor's requition and that the capsules are identical which may lead to better patient comliance.

4.5. Hardness test- Friability test- Disintegration time for sucralfate – Mastic tablets

The characteristics of the modules after the three tests (Friability, Disintegration and Hardness), are summarized in tables 5 and 6.

Friability (%)	0.35
Disintegration Time (sec)	180
Hardness (kg, axial)	2.8 ±0.5
Hardness (kg, diametral)	1.5 ± 0.4

Tablet 5: Characteristics of Sucralfate Immediate Release

Friability (%)	0.86
Disintegration Time (sec)	360
Hardness (kg, axial)	5.4 ±0.5
Hardness (kg, diametral)	3.2 ± 0.2

Tablet 6: Characteristics of Mastic Immediate Release

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

4.6. Dissolution Test for MND-TTC individual and assemled systems

Dissolution test of the individual and assembled systems of antibiotics was conducted according to the procedure in 3.2.6., in order to assay MND and TTC release.

The experiments were done in triplicate and the results (figure 9-13) are expressed as percentage of antibiotic release calculated on the drug content, per time.

- <u>Dissolution test of Metronidazole Female modules</u>

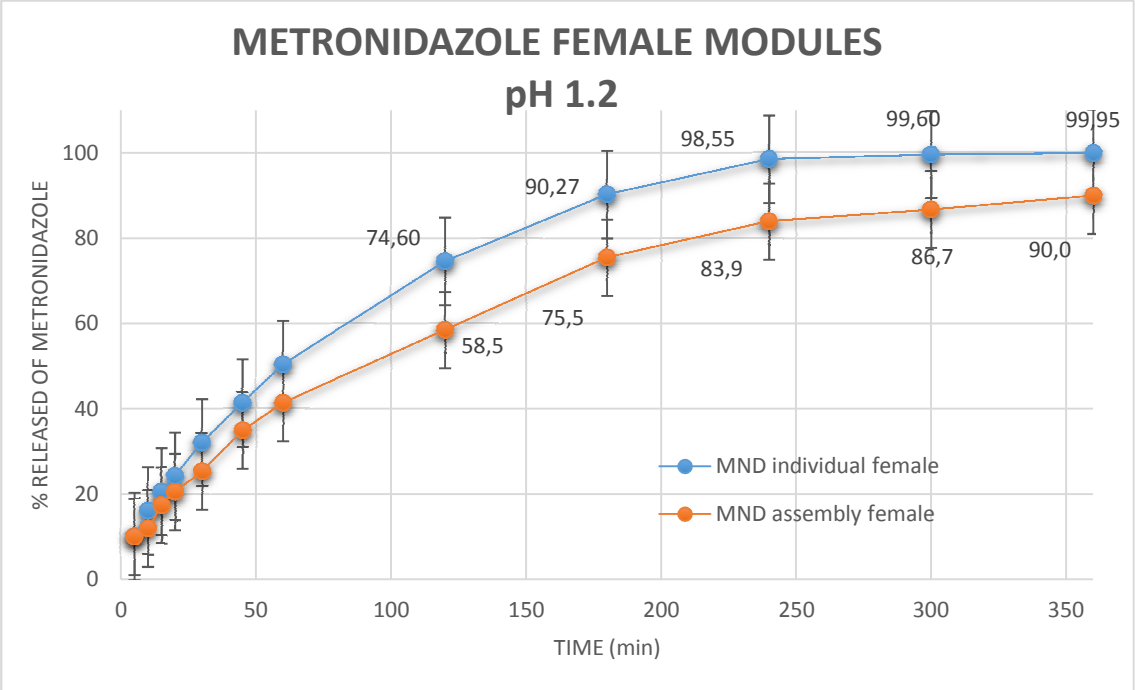


Figure 9 **:** Dissolution profile of Metronidazole female individual and female from assemled system at pH 1.2

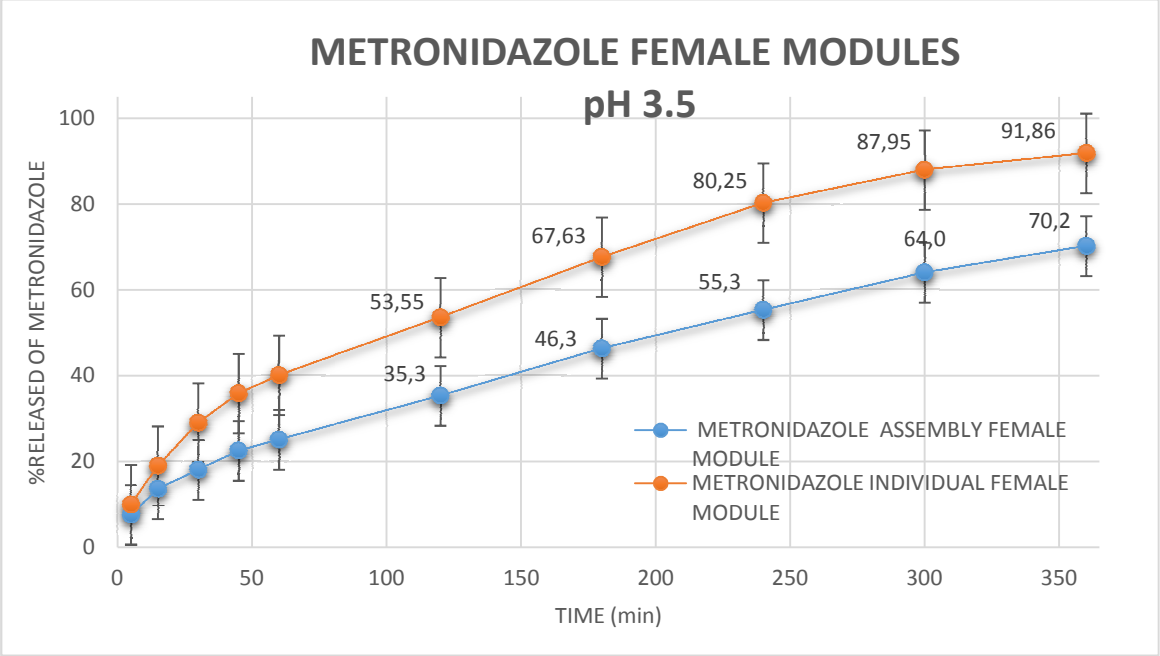


Figure 10: Dissolution profile of Metronidazole female individual and female from assemled system at pH 3.5

The release of metronidazole female individual module in pH 1.2 at 360 minutes was 99.95 %, while from assembed system was 90 %. In pH 3.5 metronidazole as individual module released at 91.86% at 360 minutes, while from assemled system the released was 70.2 %.

In the case of the two-module void assembly system, the release rate of both antibiotics was slower and smaller compared to the cumulative drug released from the male and female modules individually. This was attributed to the fact that, upon assembly by ‘‘clicking’’ together the male and female modules, the internal surface was no more accessible to the solvent.

- Dissolution profiles of Tetracycline Male modules

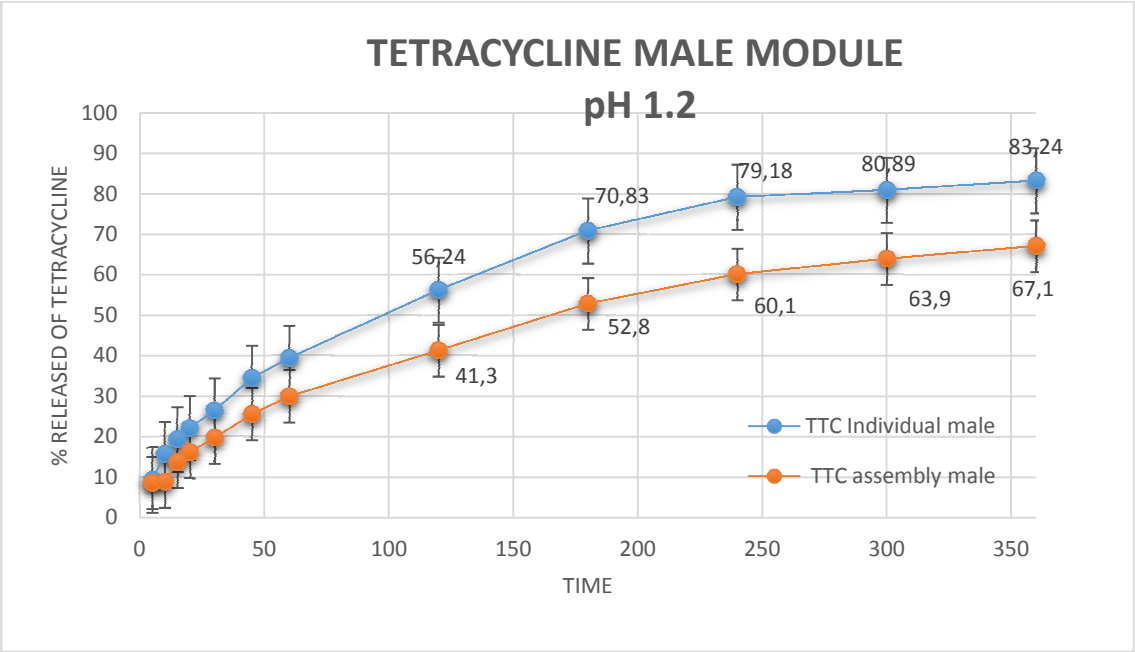


Figure 11 : Dissolution profile of Tetracycline male individual and male from assemled system at pH 1.2

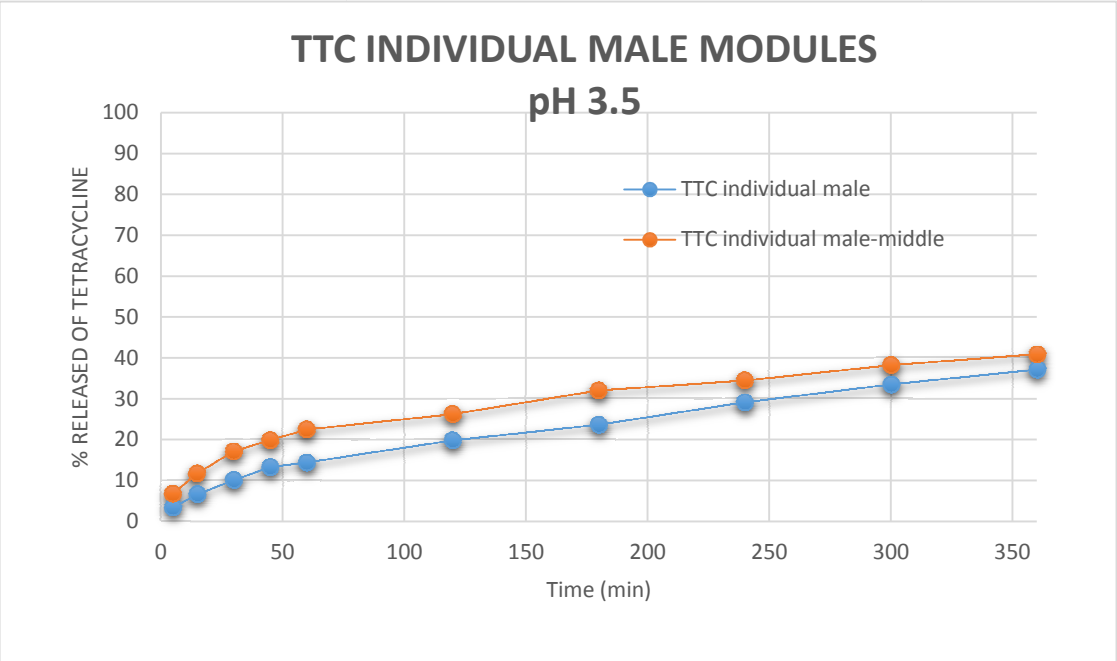


Figure 12 : Dissolution profile of Tetracycline male individual and male-middle individual module at pH 3.5

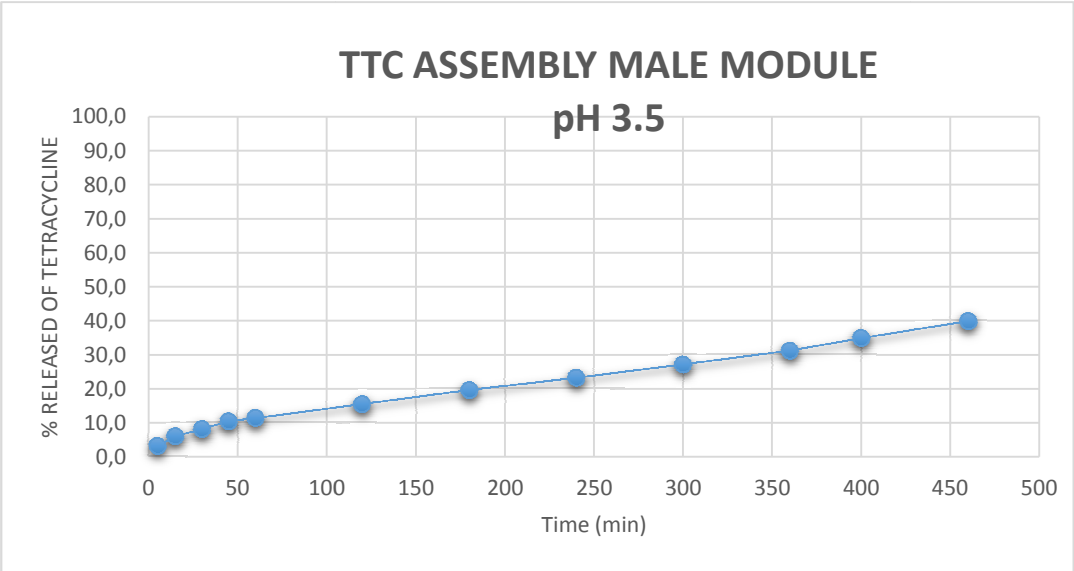
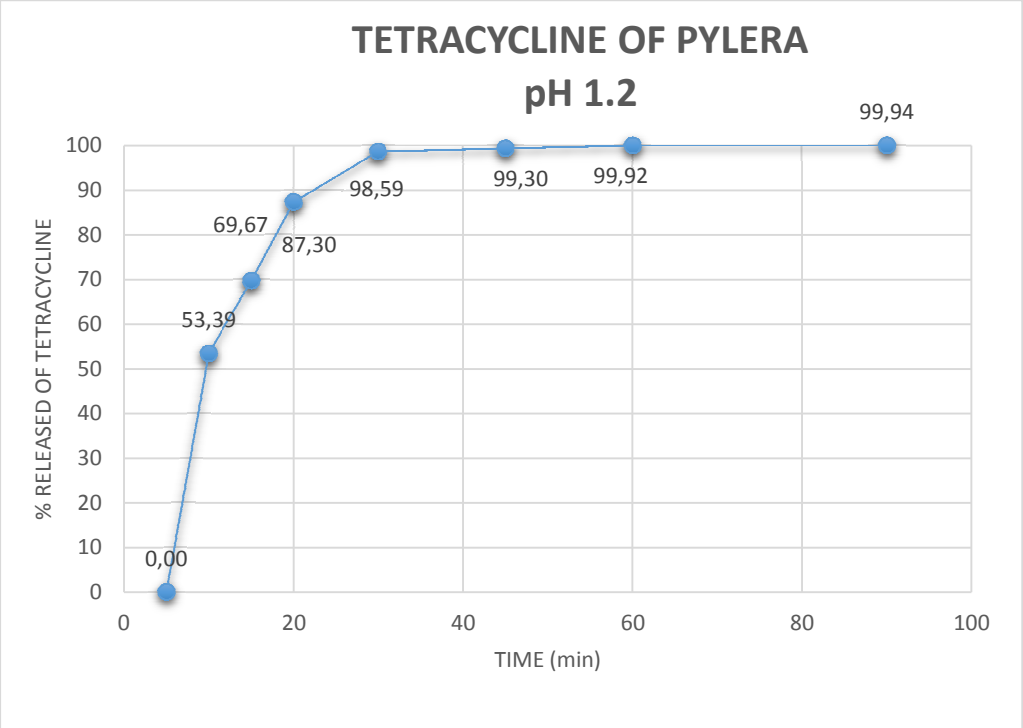
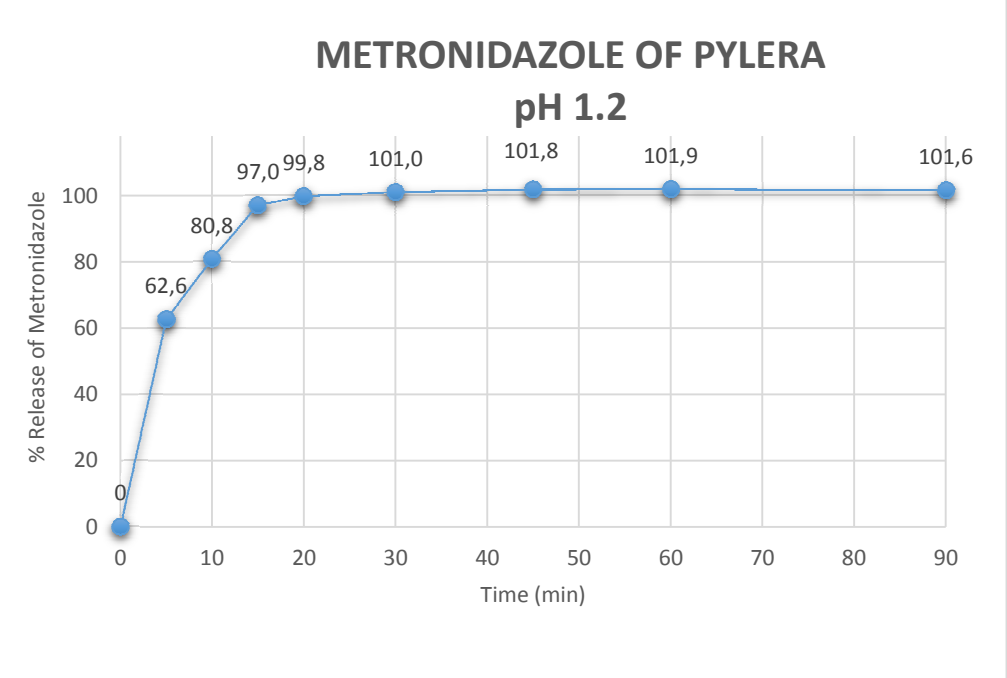


Figure 13 : Dissolution profile of Tetracycline male from assemled system at pH 3.5

The release of tetracycline male individual module in pH 1.2 at 360 minutes was 83.24 %, while from assembed system was 67.1 %. Tested at pH 3.5, where tetracycline is less soluble ref, the module showed a lower drug release . Specifically, tetracycline as individual male module released 37.06 %, from individual male-middle module released 40.08 % at 360 minutes and from assemled system the released was 39.8 % at 480 min.

4.7. Dissolution Profile of Pylera





Pylera capsule show an immediate release as predicted, reaching the 100% of antibiotic dissolved at about 30 minutes

4.8. Analysis of Dissolution Profiles- Release Kinetics

The release kinetics of the various individual modules and the assembled system has been described by Ritgel and Peppas equation (eq.1), in which the value of exponent (n) gives indication on the mechanism of drug release.

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

Exponent, n			Release mechanism
Fine film	Cylinders	Spheres	
0.5	0.45	0.43	Fickian diffusion
$0.5 < n < 1.0$	$0.45 < n < 0.89$	$0.43 < n < 0.85$	Anomalous transport
1.0	0.89	0.85	Polymer swelling

Source: Siepmann and Siepmann (2008).

Table 7: Values for the exponent n of the Peppas equation and the drug release mechanism from the polymeric systems of different geometries

The various individual modules and the assembly configurations, all substantially hydrophilic matrices, were studied with respect to drug release rate and kinetics.

All the release profiles were fitted to the power equation (eq.1), and the release kinetics were identified from the values of the exponent n. The n values obtained with these hydrophilic matrices were all different from 0.45. So, mainly diffusion through the gel layer (but also relaxation) is the mechanism that controls the release of both antibiotics as observed with several other hydrophilic matrices containing small amounts of polymer or soluble drugs and polymers.

4.8.1. Release kinetics -Metronidazole female module pH 1.2

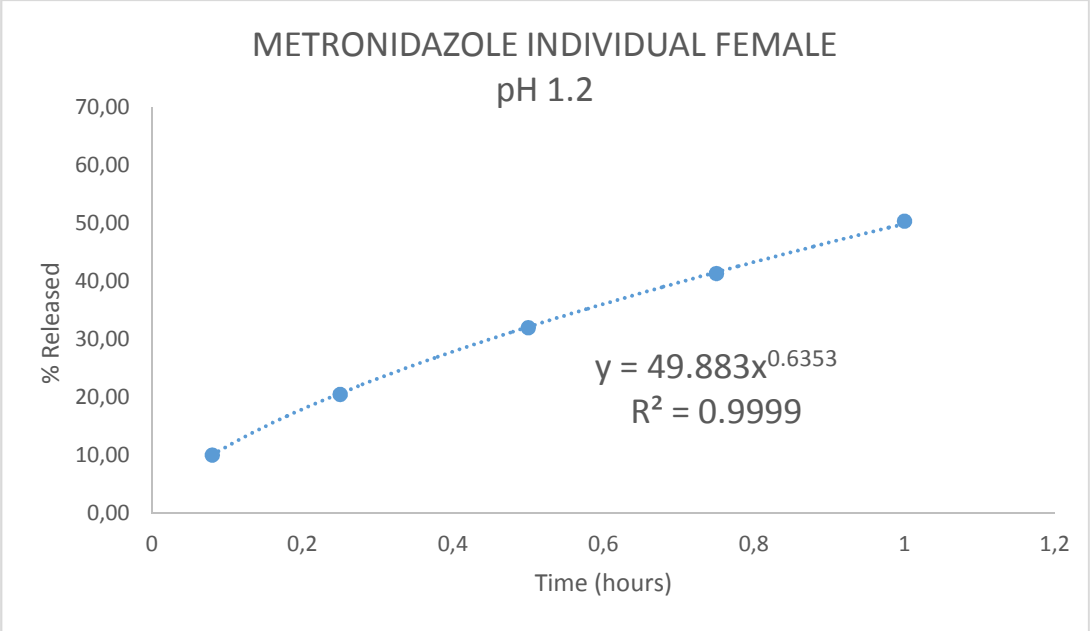


Figure 14: Percent release profile of metronidazole (n=3) as individual module in pH 1.2. Lines represent the fitting to the power equation, where n=0.6353

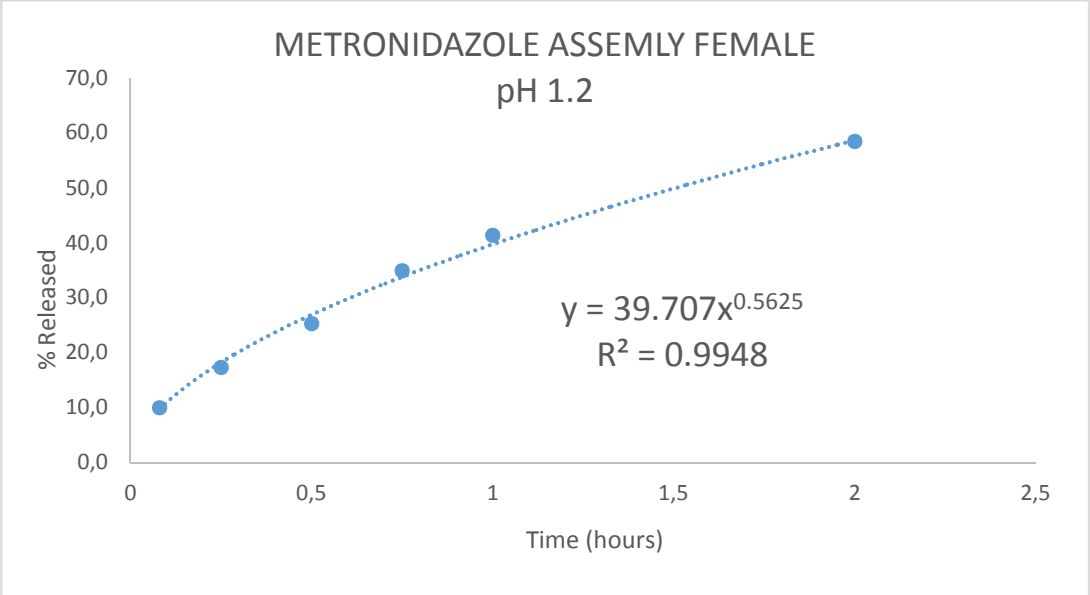


Figure 15: Percent release profile of metronidazole (n=3) from assembled system in pH 1.2. Lines represent the fitting to the power equation, where n=0.5625

4.8.2. Release kinetics -Tetracycline male module pH 1.2

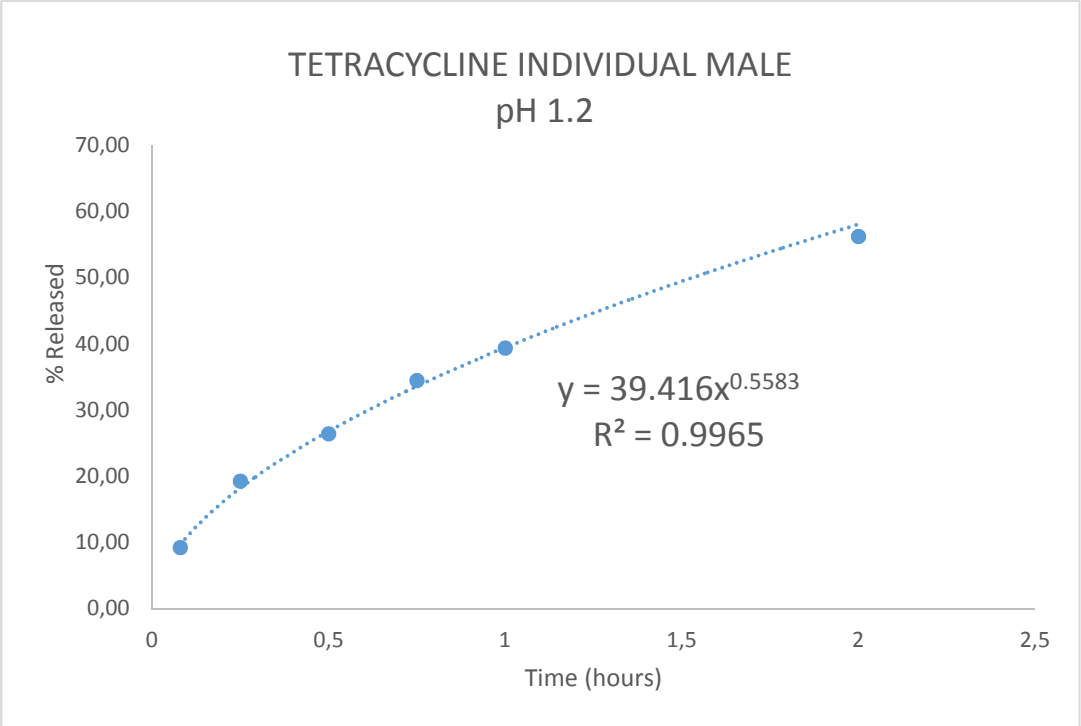


Figure 16: Percent release profile of tetracycline (n=3) as individual module in pH 1.2. Lines represent the fitting to the power equation, where n=0.5583

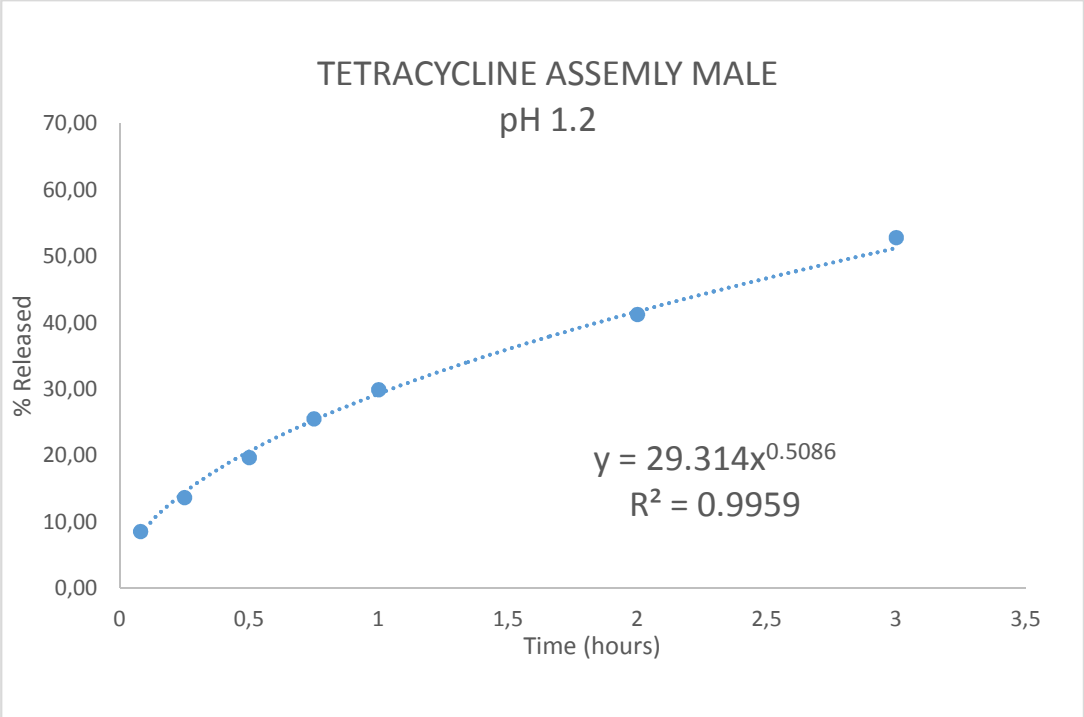


Figure 17: Percent release profile of tetracycline (n=3) from assembly system in pH 1.2. Lines represent the fitting to the power equation, where n=0.5086

4.8.3. Release kinetics -Metronidazole female module pH 3.5

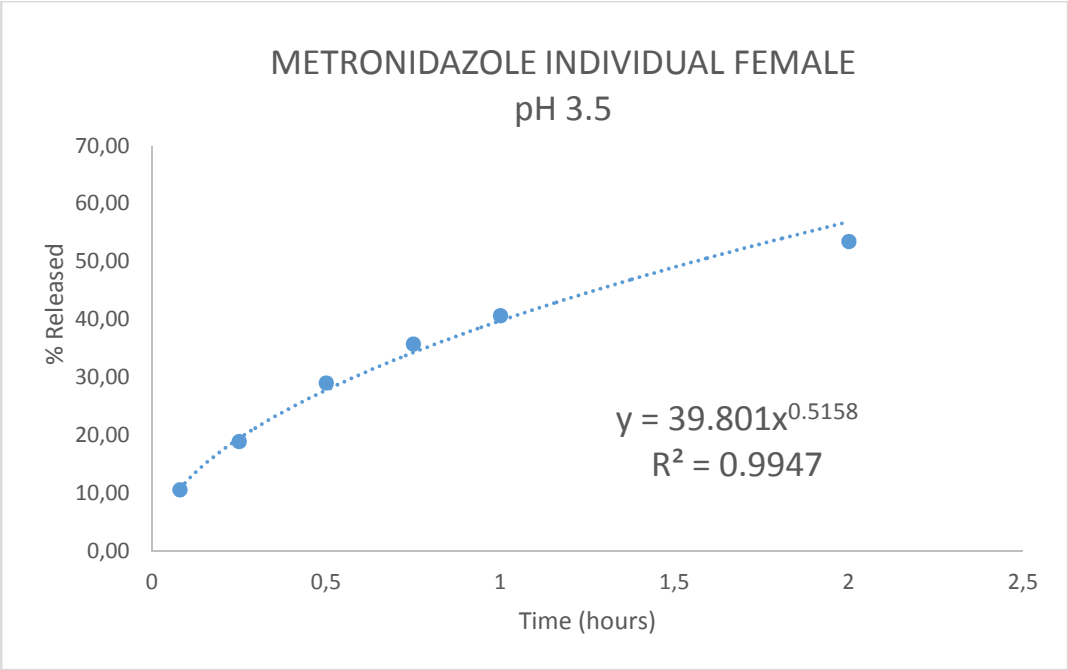


Figure 18: Percent release profile of metronidazole (n=3) as individual female module in pH 3.5. Lines represent the fitting to the power equation, where n=0.5158.

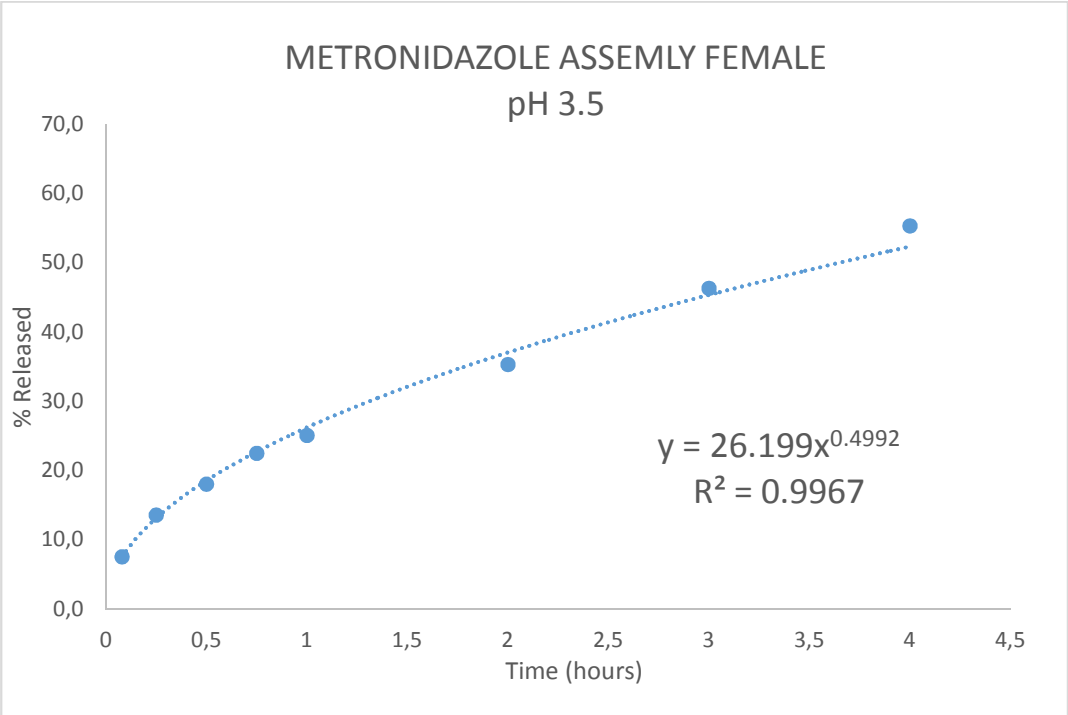


Figure 19: Percent release profile of metronidazole (n=3) from assembly system in pH 3.5. Lines represent the fitting to the power equation, where n=0.4992.

4.8.4. Release kinetics -Tetracycline male module pH 3.5

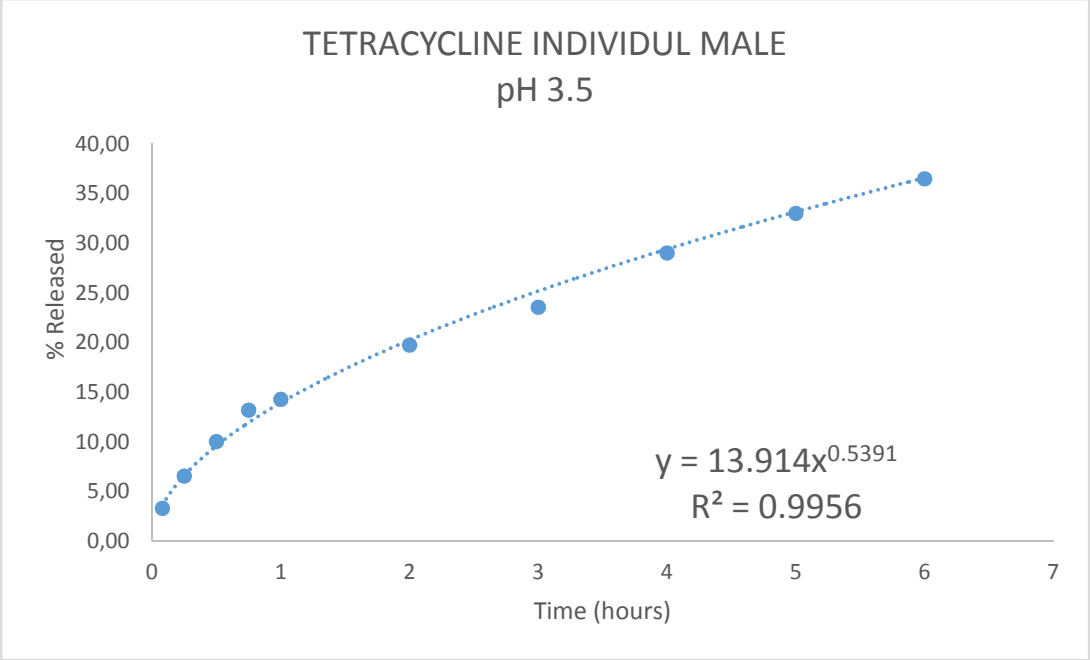


Figure 20: Percent release profile of tetracycline (n=3) as individual male module in pH 3.5. Lines represent the fitting to the power equation, where n=0.5391.

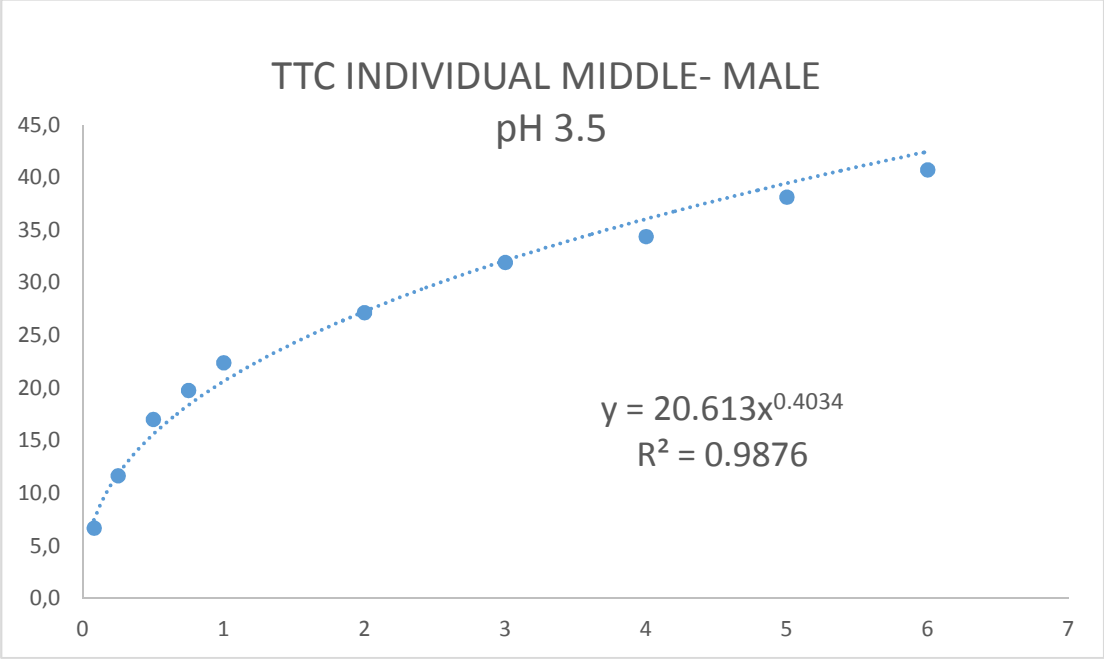


Figure 21 : Percent release profile of tetracycline (n=3) as individual male-middle module in pH 3.5. Lines represent the fitting to the power equation, where n=0.4034.

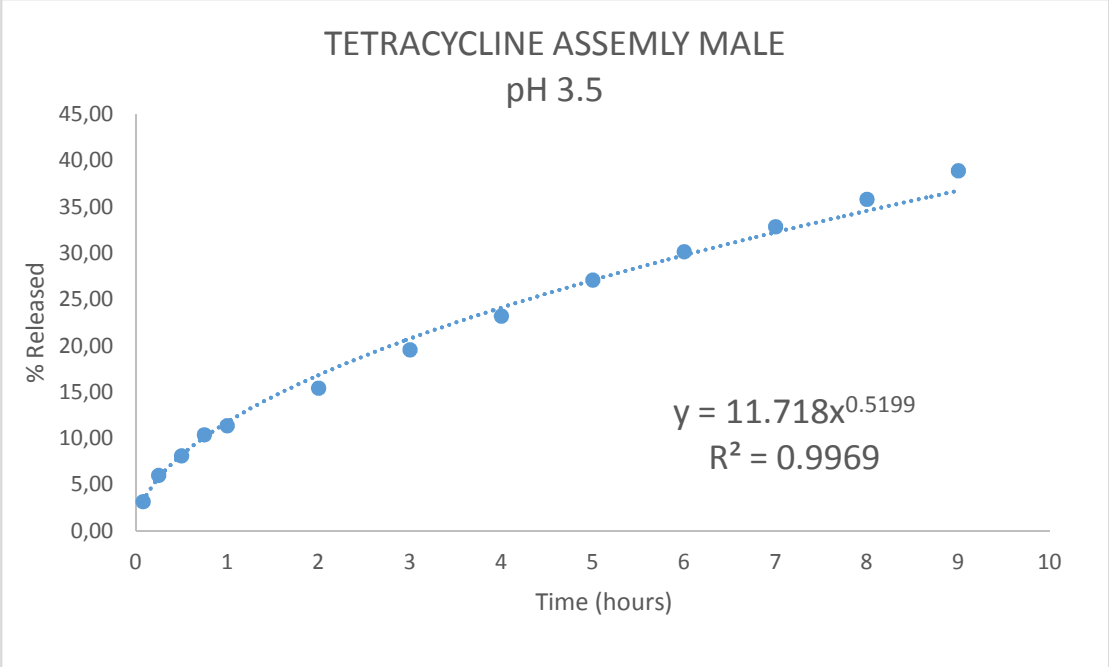


Figure 22: Percent release profile of metronidazole (n=3) from assembly system in pH 3.5. Lines represent the fitting to the power equation, where n=0.5199

pH	Type of Module	Equation by Ritgel and Peppas model	R^2	n value
1.2	MND INDIVIDUAL FEMALE	$y = 49{,}883x^{0{,}6353}$	0.9999	0.6353
1.2	MND ASSEMLY FEMALE	$y = 39.707x^{0{,}5625}$	0.9948	0.5625
1.2	TTC INDIVIDUAL MALE	$y = 39.416x^{0{,}5583}$	0.9965	0.5583
1.2	TTC ASSEMLY MALE	$y = 29.314x^{0{,}5086}$	0.9959	0.5086
3.5	MND INDIVIDUAL FEMALE	$y = 39.801x^{0{,}5158}$	0.9947	0.5158
3.5	MND ASSEMLY FEMALE	$y = 26.199x^{0{,}4992}$	0.9967	0.4992
3.5	TTC INDIVIDUAL MALE	$y = 13.914x^{0{,}5391}$	0.9956	0.5391
3.5	**TTC INDIVIDUAL MALE-MIDDLE**	$\mathbf{y = 20.613x^{0{,}4034}}$	**0.9876**	**0.4034**
3.5	TTC ASSEMLY MALE	$y = 11.718x^{0{,}5199}$	0.9969	0.5199

Table 8: The Peppas equation, the R^2 and n value respectively to the type of Dome Matrix module.

The exponent n value is higher in all cases for the individual modules in comparison with the assemby, indicating an effect of the module geometry on the release kinetics (see n values in Table 8).

In all cases (except the one of the male-middle module) the value n is between 0.45 and 0.89, so anomalous transport takes place.

The case of Tetracycline individual male-middle module at pH 3.5, is the only in which the n value is <0.45. This is because of the geometry effect of the system.

5. Conclusion

A modular unit dosage form containing tetracycline and metronidazole for H. pylori therapy was constructed by interlocking two drug modules. The final dosage form included two assembled tetracycline-metronidazole modules and one bilayer immediate release tablet consisted of sucralfate-mastic of Chios. The assembled system of antibiotics floated in vitro for more than 5 h on the surface of a dissolution medium at pH 1.2 and 3.5. Analysis of the release kinetics was done, showing that mainly diffusion through the gel layer (but also relaxation) is the mechanism that controls the release of both antibiotics.

The ability of the assembled system to float in the stomach content does not interfere with the drug release control mechanism since the floating mechanism is determined by the internal void of the assembly and the drug release control by the walls of the system. This is different and new compared to other technologies in which floatation depends on the same mechanism that controls drug release (e.g. hydrophilic matrices entrapping air/gas bubbles in the swollen polymer). By maintaining a prolonged direct contact between H. pylori and the antibiotic molecules, the assembled system has the potential to reduce the drug doses and the number of dosage forms administered daily.

The assembly of modules having different drug content and/or different kinetics in a single dosage unit leads to the assumption of an increase in patient compliance and adherence to the therapeutic regimen.

References

1. Kato, I., et al., A Prospective Study of Gastric and Duodenal Ulcer and its relation to smoking, Alcohol, and Diet. American Journal of Epidemiology, 1992. 135(5): 521-530.

2. Livingston, E.H., The stomach as a system and the pathogenesis of experimental ulcer. Med Hypothese, 1993. 41(2): p.173-6

3. Kumar, V., A.K.Abbas, and J.C Aster, eds. Robbins and Contran- Pathologic basis of disease. 8 ed., Saunders.

4. Chan, F.K.L and W.K Leung, Peptic-ulcer disease. The Lancet,2002. 360(9337): p.933-941.

5. Carmelo, S., New drugs to suppress acid secretion: current and future developments. Drug Discovery Today: Therapeutic Strategies, 2007. 4(3): p. 155-163.

6. C, S., Antisecretory drugs, Helicobacter Pylori infectionand symptom relief in GORD: Still an unexplored triangle. Digestive and liver disease, 2005. 37(7): p. 468-474.

7. El-Zahaby, S.A., A.A. Kassem, and A.H. El-Kamel, Design and evaluation of gastroretentive levofloxacine floating mini-tablets-in-capsule system for eradication of Helicobacter Pylori. Saudi Pharmaceutical Journal, (0).

8. Rajinikanth, P.S., J. Balasubramaniam, and B. Mishra, Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter Pylori. International Journal of Pharmaceutics, 2007. 335(1-2): p.182-205.

9. Baldwin DN, Shepherd B, Kraemer P, et al. (February 2007). "Identification of Helicobacter pylori genes that contribute to stomach colonization". Infect Immun 75 (2): 1005–16.

10. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, et al. An African

origin for the intimate association between humans and Helicobacter pylori. Nature

2007 Feb 22;445(7130):915-8.

11. Moodley Y, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, et al. The peopling

of the Pacific from a bacterial perspective. Science 2009 Jan 23;323(5913):527-30.

12. Konturek JW. Discovery by Jaworski of Helicobacter pylori and its pathogenetic role in peptic ulcer, gastritis and gastric cancer. J Physiol Pharmacol 2003 Dec;54 Suppl 3:23-41.

13. Bizzozero G. Uber die schlauchformigenDrusen des Magenkanals und die Beziehungen ihres Epitels zu dem Oberflachenepitel der Schleimhaut. Arch Mikr Anat 1893;42:82-152.

14. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983 Jun 4;321(8336):1273-5.

15. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984 Jun 16;1(8390):1311-5.

16. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med J Aust 1985 Apr 15;142(8):436-9.

17. Borody TJ, Carrick J, Hazell SL. Symptoms improve after the eradication of gastric Campylobacter pyloridis. Med J Aust 1987 Apr 20;146(8):450-1.

18. George LL, Borody TJ, Andrews P, Devine M, Moore-Jones D, Walton M, et al. Cure of duodenal ulcer after eradication of Helicobacter pylori. Med J Aust 1990 Aug 6;153(3):145-9.

19. Goodman K, Correa P (March 1995). “The Transmission of Helicobacter pylori. A Critical Review of the Evidence” International Journal of Epidemiology.

20. Ottemann KM, Lowenthal AC (April 2002). "Helicobacter pylori uses motility for initial colonization and to attain robust infection". Infect. Immun. 70 (4): 1984–90.

21. Schreiber S, Konradt M, Groll C, et al. (April 2004). "The spatial orientation of Helicobacter pylori in the gastric mucus". Proc. Natl. Acad. Sci. U.S.A. 101 (14): 5024–9.

22. Petersen AM, Krogfelt KA (May 2003). "Helicobacter pylori: an invading microorganism? A review". FEMS Immunol. Med. Microbiol. 36 (3): 117–26.

23. Ilver D, Arnqvist A, Ogren J, et al. (January 1998). "Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging". Science (journal) 279 (5349): 373–7.

24. Smoot DT (December 1997). "How does Helicobacter pylori cause mucosal damage? Direct mechanisms". Gastroenterology 113 (6 Suppl): S31–4; discussion S50.

25. Dixon MF (February 2000). "Patterns of inflammation linked to ulcer disease". Baillieres Best Pract Res Clin Gastroenterol 14 (1): 27–40.

26. Kusters JG, van Vliet AH, Kuipers EJ (July 2006). "Pathogenesis of Helicobacter pylori infection". Clin Microbiol Rev 19 (3): 449–90.

27. Blaser MJ, Atherton JC (February 2004). "Helicobacter pylori persistence: biology and disease". J. Clin. Invest. 113 (3): 321–33.

28. Schubert ML, Peura DA (June 2008). "Control of gastric acid secretion in health and disease". Gastroenterology 134 (7): 1842–60.

29. Suerbaum S, Michetti P (October 2002). "Helicobacter pylori infection". N. Engl. J. Med. 347 (15): 1175–86.

30. Peek RM, Crabtree JE (January 2006). "Helicobacter infection and gastric neoplasia". J. Pathol. 208 (2): 233–48.

31. Viala J, Chaput C, Boneca IG, et al. (November 2004). "Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island". Nat. Immunol. 5 (11): 1166–74.

32. Backert S, Selbach M (August 2008). "Role of type IV secretion in Helicobacter pylori pathogenesis". Cell. Microbiol. 10 (8): 1573–81.

33. Baldwin DN, Shepherd B, Kraemer P, et al. (February 2007). "Identification of Helicobacter pylori genes that contribute to stomach colonization". Infect Immun 75 (2): 1005–16.

34. Broutet N, Marais A, Lamouliatte H, et al. (April 2001). "cagA Status and eradication treatment outcome of anti-Helicobacter pylori triple therapies in patients with nonulcer dyspepsia". J Clin Microbiol 39 (4): 1319–22. doi:10.1128/JCM.39.4.1319-1322.2001.

35. Tsuji S, Kawai N, Tsujii M, Kawano S, Hori M (July 2003). "Review article: inflammation-related promotion of gastrointestinal carcinogenesis--a perigenetic pathway". Aliment. Pharmacol. Ther. 18 Suppl 1: 82–9.

36. Suganuma M, Yamaguchi K, Ono Y, et al. (July 2008). "TNF-α-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells". Int. J. Cancer 123 (1): 117–22

37. Korman MG1, Bolin TD, Engelman JL, Pianko S. (September 1997). “Sucralfate as an Alternative to Bismuth in Quadruple Therapy for Helicobacter pylori Eradication” Helicobacter. 1997 Sep;2(3):140-3.

38. Jewell, R., Sucralfate, in xPharm: The Comprehensive Pharmacology Reference, S.J. Enna and D>B Bylund, Editors. 2007, Elsevier: New York. P.1-5.

39. Assimopoulou A.N., Zlatanos S.N., Papageorgiou V.P. [2005]: Antioxidant activity of natural resins and bioactive triterpenes in oil substrates. Food Chemistry, 92: 721–727.

40. Dimas K., Hatziantoniou S., Wyche J.H., Pantazis P. [2009]: A mastic gum extract induces suppression of growth of human colorectal tumor xenografts in immunodeficient mice. In Vivo, 23 (1): 63-68.

41. Dedoussis G.V.Z., Kaliora A.C., Psarras S., Chiou A., Mylona A., Papadopoulos N.G., Andrikopoulos N.K. [2004]: Antiatherogenic effect of Pistacia lentiscus via GSH restoration and downregulation of CD36 mRNA expression. Atherosclerosis, 174: 293–303.

42. C Koutsoudaki et al. “Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of Pistacia Lentiscus Var. Chia”, J Agric Food Chem 53 (20), 7681-7685. 2005 Oct 05.

43. Al-Habbal M.J., Al-Habbal Z., Huwez F.U. [1984]: A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. Clin Exp Pharmacol Physiol., 11 (5): 541-544.

44. Al-Said M.S., Ageel A.M., Parmar N.S., Tariq M. [1984]: Evaluation of mastic, a crude drug obtained from Pistacia lentiscus for gastric and duodenal antiulcer activity. J Ethnopharmacol., 15 (3): 271-278.

45. Huwez F.U., Thirlwell D., Cockayne A., Ala'Aldeen D.A.A. [1998]: Mastic Gum Kills Helicobacter pylori. N. Eng. J. of Med., 339 (26): 1946

46. Paraschos S., Magiatis P., Mitakou S., Petraki K., Kalliaropoulos A., Maragkoudakis P., Mentis A., Sgouras D., Skaltsounis A.-L. [2007]: In vitro and in vivo activities of Chios mastic gum extracts and constituents against Helicobacter pylori. Antimicrob. Agents Chemother.,51 (2): 551–559.

47. Magiatis P, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S. Chemical composition and antimicrobial activity of the essential oils of Pistacia lentiscus var. chia. Planta Med. 1999;65:749–752. doi: 10.1055/s-2006-960856.

48. Triantafyllou A, Bikineyeva A, Dikalova A, Nazarewicz R, Lerakis S, Dikalov S. Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress. Nutrition Journal. 2011;10:64. doi:10.1186/1475-2891-10-64.

49.

50. Bebb JR, Bailey-Flitter N, Ala'Aldeen D, Atherton JC. Mastic gum has no effect on Helicobacter pylori load in vivo. J Antimicrob Chemother. 2003;52:522–523.

51. A. A. Deshpande, C. T. Rhodes, N. H. Shah & A. W. Malick Floating Controlled Drug Delivery Systems for Prolonged Gastric Retention: A Review. Drug Dev. Ind. Pharm. 22, . 531-539 (1996).

52. Joseph R. Robinson, Vincent H. L. Lee, Influence of Drug Properties and Routes of Drug Administration on the Design of Sustained and Controlled Release Systems, Controlled Drug Delivery Fundamentals and Applications, Second Edition, Marcel Dekker Inc., New York, (1987).

53. P. Colombo, R. Bettini. P. Santi, N. A. Peppas, Drug release from swelling controlled systems, in Wise D. (ed), Handbook of Pharmaceutical Controlled release Technology, Dekker, New York, 9 p. 183-206 (2000).

54. RW Korsmeyer, NA Peppas, "Macromolecular and modeling aspects of swelling-controlled systems", Rosemann T.J., Mansdorf S.Z., Controlled Release Delivery Systems, Dekker ed., New York and Basel, (1983).

55. P. Colombo, “Swelling-Controlled Release in Hydrogel Matrices for Oral Route” Adv Drug Del, Review, 11, p. 37-57 (1993).

56. Borgquist, Körner A, Piculell L, Larsson A, Axelsson A., A model for the drug release from a polymer matrix tablet--effects of swelling and dissolution. J Control Release, 113 (3), p.216-25 (2006).

57. A. Frank, S. K. Rath, S. S. Venkatraman, “Controlled release from bioerodible polymers: effect of drug type and polymer composition” . J Control Release, 102 (2), p. 333-44 (2005).

58. Colombo, Bettini R, Catellani P.L, Santi P, Peppas N.A., Drug volume fraction profile in the gel phase and drug release kinetics in hydroxypropylmethyl cellulose matrices containing a soluble drug, Eur J Pharm Sci, 9 (1). P. 33-40 (1999).

59. Sandip B., J. DiNunzio, Ali Rajabi-Siahboomi, Drug–Polymer Matrices for Extended Release, Controlled Release in Oral Drug Delivery SE-7, C.G Wilson and P. J. Crowley, Editors. 2011, Springer US DA- p. 131-159 (2011).

60. T. Phaechamud, Variables Influencing Drug Release from Layered Matrix System Comprising Hydroxypropyl Methylcellulose, AAPS PharmSciTech, 9 (2), p. 668-74 (2008).

61. P. Colombo, P. Santi, J. Siepmann, G. Colombo, F. Sonvico, A. Rossi, O.L. Strusi, Swellable and rigid matrices controlled release matrices with cellulose ethers, Pharmaceutics Dosage Forms: Tablets, Third Edition, Edited by Larry L. Augsburger, Stephen W. Hoag, 2008, vol. 2, cap. 14.

62. A.T Pham, Lee P.I., Probing the mechanisms of drug release from hydroxypropylmethyl cellulose matrices, Pharmaceutical Research, 11 (10), 1994, 1379-1384.

63. Colombo P., Bettini R., Santi P., Peppas N.A., Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance, Pharm Sci Technol Today, 3 (6), June 2000, 198-204.

64. P.I. Lee, N.A. Peppas, Prediction of polymer dissolution swelling controlled-release systems, J Control Release, 1987, 207-215.

65. P. Colombo, R. Bettini, N.A. Peppas, Observation on swelling process and diffusion front position during swelling in hydroxypropylmethylcellulose (HPMC) matrices containing a soluble drug, J Control Release, 61, 1999, 83-91.

66. P. Colombo, R. Bettini, G. Massimo, P.L. Catellani, P. Santi, N.A. Peppas, Drug diffusion front movements is important in drug release control from swellable matrix tablets, J Pharm Sci, 84, 991-997 (1995).

67. P. Colombo, R. Bettini, N.A. Peppas, “Observation of swelling process and diffusion front position during swelling in HPMC matrices containing a soluble drug” , Journal oof Controlled Release 61, p. 83-91 (1999).

68. N. A. Peppas, P. Colombo, Analysis of drug release behavior from swellable polymer carriers using dimentionality index. Journal of Controlled Release, 1997. 45 (1), p. 35-40.

69. N.A. Peppas, J.J Sahlin, A simple equation for the description of solute release III. Coupling of diffusion and relaxation. International Journal of Pharmaceutics, 1989. 57 (2), p. 169-172.

70. Ritger, P.L. , N.A Peppas, A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. Journal of Controlled Release, 1987. 5(1), p. 37-42.

71. Ritger, P.L. , N.A Peppas, A simple equation for description of solute release I. Fickian and non- Fickian release from non-swellable devices in the form of slabs, spheres, cylindres or discs. Journal of Controlled Release, 1987. 5(1), p. 23-36.

72. Losi, E., et al., Assemblage of novel release modules for the development od adaptable drug delivery systems. Journal of Controlled Release, 2006. 111 (1-2) p. 212-218.

73. Casas M., et al., Tapioca starch graft copolymers and Dome Matrix modules assembling technology. I Effect of module shape on drug release. European Journal of Pharmaceutics and Biopharmaceutics, 2010. 75 (1) p. 42-47.

74. Hascicek, C., et al., Assemblage of drug release modules: Effect of module shape and position in the assembled systems on floating behavior and release rate. European Journal of Pharmaceutics and Biopharmaceutics, 2011. 77 (1) p. 116-121.

75. Strusi O.L, et al., Module assemblage technology for floating systems: In vitro flotation and in vivo-gastro-retention. Journal of Controlled Release, 2008. 129 (2) p. 88-92.

76. Oliveira P.R., et al., Assembled modules technology for site- specific prolonged delivery of norfloxacin. International Journal of Pharmaceutics, 2011. 405(1-2) p. 90-96.

77. El-Zahaby, S. A., A.A. Kassem, A.H. El-Kamel, Design and evaluation of gastoretentive levofloxacin floating mini-tablets-in-capsule system for eradication of Helicobacter Pylori. Saudi Pharmaceutical Journal, (0).