Biopharmaceutical Studies on Gastroretentive Oral Dosage Forms for Eradication of Helicobacter pylori

Thesis Submitted by

Aya Rashad Ahmed Abdou

M.Sc. Pharmaceutics,
Assistant Researcher,
Medical and Pharmaceutical Chemistry Department,
Division of Pharmaceutical Industries,
National Research Centre

For Degree of Doctor of Philosophy in Pharmaceutical Sciences "Pharmaceutics"

Under the Supervision of

Prof. Dr. Nadia M. Mursi
Professor of Pharmaceutics,
Medical and Pharmaceutical Faculty of Pharmacy,
Cairo University.

Prof. Dr. Laila H. Emara
Professor of Pharmaceutics,
Ex - Dean,
Chemistry Department,
Division of Pharmaceutical Industries,
National Research Centre.

Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy
Cairo University

2015

Abstract

Helicobacter pylori (*H. pylori*) is currently recognized as one of the most common chronic bacterial infections worldwide. *H. pylori* is the main cause of chronic gastritis, peptic ulcer disease, and gastric malignancies not associated with the use of non-steroidal anti-inflammatory drugs. It is found in half the population of the world. Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors, being high in developing countries and lower in the developed world. The ecological niche of *H. pylori* is the human stomach, where it establishes long term colonization of the gastric mucosa. Transmission can occur by contaminated water and indirect person to person contact. The high prevalence of the infection means that public health interventions may be required. The short term approach, however, provided that resources allow for this would be a test and treat strategy for those who are at risk for peptic ulcer disease or gastric cancer, as well as for those with troublesome dyspepsia. Consequently, *H. pylori* eradication is recognized to be the correct approach along with conventional therapies in the treatment of these diseases.

For effective *H. pylori* eradication, therapeutic agents have to penetrate the gastric mucus layer to disrupt and inhibit the mechanism of colonization. This requires targeted drug delivery within the stomach wall to ensure effective local action. Although most antibiotics have very low *in vitro* minimum inhibitory concentrations (MIC) against *H. pylori* (MIC90 ≤ 1 mg/L), no single antibiotic has been able to eradicate this organism effectively. The regimen most widely used today to eradicate *H. pylori* is combination therapy with two antibiotics combined with bismuth or an acid pump inhibitor. A major reason for *H. pylori* eradication failure is resistance to Metronidazole or Clarithromycin (main antibiotics used for treatment of microorganism). In contrast, *H. pylori* does not appear to develop high resistance to Amoxicillin.

Metronidazole is a 5-nitroimidazole derivative with activity against anaerobic bacteria and protozoa. It is used to eradicate *H. pylori* in peptic ulcer disease (in combination with antimicrobials, and either bismuth compounds or proton pump inhibitors). Amoxicillin is the 4-hydroxy analogue of Ampicillin, and it is used in a similar variety of susceptible infections. It is given as part of treatment regimens to eradicate *H. pylori* infection in patients with peptic ulcer

disease. The associated limitations of administering dosage forms of these drugs are complex dosing regimen/frequency, and reduced patient compliance.

It is therefore important to develop a suitable dosage form for both antibiotics that will remain in contact with the upper GIT mucosal cell for sufficient period of time and that will deliver and sustain a continuous effective dose of both antibiotics over an extended period of time. Thus both efficiency of therapy and patient compliance will be increased. Among the attempts for developing modified release dosage forms of Amoxicillin and Metronidazole reported in literature for the specific target of eradicating *H. pylori* are the gastro-retentive dosage forms including the floating formulations.

- **The objective of the present study is to:** formulate Metronidazole and Amoxicillin (as the mostly recommended antimicrobials in *H. pylori* eradication guidelines) in different sustained-release gastroretentive floating tablets to improve the efficiency of therapy and patient compliance. Natural & synthetic polymers were used. *In vitro* evaluation for such dosage forms will be done regarding, floating and release. The effect of storage on both floating properties and release from the prepared tablets will also be investigated. Finally, a pilot clinical study will be done using a selected floating formula on *H. pylori* infected patients.

To achieve this goal, work in this thesis is divided into the following parts:

Part I:

Formulation and Evaluation of Metronidazole Sustained-Release Floating Tablets.

Part II:

Formulation and Evaluation of Amoxicillin Sustained-Release Floating Tablets.

Part III:

Clinical Outcomes of Two-Week Standard Triple Therapy Employing a New Floating Amoxicillin Tablet for *Helicobacter Pylori* Eradication: A Randomized Pilot Study on an Egyptian Infected Sample.

General Conclusion

This study concluded the following:

- Based on our study, the Egyptian Ministry of Health must pay attention to the issue of the wide spread of *H. pylori* infection in Egypt and the importance of early diagnosis due to its high impact on the general health of Egyptians. In such a way, applying *H. pylori* screening and treatment strategies would be cost-effective, as improper treatment of *H. pylori*-associated conditions, will be saved.

- It is well documented worldwide the association of *H. pylori* colonization of the stomach with chronic gastritis, peptic ulcer disease, and gastric malignancies in both adults and children. A lack of proper sanitation, of safe drinking water, and of basic hygiene, as well as poor diets and overcrowding, all play a role in determining the overall prevalence of *H. pylori* infection. Therefore, if a nationwide screening was done for patients suffering from such diseases, it will be found that the majority of them are positively infected with *H. pylori*, without even knowing it. This represents a potential risk for developing gastric cancer, if such bacterium is not successfully eradicated at the early beginnings of the infection.

- Large-scale production requires more easiness in the formulation accompanying economic drug delivery systems. Therefore, these concepts were kept in mind before starting this research, so that ultimately the development of a reproducible high quality and low cost drug product can be possible.

- Sustained release single layer floating tablets for both Metronidazole and Amoxicillin were successfully prepared using direct compression technique. For such tablets, minimal number of ingredients (3-6) and a low percentage of polymer(s) were used. A good compromise between floating properties and the drug release pattern was done as targeted.

- These floating tablets showed spectacular floating properties. Floating lag time of few seconds and a floating duration of more than 24 h were recorded for such tablets. Moreover, good matrix integrity was retained. These floating properties will allow the proposed tablets to retain in the stomach for a long time for better eradication of *Helicobacter Pylori*.

- Our proposed natural polymers based floating formulae gave comparable performance regarding *in vitro* floating and release to HPMC-K4M (the most commonly used synthetic polymer) based tablets. This would provide added advantage as natural polymers are known to be safe, nontoxic, biocompatible, inexpensive and readily available compared to synthetic polymers.

- The simplicity of the adopted formulation technique, together with the promising qualities of the proposed tablets, provides the principles of scale up and process validation to improve the quality and market availability of such GRDDS and could help to deliver a product to the market within a reasonable timeframe and in a cost-effective manner.

- The re-validation of the stability indicating HPLC/UV method for the *in vitro* determination of Amoxicillin in floating tablets (Emara, Abdou et al. 2013) proved that the method was selective, specific, accurate, precise, linear and sensitive. This HPLC method was adopted to estimate Amoxicillin content in both fresh and bench stored (for up to one month) Amoxicillin floating tablets (A8), "Bio-batch". Results indicated that all the stored Amoxicillin floating tablets were within the accepted drug content limits of US-Pharmacopoeia and would thus remain stable and safe to be administered by the patients who participated in the following clinical study.

- Clinical outcomes of two-week standard triple therapy employing our new floating Amoxicillin tablet (prepared in our Industrial Pharmacy Lab, NRC) for *H. Pylori* eradication revealed that: conventional Amoxicillin capsules (E-Mox®, E.P.I.C.O, Egypt) - based standard triple therapy gave suboptimal efficacies. After treatment, the overall eradication rate of the *H. pylori* infection was 81.3% (13/16), distributed as 75% (6/8) and 87.5% (7/8) for Group A (Reference group) and B (Test group), respectively.

- In contrast, floating Amoxicillin tablets (prepared in our Industrial Pharmacy Lab, NRC) - based triple therapy of *H. pylori* increased the eradication rate by 12.5%, significantly reduced both the frequency of adverse effects and the baseline Stool Antigen Test values in *H. pylori* infection for an Egyptian population sample in comparison with conventional triple therapy. This makes our new floating Amoxicillin tablets a better choice for eradication of *H. pylori* infections, after elaborative, integrated, multicenter study with a large sample size.

General Introduction

Peptic Ulcer and the Discovery of *Helicobacter pylori*:

Since 1982 the cause of peptic ulcer seemed to be already known. Ulcers were thought to be caused by excessive amounts of acid secretion secondary to personality, stress, smoking, or an inherited tendency. The successful introduction of H_2 receptor antagonists five years earlier seemed to confirm this idea because nearly all ulcers could be healed, at that time, by lowering stomach acid secretion with these drugs. Thus, when *Helicobacter* was revealed, doctors were not looking for a new cause of peptic ulcer; that territory had already been taken by the illusion of knowledge (Marshall 2006).

Helicobacter pylori Epidemiology and Routes of Transmission:

Helicobacter pylori (*H. pylori*) is currently recognized as one of the most common chronic bacterial infections worldwide (Everhart 2000). It is an important human pathogen causing a variety of gastroduodenal diseases. While the majority of infections are asymptomatic (Oztürk, Senocak et al. 1996; Parsonnet 1998), the association of *H. pylori* colonization of the stomach with chronic gastritis, peptic ulcer disease, and gastric malignancies is now well documented in both adults and children (Suerbaum and Michetti 2002). It is also associated with the development of Mucosa associated lymphoid tissue (MALT) lymphoma (Sherif, Mohran et al. 2004; Fennerty 2005).

Helicobacter pylori and Related Gastroduodenal Diseases:

H. pylori is a fastidious, microaerophilic, spiral gram-negative bacterium. The ecological niche of *H. pylori* is the human stomach, where it establishes long term colonization of the gastric mucosa. Lifetime persistence of *H. pylori* within its host is the normal, with rare spontaneous clearance. Transmission can occur by contaminated water and indirect person to person contact e. g., via poorly disinfected endoscopes. *H. pylori* possess several potential virulence factors. The imbalance between the host defense mechanisms and these factors leads to ailment. The variation in disease outcome is probably due to differences in bacterial virulence genes. The most intensively studied virulence factors are the cytotoxin- associated antigen (cagA), and the vacuolating cytotoxin (vacA) (Amer, El-Sokkary et al. 2013).

H. pylori is found in half the population of the world. Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors, being high in developing countries and lower in the developed world. In general, however, there has been a decreasing trend in the prevalence of *H. pylori* in many parts of the world in recent years. Direct epidemiologic comparisons of peptic ulcer disease between developing and developed countries are complex, as peptic ulcers may be asymptomatic and the availability and accessibility of the tests required for diagnosis vary widely. In developing countries, *H. pylori* infection is a public health issue. The high prevalence of the infection means that public health interventions may be required. Therapeutic vaccination is probably the only strategy that would make a decisive difference in the prevalence and incidence of *H. pylori* throughout the world. The short term approach, however, provided that resources allow for this would be a test and treat strategy for those who are at risk for peptic ulcer disease or gastric cancer, as well as for those with troublesome dyspepsia. Eradication of bacteria is effective in healing peptic ulcers, preventing ulcer relapses, and potentially decreasing the risk of progression to gastric carcinoma (Murray, DuPont et al. 1992; Feldman 2001; Sepulveda and Coelho 2002; Hunt, Xiao et al. 2011).

Helicobacter pylori **and the Risk of Gastric Cancer:**

Gastric cancer is the fourth commonest malignant disorder and the second commonest cause of cancer-related death worldwide (Kamangar, Dores et al. 2006). It represents 1.64% of all cancers in Egypt, according to the National Cancer Institute registry and the median age is 55 years with male predominance (Anwar, Youssef et al. 2012). Several studies have linked *H. pylori* and gastric cancer. The etiopathogenetic cascade of the infection leads to various errors in the genome of dividing gastric epithelial cells, which leads to abnormal differentiation of cells (Vauhkonen, Vauhkonen et al. 2006). In addition, chronic inflammation associated with the infection leads to damage in proteins, the production of reactive oxygen species and deficient repair of replication errors of DNA. All increase the risk of gastric cancer (Kim, Tao et al. 2002; Vauhkonen, Vauhkonen et al. 2006). Among the pathogenic virulence factors in *H. pylori* infection is CagA protein, which is produced by most strains into the gastric cells and phosphorylated by the host kinases, resulting in morphological changes in the epithelial cells (Huang, Zheng et al. 2003). Researchers have documented that *H. pylori* eradication therapy attenuates the endoscopic and histological lesions (Ito, Tanaka et al. 2006). Besides, many

studies have consistently reported that *H. pylori* eradication can lead to a regression of acute gastritis and intestinal metaplasia, and that such intervention could reduce the risk for adenocarcinoma of the distal stomach (Leung, Lin et al. 2004; Mera, Fontham et al. 2005). When clinically manifested, gastric cancer has an extremely poor prognosis since the 5 year survival rate using currently available treatments, surgery and radio-chemotherapy, is less than 20% (Egan and O'Morain 2007). Therefore, a potential benefit could be expected if we had a feasible diagnostic test for follow-up of patients with *H. pylori* gastritis and were able to predict the risk of development of stomach cancer (Anwar, Youssef et al. 2012).

Eradication of *Helicobacter pylori* and Associated Limitations:

Current practice dictates treatment of symptomatic individuals with a regimen containing two antimicrobial agents along with a proton pump inhibitor (Suerbaum and Michetti 2002). Although many antibacterial agents have a very low minimum inhibitory concentration (MIC) against *H. pylori in vitro* (Ateshkadi, Lam et al. 1993), no single agent is effective in the eradication of the infection *in vivo* when administered alone (Murray 1993). In addition, single antibiotic therapy is strongly discouraged to prevent the development of resistant strains (Hunt 1995). There could be one or several reasons for the failure of single antibiotic therapy against *H. pylori*. Firstly, the organism resides in the mucus gel close to the acidic environment of the gastric fluid. Many antibacterial agents, such as penicillin and erythromycin, degrade rapidly in acidic medium. Secondly, the drug must diffuse into the mucus layer to furnish concentrations sufficient for antibacterial activity. Lastly, the contact time of antibacterial drugs with the organism needs to be sufficiently long for successful eradication of *H. pylori* from the gastric mucosa (Shah, Qaqish et al. 1999), which can be achieved through a gastroretentive drug delivery system (GRDDS).

Anatomy of the Stomach and Gastrointestinal Motility:

Fundus, body and antrum are three anatomical features of the stomach (Figure 1). The ingested materials reside in proximal stomach which is made up of fundus and body regions whereas distal region (antrum) is responsible for providing mixing motion and acts as a pump for gastric emptying (Pawar, Kansal et al. 2012). Fasted and fed states show two distinct patterns of gastrointestinal motility. The fasted state is characterized by inter-digestive myoelectric cycle or

migrating motor complex (MMC). The MMC is inter-digestive series of electrical event and cycle of 2-3 h through the stomach and small intestine. The MMC is composed of four consecutive phases: Phase I (basal phase; that lasts from 45 - 60 min with rare contractions), Phase II (preburst phase; lasts for 3-45 min with intermittent action potential and contractions), Phase III (burst phase; lasts for 5-15 min and includes intense and regular contractions for short period) and Phase IV (lasts for 0-5 min and occurs between Phases III and I for two consecutive cycles) (Streubel, Siepmann et al. 2006).

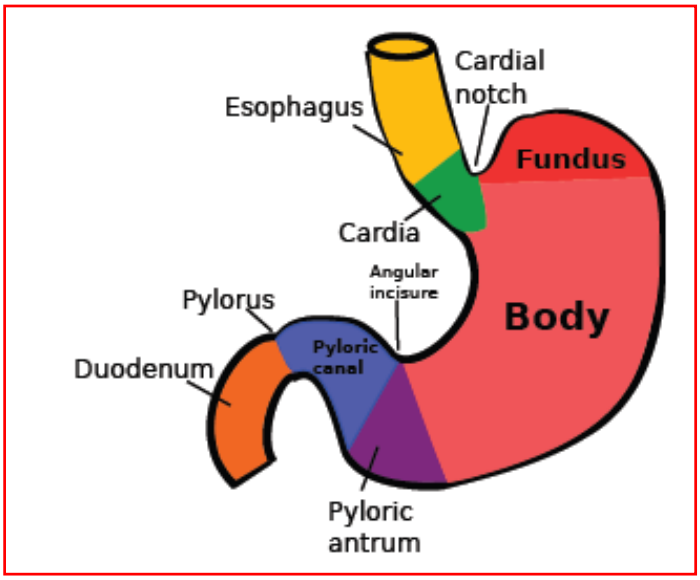

Figure 1. Anatomical Features of the Stomach.

Physiological Factors Affecting Bioavailability of Oral Sustained Release Dosage Forms:

The important physiological factor which is responsible for the reduction in efficacy of oral sustained release dosage forms (OSRDF) is gastric residence time (GRT). GRT considerably affects the bioavailability of pharmaceutical dosage forms (Bardonnet, Faivre et al. 2006). Variable and short gastric emptying time results in incomplete drug release from the OSRDF which leads to diminished efficacy of the administered dose (Adibkia, Hamedeyazdan et al. 2011). GRT is affected by both the fasting as well as fed states of the stomach. State of feeding gives direct reflection on bioavailability of the orally administered drugs. Gastric emptying studies revealed that the short GRT and unpredictable gastric emptying rate have altered the performance of OSRDF (Chawla and Bansal 2003).

Gastric residence time of an oral dosage form is affected by several factors. To pass through the pyloric valve into the small intestine, the particle size should be in the range of 1-2 mm (Arora, Ali et al. 2005). The rate of gastric emptying depends mainly on viscosity, volume, and caloric content of meals. The nutritive density of meals helps to determine gastric emptying time. It does not make any difference whether the meal has high protein, fat, or carbohydrate content as long as the caloric content is the same. However, an increase in acidity and caloric value slows down gastric emptying time. Biological factors such as age, body mass index (BMI), gender, posture, and diseased states (diabetes, Crohn's disease) influence gastric emptying. In the case of elderly people, gastric emptying is slowed down. Generally, females have slower gastric emptying rates than males. Stress increases gastric emptying rates while depression slows it down (Timmermans and Moes 1994; Arora, Ali et al. 2005; Vijayakumar, Senthilnathan et al. 2012; Emara, Abdou et al. 2013).

Gastroretentive Drug Delivery Systems:

To improve the performance of OSRDF, scientists have discovered a new concept in drug delivery, that is, gastroretentive drug delivery systems (GRDDS). An optimum GRDDS can be defined as a system which retains in the stomach for a sufficient time interval against all the physiological barriers, releasing active moiety in a controlled manner, and finally metabolized in the body (Pawar, Kansal et al. 2011). Over the last two decades, numerous GRDDS have been designed to prolong GRT. The major objective is to minimize the drawbacks associated with existing OSRDF and optimizing therapy coupled with substantial patient comfort (Chavanpatil, Jain et al. 2005; Hu, Li et al. 2011; Mostafavi, Emami et al. 2011; Pawar, Kansal et al. 2012).

❑ Rationale for Gastro-retention of Drugs:

GRDDS is superior for (1) drugs that are locally active in the stomach (e.g., misroprostol, antacids); (2) drugs that have narrow absorption windows in the gastrointestinal tract (GIT) (e.g., L-DOPA, para aminobenzoic acid, furosemide, riboflavin); (3) drugs that are unstable in the intestinal or colonic environment (e.g., captopril, ranitidine HCl, metronidazole); (4) drugs that disturb normal colonic microbes (e.g., antibiotics against *H. pylori*); and (5) drugs that exhibit low solubility at high pH values (e.g., diazepam, chlordiazepoxide, verapamil HCl) (Nayak,

Malakar et al. 2010; Sabale, Sakarkar et al. 2010). In practice, drugs that are less soluble in a high pH environment require more retention time in the stomach to improve solubility and bioavailability and to reduce drug waste. Prolonging gastric retention also has applications for local drug delivery to the stomach and proximal small intestine (Sabale, Sakarkar et al. 2010; Emara, Abdou et al. 2013). The rationale for the selection of active pharmaceutical ingredients for fabrication as a GRDDS is described in Table (1) (Parikh and Amin 2008).

Table 1 . Rationale for Gastro-retention of Drugs.

Rationale for gastro-retention	**Name of drug**
Drugs for local action : antacids, anti-ulcer drugs, antibacterials for *H. pylori* infection	Metronidazole Amoxicillin Clarithromycin Acetohydroxamic acid Misoprostol
Narrow absorption window at upper part of GIT	Levodopa Riboflavin Calcium Repaglinide Atenolol Theophylline Diltiazem Risedronate
pH-dependant absorption from stomach (acidic drugs)	Furosemide
Degradation at higher pH (higher stability at lower pH)	Captopril
Degradation in intestine or colon	Ranitidine
Higher solubility at lower pH or weakly basic drugs	Cinnarizine Diazepam Verapamil Cefpodoxime proxetil Dipyridamole Rosiglitazone maleate

Scope of Work

Helicobacter pylori (*H. pylori*) is currently recognized as one of the most common chronic bacterial infections worldwide. *H. pylori* is the main cause of chronic gastritis, peptic ulcer disease, and gastric malignancies. It is found in half the population of the world. The high prevalence of the infection means that public health interventions may be required. The short term approach for this would be a test and treat strategy. Consequently, *H. pylori* eradication is recognized to be the correct approach along with conventional therapies in the treatment of these diseases.

The ecological niche of *H. pylori* is the human stomach, where it establishes long term colonization of the gastric mucosa. For effective *H. pylori* eradication, therapeutic agents have to penetrate the gastric mucus layer to disrupt and inhibit the mechanism of colonization. Metronidazole and Amoxicillin are given as part of treatment regimens to eradicate *H. pylori* infection (in combination with other antimicrobials, and either bismuth compounds or proton pump inhibitors). The associated limitations of administering dosage forms of these drugs are complex dosing regimen/frequency, and reduced patient compliance.

It is therefore important to develop a suitable dosage form for both antibiotics that will remain in contact with the upper GIT mucosal cell for sufficient period of time and that will deliver and sustain a continuous effective dose of both antibiotics over an extended period of time. Thus both efficiency of therapy and patient compliance will be increased. Among the attempts to reach this target are the gastro-retentive drug delivery systems including the floating dosage forms.

The objective of this thesis is:

1. Formulation of Metronidazole and Amoxicillin (as the mostly recommended antimicrobials in *H.pylori* eradication guidelines) in different sustained-release floating tablets, using natural & synthetic polymers in order to improve the efficiency of therapy and patient compliance.
2. Studying the floating properties and *in vitro* release of Metronidazole and Amoxicillin from the prepared tablets.
3. Studying the effect of storage on the prepared tablets.
4. Pilot clinical study to be done using a selected floating formula on *H. pylori* infected patients.

The work in this thesis is divided into the following parts:

PART I

Formulation and Evaluation of Metronidazole Sustained-Release Floating Tablets.

Work in this part deals with:

1- Preparation of Metronidazole sustained-release effervescent floating tablets.
2- Determination of floating lag time and floating duration of Metronidazole tablets.
3- *In Vitro* Release Study of Metronidazole Floating Tablets.
4- Dissolution Efficiency Determination of Metronidazole Tablets.
5- Kinetic Study of Metronidazole Release Data.
6- Physico-chemical Interaction Studies (DSC, FT-IR and XRD).
7- Stability Study of Metronidazole Floating Tablets.

PART II

Formulation and Evaluation of Amoxicillin Sustained-Release Floating Tablets.

Work in this part deals with:

1- Preparation of Amoxicillin Sustained-Release Floating Tablets.
2- Determination of Floating Lag Time and Floating Duration of Amoxicillin Tablets.
3- *In Vitro* Release Study of Amoxicillin Floating Tablets.
4- Dissolution Efficiency Determination of Amoxicillin Tablets.
5- Kinetic Study of Amoxicillin Release Data.
6- Physico-chemical Interaction Studies (DSC, FT-IR and XRD).
7- Re-validation of a Stability Indicating HPLC/UV Method for the *In Vitro* Determination of Amoxicillin.
8- Stability Study of Amoxicillin Floating Tablets "Bio-Batch".

Part III

Clinical Outcomes of Two-Week Standard Triple Therapy Employing a New Floating Amoxicillin Tablet for *Helicobacter Pylori* Eradication: A Randomized Pilot Study on an Egyptian Infected Sample

The work in this part includes:

Comparing the performance of the standard triple therapy containing conventional Amoxicillin dosage form with that containing the selected modified-release Amoxicillin floating tablets (prepared in part II) for better eradication of *H. pylori* in infected patients.