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Development and In-Vitro Evaluation of Novel Floating Bi-Layer Gastroretentive Tablet of (Metronidazole, Clarithromycin and Esomeprazole) For the Treatment of H. pylori Induced Peptic Ulcer

Sa`ida Mohammad Abdel-Qadir

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Prepared By:

Sa`ida Mohammad Abdel-Qadir

B.Sc. Pharmacy

An-Najah National University

Supervisor: Dr. Tareq Al-Jubeh

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Prepared by: Sa`ida Abdel-Qadir

Registration number: 21210089

Supervisor: Dr. Tareq Al-Jubeh

Master thesis submitted and accepted, 28/12/2015 ,The names and signatures of the examining committee are as follows:

1- Dr. Tareq Al-Jubeh Head of Committee Signature:

2- Dr.Hussien Hallak Internal Examiner Signature:

3- Dr. Abdel Naser Ziad External Examiner Signature:

Jerusalem – Palestine

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Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed: ……..

Sa`ida Mohammad Abdel-Qadir

Date:

Dedication

I would like to dedicate to this workto my family as a simple thank for every thing they gave to me. Firstly my parents who offer permanent support to me in all steps of my life, and especially my mother which is often the bright torch to me in all circumstances. And to my great husband Abdulla Abu-Shoshah who gave me this opportunity to achieve my ambition and stood beside me during this period, thanks for your support, help and love . Special dedication with love to my children Zeinah, Muhannad and Hala for their love and patience. And I hope that God will preserve them all.

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List of Abbreviations

AUC	Area under the curve
BA	Bioavailability
CLA	Clarithromycin
C_{max}	Maximum concentration
Conc.	Concentration
CR	Controlled release
d	Days
D	Direct compression formulations.
DG	Dry granulation formulations
DDS	Drug delivery system
DU	Duodenal ulcer
EZO	Esomeprazole
FDDS	Floating drug delivery system
FLT	Floating lag time
GET	Gastric emptying time
GIT	Gastro intestinal tract
GORD	Gastroesophageal Reflux Disease
GRDDS	Gastroretentive drug delivery system
GRT	Gastric residence time
GU	Gastric ulcer
H	Hours
H. pylori	Helicobacter pylori
HBC	Hydrodynamically balanced system
HPC	Hydroxypropylcellulose
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropylmethyl cellulose
ICH	International Conference of Harmonization
IR	Immediate release
LC	Liquid chromatography
MALT	gastric lymphoma of mucosa associated lymphoid tissue
MCC	Microcrystalline cellulose

MEC	Minimum effective concentration
MIC	Minimum inhibitory concentration
Min	Minutes
MMC	Migrating motor complex
MOH	Ministry of Health
MTC	Minimum toxic concentration
MTZ	Metronidazole
NLT	Not less than
NMT	Not more than
NSAIDs	Nonsteroidal anti-inflammatory drugs
NUD	Non-ulcer dyspepsia
PCA	PPI-Clarithromycin-Amoxicillin
PCM	PPI-Clarithromycin-Metronidazole
PD	Pharmacodynamics
PK	Pharmacokinetic
PPI	Proton pump inhibitor
PVP	Polyvinylpyrrolidone
QC	Quality control
RS	Reference standard
RSD	Relative standard deviation
RDL	Rapidly dissolving layer
SD	Standard deviation
SR	Sustained release
TFT	Total floating time
W	Wet granulation formulations
Wt.	Weight
WU	Water uptake
USP	United States Pharmacopoeia
UV	Ultraviolet

Abstract

H pylori infection is one of the most prevalent infectious diseases worldwide which affects nearly 40%-50% of the world population. Eradication of *Helicobacter pylori (H. pylori)* remains a worthwhile goal to treat the associated diseases, especially the peptic ulcer, and to reduce the lifetime risk of the infection, mainly the gastric cancer.

The first line therapy for Helicobacter pylori infection and induced peptic ulcer is a 'triple therapy' consisting of two antibiotics (amoxicillin or metronidazole with clarithromycin) and a proton pump inhibitor (i.e. omeprazole, esomeprazole, lansoprazole, etc.). Treatment of H. pylori with the conventional dosage forms may fail, due to the short time within which the antibiotics reside within the main site of the bacteria colonization in the stomach, since they may be emptied rapidly during gastric emptying event. Disadvantages of current treatments also include poor patient compliance due to the requirement of multiple dosing and the high prevalence of side effects.

Improvement in the treatment of gastric ulcer requires providing alternative delivery systems that ensure the prolongation of the local availability of the antibiotics with a sustained and high concentration within the stomach (the site of infection), thus increasing the efficacy of the treatment, decreasing dosing frequency; hence increasing the patient compliance and decreasing side effects. New gastroretentive drug delivery systems for the treatment of H. pylori have received great interest from researchers worldwide, since these systems may provide the aforementioned advantages.

In this research, a novel dosage form is designed for the first-line triple therapy treatment (clarithromycin (CLA), metronidazole (MTZ) and esomeprazole (EZO)) of *H. pylori*

associated peptic ulcers. The design of the delivery system was based on single, bilayer gastroretentive tablet approach, with floating, swelling and sustained release (SR) properties. One layer included esomeprazole, which is a rapidly disintegrating layer that contained enteric coated pellets. The other layer is a floating-sustained release matrix of metronidazole and clarithromycin.

Formulations were developed using hydroxypropylmethylcellulose (HPMC) with different viscosity grades (K-4M, K-15M and K-100M) and ethyl cellulose (EC) as the major swellable matrix-forming and rate-controlling polymeric excipients, while sodium bicarbonate (NaHCO3) and citric acid were used as the gas generating agents. Different manufacturing techniques were applied including, direct compression, wet granulation and dry granulation in order to choose the most suitable one. The choice of the best formula was based on the requirements of short floating time, long floating duration and sustained-synchronous release of the two antibiotics. In the optimized formula, metronidazole was incorporated inside granules within the SR- layer, while clarithromycin was distributed within the matrix of the SR-layer itself which also included the gas generating agents NaHCO3 and citric acid in ratios 4:1. Esomeprazole pellets were incorporated within the rapidly dissolving layer to be separated from the tablet within two minutes. Physical properties of the tablets, namely, hardness, friability, weight variation, content assay and powder properties all meet the qualifications of the USP.

The drug content (assay) and the release over time of both clarithromycin and metronidazole were determined simultaneously on a gradient high-performance liquid chromatography (HPLC) method that was developed and validated in this research. The developed HPLC method for the concurrent determination of CLA and MTZ was proved to be linear, accurate, precise and selective.

Single tablet of two layers, combining the first line therapy for treatment of H. pylori induced ulcer was formulated. DG2 fotmula exhibited excellent floating properties and a synchronous sustained release of both antibiotics. The swelling index of the tablets, and the kinetics of the release were also elucidated. They indicated that the release mechanism of both antibiotics was mainly controlled by diffusion, polymer relaxation and erosion.

This final optimized formula was manufactured by dry granulation technique and contained (23%) HPMC-K100M, (17%) EC, (12%) NaHCO3 and (3%) citric acid within the SR-floating layer. In this formula the two layers separated with less than 20 seconds, the SR-layer has floating lag time (FLT) less than 30 seconds , and total floating time (TFT) more than 24 h. The drug release after 20 h was 85% of MTZ and 76% of CLA. These results applied to our goals of producing a delivery system that stays in the stomach for a long period while releasing the two antibiotics which will exert a local effect on the bacteria. As a result, this proposed formula of bilayer-triple therapy delivery system could be very promising in providing a better treatment of H. pylori induced ulcer than the existing ones.

Part one

1. Introduction

1.1. Helicobacter Pylori

1.1.1. Overview and epidemiology of H .pylori:

Helicobacter pylori *(H .pylori)* infection is one of the most common bacterial infections in the world, it affects nearly half of the world's population and, thus, is one of the most frequent and persistent bacterial infections worldwide(1). It is a chronic infection and once acquired it remains life long, unless it is eradicated by suitable antibiotic treatments (2).

Globally, different strains of H. pylori exist & are associated with differences in virulence, and the resulting interplay with host factors and environmental factors leads to differences in the expression of disease. Age, ethnicity, gender, geography and socioeconomic status, are all factors that influence the incidence and prevalence of H. pylori infection. The overall prevalence is high in developing countries and lower in developed countries(3).

In developing countries, the prevalence of infection is as high as 90%, whereas in developed countries the prevalence is below 40% (4). In asymptomatic patients, the prevalence of *H. pylori* infection varies from 31%-84%(2).

Several studies reported data on the worldwide prevalence of *H. pylori* infection; these data are summarized in **Table 1.1.** (5).

Other studies reported data on the Middle East prevalence which has a high rate prevalence of *H. pylori* infection , with percentages varies from >80% in Egypt, 60-70% in Saudi Arabia and < 50 % in Palesine (6).

H. *pylori* were firstly discovered by Warren and Marshall in 1982, after they isolated it from patients with peptic ulcer. Then they published a research paper in 1984 that has firmly established H. pylori as an important etiologic agent in many diseases related to stomach(7).

Table1.1. Prevalence of *H. pylori* infection in adults reported by studies published in 2013.(5)

Country (Reference)	Setting	Number	Diagnostic method	Prevalence of *Helicobacter pylori* (%)
Western Europe				
The Netherlands [3]	Blood donors	1550	Serology	31.7
The Netherlands [4]	Pregnant women	6837	Serology	46
Portugal [5]	General population	2067	Serology	84.2
Eastern Europe				
Cyprus [35]	Patients with dyspepsia	103	PCR	39.8
Turkey [6]	General population	4622	UBT	82.5
America				
Canada [7]	Aboriginal population	203	Histology	37.9
Mexico [8]	Pregnant women	343	Serology	52.2
Asia				
Saudi Arabia [17]	Healthy individuals	456	Serology	28.3
Korea [10]	Routine health check-up	10796	Serology	54.4
India [12]	Patients with dyspepsia	2000	Histology RUT	58
India [13]	Patients with dyspepsia	530	Histology Urease test	62
China [11]	Healthy individuals	5417	UBT	63.4
Bhutan [15]	Volunteers	372	Histology RUT Culture Serology	73.4
Bhutan [16]	Patients with dyspepsia	244	Serology	86
Kazakhstan [14]	Asymptomatic and patients with dyspepsia	835	Serology	76.5
Africa				
Ethiopia [21]	Selected population	1388	Serology	65.7
Morocco [20]	Patients with dyspepsia	429	Histology RUT Culture	75.5
Nigeria [22]	Patients with dyspepsia	125	Serology Histology	93.6 80

UBT, urea breath test; RUT, rapid urease test.

H. pylori is a gram-negative bacilli, microaerophilic bacteria, resides mainly in the gastric mucosa, associated to the mucus layer, living both within and beneath it, and adhering to the gastric epithelial cells , mainly at the antral region of the human stomach, it can also be found on the epithelial cells of the duodenum (8) (**Figure 1.1**).

The organism is catalase positive, oxidase positive and urease positive. As a result, urea is broken down into bicarbonate and ammonia, which protects the bacterium in the acid medium of stomach and causes gastric epithelial injury(9).

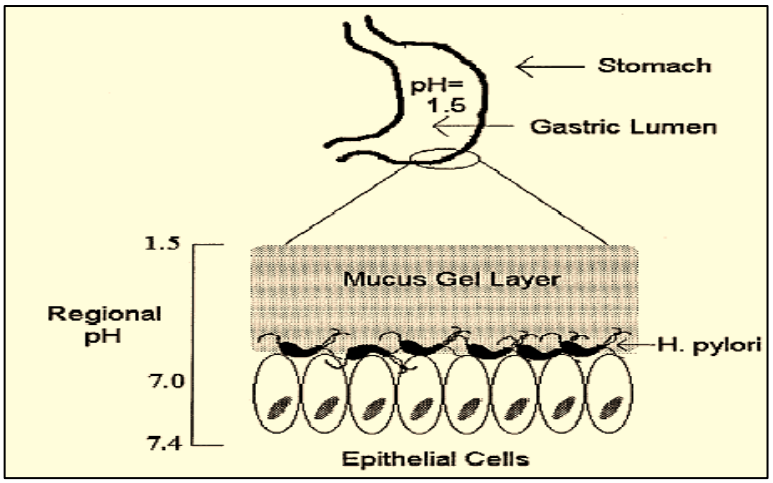


Figure1.1. H. pylori location in the stomach(10)

1.1.2. Proposed mechanisms of pathogenesis of H. pylori (11) (12), (13).

By direct contact of H. pylori with the gastric mucosa, elaboration of different enzymes and cytotoxins by H. pylori occur, this can directly cause gastric cells injury and, therefore is responsible for the most of gastric diseases associated with gastric cells damage, this process of pathogenesis can be mediated by (**Figure 1.2**)

- Bacterial Adherence: This process is mediated by specialized adhesion molecules that allow H. pylori to maintain themselves within their habitat. This adhesion prevents bacteria from being removed by the host-defense mechanisms (e.g. peristalsis, ciliary activity, and turnover the mucous layer).
- Ureases: H. pylori produces large amounts of urease enzyme (localized inside and outside of the bacterium).Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia which is converted to ammonium by accepting a proton (H+), this will neutralizes surrounding media and enable the survival of H. pylori in the acidic stomach. The ammonia is toxic to the epithelial cells,

and beside other products of H. pylori including proteases and certain phospholipases, damages gastric cells.

- Lipase and Protease: H. pylori proteases activity leads to disintegration of the polymeric structure of mucin, whereas elaborated lipases and phospholipase act on the lipids degradation in the mucous layer and the gastric epithelial cell membranes, so bacterial colonies damage the mucous linings and undermine the gastric mucosal cytoprotection.
- Bacterial Virulence factors: the two most important virulence factors of H. pylori are Cytotoxin-associated gene A (cagA) and vacuolating cytotoxin gene A (vacA).

  cagA is related to peptic ulcer and gastric malignancy in certain populations ,vacA can induce host cell vacuolation and eventually cell death.

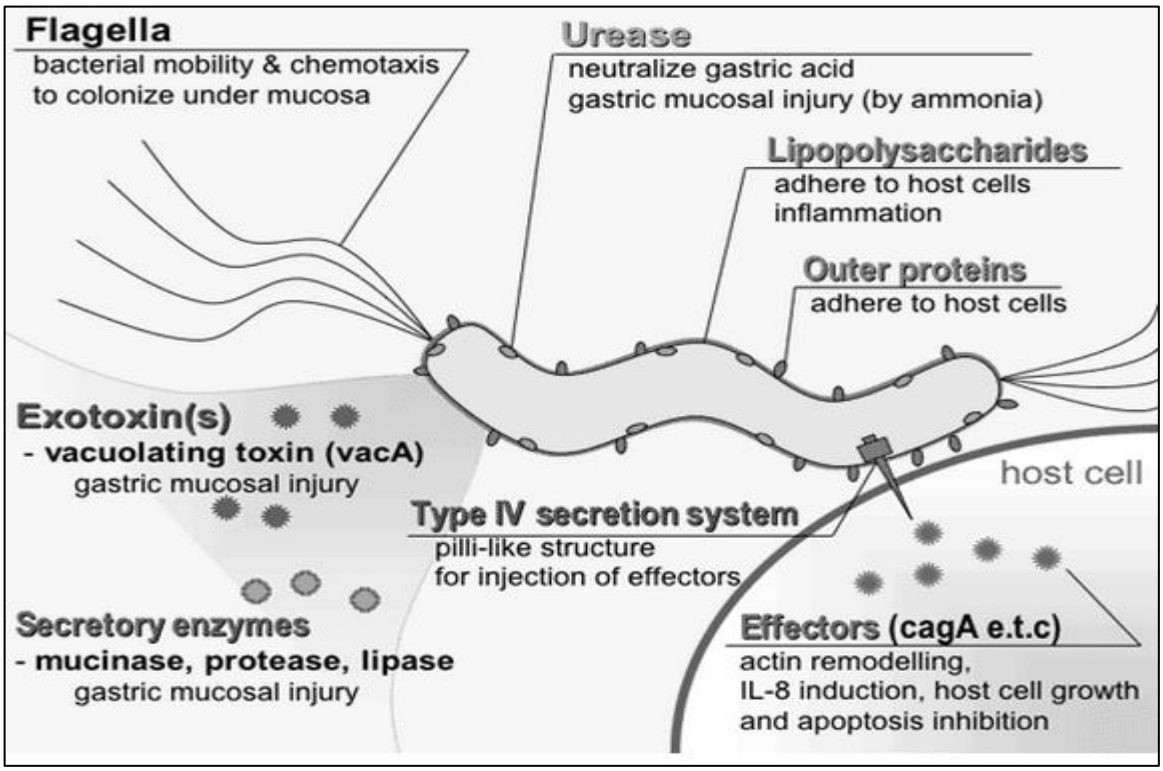


Figure 1.2: H. pylori pathogenesis factors

1.1.3. Symptoms of H. pylori infection:

H. pylori infection generally is asymptomatic and most people with H. pylori don't have any symptoms. Although number of symptoms may be associated with *H. pylori* infection, including: Gnawing pain, Nausea, Vomiting, Loss of appetite, Bloating, Burping, Weight loss, Bleeding (14) (15).

1.1.4. Diagnosis of H. pylori infection (16),(17), (18):

1.1.4.1. **Serological test:**

It is a blood test to find out antibodies against H pylori and especially against its most specific antigen CagA. If antibodies to H. pylori present in the blood, it means the patient either currently infected or has been infected in the past. Its sensitivity is 92% but only a specificity of 83%.

1.1.4.2. **Urea breath test (UBT):**

The C13 or C14 urea breath test (UBT) has been recognized as an excellent test because of its accuracy as well as of its robustness is easy to perform.

Its sensitivity is 88- 95% and specificity 95%-100%.

1.1.4.3. **Stool antigen test:**

A stool antigen test is used to see if antigens that trigger the immune response & produced by H. pylori (CagA & VagA) are present in the feces (stool). This test is considered as a valuable noninvasive alternative to diagnose H. pylori when UBT is not available.

It has a sensitivity of 94% and a specificity of 92%.

1.1.4.4. **Stomach biopsy:**

This test is invasive because a small sample (biopsy) is taken from the lining of the stomach and small intestine during an endoscopy procedure.

This test is important to perform susceptibility testing to antimicrobial agents in a regions of high clarithromycin resistance before prescription of the first-line treatment(19), and after a second line therapy failure, it should be performed in all cases(18).

1.1.5. Transmission of H. pylori (20), (5):

H. pylori transmission occurs via several routes including:

1. Oral-oral (e.g. mothers could transmit the infection via mouth secretions when tasting the Child's food).

2. Fecal-oral (e.g. contaminated ground-water and plants with feces, lack of personal hygiene).

3. Gastro-oral (iatrogenic spread with inadvertent use of unsterile endoscopes).

1.1.6. Risk factors related to H. pylori infection:

1. Low socioeconomic conditions are the most important risk factors for H. pylori infection(7).
2. living in a rural area(21).
3. Living in crowded homes(22).
4. Having contaminated sources of drinking water(23).

1.1.7. Diseases associated with H. pylori.

1.1.7.1. **Possible diseases caused from H. pylori infection.**

Occurrence of many diseases can be caused *by H. pylori* infection, those are:

Gastritis, non-ulcer dyspepsia (NUD), peptic ulcer diseases (PUD) including duodenal ulcer and gastric ulcer, gastric atrophy, gastric carcinoma, gastric lymphoma of mucosa associated lymphoid tissue (MALT) and even coronary heart diseases(**24**). It was found from a number of studies to that H. *pylori* may play a role in iron deficiency anemia (**25**).

It has been approved that *H .pylori* is the cause of almost all peptic ulcer diseases (i.e. duodenal ulcers (DU) and gastric ulcers (GU)) that are not related to nonsteroidal anti-inflammatory drugs (NSAID)(**26**).

H. pylori appears to be associated with 95% of the gastritis cases, 90-95% of DU cases and 70-90% of GU cases (27), (28).

H. pylori-positive patients have at least a six-fold greater risk of developing gastric adenocarcinoma than do those without infection(29).

1.1.7.2. **Peptic ulcer disease:**

1.1.7.2.1. Overview:

Peptic ulcer disease (PUD) represents a serious medical problem affects about 5% and 10% of adults globally, with a 4 million new cases every year (30).

It affects the stomach & the upper part of the duodenum, consisting of a distinct breach (open sore) in the gastrointestinal mucosa of the stomach or the duodenum, causing burning sensation or pain in the area between the chest and umbilicus, this pain becomes more intense when the stomach is empty. It lasts from few minutes to several hours. A 2%-14% of the these ulcers will perforate, and severe complications that cause mortality rates varies from 10%-40%(31).

Duodenal ulcers are rarely malignant but gastric ulcers are more commonly associated with malignancy. People can have both gastric and duodenal ulcers at the same time. They also can develop peptic ulcers more than once in their lifetime.

The majority of peptic ulcers are caused by infection with H. pylori. Globally, different strains of H. pylori exist & are associated with differences in virulence, and the resulting

interplay with host factors and environmental factors leads to differences in the expression of disease. Age, ethnicity, gender, geography and socioeconomic status, are all factors that influence the incidence and prevalence of H. pylori infection. The overall prevalence is high in developing countries and lower in developed countries(**3**).

Most H. pylori induced ulcers can be cured with proper treatment that consists usually of a combination of drugs, including antibiotics and a proton pump inhibitor.

1.1.7.2.2. Pathophysiological & etiological factors of peptic ulcer:

- The majority of duodenal and gastric ulcers are caused by Helicobacter infection, 95% of duodenal and 70% of gastric ulcers are associated with Helicobacter pylori. Eradication of H pylori reduces the relapse rate of ulcers but the magnitude of this effect is uncertain(32).

- Acid secretion: auto-digestion of the gastro-duodenal mucosa by acid secretion. In variety of diseases known to cause peptic ulcer, including Zollinger-Ellison syndrome (ZES /gastrinoma), antral G-cell hyper function.

- Abnormalities of normal mucosal defense mechanisms(33), and reflux of duodenal contents into the stomach or delayed gastric emptying may also be involved

- Drugs (mainly NSAIDs, corticosteroids) .Taking NSAIDs causes 15 to 20 % annual incidence of peptic ulcer (34). It is reported that there is a synergism between Helicobacter infection and NSAID use for the development of peptic ulcer as well as ulcer bleeding(35).
- Cigarette smoking: smokers are approximately twice as likely to develop peptic ulcer disease as non -smokers (36).Smoking increases gastric acid secretion and duodenogastric reflux. Smoking decreases gastro-duodenal prostaglandin production.

- Drinking alcohol.
- Stress(both physiological and psychological stress play a role in the development of peptic ulcer in some patients) (37).

1.1.7.2.3. Complications of peptic ulcer associated with H. pylori (14):

1. Bleeding: when a peptic ulcer breaks through the blood vessels.
2. Obstruction: ulcer progression may block the pyloric region and prevents food from leaving the stomach.
3. Perforation, can happen when an ulcer breaks through the stomach wall.
4. Peritonitis: infection of the peritoneum, or the lining of the abdominal cavity

1.1.8. Treatment of H. pylori induced Peptic Ulcer Disease:

1.1.8.1. **Background and General Considerations:**

The main goals of peptic ulcer treatment are:

- Relief of symptoms.
- Healing of ulcer.
- Preventing ulcer recurrences.
- Reducing ulcer related complications.
- Reducing the morbidity and mortality rates.

Primarily, before the bacterium was found, it was believed that stomach ulcers occur when excess acid damage the gastric mucosa so the treatment was based on reduction or neutralization of that acid(38). Patients were treated with H2-blockers and, more recently, proton pump inhibitors, these drugs include:

- H_2-receptor antagonists (i.e. ranitidine, cimetidine, famotidine).
- PPI (i.e. omeprazole, pantoprazole, esomeprazole).
- Cyto-protective agents (i.e. sucralfate)

- Prostaglandin agonists (i.e. misoprostol)
- Antacids (aluminum hydroxide, magnesium hydroxide, calcium carbonate, sodium carbonate).

This kind of treatment could certainly relieve ulcer-related symptoms, but has a high recurrence rate(39).

The identification of H. pylori and understanding of H. pylori associated peptic ulcer disease (GU and DU) have greatly changed therapeutic regimens covering peptic ulcer disease. Eradication of H. pylori is now recognized to be the most correct approach in the treatment of the disease(40).

H pylori eradication is highly recommended for both DUs and GUs, as it has been proved that H. pylori eradication effectively heals the ulcer rates of >90%(41).

The presence of H. pylori should usually be confirmed before starting the eradication therapy, "test-and-treat strategy" which is highly recommended by international communities (42).

Although most antibiotics have very low in-vitro minimum inhibitory concentrations (MIC) against H. pylori (43), no single antibiotics has been able to eradicate this organism effectively, and effective eradication rate need a combination of different antibiotics(44), since the cross-resistance present within each family of antibiotics but no cross-resistance between different families of antibiotics which have different resistance mechanisms(29).

Prerequisite for clinically effective H. pylori eradication regimens is at least 80-90 % eradication rate, without induction of major side effects nor bacterial resistance (45).

1.1.8.2. **Regimens available for treatment of H. pylori induced ulcers:**

1.1.8.2.1. First line therapy:

The triple treatment including *Proton pump inhibitor (PPI)-clarithromycin and amoxicillin or metronidazole* proposed at the first Maastricht conference1 to treat H pylori infection has become universally the 1st line therapy since it was recommended by all the consensus conferences held around the world (42) (46),(47).

Table 1.2: First- line seven day triple therapy regimen(46)

ANTIBIOTIC	PROTON PUMP INHIBITOR
Amoxicillin 1g twice daily and Clarithromycin 500mg twice daily	Esomeprazole 20mg twice daily or Lansoprazole 30mg twice daily or Omeprazole 20mg twice daily or Pantoprazole 40mg twice daily or Rabeprazole 20mg twice daily
Metronidazole 400mg twice daily and Clarithromycin 250mg twice daily	Lansoprazole 30mg twice daily or Omeprazole 20mg twice daily or Pantoprazole 40mg twice daily

- After completion of triple therapy, the proton pump inhibitor has been recommended to be continued once daily for a total of 4–6 weeks to ensure complete ulcer healing(42)

- In regions with high resistance to clarithromycin, PPI-clarithromycin-containing triple therapy should be abandoned without prior susceptibility testing(42).

How to improve the 1st line therapy?

1- Increase the length of treatment.

Eradication rate for 7days is 70-85% (47), while increasing the duration of the treatment to 10-days, improves the eradication rate by 4%, and a 14-days treatment improves the eradication rate by 5-6%, in comparison to a 7-day treatment(48), (42).

2- Increase the dose of PPI:

Using twice-a-day PPI was better than a single daily dose in triple therapy and increases the efficacy of the triple therapy(49), also increases the cure rate by 6-10% (50).

3- Use metronidazole instead of amoxicillin as the second antibiotic.

PPI-clarithromycin-metronidazole (PCM) and PPI-clarithromycin-amoxicillin (PCA) regimens are equivalent but PCM is less expensive & has less side effects than PCA as Amoxicillin sometimes is associated with allergic reactions (42)

Pooled data for PAC regimens show eradication rates of 79.8% with clarithromycin 250 mg compared with 89.6% with clarithromycin 500 mg, while in PCM regimens, doubling the dose of clarithromycin had no statistically significant effect: eradication rates were 87.4% for clarithromycin 250 mg and 88.9% for clarithromycin 500 mg(51), this is beneficial as the lower dose is also better tolerated and less costly

What are the reasons for the decreased efficacy of the standard triple therapy?

The low patient compliance, the high gastric acidity, the high bacterial load, the type of H. pylori strains and the most important is the increase in H pylori resistance to clarithromycin are the main reasons for the decreased efficacy of the standard therapy(42). In addition, in high BMI persons, especially the obese people, the distribution volume of the drugs being higher, which means that the concentration at the drug in the gastric mucosa will be lower and the risk of treatment failure is higher(52).

In case of 1st line therapy failure or in case of high resistance to clarithromycin (> 15-20%) the 2nd line therapy is recommended (42).

1.1.8.2.2.Second line therapy:

- (Bismuth 120mg qds + tetracycline 500mg qds+ metronidazole 400mg tds+ PPI OD) : (*Bismuth based therapy*) for 7days. (eradication rate 70-85%) (47)

OR

(PPI-levofloxacin 500mg OD- amoxicillin 1G BID) for 10-day is the other alternative second-line treatment based on the results obtained in recent years(53). (eradication rate 75-90%)(47).

- *In regions of low clarithromycin resistance:*

After the failure of the 1st line triple therapy, one of the two regimens, either bismuth based therapy or levofloxacin quadruple therapy can be used as a second line therapy (42).

- *In regions of high clarithromycin resistance:*

Bismuth-containing quadruple therapies are the first choice (the 1st line therapy). After failure of bismuth containing quadruple therapy, levofloxacin containing triple therapy is recommended as the 2nd line therapy(42).

- Also Bismuth-containing quadruple therapy could be used as alternative first choice therapy regardless resistance to clarithromycin (18) , (54).

- Rising rates of levofloxacin resistance should be taken into account before taking levofloxacin triple therapy, and it is recommended to test levofloxacin susceptibility before prescribing it (55). Also it is strongly advised not to use levofloxacin in a patient with chronic infections who may have received fluoroquinolones (42).

1.1.8.2.3.Third line therapy:

- No standard third-line therapy exists. After failure of second-line therapy, European guidelines recommend that treatment should be based on the antibiotic sensitivity (56),(42).

- Usually, after two treatment failures, it is recommendable to prescribe antibiotics not previously used , but whenever possible it is better to obtain gastric biopsy and perform susceptibility testing(57).

- Different combinations have been prescribed after the failure of the 2nd line therapy, examples of these combinations are: (3)

  - PPI + amoxicillin + rifabutin for 10 d.

  - PPI + furazolidone + levofloxacin for 7–10 d.

1.1.8.2.4. Alternative therapies:

1.1.8.2.1.1. Sequential therapy:

- It is considered as an alternative therapy for the standard 1st line therapy in case of clarithromycin resistance.
- It involves treatment with: PPI and amoxicillin for 5 d, followed by the PPI and a nitroimidazole antibacterial (metronidazole or tinidazole) and clarithromycin for a further 5 days, is more effective than a standard 7 or 10 d triple therapy regimen in treatment naïve-patients(58, 59).
- This therapy showed excellent eradication rates 90-94%(60).
- Sequential therapy is effective despite clarithromycin resistance, due to the cell wall disruption by amoxicillin preventing the development of clarithromycin efflux

channels& due to the larger number of antibiotics to which the microorganism is exposed (61).

1.1.8.2.1.2. Concomitant therapy:

- A novel alternative regimen for first line therapy in case of clarithromycin resistance(62).
- A 4-drug regimen contains a PPI, clarithromycin ,amoxicillin and metronidazole (63).
- Both the sequential therapy and the concomitant therapy showed that they were equivalent in eradication rates, but the concomitant therapy is less complex as it does not involve changing drugs halfway through(64).

1.1.8.2.1.3. Hybrid (dual therapy):

- A recent hybrid (dual-concomitant) therapy has been reported, consisting of a dual therapy; starting on a PPI (standard dose, *b.i.d.*) and amoxicillin (1 g, *b.i.d.*) for 7 d followed by a concomitant quadruple therapy with a PPI , amoxicillin , clarithromycin and metronidazole for 7 d (65).
- This therapy provided excellent eradication rates of 97% -99%. Also it is noticed that the new therapy has a high efficacy in the treatment of *H. pylori* strains harboring dual resistance to clarithromycin and metronidazole on the contrary to the sequential therapy (66).

1.1.8.3. **Future therapeutic strategies:**

1.1.8.3.1. Vaccination

The host response plays an important role during *H. pylori* colonization. Thus immunization against *H. pylori* may be considered as a strategy to potentiate the host immune response to be capable of attenuating or eliminating *H. pylori* and its associated gastric inflammatory sequelae(67).So vaccination is expected be very effective for elimination of H. pylori infection and would be cost-effective strategy(68).

H pylori is vaccine is feasible in animal models, but requires further research, and efforts to be applied against H pylori in humans (69).

1.1.8.3.2. Genome-based drug discovery:

The principle of genome based drug development is identifying the essential proteins which are specific to *H. pylori*, and then to isolate, identify and synthesize a small molecule chemical which inhibits the essential activity of such proteins(70).

1.1.8.3.3. Novel drug delivery approaches:

One of the reasons for the failure in eradication of *H .pylori* is the short residence time of the antimicrobial agents in stomach, so the effective antimicrobial concentration cannot be achieved in the mucus layer or epithelial cell surfaces where *H. pylori* colonizes(71), (72).

To overcome the problem, a new site specific drug delivery system (DDS) has been proposed based on increasing the residence time in stomach; this DDS is known as "gastroretentive drug delivery systems (GRDDS)"(73)

Table1.3: Summary of the recommended regimens for Helicobacter pylori therapy(63).

Treatment	Regimen
First-line therapy	
Standard triple therapy[1]	A PPI (standard dose, *b.i.d.*), clarithromycin (500 mg, *b.i.d.*) and amoxicillin (1 g, *b.i.d.*) for 7-14 d
Sequential therapy	A 5-d dual therapy with a PPI (standard dose, *b.i.d.*) and amoxicillin (1 g, *b.i.d.*) followed by a 5-d triple therapy with a PPI (standard dose, *b.i.d.*), clarithromycin (500 mg, *b.i.d.*) and metronidazole (500 mg, *b.i.d.*)
Concomitant therapy	A PPI (standard dose, *b.i.d.*), clarithromycin (500 mg, *b.i.d.*), amoxicillin (1 g, *b.i.d.*) and metronidazole (500 mg, *b.i.d.*) for 7-10 d
Hybrid therapy	A 7-d dual therapy with a PPI (standard dose, *b.i.d.*) and amoxicillin (1 g, *b.i.d.*) followed by a 7-d quadruple therapy with a PPI (standard dose, *b.i.d.*), amoxicillin (1 g, *b.i.d.*), Clarithromycin (500 mg, *b.i.d.*) and metronidazole (500 mg, *b.i.d.*)
Bismuth-containing quadruple therapy	A PPI (standard dose, *b.i.d.*), bismuth (standard dose, *q.i.d.*) tetracycline (500 mg, *q.i.d.*) and metronidazole (250 mg, *q.i.d.*) for 10-14 d
Second-line therapy	
Bismuth-containing quadruple therapy	A PPI (standard dose, *b.i.d.*), bismuth (standard dose, *q.i.d.*) tetracycline (500 mg, *q.i.d.*) and metronidazole (500 mg, *t.i.d.*) for 10-14 d
Levofloxacin-based triple therapy[2]	A PPI (standard dose, *b.i.d.*), levofloxacin (500 mg, *q.d.*) and amoxicillin (1 g, *b.i.d.*) for 10 d
Third-line therapy	
Culture-guided therapy	A 10-d quadruple therapy comprising a PPI (standard dose, *b.i.d.*), bismuth (standard dose, *q.i.d.*) and two antibiotics selected by antimicrobial sensitivity tests
Levofloxacin-based quadruple therapy	A PPI (standard dose, *b.i.d.*), bismuth (standard dose, *q.i.d.*), levofloxacin (500 mg, *q.d.*) and amoxicillin (500 mg, *q.i.d.*) for 10 d
Rifabutin-based triple therapy	A PPI (standard dose, *b.i.d.*), rifabutin (150 mg *b.i.d.*) and amoxicillin (1 g *b.i.d.*) for 14 d
Furazolidone-based quadruple therapy	A PPI (standard dose, *b.i.d.*), tripotassium dicitratobismuthate (240 mg, *b.i.d.*), furazolidone (200 mg, *b.i.d.*) and tetracycline (1 g, *b.i.d.*)

[1]Employed in areas with clarithromycin resistance < 10% and abandoned in areas with clarithromycin resistance ≥ 20%. [2]Employed in patients who fail to eradicate *Helicobacter pylori* with standard triple therapy, sequential therapy, concomitant therapy or hybrid therapy. PPI: Proton pump inhibitor.

1.1.9. Risk of Recurrent Helicobacter pylori Infection:

- The long-term effectiveness of H. pylori eradication programs will depend on recurrence risk after 12 months of eradication. Recurrence of H. pylori infection is the result of recrudescence (due to ineffective treatment regimen), reinfection, individual factors and community factors. Recrudescence is considered to be more likely responsible for most of cases than reinfection(14).
- Annual recurrence rates per patient-year of follow-up have been reported to vary across countries(74).The percentage of H pylori annual recurrence was 2.67% and 13.00% in developed and developing countries respectively, this confirming that low

socioeconomic development areas are more likely to have higher rates of H. pylori infection recurrence. (75), (76).

- Risk of Recurrent H. pylori Infection 1 Year after Initial Eradication Therapy in 7 Latin American Communities was studied in 2013; recurrence occurred in 11.5% of participants who had negative post treatment UBT results. Recurrence reasons were, non-adherence, demographics, and the most important was the non- specific antibiotic regimen(77).

1.2. Anatomy and physiology of the stomach:(78) ,(79),(80)

The stomach is an important part of the digestive system. It is a muscular, hollow, sac-like, dilated organ located in the left upper part of the abdominal cavity just below the diaphragm and liver. The volume of empty stomach is 20–25 ml, which can get expanded up to 1.5 L after meal consumption. The stomach locates between the esophagus and to the small intestine.

1.2.1. Parts of the stomach:

The stomach is divided into 5 regions, as shown in **Figure 1.3**:

1. The cardia: A small area in the upper portion of the stomach where the esophagus and stomach join (in gastroesophageal (GE) junction).This region includes the cardiac sphincter, which acts as a valve that prevents the stomach contents from going back up into the esophagus.
2. The fundus: Is the area below the cardia, but it balloons out above it and serves as a temporary storage area for food.
3. The body: Is the main part of the stomach where the food becomes mixed and broken down.

4. The antrum: Is the lower part of the stomach which holds the broken down food, until it is ready to be released into the small intestine.
5. The pylorus: Is the narrow, bottom part of the stomach near the small intestine it includes the pyloric sphincter that acts as a valve to control the emptying of stomach contents into the small intestine.

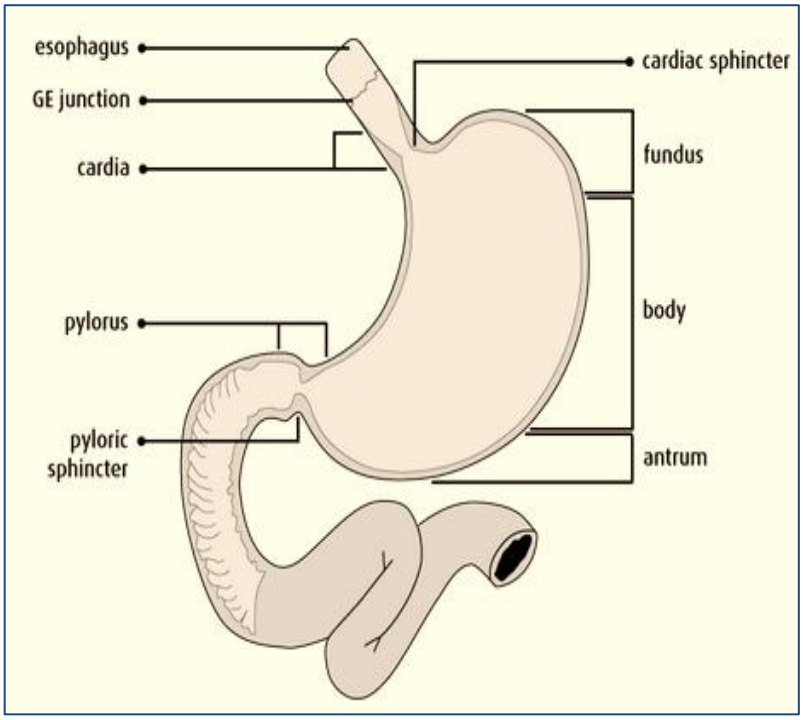


Figure 1.3: Stomach regions

1.2.2. Functions of stomach:

There are three major functions of stomach:

- Physical digestion (churning function).
- Chemical digestion.
- Limited absorption (some water, alcohol, certain drugs).

1.2.3. Anatomical structure of stomach:

*The stomach is made up of several layers of tissue that facilitate its functions(see **Figure 1.4**):*

1. The mucosa (mucous membrane) is the inner lining of the stomach. It has many glands that produce mucus , hydrochloric acid and .digestive enzymes

2. The submucosa is a layer of connective tissue that has large blood vessels, lymph glands, nerve cells and fibers, and glands that secrete digestive hormones**.**
3. The muscularis propria (or muscularis externa) is the next layer. It is the main muscle of the stomach, and is made up of 3 layers of muscle which help in breaking up the food by churning action resulting in milky white liquid chime.
4. The serosa is the fibrous membrane covering the outside of the stomach, consisting of layers of connective tissue continuous with the peritoneum.

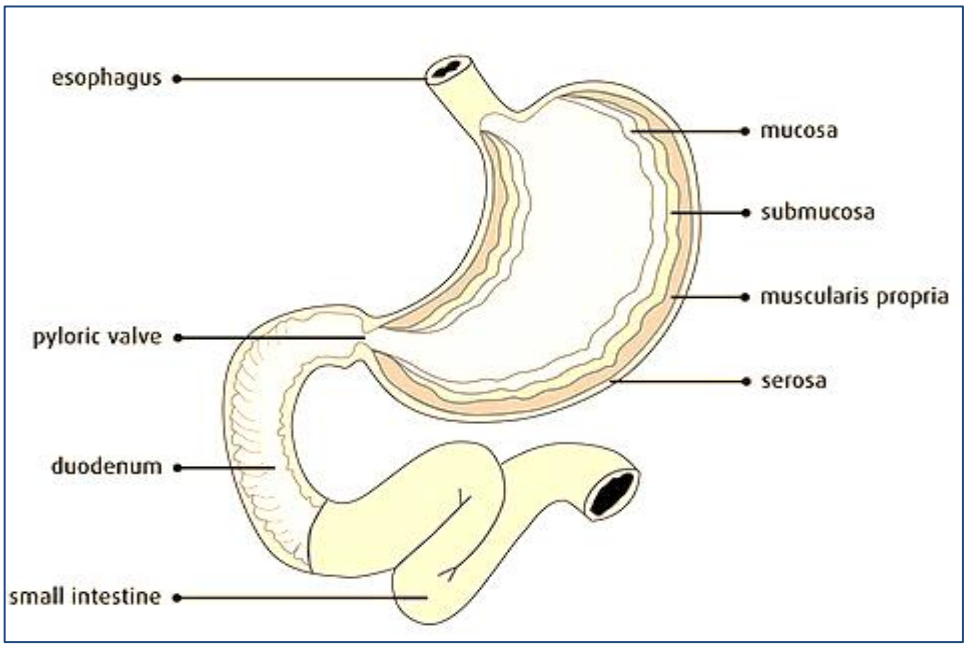


Figure 1.4: Layers of the stomach

1.2.4. Gastric motility and gastric emptying time:(81),(82),(83)

The emptying process of the stomach is caused by tonic contraction of the stomach and peristaltic waves moving over the gastric region. Two types of GI motility and secretion exist, including the fasted and the fed states. As a result, the bioavailability (BA) and the efficacy of orally administered drugs vary depending on the state of feeding. The gastric motility associated with various cyclic events, known as the migrating motor complex (MMC), which regulates its motility. The MMC is divided into four phases.

i. Phase I (basal phase) is a quiescent period with virtually no contractions, characterized by lack of secretory, electrical, and contractile activity, lasts for 30–60 min.

ii. Phase II (pre-burst phase) lasts for 20-40 min with intermittent, irregular low amplitude contractions. Bile secretion occurs during this phase, and mucus discharge occurs during the latter part of Phase II and throughout Phase III.

iii. Phase III (burst phase) consists of intense, large, and regular contractions known as (house-keeper waves) in which all the undigested material is swept out of the stomach to the small intestine. This phase lasts for 10-20 min.

iv. Phase IV is a short transition period between phases III and I of two consecutive cycles and lasts for 0–5 min with very little or no contractions.

This cycle of electrical events is repeated every 2-3 hours in the fasted state. In the fed state a continuous pattern of spike potentials and contractions called postprandial motility occur. The larger the ingested amount of food, the longer the period of feeding activity, with usual duration of 2–6 hours.

The performance of the peroral DDS generally and the GRDDS specifically is affected by the state and the phase during which it is administered. When the DDS is administered in the fasted state, the phase of the MMC can significantly influence the gastric residence time (GRT) and transit time in the gastro intestinal tract (GIT). This will have greater effect on the drugs with narrow absorption window in the upper GIT; the lesser time spent in that region, the lower degree of absorption. Therefore, the design of GRDDS should take into consideration the resistance of the dosage form to gastric emptying during Phase III of the MMC in the fasted state and to continuous gastric emptying through the pyloric sphincter in the fed state. Therefore, the GRDDS must be functional quickly and able to resist the aggressive gastric physiological events for enough time.

1.3. Gastroretentive Drug Delivery Systems (GRDDS):

1.3.1. Overview:

Oral route remains the most desired, convenient and preferred drug delivery system (DDS), due to its ease of administration, low cost, patient compliance and flexibility in formulation and manufacturing that gives it more attention in the pharmaceutical field(84). About 50% of the drug delivery systems available in the market are oral systems(85).

Optimal DDS ensures the presence of the drug in its active form at the site of action within its therapeutic range (concentration above the minimum effective concentration (MEC) and below the minimum toxic concentration (MTC))for the correct time an duration(86).

It is necessary to take the drug several times a day to maintain its concentration within the therapeutic range, which could result in significant fluctuation in plasma drug concentration(87).

This has led to the development of sustained releases (SR) dosage forms, where the dosage form is designed to control the drug release such that its plasma profile is maintained within the therapeutic range for prolonged time(88).

Sustained release dosage forms are DDS that provide the drug release over extent period of time after administration, thereby extending the dosing interval (decreasing the dosing frequency) and reducing fluctuations in the drug plasma concentration (**Figure 1.5**).

This can be achieved by using of suitable polymers which are used as reservoir systems (coating of granules or tablets) or matrix systems (in which drug is dispersed).(86)

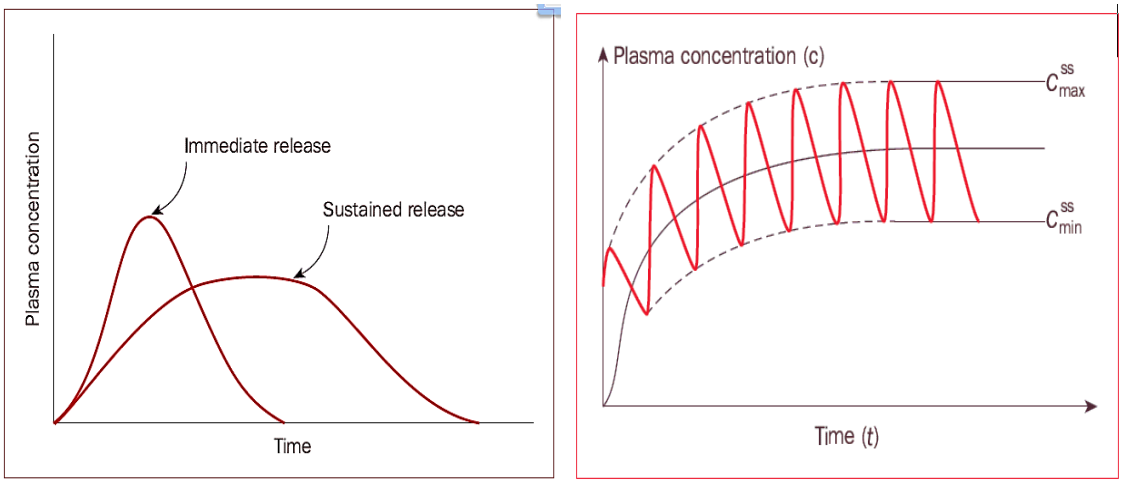


Figure 1.5: Drug plasma concentration and dosing intervals from sustained release and immediate release systems.(86)

Oral sustained release delivery system has been developed due to its therapeutic advantages, mainly for drugs that have short half-lives and are easily absorbed throughout the GIT (89). A common property of conventional sustained release dosage forms is that a large amount of the drug loaded in it is released in the colon where it stays for a relatively long time compared to other parts of the GIT. So while this DDS is suitable for many drugs, it is inappropriate for drugs that are poorly absorbed from the lower part of the GI tract, drugs with narrow absorption window or drugs having local effect in the upper GIT part(90).

This approach has many difficulties; The inability to restrain and locate within this desired region of the GIT due to variable gastric emptying and motility and the relatively short gastric emptying time (GET) result in incomplete absorption of the drug from the drug delivery system(89). Also the extent of drug absorption from duodenum and jejunum is limited despite their high absorption properties as the passage through them is rapid. After crossing this absorption window, the released drug goes to waste with low absorption. This phenomenon significantly decreases the time available for drug absorption and limits the success of the delivery system (90).

In order to increase the bioavailability and the efficacy of such drugs (drugs characterized by a narrow absorption window or local effects in the upper part of GIT, the residence time of the drug delivery system (DDS) in the upper GIT needs to be prolonged; this will offer better option for drug therapy. This can be achieved by the development of a unique oral sustained or controlled release dosage form, that can withstand the vigorous gastric motility and gastric emptying and exhibits extended release of the drug in the gastric environment, this system is called "gastroretentive drug delivery system".(91)

1.3.2. Definition of GRDDS:

Orally administered, site-specific, controlled release drug delivery systems that could potentially prolong the gastric residence time of the drug at the gastric region (stomach) and can withstand the barriers that affect their residence in the stomach.

Gastroretentive drug delivery systems control/sustain the release of a drug over a long period, thereby it could be supplied continuously to its target site in the stomach and the upper part of small intestine, this could potentially improve its bioavailability and its therapeutic effect; as a result the drug necessary dose can be reduced, and its side effects will be minimized.(92)

A gastroretentive delivery system must be stable in the acidic environment of the stomach for prolonged time and should have good absorption in the upper GIT (93).

1.3.3. Drug candidate for GRDDS:

Many processes occur in the GIT to the drug after its release from a dosage form, including: degradation (chemical, enzymatic or bacterial), absorption (passive and/or active), precipitation, efflux by P-glycoprotein pump, and metabolism by Cyp450 enzymes. As a result this will affect the pharmacokinetics (PK) and the pharmacodynamics (PD) of the drug depending on its delivery site(94).

Taking into considerations the above mentioned processes, the best PK and PD properties would be achieved for the following drug candidates when formulated as GRDDS:

- Drugs that have narrow absorption window in the upper part of GIT (mainly the duodenum).

  E.g. L-DOPA, Furosemide, Riboflavin, Para-aminobenzoic acid, Cyclosporine, Atenolol, Theophylline, Dilitazem, Risedronate.(95),(96).

- Drugs those are locally active in the stomach.

  E.g. Antacids and Misoprostol.(97)

- Drugs that are used for eradication of H. pylori in the stomach.

  E.g. Clarithromycin, Metronidazole, Amoxicillin(97).

- Drugs that are substrates for Pg-p (which have highest levels in the colon).

  E.g. Digoxin(98).

- Drugs those are unstable and undergo degradation at the intestinal or colonic environment (high pH).

  E.g. Captopril, Ranitidine-HCl, Metronidazole.(96),(99)

- Drugs with high solubility at low pH values.

  E.g. Chlordiazepoxide, Cinnarizine, Diazepam, Verapamil, Cefpodoxime-proxetil, Rosiglitazone maleate.(100)

- Drugs that are mainly absorbed from the stomach.

  E.g. amoxicillin(97), alendronate(98).

1.3.4. Advantages of GRDDS:

1. Appropriate dosage forms for the drugs that are primarily absorbed through the stomach and the duodenum, for drugs with local effect in the stomach, for gastric H. pylori medications, and for drugs which are unstable in intestinal pH .(101)

2. Enhance the absorption and the bioavailability of drugs which are absorbed or solubilized only in the upper part of GIT compared to the administration of non-gastroretentive drug delivery. i.e. the bioavailability of riboflavin CR-GRDF was significantly enhanced compared to the administration of non-GRDF CR formula.(102)
3. Reduce fluctuations of drug concentration by the continuous input of the drug from this sustained release dosage form. When fluctuations are minimized, the concentration dependent adverse effects associated with peak concentrations can be decreased. (103)
4. Improved selectivity in receptor activation as a result of minimizing fluctuations in drug concentration, this is especially for drugs that activate different types of receptors at different concentrations(103).
5. Enhance the pharmacological effects and improves the clinical outcomes of drugs that have non-concentration dependent pharmacodynamics, such as some antibiotics, for which their response is not associated with peak concentration, but rather with the duration of time over a critical therapeutic concentration.(91)
6. Improve the patient compliance by decreasing dosing frequency and thereby improves the therapy .(101)
7. Enhance the therapeutic effect of the drugs with short half-life(101).
8. Avoid gastric irritation because of the sustained and uniform release of the drug from this delivery system.(101)
9. Minimize the adverse activity at the colon by decreasing the amount of drug that reaches to it. Thus, undesirable side effects of the drug in colon would be reduced.(91)
10. Site-specific gastroretentive dosage forms provide sufficient local action at the target site, and minimizing the systemic exposure of drugs, thus will increase the local activity and reduce undesirable side effects. So they are useful in the treatment of

disorders related to stomach and small intestine (e.g. eradication of Helicobacter pylori) (95).

11. In case of abnormal intestinal movement and a short intestinal transit time such as in case of diarrhea, poor absorption is expected. In this case it may be advantageous to retain the drug in the stomach to get a relatively better response; based on type of drug (101).

1.3.5. Disadvantages of GRDDS:

1. They require continuous food intake for extension of gastric emptying time and high levels of fluid to achieve buoyancy in the stomach.(104)
2. GRDDS are affected by many factors such as motility and pH of the stomach, the age, gender and posture of the patient….etc (104).
3. They are not suitable for the following drug candidates:(105)
   - Drugs with stability problems in the stomach, e.g. Erythromycin.
   - Drugs which undergo extensive first pass metabolism.
   - Drugs with irritant effect to the stomach, e.g. NSAIDS.
   - Drugs experience high first pass metabolism, e.g. Nifedipine
   - Drugs that have good absorption throughout the different GIT regions.
   - Drugs with poor solubility at acidic pH of the gastric region, e.g. Phenytoin.
   - Drugs intended for selective release in the colon, e.g. 5- amino salicylic acid and corticosteroids.
4. Poor in-vitro and in-vivo correlation.(106)
5. Higher cost formulations (106).
6. Difficult Retrieval of drug in case of overdose or poisoning (106).
7. Large size of the tablets due to many excipients have to be used in their formulation.

1.3.6. Factors affecting gastric retention of dosage forms:

1. Age and gender :

   People over 70 years usually have a longer gastric residence time than younger peoples. The mean gastric residence time differs between males and females; in males it is (3.4±0.6 h) while in female counterparts (4.6±1.2 h) (107).

2. Fed or fast state and frequency of feeding:

   Under the fast state, the GI motility is characterized by MMC which act to remove the undigested food from the stomach. The retention time of the dosage form is very short if its administration coincides with that of the housekeeper waves of the MMC.

   GRT increases in the presence of food. Successive meals can increase the gastric retention time by 6–7 h. The presence of food in the stomach maintains the FDDS away from the gastro-duodenal junction, which prevents the early emptying during the digestive phases(108).

3. Nature of food:

   The nature of food and the caloric content affect the GRT of the dosage form. GRT increases by 4–10 h after high fats and proteins meal (109).

4. Subject posture (standing or supine):

   In the upright (standing) position, the floating systems floated to the top of the gastric contents and are protected from postprandial emptying, showing prolonged GRT than non-floating units. However, in supine position, the floating units are emptied faster than non-floating units of similar size(110).

5. Concomitant drug administration :

   Concomitant administration of anticholinergics like atropine and propentheline , opiates like codeine and prokinetic agents like metoclopramide, can prolong gastric retention time(111).

6. Density of the dosage form:

   Dosage forms having a density lower than that of gastric fluid experience floating behavior and hence good gastric retention time. A density of <1.0 gm/cm3 is required to exhibit floating property. However, sometimes the bulk density of a dry dosage form is not an appropriate parameter for describing the buoyancy due to the floating force kinetics of these dosage forms which can be estimated from the resultant-weight measuring versus time(112).

7. Size of the dosage form:

   The dosage forms which are less than 10mm in size can get emptied faster from the stomach than larger dosage form(113).

1.3.7. Approaches for GRDDS:

"The first pioneering GRDDS was suggested as far as back in 1957"(93). Over the last 2–3 decades, numerous gastroretentive dosage forms have been designed to prolong the gastric residence time (93), those included in **Figure 1.6**:

(1) High-density systems

(2) Swelling and expandable systems

(3) Mucoadhesive or bioadhesive systems

(4) Superporus hydrogels

(5) Magnetic systems

(6) Floating systems

(7) Raft forming systems

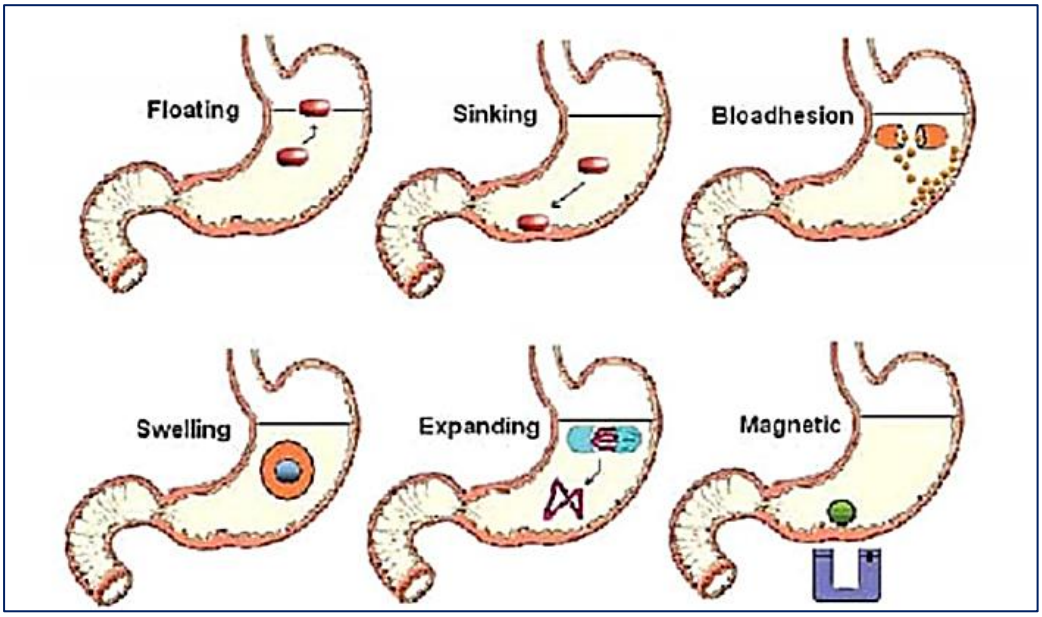


Figure 1.6: Different approaches of GRDDS.

1.3.7.1. **High-density systems:**

The density of gastric content is about (<1.004 g/cm^3), whereas the density of these systems is about (3 g/cm^3). So these systems retained in the bottom of the stomach due to their high density, they withstand the gastric motility and gastric emptying and hence increase their gastric residence time (113),(114).

The major drawback of these systems is the difficulty of their manufacturing with high drug capacity, because when the drug is released from the matrix, its weight decreases progressively and this will decrease its density and accordingly affect its residence time (115).

Some studies declared that high-density systems did not extend the gastric residence time significantly(116).Till date, these systems are not available in the market(93).

1.3.7.2. **Swelling and expandable systems:**

The size of these systems increases significantly and rapidly when they reach the stomach and come in contact with its contents and this will prevent premature emptying through the

pyloric sphincter. The initial size of these systems should enable their oral swallowing, and the size of these systems needs to decrease after completing the drug release to enable their evacuation from the stomach(117).

The stomach must be filled with fluids for these systems because their swelling and expansion is due to the fluid uptake. The expansion can be achieved by swelling or unfolding of the system in the stomach(118).

The expandable systems should not interfere with gastric motility or cause any local damage to the gastric mucosa. They should be biodegradable because their long retention time in the stomach may cause bowel obstruction (119).

The major drawbacks of these systems are the stability problems in their integrity and their mechanical shape during storage due to the presence of hydrolysable, biodegradable polymers. Also their manufacturing is costly and not easy(93).

1.3.7.3. **Mucoadhesive or bioadhesive systems:**

Another approach to increase the retention time of the dosage form in the stomach or upper small intestine is the adhesion of the dosage forms to the mucosal membrane of the stomach(120) . These formulations incorporate bioadhesive materials which enable the system to adhere to the gastric mucosal wall by forming strong non-covalent bonds with the mucin-epithelial cells surface of the GIT. The bioadhesion of polymers to the mucus membrane is achieved by the formation of electrostatic and hydrogen bonding at the mucus-polymer boundary(121).

The main drawback of these systems is their unpredictable adherence to the gastric mucosa that controls the gastric residence time of the dosage form, this is due to the continuous renewal of mucus on the walls of the stomach (122).Another drawback of the mucoadhesive delivery systems is their local side effects due to their direct contact with the gastric mucosa for long time(123).

1.3.7.4. **Super-porous hydrogels:**

Hydrogels are cross-linked network of hydrophilic polymers that are insoluble in water, but they have the ability to swell by absorbing water(124).

The conventional hydrogels are not suitable for GRDDS as their rate of swelling is very slow, and hence, premature evacuation of the dosage form through the pyloric sphincter can take place, while super-porous hydrogels (pore size >100 μm) swell very fast due to rapid water uptake, and hence, they can be used in the development of gastroretentive delivery systems (125).

1.3.7.5. **Magnetic systems:**

These systems contain small amount of iron powder within their matrix as an internal magnet, and an external magnet piece placed on the abdomen over the position of the stomach to control the gastrointestinal position and transit of these systems(126).

These systems have prolonged residence time in the stomach, and this was proved clinically in healthy volunteers by magnetic resonance imaging (127).

The major drawback of these systems is that their efficacy depends on the position of the external magnet, which should be fixed on the abdomen at one position accurately; over the stomach, during the treatment period and this will limit the free movement of the patient and lead to poor compliance(127).

1.3.7.6. **Floating systems:**

These systems have a density less than that of the stomach fluids, which cause their floating over its contents for prolonged time and increase their gastric residence time(128).

Floating of these systems can be achieved by different techniques (discussed in details under the section 'floating drug delivery systems':

1. Effervescent systems.
2. Non-effervescent systems.

3. Raft forming systems.

The major drawbacks of these systems are their need for high level of fluids and frequent feed, also their residence in the stomach is dependent on the floating lag-time, if it was too long the system can be eliminated rapidly from the gastric region(93).

1.3.7.7. **Dual working systems:**

These systems combine both mucoadhesion and floating technologies. So they have more potential to improve the in vivo performance of the gastroretentive dosage forms. The combination of mucoadhesion and floating technologies aims to increase the gastric residence time of the system by ameliorate the major drawbacks of both systems(129),(130).

In last few years, many studies for these systems on the animals have been reported. These studies proved that these systems have prolonged gastric residence time(131).

Table.1.4.. Marketed gastroretentive drug delivery systems available in the international market(93).

Manufacturer	Technology	Brand Name	Drug
Pierre Fabre drug, France	Floating liquid form	Almagate float coat®	Al-MG antacid
Ranbaxy, India	Colloidal gel forming floating system	Conviron®	Ferrous sulfate
Ranbaxy, India	Gas generating System	Cifran OD®	Ciprofloxacin
Pharmacia Ltd., UK	Bilayer floating capsule	Cytotec®	Misoprostol
GlaxoSmithKline	Osmotic system	Coreg CR®	Carvedilo[
Reckitt Benckiser Healthcare, UK	Effervescent floating liquid preparation	Liquid Gaviscon®	Aluminium hydroxide, Magnesium carbonate
Lupin, India	Bioadhesive tablets	Xifaxan®	Rifaximin
Hoffmann-La Roche, USA	Floating CR capsule	Madopar®	Levodopa Benserazide
DURECT Corporation, USA	OROS®	Covera HS®	Verapamil HCl
Ranbaxy, India	Effervescent floating system	Riomet OD®	Metformin hydrochloride
Pierre fabre drug, France	Floating liquid alginate	Topalkan®	Al-Mg antacid
Hoffmann-La Roche, USA	HBS Floating capsule	Valrelease®	Diazapam
Ranbaxy, India	Effervescent floating system	Zanocin OD®	Ofloxacin
Skyepharma	Geomatrix™ (expandable/swelling)	Xatral OD®	Alfuzosin HCl
Skyepharma, Shionogi Phasma Inc.	Geomatrix™	Paxil CR™	Paroxitine
Skyepharma, Shionogi Phasma Inc.	Geomatrix™	Requip®	Ropinirole
Skyepharma, Shionogi Phasma Inc.	Geomatrix™	Sular®	Nisoldipine
Skyepharma, Shionogi Phasma Inc.	Geomatrix™	Zyflo CR®	Zileuton

1.4. Floating Drug Delivery Systems (FDDS):

1.4.1. Background of FDDS:

In 1968 Davis firstly described the concept of floating drug delivery system to solve gagging or choking problems in some persons while swallowing medicinal tablets. He formulated pills having density less than water density (1gm/ml), so that the pills will float on the water surface and could be administered easily in the bowed position. Since then several approaches have been proposed for ideal floating delivery devices(132).

FDDS should have a bulk density less than gastric fluids to remain buoyant in the stomach for a prolonged period. But this bulk density of the dry dosage form is not the most appropriate parameter for determining of the floating capacity, because during the floating time of the system a minimal level of floating force (F) is required to keep the dosage form buoyant, this force could be estimated from the total vertical forces affecting the immersed system(112) (**Figure 1.7**). To measure the floating force (resultant weight), a novel

apparatus has been designed(133) .The principle of this apparatus based on measuring continuously the force equivalent to F (as a function of time) that is required to maintain the system floating. The object floats better if F is on the higher positive side, whereas it will sink if the value of F is in minus(112).

$$F = F_{buoyancy} - F_{gravity} \quad \text{.....(85)}$$

$$= Df \cdot g \cdot V - Ds \cdot g \cdot V$$

$$= (Df - Ds)\, g.V$$

Where, F = total vertical force, Df = fluid density, Ds = system density, V = volume

and g = acceleration due to gravity.

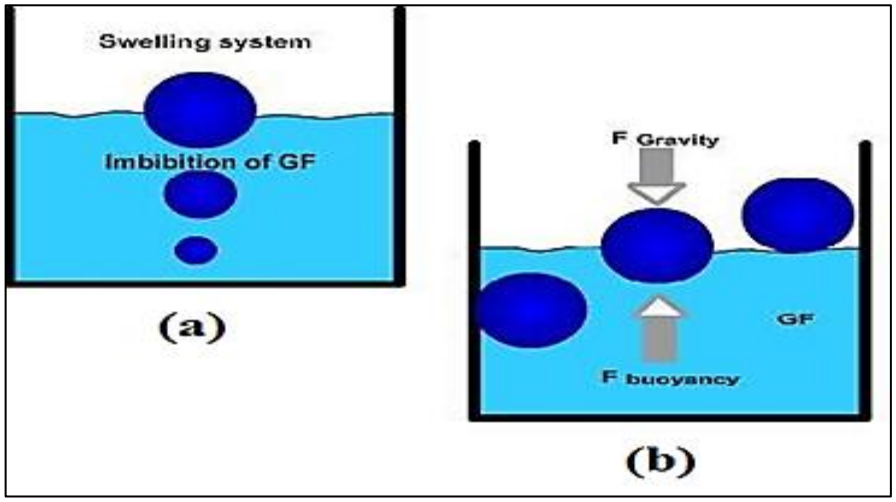


Figure 1.7 Measuring of the floating force.

1.4.2. Technologies of FGRDDS:

Different technologies have been utilized in the development of FDDS, based on the buoyancy mechanism:

1.4.2.1. Non-effervescent systems:

These systems are developed using a high level of one or more gel-forming, highly swellable polymers (i.e. hydroxypropylmethyl cellulose (HPMC), hydroxethyl cellulose

(HEC), hydroxypropyl cellulose (HPC), sodium carboxymethyl cellulose (Na-CMC), polyacrylate, polystyrene, agar, carrageenans and alginic acid) that swell to a great extent by hydration when come in contact with the gastric fluid; the entrapment of air within the swollen polymer framework maintains a bulk density for these systems of less than one, and provides buoyancy to the dosage form(134).

These systems can be divided into many subtypes:

1.4.2.1.1. Hydrodynamically balanced systems (HBS):

Sheth and Tossounian were the first designators of the 'hydrodynamically balanced systems(135).These systems are single-unit dosage forms. Their incorporation of swellable gelatinous polymer enables the gel barrier formation on the outer surface that maintains good system integrity and low apparent density less than the gastric fluid to remain buoyant on the stomach content for longer periods. The drug is released by diffusion and erosion from this gel barrier (**Figure 1.8**). Incorporating of fatty excipients gives low-density to the system and reducing its erosion (135), (136).

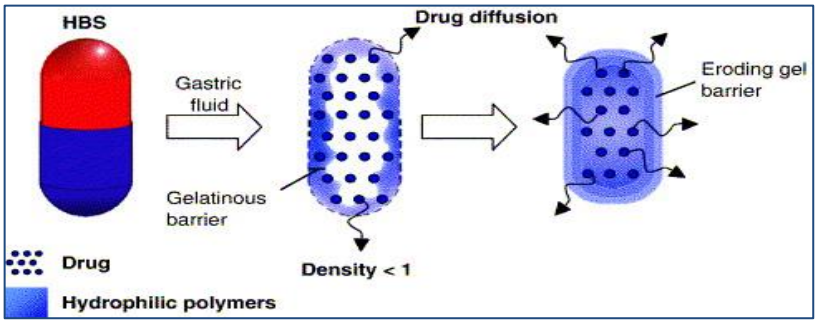


Figure1.8. Hydrodynamically balanced systems (HBS).

1.4.2.1.2. Microporous compartment systems:

These systems consist of floating chamber attached to microporous compartment which encapsulates a drug reservoir (**Figure 1.9**). The floating occurs due to the presence of the floatation chamber, which may be filled with a vacuum or filled with air or a harmless gas.

The microporous compartment contains pores along its top and bottom walls, these micropores enable the entry of the gastric fluid to the drug reservoir to dissolve the drug, while the peripheral walls of the drug reservoir compartment are completely sealed to prevent direct contact with the undissolved drug(137).

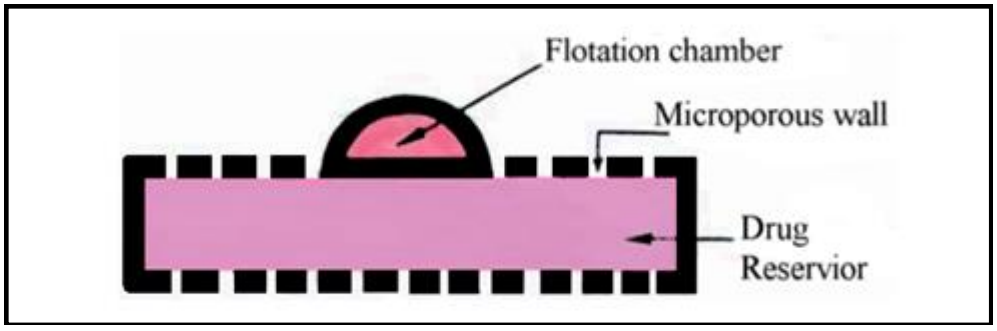


Figure1.9: Microporous compartment system

1.4.2.1.3. Alginate Beads:

Multi units floating dosage forms that are developed from freeze dried calcium alginate. They are spherical shape beads with diameter of about 2.5 mm.

These beads can be prepared by dropping sodium alginate solution into aqueous solution of calcium chloride causing precipitation of calcium alginate beads; these beads are then separated, frozen in liquid nitrogen, and finally freeze-dried at -40ºC for 24 hours, this process leads to the formation of porous beads, which have longer residence time (about5.5 h), compared to the solid beads which have shorter floating time (1 h)(138),(139).

1.4.2.1.4. Hollow microspheres/ Microballons:

These are multiple-units systems that load the drug in their outer polymer shelf.

They are prepared by emulsion solvent diffusion method to create a hollow in their inner core; this hole will prolong the GRT of the system. The most common used polymers in these systems are: polycarbonate, cellulose acetate, agar, Eudragit S, etc. The polymer is dispersed/ dissolved in the organic solvent and the drug is dissolved/ dispersed in the polymer solution. The resultant solution emulsified into an aqueous phase containing

polymers to form oil-in-water emulsion. Then the organic solvent is evaporated leading cavity formation, and thus, hollow microspheres are formulated (**Figure 1.10**). These microballoons can float over the surface of an acidic media for 12h(140).

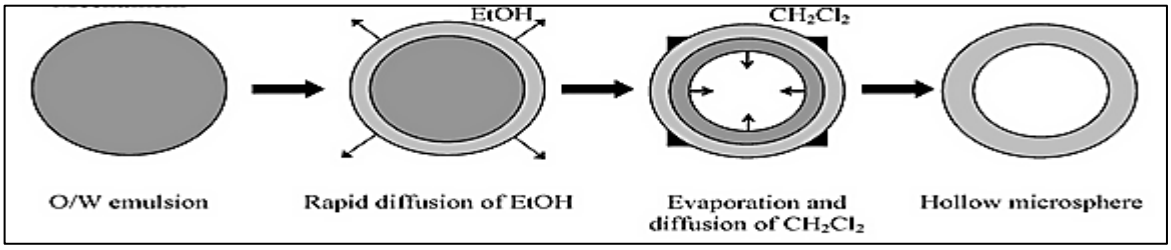


Figure 1.10: Example on hollow microsphere system.

1.4.2.2. **Effervescent systems:**

The matrix of these systems contains two main components; swellable polymers (i.e. methocel, polysaccharides (e.g., chitosan) etc.) and effervescent components (i.e. sodium bicarbonate, calcium carbonate, citric acid or tartaric acid, etc.), these systems generate carbon dioxide when they come in contact with stomach fluids, causing the system to float over the stomach contents (141).

Another technology is the incorporation of liquid which produce gas that evaporates at body temperature (142).

These systems further divided into two main types:

1.4.2.2.1. Volatile liquid containing systems:

These systems incorporate inflatable chamber which contains a liquid that gasifies at body temperature (e.g. ether, cyclopentane) and cause the inflation of the chamber in the stomach and floatation for the system. The system may also consist of bioerodible materials that dissolve gradually causing the inflatable chamber to collapse after a predetermined time to permit its evacuation from the stomach(143).

1.4.2.2.2. Gas-generating Systems:

These systems are composed of: gas (usually CO_2) generating components, the drug and the matrix that contains hydrophilic swellable polymers. The floating of these systems depends on the reaction between the gas generating components and the gastric acids or the acids incorporated within the system to liberate a gas in this system, which gets entrapped in the gelled hydrocolloid layer formed by the hydrophilic swellable polymer; thus decreasing its density lower than the density of the gastric content and enables its floatation over these contents for prolonged time that gives the desired sustained drug release from the system. These systems have to be biodegradable after completion of the drug release, and they could be:

- *Single layer tablet (Intragastric floating tablet).*

This system contains the drug, the CO_2 generating components and the hydrocolloidal polymer in one sustained release layer (**Figure 1.11**) (144).

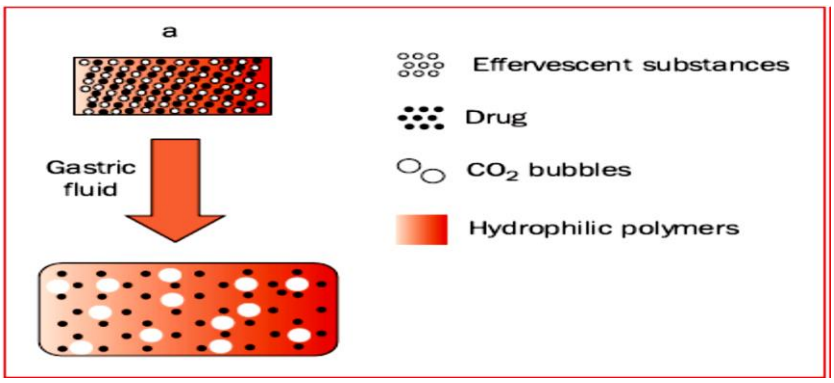


Figure1.11: Intragastric floating tablet

- *Bilayer tablet (Intra gastric floating bilayer tablet).*

This system consists of two layers; one layer for SR contains the CO2 generating agents with the drug and the hydrocolloid polymer, while the other layer is for immediate release (**Figure 1.12**) (145).

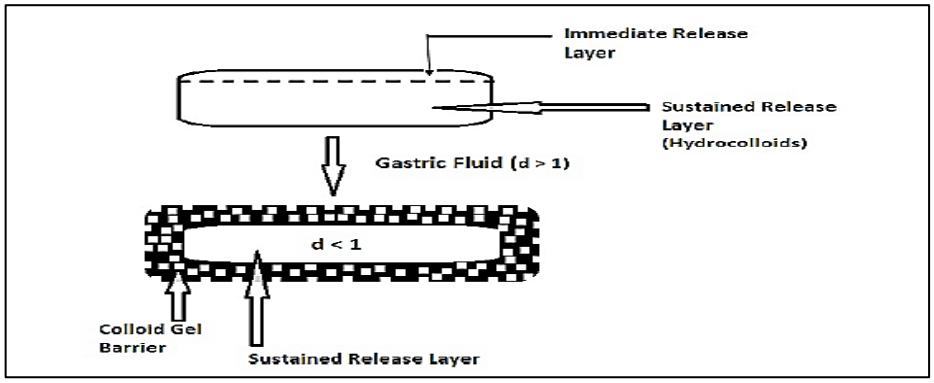


Figure 1.12: Intra gastric floating bilayer tablet.

- *Multiple-unit type of floating pills.*

This system consists of multiple units (pills) which all have tha ability to generate CO2 and float(146).

1.4.2.2.3. Raft systems.

These systems are gel forming solutions (e.g. sodium alginate solution containing carbonate) that swell and forms a viscous gel containing entrapped CO2 bubbles that are formed by contact with gastric fluid .This viscous, continuous, floating layer of cohesive gel above the gastric contents (called raft). The floated raft on the gastric fluids will not only increases the gastric residence time of the system, but also will act as a barrier between the stomach and the esophagus and prevents the reflux of the gastric contents into the esophagus(147, 148), so they can be also used for treatment of gastroesophageal reflux(149)

1.4.3. The major requirements for floating drug delivery system (150):

- It must be convenient for intake to enhance patient compliance.
- It should maintain an overall density lower than that of gastric contents (1.004 – 1.01 g/cm^3).
- It should form a swellable and cohesive gel barrier.

- It should act as reservoir, that dissolves slowly and release the drug in a controlled manner.

1.4.4. Formulation aspects of FDDS (104, 151-153):

Specific polymers and suitable excipients should be utilized in the formulation of floating drug delivery systems to give the major requirements of these systems, these are:

- *Hydrocolloidal polymers.*

These polymers have the ability to be hydrated when come in contact with water, forming swellable, cohesive gel barriers in the floating systems, to retard drug release, entrap gases within the systems to decrease their densities.

Examples: Acacia, Pectin, agar, alginates, gelatin, casein, veegum, methylcellulose, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), hydroxyethylcellulose (HEC) and carboxymethylcellulose sodium (CMC-Na).

- *Inert fatty materials.*

Nontoxic, edible inert fatty material that have a specific gravity less than one can be incorporated within these systems to decrease their hydrophilic property and their density, and hence increase their floatation.

Examples: Purified grades of beeswax, fatty acids, glycerides, and mineral oils.

- *Effervescent agents.*

These are the gas generating component (produce CO2 after their reaction), the entrapment of the gas bubbles within the system caused decrease in its density and so enable its buoyancy.

Examples: Calcium and Sodium bicarbonate, calcium carbonate, tartaric acid, citric acid, Di-Sodium Glycine Carbonate.

- *Release rate accelerants.*

These agents can increase the release rate of the drug from its delivery system.

They can be used up to 60% of the system weight.

Examples: lactose and mannitol.

- *Release rate retardants.*

These are insoluble substances that decrease the solubility of the system and hence retard the release of drug from it.

Examples: dicalcium phosphate, ethyl cellulose (EC), talc and magnesium Stearate.

- *Buoyancy increasing agents.*

These agents have bulk density less than one, so when added to the system they decrease its density and enhance its buoyancy. Can be used up to 80 % weight by weight.

Example: ethyl cellulose (EC).

- *Miscellaneous*.

Other pharmaceutical excipients could be used, like diluents, preservatives, emulsifiers, and lubricants can be incorporates in the system as needed. They shouldn't adversely affect the floatation of the systems.

1.4.5. Advantages of floating systems over other systems:

Floating systems are the most preferable GRDDS; this is due to the following specific advantages they have:

1. Have all the advantages of GRDDS (section 1.3.5.).
2. Have simple procedures and conventional equipment for manufacturing(154).
3. They are advantageous over other GRDDS as many drawbacks of other systems can be avoided with FDDS, example (155):

- Have higher drug loading capacity than high density systems.
- Avoid chocking problems caused by the increased size, and the economical manufacturing economical difficulties of the expandable systems, and have lower storage problems

- Don`t cause gastric mucosa is irritation as mucoadhesion systems.
- No compliance problems as magnetic systems

4. Can be used specifically and effectively for the treatment of upper gastrointestinal disorders such as gastro-esophageal reflux (156).

1.4.6. Disadvantages of floating systems:

1. The gastric retention time of these systems is influenced by many factors such as: floating lag-time, pH and gastric motility. These factors are not constant and hence the floating time cannot be predicted(157).
2. The ability of the system to remain in the stomach depends on the patient`s posture, which need to be positioned upright(153).
3. They need high level of fluids and continuous feeding to remain afloat over the stomach contents(158).

1.4.7. Different manufacturing processes of FDDS (153):

1.4.7.1. Direct compression:

This procedure involves the compressing of tablets directly from the mixture of the components in the powder form. The vehicles that form the matrix must have good flow and compressibility properties. Direct compression is the most preferable technology due to the minimal number of manufacturing steps it requires, thus offering advantage in terms of speedy production.

1.4.7.2. Wet granulation:

Wet granulation process involves wetting of the formula powders, granulating, drying and compression. Granules are formed by binding the powders together by addition of solution or suspension containing the binder or adding the binder to the dry powder mixture and the liquid then added by itself. The result mass should be moist rather than wet or pasty, and solvent that is used should have a limited quantity. Once the granulating liquid has been

added mixing continues until a uniform dispersion is attained and all the binder has been activated. Then the wet mass is granulated by sieving. The wet granules undergo drying, after completion of the drying; lubricant is blended (in case of tablets) with dried granules and finally compressed.

1.4.7.3. **Dry granulation:**

This process is used to form granules without using a liquid solution in case that the drug granulated may be sensitive to moisture and heat. This process requires compacting of the powders as one bulk under high pressure instead of wetting, then dry granulation with no need for drying.

1.4.7.4. **Melt granulation.**

This process involves the agglomeration of the formula powders within melt-able material when it is in the molten state. Then cooling of the resultant agglomerate takes place with granulation process. Both the agglomeration and granulation accomplished in high shear mixer equipment supplied with a jacketed bowl. The process is less time consuming and uses less energy.

1.4.7.5. **Spray drying:**

It involves dispersing of the formula components in a liquefied coating material, then spraying this mixture into a drying chamber and mixing it with a heated gas to rapidly evaporate the solvent from the coating material and cause solidification to the mixture.

Spray drying process has good control on the resultant powder properties such as density, particle size, flow properties and moisture content.

1.4.7.6. **Ionotropic gelation technique:**

Ionotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogel beads (gelispheres). Gelispheres are spherical crosslinked hydrophilic polymeric entity capable of extensive gelation and swelling in simulated

biological fluids and the release of drug through it controlled by polymer relaxation. The hydrogel beads are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations. The cations diffuses into the drug-loaded polymeric drops, forming a three dimensional lattice of ionically crosslinked moiety. Biomolecules can also be loaded into these gelispheres under mild conditions to retain their three dimensional structure(159).

1.5. Quality control of floating gastroretentive tablets:

In-vitro evaluation of physical and chemical properties is important in controlling the quality of any dosage form. The most important parameters for evaluation of floating gastroretentive tablets that has to be tested are described in details in methodology, section 5.2.

1.6. Kinetic mathematical models of drug dissolution/release:

1.6.1. Overview

Drug release/dissolution from a solid dosage forms is necessary to ensure that drug dissolution occurs in an appropriate manner and in vitro dissolution has been recognized as an important element in drug development. Recently both the industry and the health authorities focus on drug dissolution studies which could be used as a surrogate for the Bio- equivalence studies. The quantitative analysis of the values obtained in the dissolution/release tests is easier when using mathematical models that express the dissolution results as characteristic functions of some of the dosage forms. Hence, the use of mathematical models is useful in the prediction of release kinetics and enables the measurement of some important physical parameters, such as the type of drug diffusion and resorting to model fitting on experimental release data. The principle for evaluation of the kinetics of drug release was offered by Noyes and Whitney in 1897 (160). Nowadays

there are several kinetic models describing the drug release from solid dosage forms in which the dissolved amount of drug (Q) is a function of the test time (t) or Q = f(t)

The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the usage of generic equations that mathematically translates the dissolution curve in function of some parameters related with the pharmaceutical dosage forms(161).

The drug polymorphic form, crystalinity, particle size, solubility and its amount in the pharmaceutical dosage form can influence the kinetics of the release(162). A water-soluble drug incorporated in a matrix is mainly released by diffusion, while for a low water-soluble drug the self-erosion of the matrix will be the principal release mechanism. To compare dissolution profiles between two drug products model dependent (curve fitting), statistical analysis and model independent methods can be used(161).

Mathematical models have been used extensively for the parametric representation of drug release kinetics from formulations. Models that have been used include the zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell (161).

1.6.2. Objectives of kinetic of mathematical modeling (161), (160)

The major objectives of mathematical modeling are listed below:

1. Designing the new drug delivery system based on general release expression.
2. Prediction of the exact drug release rates and drug release behavior through the matrix, thus avoid excessive experimentation.
3. Optimization of the drug formulations.
4. Elucidation of the physical mechanism of drug transport by simply comparing the release data to mathematical models.
5. Compare the release profiles between two drugs.

1.6.3. Kinetic of mathematical models for slow release dosage forms.

1.6.3.1. Zero order model

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation:

$$\mathbf{Q_t = Q_0 + K_0\, t}$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution and K is the zero order release constant(163).

Application: This model can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms. (E.g. transdermal systems, matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.) (164).

1.6.3.2. First order model

This model was first proposed by Gibald & Feldman (1967) later by Wagner (1969). The pharmaceutical dosage forms containing water-soluble drugs in porous matrices follow first order release kinetics, and can be expressed by the equation:

$$\mathbf{Q_t = Q_0 e^{-kt}}$$

Where Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in the solution and k is the 1st order release constant. The above equation in decimal logarithm will take the form,

$$\mathbf{\ln Q_t = \ln Q_0 + kt}$$

This equation implies that a graphic of the decimal logarithm of the amount of drug versus time will be linear. The dosage forms that follow this dissolution profile release the drug in a way that is proportional to the amount remaining in the interior of the dosage form, in such a way that the amount of drug released by unit of time diminishes. Thus any system

obeying this model releases the drug in such a way that the remaining amount in the system governs the rate of release of drugs (161).

Application: This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices(165).

1.6.3.3. **Higuchi Model.**

In 1961 Higuchi introduced the most famous and often used mathematical equation to describe the release rate of drugs from matrix system initially; it was valid only for planar systems(166). It was later modified and extended to consider different Geometries and matrix characteristics including porous structure (167). Higuchi developed an equation for the release of a drug from an ointment base and later applied it to diffusion of solid drugs dispersed in homogeneous and granular matrix dosage system. In this model, it is assumed that solid drug dissolves from the surface layer of the device first; when this layer becomes exhausted of drug, the next layer begins to be depleted by dissolution through the matrix to the external solution. In this way the interface between the regions containing dissolved drug and that containing dispersed drug moves into the interior as a front(161). In a general way it is possible to resume the Higuchi model to the following expression (generally known as the simplified Higuchi model):

$$Q_t = K_H t^{0.5}$$

Where, K_H is the Higuchi dissolution constant. Higuchi describes drug release as a diffusion process based on the Fick's law, square root time dependent.

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs(166).

1.6.3.4. **Korsmeyer- Peppas Model (The Power Law)**

Power law equation is more comprehensive very simple and semi-empirical equation developed by Korsmeyer- Peppas which can be used to analyze data of drug release from polymers. The equation implies that; the fractional release of drug is exponentially related to release time(162).

$$\frac{M_t}{M_\infty} = kt^n$$

Where, M_t & $M\infty$ are the absolute cumulative amounts of drug released at time t and infinity respectively, k is a constant incorporating structural and geometrical characteristics of the device, the k value is experimentally determined, and n is the exponent, indicative of the mechanism of drug release. The numerical value of the release exponent, n, is characteristic of the mechanism of diffusion release from delivery system. Peppas used the n value to characterize different release mechanisms from polymeric systems, and the data are summarized in Table (1.5).

A value of n = 1,however, means that the drug release is independent of time, regardless of the geometry. Thus, zero-order release can exist for any geometry; only for slabs does this release coincidered with Case-II transport.

Table 1.5: Exponent (n) of the power law and drug release mechanism from polymeric controlled delivery systems of cylindrical and spherical geometry(168).

Exponent, n			
Thin Film	Cylinder	Sphere	Drug Release Mechanism
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	Case II transport

When the release mechanism is not well known or when more than one type of release phenomena could be involved, this model can be used to analyze the release of poly-metric dosage form. This equation was later modified to accommodate the lag time (L) in the beginning of the drug release from the pharmaceutical dosage form(169):

$$\frac{M_{t-l}}{M_{\infty}} = a\,(t-l)^{n.}$$

And when there is possibility of burst effect(170) (b),

$$\frac{M_{l}}{M_{\infty}} = at^{n.} + b$$

Whenever there is absence of lag time and burst effect L and b value would be zero and only Kt^n is used. This mathematical model has been frequently used to describe the drug release from different modified release dosage forms(161).

1.6.3.5. **Hixson-Crowell model**

Hixson and Crowell (1931) recognized that the release of drug from particles with regular area is proportional to the cube root of its volume. They derived the equation:

$$W_0^{1/3} - W_t^{1/3} = \kappa t$$

Where W_0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the remaining amount of drug in the pharmaceutical dosage form at time t and κ (kappa) is a constant incorporating the surface-volume relation. The equation describes the release from systems where there is a change in surface area and diameter of particles or tablets(171).

Application: This model applies to pharmaceutical dosage form such as tablets, where the dissolution occurs in planes that are parallel to the drug surface if the tablet dimensions

diminish proportionally, in such a manner that the initial geometrical form keeps constant all the time(172).

1.6.4. Selection of the Best Model

The selection of the appropriate model in the drug release studies is critical to ensure the effectiveness of the study. There are various criteria for the selection of the mathematical models which are based on the statistical treatments. The most widely used method employs the coefficient of determination, R^2, to assess the fit of the model equation. This method can be used when the parameters of the model equations are similar. But when the parameters of the comparing equations increased; a modification is incorporated in this technique where an adjusted coefficient of determination (R^2 adjusted) given by:

$$R^2_{adjusted} = 1 - \frac{n-1}{n-p}(1-R^2)$$

Where (n) is the number of dissolution data points and (p) is the number of parameters in the model. Hence, the best model is the one with the highest adjusted coefficient of determination. A value for R^2 adjusted > 0.950 is considered acceptable for the purposes of comparison of modeling dissolution profiles generated(161).

Similarly other statistical tools like correlation coefficient (R), Analysis of Variance (ANOVA) and Multivariate analysis of variance (MANOVA) are used for the comparison and selection of the models(161).

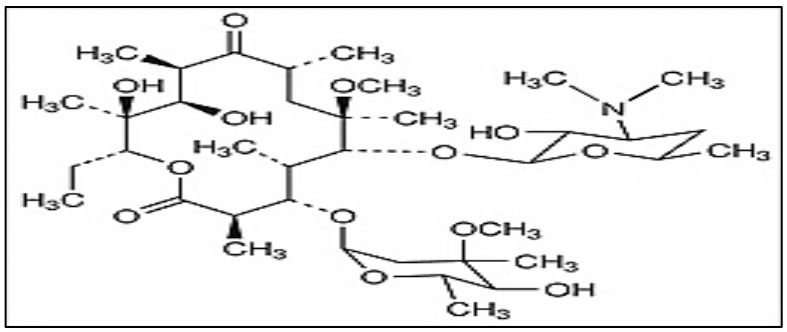
1.7. Background Information for the drugs of choice:

1.7.1. Clarithromycin (CLA):

1.7.1.1. **Description (173):**

- Molecular formula: $C_{38}H_{69}NO_{13}$
- Molecular weight: 747.95

- CAS: 81103-11-9
- Chemical structure:



- **IUPAC Name:** (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-{(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl}oxy-14-ethyl-12,13-dihydroxy-4-{(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl}oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione.

- **Content:** Clarithromycin contains NLT 96.0% and NMT 102.0% of clarithromycin (C38H69NO13), calculated on the anhydrous basis.

1.7.1.2. **General properties:**

- Appearance: White to off-white, crystalline powder(173).
- Solubility: Soluble in acetone; slightly soluble in dehydrated alcohol, in methanol, and in acetonitrile, and in phosphate buffer at pH values of 2 to 5; practically insoluble in water (0.33 mg/L) (173).
- log P: 3.16 (14)
- pKa: 8.99 at 25 ^{o}C (14)
- Stability :Clarithromycin is stable in aqueous solutions of pH (5.0-8.0) (174).

1.7.1.3. **Pharmacodynamics (175),(176),(177).**

- *Pharmacological-therapeutical group*: ATC code J01F A09

Clarithromycin is a macrolide antibiotic, semi-synthetic derivative of erythromycin.

- *Mechanism of action*:

  Clarithromycin binds to the 50s ribosomal sub-unit of susceptible bacteria and suppresses protein synthesis. It is highly potent against a wide variety of aerobic and anaerobic gram-positive and gram-negative organisms. The 14-hydroxy metabolite of clarithromycin also has antimicrobial activity, its MICs equal or two-fold higher than the MICs of the parent compound.

1.7.1.4. **Pharmacokinetic (175),(176),(177):**

- Clarithromycin is rapidly absorbed from the gastrointestinal tract, mainly in the jejunum.
- The bioavailability of the parent drug is about 55%.
- Peak plasma concentration occurs 2 to 3 hours after an oral dose.
- The extent of absorption is relatively unaffected by the presence of food.
- Clarithromycin distribution levels in the tissues are several times higher than the circulating drug levels since it has good penetration into different compartments. Clarithromycin is 80 % bound to plasma proteins at therapeutic levels.
- Clarithromycin is rapidly and extensively metabolized in the liver.
- Elimination half-life is 3 to 4 hours for 250mg and 5-6 hours for 500mg dose.
- Clarithromycin PK is non-linear.

1.7.1.5. ***Clinical uses and indications*,(175),(176),(177).**

- Acute bacterial exacerbation of chronic bronchitis.
- Mild to moderate community acquired pneumonia.
- Acute bacterial sinusitis.
- Bacterial pharyngitis.
- Skin infections and soft tissue infections of mild to moderate severity.

- Clarithromycin used in appropriate combination with antibacterial therapeutic regimens and an appropriate ulcer healing agent for the eradication of Helicobacter pylori in patients with Helicobacter pylori associated ulcers.

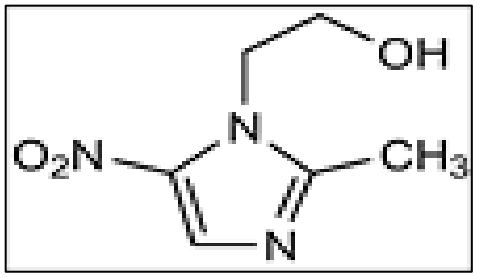
1.7.1.6. **Special considerations**

- Clarithromycin can penetrate the gastric mucus(175).
- In gastric juice samples of pH 2.0, clarithromycin degradation half-live is1.0 +/- 0.04 h. The co-administration of omeprazole with clarithromycin is likely to increase its chemical stability in gastric juice (174).
- The eradication of H. pylori needs a high concentration of clarithromycin in the stomach to ensure effective localized treatment for the pathogen (100)
- Clarithromycin was the most commonly used single potent antibiotic in anti-helicobacter treatment and penetration through gastric mucus at a 9-folds higher than amoxicillin and 48-folds higher rate than tetracycline(178).

1.7.2. Metronidazole (MTZ):

1.7.2.1. ***Description* (173):**

- Molecular formula: $C_6H_9N_3O_3$
- Molecular weight: 171.15
- CAS : 443-48-1
- Chemical structure:



- **IUPAC Name:** 1*H* -Imidazole-1-ethanol, 2-methyl-5-nitro-;

  2-Methyl-5-nitroimidazole-1-ethanol

  2-(2-methyl-5-nitroimidazol-1-yl) ethanol

- **Content:** Metronidazole contains NLT 99.0% and NMT 101.0% of metronidazole (C6H9N3O3), calculated on the dried basis.

1.7.2.2. **General properties:**

- Appearance: White to pale yellow, odorless crystals or crystalline powder (173).
- Solubility: Soluble in dilute hydrochloric acid (1 in 2); sparingly soluble in water and in alcohol; slightly soluble in ether and in chloroform. (173).

  Solubility g/100 ml at 20 °C: 1.0 in water, 0.5 in ethanol, less than 0.05 in ether, chloroform; soluble in dilute acids; sparingly soluble in dimethylformamide (14).
- pH : of saturated aqueous solution is 5.8 (14).
- PKa: 2.38 (14).
- log P : - 0.02 (14).
- Stability: Is stable in air, but darkens on exposure to light(173).

  It is stable in aqueous solutions of pH 2.0-7.0

1.7.2.3. ***Pharmacodynamics*** **(175),(176),(177).**

- *Pharmacological-therapeutical group*: J01XD01.

  Metronidazole is a 5-nitroimidazole derivative with has activity against both anaerobic bacteria and protozoa
- *Mechanism of action*:

  Its mechanism of action is thought to involve interference with DNA by a metabolite in which the nitro group of metronidazole has been reduced.

1.7.2.4. ***Pharmacokinetic* (175),(176),(177).**

- Metronidazole is readily and almost completely absorbed (>80%) after oral doses.
- Peak plasma concentration occurs 1 to 2 hours after an oral dose.
- The absorption is relatively unaffected by the presence of food.
- Less than 20% of the circulating metronidazole is bound to plasma proteins. Metronidazole appears in cerebrospinal fluid, saliva, and human milk in concentrations similar to those found in plasma.
- The major route of elimination of metronidazole and its metabolites is via the urine (60 to 80% of the dose).
- Elimination half-life is 8.5 ± 2.9 hours.

1.7.2.5. ***Clinical uses and indications*,(175),(176),(177).**

- The prevention and treatment of post-operative infections due to anaerobic bacteria, (i.e. septicaemia, bacteraemia, peritonitis, etc.).
- Urogenital trichomoniasis in the female (trichomonal vaginitis) and in the male.
- All forms of amoebiasis.
- Giardiasis.
- Acute ulcerative gingivitis.
- Acute dental infections (e.g. acute pericoronitis and acute apical infections).
- Eradicate of Helicobacter pylori in peptic ulcer disease (with other antimicrobials, and either bismuth compounds or proton pump inhibitors).

1.7.2.6. ***Special considerations***.

Metronidazole is bactericidal at low concentrations (0.78–6.25 µg/ml) for most anaerobes(179).

1.7.3. Esomeprazole (EZO):

1.7.3.1. **Description (173):**

- Molecular formula: $C_{34}H_{36}MgN_6O_6S_2$ (anhydrous).

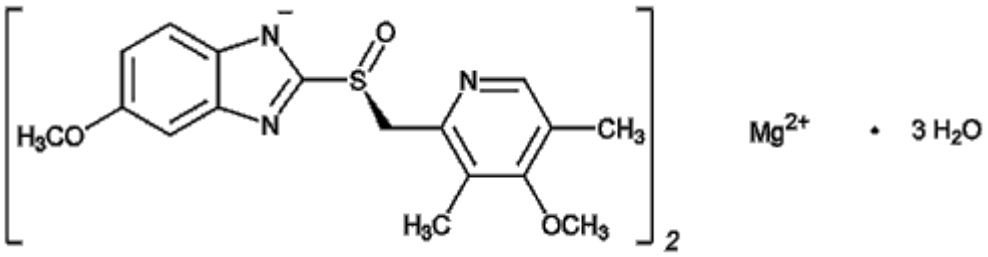
  $C_{34}H_{36}MgN_6O_6S_2 \cdot 3H_2O$ (trihydrate).

- Molecular weight: 713.12 g/mole (anhydrous)

  767.17 g/mole (trihydrate)

- CAS : 217087-09-7

- Chemical structure:



- **IUPAC Name:** 1*H* -Benzimidazole,5-methoxy-2-{(*S*)-{(4-methoxy-3,5-dimethyl-2-pyridinyl)methy}lsulfinyl}, magnesium salt (2:1), trihydrate; 5-Methoxy-2-{(*S*)-{(4-methoxy-3,5-dimethyl-2-pyridyl)methyl}sulfinyl}benzimidazole, magnesium salt (2:1), trihydrate .
- **Content:** Esomeprazole Magnesium contains NLT 98.0% and NMT 102.0% of $C_{34}H_{36}MgN_6O_6S_2$, calculated on the anhydrous basis

1.7.3.2. **General properties:**

- Appearance: White to slightly colored powder. (173).
- Solubility: Soluble in methanol; very slightly soluble in water; practically insoluble in heptane.(173).
- It is a weak base.

- log P: 0.6 (14).
- Stability: It is light sensitive, should be protected from light(173).Also it is acid labile(175).

1.7.3.3. ***Pharmacodynamics*** **(175),(176),(177).**

- *Pharmacological-therapeutical group*: (ATC code: A02B C05)

  It is a proton pump inhibitors (S-isomer of omeprazole).
- *Mechanism of action*: Esomeprazole specifically inhibits the proton pump (H+ /K+ - ATPase) in the gastric parietal cells, and thus inhibits acid secretion and decreases both basal and stimulated acid secretion.

1.7.3.4. ***Pharmacokinetic*** **(175),(176),(177):**

- Esomeprazole is rapidly absorbed after oral doses.

(Food delays and decreases its absorption, but this does not significantly change its therapeutic effect).

- The peak plasma levels occur after 1 to 2 hours.
- The absolute bioavailability is 64% after a single dose of 40 mg and 50% for 20 mg and it increases with repeated doses.
- With repeated dosage, there is a decrease in first-pass metabolism and systemic clearance.
- Esomeprazole is 97 % plasma protein bound.
- Esomeprazole is completely metabolized in the liver (mainly by CYP2C19).
- Elimination half-life is about 1.3 hours.
- The mean steady state AUC and C_{max} of both esomeprazole & clarithromycin increase in case of co-administration.
- The PK of esomeprazole is dose-dependent (non-linear).

1.7.3.5. **Clinical uses and indications,(175),(176),(177).**

- Gastroesophageal Reflux Disease (GORD)
- In combination with appropriate antibacterial therapeutic regimens for the eradication of Helicobacter pylori and healing of Helicobacter pylori associated duodenal ulcer and prevention of relapse of peptic ulcers in patients with Helicobacter pylori associated ulcers.
- Patients requiring continued NSAID therapy (for prevention of gastric and duodenal ulcers associated with NSAID therapy, in patients at risk).
- Treatment of Zollinger Ellison Syndrome.

1.7.3.6. **Special considerations**

- The chemical stability of the clarithromycin and amoxicillin has been approved to be increased when co-administered with PPI, thus their degradation half-lives will increased and will maintain them within the effective antibacterial concentration in the stomach(180), this could be justified by the median gastric pH which is maintained >4 (4±0.8) for 14 h after treatment with 40mg daily of EZO(181).
- After oral dosing with esomeprazole 20 mg and 40 mg the onset of effect occurs within one hour(175).
- Esomeprazole is the (S)-isomer of the proton pump inhibitor (PPI) omeprazole. esomeprazole and omeprazole have the same mechanism of action and are subjected to the same metabolic transformations with less first pass metabolism for esomeprazole resulting in a higher area under the concentration-time curve (AUC) after administration of the same dose(182). Esomeprazole has been clinically studied versus omeprazole for a variety of acid-related conditions, showing that the compound is as well tolerated and more effective with regard to healing and symptom relief than the recommended treatment with omeprazole(183). Esomeprazole provided better control

of intragastric pH than omeprazole, lansoprazole and pantoprazole in trials conducted in patients with gastro-oesophageal reflux disease (GORD) or healthy volunteers(184). these reasons let us chose esomeprazole instead of omeprazole in our research as a component of the 1st line therapy for the treatment of H.pylori induced ulcers.

Part two

2. Literature Review

After more than30 years of discovering H. pylori, clinicians and researchers are still exploring the ideal treatment with the ideal dosage form for eradication of this pathogen. Recent researches have focused on the gastroretentive drug delivery systems as novel approaches to increase the eradication of H. pylori which colonize the stomach, in order to release drugs as long as possible in the ecological niche of the bacterium and hence increasing eradication (100).

Recently, GRDDS for treating H. pylori infection and H. pylori associated peptic ulcer have shown special importance and interest, as the prolongation of the local availability of the antibacterial agents within the stomach has been reported to be an important factor in increasing the eradication of H. pylori(185). Here we will focus on the works done on the first line therapy drugs:

- 1994-*Dettmar and Lloyd-Jones(186)*

A patent raft-forming formulation using triclosan was developed. The drug was mixed with alginic acid, sodium bicarbonate, calcium carbonate and mannitol. The mixture was granulated, citric acid added, and then packed into sachets or compressed to tablets. In contact with the stomach acidic media, CO_2 bubbles were produced, within the raft structure formed by the alginates, causing it to float.

- **1998**- *Libo Yanga, Jamshid Eshraghib, Reza Fassihia* (187).

  Developed an intragastric delivery system based on a swellable asymmetric triple layer tablet, incorporating the triple drug therapy (tetracycline, metronidazole and bismuth salt). The first layer contained the gas generating material, the second layer contained both antibiotics in a SR-matrix (main polymers were Hydroxypropylmethylcellulose-K4 and polyethylene oxide) and the third layer was rapid dissolving contained the bismuth salt. This system was manufactured by "*direct compression technique*".

The in-vitro results showed that, the lag floating time was in the range of 17–28 min, the rapidly dissolving layer disappeared after 30 min during dissolution, and both drugs were able to be delivered at constant rates up to 90% of the total loading dose within 8 h.

- Main drawback of this system is the long floating lag time.

- **2007**-*Rania A.H. Ishak, Gehanne A.S. Awad, Nahed D. Mortada, Samia A.K. Nour*(185).

Developed metronidazole-loaded alginate beads, chitosan- alginate based beads (with different polymers, methyl cellulose, carbopol 934P and κ-carrageenan incorporated) using "the *ionotropic gelation technique"* in the manufacturing process.

The in vitro studied showed that the beads have immediate floating, good entrapment efficiency (about 89%) and acceptable release and good SR of the drug (100% drug release after 4 h).

The in vivo H. pylori eradication tests showed that MTZ floating beads with a dose of 15 mg/kg provided 100% eradication rate whereas the MTZ oral suspension with a dose of 20 mg/kg gave only 33.33% eradication rate.

- Main drawback of this system is the rapid release time.

- **2008**-*Muralidhar Nama, Chandra Sekhar Rao Gonugunta, and Prabhakar Reddy Veerareddy(188)*

A hydrodynamically balanced system of Clarithromycin (CLA) was developed, using "*Wet granulation technique*". Different polymers were used in the SR matrix (Carbopol 934P, HPMC K4M, Xanthan gum, HPC-LF and sodium alginate), but desired properties achieved for with the formula having 12% HPMC K4M polymer and 8% sodium bicarbonate. The floating lag time was less than 3 min with a floating time

of 12 h, and an in vitro release profile very near to the desired release (100% within 12h)..

- **2011**- *Juárez-Soberanez ,Villafuerte-Robles,(189)*

  Metronidazole-Gelucire 39/01 (wax matrix) granules and tablets were prepared by "*melt granulation technique*", alone and after addition of HPMC-K15M or sodium cross-linked carboxymethylcellulose (Carmacel). Addition of Carmacel and HPMC-K4 enhances the drug release but Carmacel formulations have no floating. The single-unit systems (tablets) gave more gradual drug release than granules (both give 100% within 3h), and the total floating time was 6h for the tablets while was 3h only for the granules. Gelucire 39/01, can be used as a matrix for floating drug delivery systems only when mixed with dissolution enhancers to increase the permeability of the impermeable wax matrix.

- **2013**- *ANILKUMAR (Ph. D Thesis)* (190).

He developed floating Chitosan *microparticles* containing Clarithromycin by a "*capillary extrusion procedure" and* Eudragit coated Pantoprazole alginate beads by "*ionotropic gelation method".* Other polymers used (Pectin (low methoxy).

The in vitro studies conducted on the clarithromycin loaded chitosan formulations proved that it has a potential as a floating delivery system. The best floating of clarithromycin formulation in-vitro was that 55.6% of the beads floated for 8 hours. This has been proved *in situ* where 45.40 % of the floating beads retained in the stomach at the end of 4 h.

The in vivo and in situ animal studies have shown an enhanced gastric mucosal and plasma concentration of clarithromycin in the presence of enteric coated pantoprazole formulations.

- **2014**-*Porntip Pan-Ina, Wijit Banlunarab, Nuntaree Chaichanawongsarojc, Supason Wanichwecharungruang(191).*

Clarithromycin-loaded EC nanoparticles were developed using "*anti-solvent particle induction method"* that gave submicron-sized semi-spherical particles (223 ± 50 nm) with EE (entrapment efficiency) of 86 ± 0.5%and the clarithromycin loading of the particles was 22.3 ± 0.17% weight by weight. In vivo studies showed that the efficiency of these particles on H. pylori clearance in C57BL/6 mice infected with these bacteria was significantly improved although their MIC was greater than the free CLA.

- **2015-** *Nancy Abou Youssef , Abeer Kassem , Magda EL-Massik a, Nabila Boraie*(192)

Gastroretentive floating raft system of metronidazole was developed, using ion-sensitive, in-situ gel forming polymers and gas generating agents (i.e. sodium alginate, gellan gum, sodium citrate and calcium carbonate, glyceryl mono stearate). The final system is a liquid dispersion with in situ gelling and floating properties.

The best formula exhibited short gelation lag time (3 s), long duration (>24 h), floating lag time 1 min and duration >24 h, and sustained drug release with mean dissolution time of ˜6 h.

- 2015-*Alessandra Rossi, Chiara Conti, Gaia Colombo, Luca Castrati, Carmelo Scarpignato, Pedro Barata, G. Sandri, Carla Caramella, Bettini, Francesca Buttini & Paolo Colombo*(193).

Floating *modular drug delivery systems* of two antibiotics together (Amoxicillin and Clarithromycin) has been developed. The final dosage form consisted of three modules of clarithromycin and two of amoxicillin (each has100mg of the antibiotic). Clarithromycin modules were prepared by *wet granulation* technique with HPMC-K15 as a matrix, while

Amoxicillin modules were prepared by *direct compression* technique. The modules were compressed in different geometrical shapes and then manually attached to each other to form one system. The assembled system floated immediately in vitro for more than 5 h. The floating mechanism is determined by *the internal void of the assembly system.*

The drugs release profiles from individual modules and assembled systems exhibited *quasi-linear* release rate (erosive release mechanism) during buoyancy and about 80% of drugs were released within 4-6h. The predicted simulated drug Intra-gastric concentrations over time were higher than the MIC values of both antibiotics. An experiment in dogs showed prolonged plasma concentrations of clarithromycin and amoxicillin.

- Main drawback: The high doses of antibiotics required in therapy, restricted the formulation design, due to its limited drug loading capacity.

Part three

3. Problem statement

- The objective an optimal *H. pylori* treatment is the complete eradication of the bacteria from its colonization site. It is well known that the ecological niche of H. pylori is the human stomach, where it colonizes for long periods within the gastric mucosa. Hence, for effective H. pylori eradication, therapeutic agents have to penetrate the gastric mucus layer to disrupt and inhibit the mechanism of colonization (10).
- Until today, the available DDS for *H. pylori* infection have many problems, and the major problem associated with the first line therapy for treatment of H. pylori is the incomplete eradication achieved from the conventional dosage forms available (42).
- Conventional drug delivery systems including immediate release (IR) and sustained release (SR) do not remain in the stomach for prolonged periods; they are unable to deliver the antibiotics to the site of infection in effective concentrations and in fully active forms. It was proved that the absorption of antibiotics into the epithelial cells through the mucus layer (from the gastric lumen) is believed to be more effective for H. pylori eradication than absorption through the basolateral membrane, i.e. from blood(194, 195).
- The conventional (IR) dosage forms of the therapies available for H. pylori eradication, typically, possess several disadvantages that lead to incomplete eradication (196),(197), (198):
    - Poor patient compliance due to the large doses needed to be administered multiple times daily.
    - Incomplete and non-uniform drug absorption profiles at the site of infection.
    - The residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be maintained for prolonged time in the gastric mucous layer or epithelial cell surfaces where H. pylori exists

- Large amounts of excipients incorporated with the large daily doses will increase their associated side effects.

- Sustained release (SR) dosage forms have no remarkable benefit in efficacy over the conventional immediate release dosage forms due to the following reasons(199),(197) :
  - Their variable and short gastric residence time at site of infection.
  - Incomplete drug release at the targeted site.
  - They extend the time for the drug release along GIT and thus increase the absorption to the plasma of some antibiotics, while the absorption of antibiotics into the mucus through the mucus layer (from the gastric lumen) is approved to be more effective for H. pylori eradication than absorption through the basolateral membrane (i.e. from blood).
- As a result, eradication of H. pylori with conventional systems (IR, SR and CR) may fail due to their systemic effect all over the gastro intestinal tract and not focusing in the gastric region (200). So a logical way to improve the effectiveness of these therapies was to develop a dosage forms that maintains in the stomach for a sufficient time and deliver and sustain a continuous effective dose of the drug over an extended period of time in the ecological niche of the bacterium, and thus enhance a successful treatment for H. pylori infection, such systems have been known as gastro-retentive drug delivery systems (93), (100).
- Since two decades, lot of work has been done targeting H. pylori therapeutic agents based on gastro-retentive mechanism , to minimize the drawbacks associated with the existing conventional dosage forms, and to optimize the therapy efficacy and enhance the patient compliance (201), (201), different gastroretentive techniques have been applied and developed, different manufacturing procedures were used, single and combination of drugs

were incorporated , *but:* ***Until today NO work has been done that combines a full regimen`s drugs for H. pylori eradication in one single dosage form.***

Part Four.

4. Objectives

4.1. General objectives:

- Develop a single dosage form (tablet-FDDS) that combines the first line triple therapy`s drugs for H. pylori eradication and treatment of associated peptic ulcer (Esomeprazole, Metronidazole and Clarithromycin).
- To retain the tablet in the gastric region for longer time based on buoyancy principle.
- Enhance the chemical stability of CLA by decreasing the degradation in the acidic environment by concomitant administration with the PPI which will increase the pH .
- Increase the local eradication of H. pylori by increasing the residence time of the active-stable antibiotics within their site of action (the colonization site of bacteria), thus the antibiotic concentrations in the stomach maintained for long time higher than their minimum inhibitory concentrations (MIC), (i.e. it has been approved that 60-70% of the oral dose in the gastric region was found to be distributed in the mucosal layer, mainly in the mucous layer and in surface epithelial cells following oral administration(202). Thus increasing the residence time in the stomach will increase the conc. At the site of action and improve eradication).
- Decrease the number of tablets to be administered each time, by incorporation the different drugs in one tablet.
- Increase the dosing interval (decrease the frequency of dosing) by the sustained release of these dosage forms.
- Minimizing the daily intake of the antibiotics and hence, decreasing their associated side effects.
- Enhance the patient compliance.
- Decrease the cost of manufacturing of the triple therapy on industrial scales.
- Decrease the cost of the treatment on the patient.

4.2. Specific Objective

1) To develop a method for concurrent analysis of both antibiotics in the SR-layer (Metronidazole and Clarithromycin) by HPLC for assay and dissolution.

   Note: follow the USP-36 method of analysis for the assay and dissolution of Esomeprazole.

2) To validate the new method of analysis according to International Conference of Harmonization (ICH) guidelines for Validation Q2 (R1).

3) To develop/formulate a floating bilayer tablet (contains: 300mg Metronidazole, 250mg Clarithromycin and 20 mg Esomeprazole), with different release mechanism layers (rapidly dissolving layer (RDL) of Esomeprazole and sustained release layer of Metronidazole and Clarithromycin) and acceptable size for oral administration.

4) To optimize the amounts of excipients and different types and grades of polymers to achieve the desired properties (Quick separation of layers, good floating properties, good matrix of the SR layer with sustained-synchronous release of both antibiotics.

5) To choose appropriate dissolution media.

6) To perform quality control testing for the tablets (physical & chemical tests).

7) To analyze the drug release data and test the kinetics of drug release using the kinetic mathematical models for slow release dosage forms using DDSOLVER.

Part Five

5. Methodology

(Experimental part)

5.1. Materials and reagents:

5.1.1. Materials and reagents used in the analytical parts:

(*All were supplied by Central Public Health Laboratory- Palestinian MOH).*

Clarithromycin reference standard (CLA-RS), Metronidazole reference standard (MTZ-RS), Potassium di-Hydrogen Phosphate, Phosphoric Acid, Distilled water (HPLC-grade water), Methanol (HPLC Grade), Hydrochloric acid (concentrate), Sodium hydroxide, Acetonitrile (HPLC-grade), Sodium acetate anhydrous, Glacial acetic acid, Phosphoric Acid, Mono Potassium Phosphate, Dibasic sodium phosphate, Tribasic sodium phosphate dodecahydrate, Omeprazole reference standard,

5.1.2. Materials used in the formulations:

Table 5.1: Materials used in the formulations:

Item	Manufacturer	Donated by
Metronidazole (MTZ)	***Alembic, India***	Bir Zeit Pharm. Co
Clarithromycin (CLA)	***Arti Drugs Limited, India***	Bir Zeit Pharm. Co Pharmacare Pharm. Co
Esomeprazole enteric coated pellets	***Glukem Pharmaceuticals Ltd, India***	Bir Zeit Pharm. Co Pharmacare Pharm. Co
Ethyl Cellulose (EC)	***Ashland, Japan***	Bir Zeit Pharm. Co
Avicel PH102	***FMC Biopolymer, Irland***	Bir Zeit Pharm. Co
Sodium bicarbonate (NaHCO3),	***Merck Milpore, Germany***	Bir Zeit Pharm. Co
Citric Acid	***TTCA Ltd. China***	Bir Zeit Pharm. Co
Aerosil (silicone dioxide)	***Evonik Industries, Germany***	Bir Zeit Pharm. Co
Lactose	***DFE Pharma. Germany***	Bir Zeit Pharm. Co
Polyvinylpyrrolidone K 30	***Jiaozou Zhongwei special peoducts Pharm. Co Ltd, china***	Bir Zeit Pharm. Co
Magnesium stearate (Mg-stearate)	***Magnesia, Germany***	Bir Zeit Pharm. Co
Hydroxypropylmethyl cellulose-K4M (HPMC-K4M)	***Orison Chemicals Limited, China***	Bir Zeit Pharm. Co
Hydroxypropylmethyl cellulose-K15M (HPMC-K15M)	***Orison Chemicals Limited, China***	Bir Zeit Pharm. Co
ydroxypropylmethyl cellulose (HPMC-K100).	***Shin-etsu, Japan***	Bir Zeit Pharm. Co

5.1.3. Tools, instruments and equipments used in the analytical parts:

(*All were supplied by Central Public Health Laboratory-Palestinian MOH*).

1) ***Disposables***: Syringes, HPLC-vials, filters (Nylon membrane -0.45µm and Cellulose ester filters- 0.45 µm), pipettes, plastic cups.

2) ***Glassware***: Volumetric flasks, volumetric pipettes, graduated measuring Cylinders, beakers.

3) ***Machinery***: Analytical balance, Vortex (IKA Genius 3), Glass electrode pH meter, Sonicator, Hot-plate (710 R), UV double beam spectrophotmeter with scanner, HPLC-Waters (e2695-Separation Module) equipped with photodiode array and UV detectors, Stationary phase: XBridge, C18 column-5µm (4.6*150mm) ***(****It was supplied by Dr. Saleh Abu-Lafi)*, Hardness tester (Pharma Test-PTB), Friability tester (Pharma Test-PH138), Dissolution apparatus (ERWEKA-DT700), Dissolution auto-sampler (Pharma Test-DT70).

5.1.4. Tools, Instruments and equipment used in the formulation part:

(*All were available in Al-Quds University labs*).

1) ***Disposables:*** plastic droppers, syringes, weighing papers, plastic cups, aluminum foil.
2) ***Glassware:*** graduated cylinders, beakers.
3) ***Others:*** pH-meter, Magnetic stirrors and sieves with different mesh numbers.
4) ***Machinery:*** Oven (Reichenbacher-KOM 119676), MIXER (ERWEKA -AR402), IR-Disc compression machine (RERKIN ELMER), Slugging machine (*in Jerusalem pharmaceutical company).*

5.2. Development of analytical procedure for concurrent determination of CLA and MTZ (for assay and dissolution):

5.2.1. Selection of suitable HPLC conditions and reagents:

- Prior to optimizing the HPLC conditions, a suggested method based on the single USP-monographs of CLA and MTZ were primarily used, it was as follow:

Mode	LC
Detector	UV
UV wavelength, nm	210 and 254
Flow rate, ml/min	1.0
Injection Volume (µL)	20
Temperature	40 C°±1
Mobile phase Methanol : Buffer	60 : 40

- ***Buffer preparation:***

  In order to prepare 1 Liter of 0.067 M KH2PO4 buffer:

  - Dissolve 9.1 g of KH2PO4 in 1L of purified water.
  - Filter the buffer using 0.45 µm filters.
  - Degas the buffer using the Sonicator.

- Three standards were prepared for analysis as described in each step below:
  - CLA-RS solution.
  - MTZ-RS solution.
  - Mixture of CLA and MTZ solution.

5.2.1.1. **Selection of suitable wave-length for detection of both CLA and MTZ:**

- **In this trial we aim to:**
- Select a single wavelength (λ) for detecting of both CLA and MTZ in single run of HPLC, this λ should have maximum absorbance of both two substances (C LA and MTZ).
- **Procedure:**

- Prepare two solutions, one of CLA by dissolving 27.8mg in 100ml mobile phase, and

another of MTZ by dissolving 33.3mg in 100ml mobile phase.

- Prepare mixture solution of both of CLA and MTZ with the same previous concentrations.
- Take 1ml of each solution and dilute to 25ml with the mobile phase.
- Measure the absorbance using UV-spectrophotmeter.

5.2.1.2. **Selection of HPLC-column:**

The selection of the column depends on the nature and the chemical structure of the substances to be analyzed.

5.2.1.3. **Selection of suitable mobile phase (components and percentages), column temperature and flow-rate:**

Different mobile phases (components, percentages and programs), different column`s temperature and different flow-rates were applied in order to obtain peaks of both CLA and MTZ with the following properties:

- Good shape (sharp peaks, symmetrical peaks).
- Well separated from each other (avoid overlapping problem).
- Away from the solvent peaks as much as possible.
- Well defined retention times.

Procedure:

The following HPLC-settings were applied during development of the analytical method:

5.2.1.3.1. Isocratic mobile phase trials:

Different conditions were applied by changing one variable each time, using the isocratic mode, these trials were summarized in **Table 5.2**.

5.2.1.3.2. Gradient mobile phase trials:

Different conditions were applied by changing one variable each time, using the gradient mobile phase mode, these trials were summarized in **Table 5.3**.

Table 5.2: Modifications of isocratic program during HPLC-method development

Trial No.	Mobile phase		Buffer (KH2PO4) conc. (M)	Injection V. (µl)	Column Temp. (ºC)	Flow rate. (ml/min)
	Methanol %	Buffer %				
1	80	20	0.067	20	40	1
2	60	40	0.067	20	40	1
3	60	40	0.067 (pH 4)	20	50	1
4	60	40	0.067 (pH 4)	10	50	1

Table 5.3: Modifications of gradient program during HPLC-method development

Trial No.	Mobile phase				Buffer conc. (M)	Injec. V. (µl)	Column Temp. (ºC)	Flow rate ml/min
	Methanol %	Buffer %	Acetonitrile %	Time intervals				
1	40»40»70»40	60»60»30»60	-------	0»2»6»7»10	0.02	10	50	1
2	20»20»80»80»20»20	80»80»20»20»80»80	---------	0»3»6»8»9»11	0.02	20	50	1
3	20»20»80»80»20»20	80»80»20»20»80»80	-------	0»3»9»12»14»16	0.02	20	50	1
4	20»20»60»60»20»20	80»80»40»40»80»80	-------	0»3»6»8»9»11	0.02	20	50	1
5	20»20»80»80»20»20	80»80»20»20»80»80	-------	0»3»6»8»9»11	0.017	20	50	1
6	20»20»80»80»20»20	80»80»20»20»80»80	-------	0»3»6»8»9»11	0.014	20	50	1
7	20»20»80»80»20»20	80»80»20»20»80»80	-------	0»3»6»8»9»11	0.018	20	50	1
8	10»10»70»70»10»10	90»90»30»30»90»90	--------	0»3»6»8»9»12	0.017	20	50	1
9	--------	90»90»35»35»90»90	10»10»65»65»10»10	0»3»10»11»12»15	0.017	20	50	1
10	-------	90»90»35»35»90»90	10»10»65»65»10»10	0»3»17»18»19»20	0.017	20	50	1

5.2.2. Selection of suitable dissolution media:

As recommended by USP-NF 36;

Physical and chemical data for the drug substance and dosage unit need to be determined before selecting the dissolution medium. Two key properties of the drug are the *solubility and solution state stability* of the drug as a function of the pH value. When selecting the composition of the medium, the influence of buffers, pH value, and surfactants on the solubility and stability of the drug need to be evaluated. The dissolution characteristics of an oral formulation should be evaluated at different physiologic pH range. During method development, it may be useful to measure the pH before and after a run to discover whether the pH changes during the test. Selection of the most appropriate conditions for routine testing is then based on relevance to in vivo performance, where possible.

Volume of dissolution media:

Normally, for basket and paddle apparatus, the volume of the dissolution medium is 500 mL to 1000 mL, with *900 mL* as the most common volume.

Procedure:

1. *To test the stability of CLA and MTZ in the dissolution media*:

Fresh standards solutions of both active ingredients were prepared, then the maximum dose of these drugs were dissolved in 900ml of the following different dissolution media at 37ºC, left for at least for 3 hours, then samples were withdrawn and analyzed. The resultant peak response of each sample was compared to its freshly prepared standards.

The following dissolution media were tested:

- 0.1 N HCl (pH=1.2).
- 0.1 N HCl (Adjust the pH with 1N NaOH to pH=4)
- 0.01 N HCl (adjust the pH by NaOH solution to pH=5).
- 0.1M sodium acetate media (adjusted the pH with glacial acetic acid to pH=4).

- 0.1M sodium acetate media (adjust the pH with glacial acetic acid to pH=5).

2. *To test the solubility of the drugs in the selected media:*

- Dissolve the maximum amount of the drugs present in the dosage form (250mg CLA, 300mg MTZ) in 200ml of the selected dissolution media in the previous step (1), use sonication and mechanical shaking to facilitate dissolution.

  Take 10mlof the stock solution and complete the volume to 50ml, then filter a portion and analyze using HPLC.
- Prepare standard solution by dissolving 25mg CLA and 30mg MTZ in 100ml dissolution media. Filter portion and analyze.
- Compare the peak response of both.

5.3. Validation of analytical procedures (assay and dissolution) (203),(173).

5.3.1. Introduction:

Validation of an analytical procedure is the process by which it is established, by experimental studies, that the performance characteristics of the analytical procedure meet the requirements and hence the analytical method is suitable for its intended purposes. Typical analytical performance characteristics that should be considered in the validation procedure according to the ICH-guidelines and USP-NF are: Accuracy, precision, selectivity/specificity, linearity, range, limit of quantitation (LOQ) and limit of detection (LOD) and robustness.

5.3.2. HPLC conditions:

The most suitable conditions were selected based on trials in section 5.1 and are listed in the results and discussion chapter under section 6.1.1.

5.3.3. Procedure:

This validation procedure was applied to the method of analysis that was selected in section **5.1,** as directed by ICH-Q2 (R1) and USP-36 (Chapter 1225) to ensure its suitability and accurate analysis of the assay and dissolution of both CLA and MTZ in our novel formula.

5.3.3.1. **Linearity**

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally calculated by using appropriate lest-squares regression programs.

ICH recommends that, for the establishment of linearity, a minimum of five concentrations normally be used. It is also recommended that the following minimum specified ranges should be considered:

- *For assay*, the minimum specified range is from 80-120% of the target concentration.
- *For content uniformity* testing, the minimum range is from 70-130% of the test or target concentration.
- *For Dissolution Testing:* ±20% over the specified range (e.g., if the acceptance criteria for a controlled-release product cover a region from 30%, after 1 hour, and up to 90%, after 24 hours, the validated range would be 10% to 110% of the label claim).

Acceptance Criteria:

The correlation coefficient (R^2) is not less than (NLT) 0.999 for the least squares method of assay analysis, and NLT 0.98 for dissolution.

Procedure:

Prepare standard stock solution with concentrations of 1.665 mg/ml Metronidazole and 1.385 mg/ml Clarithromycin. Transfer 166.5mg Metronidazole-RS and 138.5mg

Clarithromycin-RS to a 100 volumetric flask. Add 50ml diluent (sodium acetate pH=5) and sonicate for 5min, complete to volume with the diluent. Then 7 standard solutions with different concentrations are prepared by diluting proportions from the stock solution with the diluent according to the **table 5.4.**

The standards then will be analyzed in according to the selected HPLC analytical method.

Data Analysis:

The response of each concentration was plot versus standard concentrations prepared for linearity and Range. The least squares linear regression analysis, the slope, and Y-intercept of the data were performed.

Table 5.4: Standard solutions preparation for linearity determination

Solution No.	**Conc. %**	**Conc. MTZ (µg/ml)**	**Conc. CLA (µg/ml)**	**Volume Pipetted from Stock St Solution (ml)**	**Final Volume (ml)**
1	160	532.8	443.2	8	25
2	120	399.6	332.4	6	25
3	100	333	277	5	25
4	80	266.4	221.6	4	25
5	50	166.5	138.5	5	50
6	25	83.25	69.25	5	100
7	10	33.3	27.7	2	100

5.3.3.2. **Accuracy:**

The accuracy of an analytical procedure measures the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and value found. Accuracy is evaluated by analyzing synthetic mixtures spiked with known quantities of active pharmaceutical ingredient.

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g.3 concentrations /3 replicates each).

Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value.

Acceptance Criteria:

- The mean recovery of the assay should be within 100±2.0% at each concentration over the range of 80 – 120% of nominal concentration.
- The mean recovery of the dissolution should be within 100±5.0% at each concentration.

Procedure:

Prepare placebo mixture, by mixing excipients used in the formulation procedure.

Prepare spiked sample (stock solution) by spiking (addition) of known concentration of both MTZ and CLA to a portion of placebo sample. Try to get concentrations of CLA1.665mg/ml MTZ and 1.385mg/ml CLA by dissolving 333mg MTZ and 277mg CLA with portion of placebo powder in 200-ml volumetric flask, dissolve with 50ml methanol and 50ml of the diluent and sonicate for 10min, the volume is then completed to 200ml with the diluent, then five samples with different concentrations are prepared in triplicates, by diluting proportions from the stock solution by the diluent according to **Table 5.5,** analyze the samples according to the HPLC analytical method.

Table 5.5: Accuracy determination sample solutions:

Conc. (%)	**Concentration of MTZ (µg/ml)**	**Concentration of CLA (µg/ml)**	**Pipetted Volume of sample (ml)**	**Flask Volume (ml)**
160	532.8	443.2	8	25
100	333	277	5	25
50	166.5	138.5	5	50
25	83.25	69.25	5	100
10	33.3	27.7	2	100

Data Analysis:

The actual concentrations of the stock -spiked sample prepared were 1.55mg/ml MTZ and 1.33mg/ml CLA, so the concentrations of the prepared samples were slightly different from the theoretical ones, and hence the recovery will be calculated according to the following equation:

- Calculate the recovery data for each determination; calculate the average of recovery data and the relative standard deviation (RSD) for each level.

$$\% \text{ Recovery} = \frac{\text{Peak area}_{\text{sample}}}{\text{Peak area}_{\text{standard}}} \times \frac{\text{Conc.}_{\text{standard}}}{\text{Conc.}_{\text{sample (actual)}}}$$

Conc. : concentration

5.3.3.3. **Precision:**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Procedure: for Repeatability.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Repeatability is assessed using 9 injections covering the specified range for the procedure (3 concentrations / 3 replicates each), it can be assessed using the data of samples from the accuracy test.

Acceptance Criteria: Relative Standard Deviation between the data of the same concentration shall not be greater than 2%.

5.3.3.4. **Range**

The range is the interval between the upper and lower concentrations of analyte in the sample that have been demonstrated to have a suitable level of precision, accuracy, and linearity. The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The following minimum specified ranges should be considered:

- For the assay of an active substance or a finished product: normally from 80 to 120 percent of the test concentration.
- For content uniformity testing, the minimum range is from 70-130% of the test or target concentration.
- For dissolution testing, from below the lowest expected concentration to above the highest concentration during release.

5.3.3.5. **Selectivity:**

Specificity is the ability to assess unequivocally the analyte in the presence of components

which are expected to be present. Typically these might include excipients, degradants, impurities, diluents, etc.

Procedure:

A. No interference from excipients:

This test is conducted by preparing a placebo sample (mixture of the product excipients without the active ingredients), then prepare a 100% sample solution from this placebo same as sample preparation under accuracy test, and analyze under the same conditions.

B. No interference from the diluent:

Inject a blank sample (only contains the diluent) to the HPLC under the same conditions of analysis.

5.4. Formulations development:

5.4.1. Selection of the excipients:

1. Excipients selection for our formulations was mainly dependent on the selection of the essential components of the floating gastro retentive systems (i.e. hydrophilic swellable polymers, release retardants, gas generators, density decreasing materials) and other components of tablets (i.e. binders, glidants and lubricants) (151, 204), (205).
2. The excipients should be of pharmaceutical grades, for internal use and biodegradable.

5.4.2. Selection of the manufacturing process:

Different manufacturing procedures were applied in order to achieve a formula with the desired properties, these procedures included: Direct Compression, Wet Granulation and Dry Granulation.

5.4.3. Formulations and Manufacturing procedures:

- Different formulations were generated based on using different grades of hydrophilic polymers (i.e. HPMC K-100M, K-15M and K-4M) and hydrophobic polymers (i.e. ethyl cellulose) as SR-matrix modifiers. Sodium bicarbonate and anhydrous citric acid were used as gas generating agents. Other excipients were used such as glidants, binders, diluents,

and lubricants in order to attain the objective of designing a new floating system with acceptable floating and sustained release properties.

- During manufacturing process the amount of MTZ had been decreased from 400mg/tab to 300mg/tab, due to the large size of the tablet and based on previous studies which had shown that the efficacy of floating system of MTZ against H. pylori was nearly threefold that of the classical MTZ system(206)
- In the immediate dissolving layer, the esomeprazole enteric coated pellets were compressed directly using microcrystalline cellulose (Avicel) as the pressure absorbing matrix to prevent the destruction of the coat that protect EZO from degradation in the acidic media (207).
- The primary parameters to evaluate formulations were: the separation time of the two layers (the rapidly dissolving EZO-layer and the SR layer of CLA and MTZ), and the in-vitro buoyancy testing which includes the floating lag time and the total floating time.

The two layers should be separated immediately upon contact with the dissolution media before floating(187). The tablets should show floating lag time less than two minutes(188) and total floating time not less 24 hrs(208).

- After passing these tests, further in-vitro dissolution testing will be applied, the dissolution profile showing SR or CR manner of both CLA and MTZ will be accepted. If a synchronous SR of both API`s could be achieved this would be a superior result.
- The quantities listed are per single unit and the % is for materials in each layer.

5.4.3.1. **Direct compression Formulations:**

5.4.3.1.1. Summary of formulations manufactured by direct compression technique:

The different formulations prepared by direct compression were summarized in **Table 5.6**.

Table 5.6: Direct compression formulations.

Layer	Component	Function in the formula	D1		D2		D3	
			Mg	%	mg	%	Mg	%
1st-layer	Metronidazole	Active Ingredient	300	24.5	300	24.5	300	23.1
	Clarithromycin	Active Ingredient	250	20.5	250	20.5	250	19.1
	HPMC-K4	SR-matrix & swelling agent (hydrophilic)	250	20.5	-----	-----	-----	-----
	HPMC-K100	SR-matrix & swelling agent (hydrophilic)	-----	-----	250	20.5	250	19.1
	Ethyl cellulose (EC)	SR-matrix (hydrophobic)	250	20.5	250	20.5	250	19.1
	NaHCO3	Gas generating agents	160	12.8	160	12.8	160	12.3
	Citric acid	Gas generating agent	-----	-----	-----	----	80	6.16
	Aerosil	Glidant	10	0.8	10	0.8	10	0.76
	Mg-stearate	Lubricant	5	0.4	5	0.4	5	0.38
	1st Layer Wt.**		**1225**		**1225**		**1305**	
2nd layer	Esomeprazole pellets (22.5%)	Active Ingredient	100	49.5	100	49.5	100	49.5
	Avicel PH102 (MCC)	Diluent and binder	100	49.5	100	49.5	100	49.5
	Mg-stearate	Lubricant	2	1	2	1	2	1
	2nd Layer Wt.		**202**		**202**		**202**	
	Tablet Wt.		**1427**		**1427**		**1507**	

Table 5.6: Continued…

	Component	D4		D5		D6		D7	
		mg	%	mg	%	mg	%	Mg	%
1st-layer	Metronidazole	300	24.5	300	22.5	300	23.7	300	24.7
	Clarithromycin	250	20.4	250	19	250	19.8	250	20.6
	HPMC-K4	-----	-----	-----	-----	----	-----	-----	-----
	HPMC-K100	210	17.1	210	15.8	200	15.8	250	20.6
	Ethyl cellulose (EC)	250	20.4	250	19	300	23.7	200	16.5
	NaHCO3	160	13.1	220	16.6	160	12.7	160	13.1
	Citric acid	40	3.3	40	6	40	3.2	40	3.25
	Aerosil	10	0.8	10	0.76	10	0.79	10	0.83
	Mg-stearate	5	0.4	5	0.34	5	0.39	5	0.42
	1st Layer Wt.	**1225**		**1325**		**1265**		**1215**	
2nd layer	Esomeprazole pellets (22.5%)	100	49.5	100	49.5	100	49.5	100	49.5
	Avicel PH102 (MCC)	100	49.5	100	49.5	100	49.5	100	49.5
	Mg-stearate	2	1	2	2	2	1	2	1
	2nd Layer Wt.	**202**		**202**		**202**		**202**	
	Tablet Wt.	**1427**		**1487**		**1467**		**1417**	

**: Wt (weight).

5.4.3.1.2. Manufacturing steps of direct compression procedure:

The following steps describe the general manufacturing procedure, mentioning all the ingredients were used during different formulation trials; so one can only consider the ingredients those were present in each formula as listed in the table of "Direct Compression Formulations".

1st-layer

1. Pass EC and citric acid through sieve mesh # 20.
2. Pass MTZ, HPMC-K4, HPMC-K100, NaHCO3, and Avicel PH102 through sieve mesh#40.
3. Mix ingredients in steps 1 and 2 for *5minutes (min)**.
4. Pass talc, aerosil via sieve mesh# 20.
5. Mix ingredients in steps 3 and 4 for *5min**.
6. Add Magnesium stearate to the mixture in step 5and mix for additional *10 min**.

**: mixing was done using ERWEKA-Mixer.*

2nd-layer:

1. Pass Avicel PH-102 through sieve mesh #40.
2. Mix the esomeprazole pellets with Avicel PH-102 manually using polyethylene bag for 5 min.
3. Add 1% magnesium stearate and mix for additional *5minutes* manually using the polyethylene bag.

Tablet compression:

1. Fill the IR-disc hopper manually with accurate weight of the first layer and compress at low pressure ***0.5 ton***.
2. Then add the accurate weight of the second layer above first layer, and compress at high pressure ***2.5 tons.***

5.4.3.2. **Wet-Granulation Formulations:**

5.4.3.2.1. Summary of formulations manufactured by wet-granulation technique:

The different formulations prepared by wet-granulation were summarized in Table 5.7.

Table 5.7: Wet granulation formulations.

Layer	**Component**	Function in the Formula.	(W1) [1]		(W2) [1]		(W3)[1]		(W4)[2]	
			mg	%	mg	%	mg	%	mg	%
1st-layer	Metronidazole	Active Ingredient	400	39.5	400	38.5	400	39	400	36.3
	Clarithromycin	Active Ingredient	250	24.5	250	24	250	24.3	250	22.7
	HPMC-K4	SR-matrix & swelling agent (hydrophilic)	122	12	122	11.7	110	10.6	122	11.1
	HPMC-K15	SR-matrix & swelling agent (hydrophilic	-----	-----	-----	-----	-----	-----	-----	-----
	HPMC-K100	SR-matrix & swelling agent (hydrophilic)	-----	----	-----	-----	-----	-----	-----	-----
	Ethyl cellulose (EC)	SR-matrix (hydrophobic)	-----	-----	-----	-----	-----	-----	-----	-----
	NaHCO3	Gas generating agents	90	9	120	11.5	120	11.7	150	13.6
	Citric acid	Gas generating agent	-----	-----	-----	-----	-----	-----	30	2.7
	Avicel PH102 (MCC)	Diluent and binder	126	12.5	126	12.2	126	12.3	126	11.5
	Aerosil	Glidant	10	1	10	0.95	10	0.96	10	0.9
	PVP K-30 (2%solution)	Binder	9	0.9	7	0.7	7	0.67	8	0.75
	Mg-stearate	Lubricant	5	0.5	5	0.45	5	0.47	5	0.45
	1st Layer Wt.		**1012**		**1040**		**1028**		**1101**	
2nd layer	Esomeprazole pellets (22.5%)	Active Ingredient	100	49.5	100	49.5	100	49.5	100	49.5
	Avicel PH102 (MCC)	Diluent and binder	100	49.5	100	49.5	100	49.5	100	49.5
	Mg-stearate	Lubricant	2	1	2	1	2	1	2	1
	2nd Layer Wt.		**202**		**202**		**202**		**202**	
	Tablet Wt.		**1214**		**1242**		**1230**		**1303**	

Table 5.7: Continued.

Layer	Component	(W5)[3]		(W6)[4]		(W7)[4]		(W8)[4]		(W9)[4]	
		mg	%	mg	%	Mg	%	mg	%	mg	%
1st-layer	Metronidazole	300	24.5	300	23.5	300	23	300	22.6	300	22.6
	Clarithromycin	250	20.5	250	19.5	250	19.2	250	19	250	19
	HPMC-K4	-----	-----	-----	-----	-----	----	-----	-----	-----	------
	HPMC-K15	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	HPMC-K100	250	20.5	300	23.5	330	22.4	300	22.6	225	17
	Ethyl cellulose (Fine powder)	200	16.3	200	15.5	200	15.4	230	17.3	230	17.3
	Lactose	-----	-----	-----	-----	-----	-----	-----	-----	75	5.6
	NaHCO3	160	13.1	160	12.5	160	12.3	180	13.5	180	13.6
	Citric acid	40	3.25	40	4	40	3.1	45	3.4	4.5	3.3
	Avicel PH102 (MCC)	-----	-----	-----	----	-----	----	-----	-----	-----	----
	Aerosil	10	0.81	10	0.75	10	0.78	10	0.75	10	0.8
	PVP K-30 (2%solution)	7	0.57	6	0.5	6	0.46	6	0.45	5	0.4
	Mg-stearate	5	0.41	5	0.36	5	0.38	5	0.38	5	0.4
	1st Layer Wt.	**1222**		**1271**		**1301**		**1326**		**1325**	
2nd layer	Esomeprazole pellets (22.5%)	100	49.5	100	49.5	100	49.5	100	49.5	100	49.5
	Avicel PH102 (MCC)	100	49.5	100	49.5	100	49.5	100	49.5	100	49.5
	Mg-stearate	2	1	2	1	2	1	2	1	2	1
	2nd Layer Wt.	**202**		**202**		**202**		**202**		**202**	
	Tablet Wt.	**1424**		**1473**		**1503**		**1528**		**1527**	

Table 5.7: Continued

Layer	Component	(W10)[4]		(W11)[5]		(W12)[4]		(W13)[4]	
		mg	%	mg	%	Mg	%	mg	%
1st-layer	Metronidazole	300	22.6	300	22.6	300	23	300	23
	Clarithromycin	250	18.9	250	18.8	250	19	250	19
	HPMC-K4	-----	-----	----	----	50	3.82	50	3.8
	HPMC-K15	-----	-----	150	11.3	-----	-----	-----	-----
	HPMC-K100	280	21.1	150	11.3	250	19	250	19
	Ethyl cellulose (Fine powder)	230	17.3	230	17.4	230	17.6	230	17.7
	Lactose	20	1.5	-----	-----	-----	------	----	-----
	NaHCO3	180	13.6	180	13.7	170	13	160	12.4
	Citric acid	45	3.4	45	3.4	40	3.04	40	3.2
	Avicel PH102 (MCC)	----	-----	-----	-----	-----	----	-----	-----
	Aerosil	10	0.8	10	0.74	10	0.76	13	1
	PVP K-30 (2%solution)	6	0.4	5	0.38	5	0.39	5	0.4
	Mg-stearate	5	0.4	5	0.38	5	0.39	6.5	0.5
	1st Layer Wt.	**1326**		**1325**		**1310**		**1304**	
2nd layer	Esomeprazole pellets (22.5%)	100	49.5	100	49.5	100	49.5	100	49.5
	Avicel PH102 (MCC)	100	49.5	100	49.5	100	49.5	100	49.5
	Mg-stearate	2	1	2	1	2	1	2	1
	2nd Layer Wt.	202		**202**		**202**		**202**	
	Tablet Wt.	1528		**1527**		**1512**		**1506**	

1, 2, 3, 4 and 5: Indicate the composition of the granules, listed in **Table 5.8.**

5.4.3.2.2. Manufacturing steps of wet-granulation procedure:

1st-layer

1. Prepare 100ml of 2% PVP solution as follow:

Dissolve 2g of PVP-K30 in 100ml isopropanol using the magnetic stirrer.

2. Sieve the following materials by passing through sieve ***mesh #40*** independently from each other: MTZ, CLA, HPMC-K4, HPMC-K15, HPMC-K100 lactose, Avicel PH102 and NaHCO3.

And sieve the following material by passing via sieve ***mesh # 20***: citric acid and aerosil.

3. Grind EC pellets into fine powder then pass through sieve ***mesh #40.***

4. Mix the components of granules (the existed ones in each formula) listed in the **Table 5.7** below very well before granulation.

5. Granulate mixture in step 4 using PVP solution.

- Wet the mixture with suitable quantity of the solution till reaching suitable end point.
- Pass the wet mass via sieve #20 to get granules.
- Dry the wet granules in oven at 50ºC for *30min.*
- Sieve the dried granules again using sieve mesh #20.

6. Mix the remaining ingredients (those not included in granules in step 4) except magnesium stearate for *5min.*

7. Mix ingredients in steps 5 (dry granules) and 6 together for *10min* using ERWEKA mixer.

8. Add Magnesium stearate to the above mixture in step 7 and mix for additional *10minutes* using ERWEKA mixer.

2nd-layer:

1. Pass Avicel PH-102 via sieve mesh #40.
2. Mix the esomeprazole pellets with Avicel PH-102 for *5 minutes*.
3. Add 1% magnesium stearate and mix for additional *5minutes*.

Tablet compression:

1. Fill the IR-disc hopper manually with accurate weight of the first layer and compress at low pressure ***0.5 ton***.

2. Then add the accurate weight of the second layer above the first layer & compress at high pressure ***(2.5tons***).

Table 5.8: Granules composition in the wet granulation

Method number	Components of the granules.
1	MTZ, CLA, HPMC-K4, NaHCO3, Avicel PH 102.
2	MTZ, CLA, HPMC-K4, ½ (NaHCO3), Avicel PH 102.
3	MTZ, CLA, ½ (HPMC-K100), ½(NaCO3), ½ (EC), Citric acid.
4	MTZ, ½ (HPMC-K100), ½ (HPMC-K4), ½ Lactose.
5	MTZ, HPMC-K15

.

5.4.3.3. **Dry-granulation method:**

5.4.3.3.1. Summary of formulations manufactured by dry-granulation technique:

The different formulations prepared by dry granulation were summarized in **Table 5.9.**

Table 5.9: Dry-Granulation Formulations.

Layer	**Component**	Function in the formula	DG1		DG2		DG3	
			mg	%	mg	%	mg	%
1st-layer	Metronidazole	Active Ingredient	300	23.44	300	23.35	300	23.35
	Clarithromycin	Active Ingredient	250	19.53	250	19.45	250	19.45
	HPMC-K100	SR-matrix & swelling agent (hydrophilic)	300	23.44	300	23.35	300	23.35
	Ethyl cellulose (EC)	SR-matrix (hydrophobic)	215*	16.8	215*	16.73	215	16.73
	NaHCO3	Gas generating agents	160	12.5	160	12.45	160	12.45
	Citric acid	Gas generating agent	40	3.12	40	3.11	40	3.11
	Aerosil	Glidant	10	0.78	10	0.78	10	0.78
	Mg-stearate	Lubricant	5	0.39	10	0.78	10	0.78
	1st Layer Wt.		**1280**		**1285**		**1285**	
2nd layer	Esomeprazole pellets (22.5%)	Active Ingredient	100	49.5	100	49.5	100	49.5
	Avicel PH102 (MCC)	Diluent and binder	100	49.5	100	49.5	100	49.5
	Mg-stearate	Lubricant	2	1	2	1	2	1
	2nd Layer Wt.		**202**		**202**		**202**	
	Tablet Wt.		**1482**		**1487**		**1487**	

*: Ethyl Cellulose fine powder.

5.4.3.3.2. Manufacturing steps of dry-granulation procedure:

1st-layer:

1. Sieve half of HPMC-K100 and MTZ via sieve mesh #40.

2. Mix ingredients in step 1 for *5 min.*

3. Compress the mixture in step 2 into a slug using a pilot press machine.

4. Mill the slug resulted in step 3.

5. Pass the milled material in step 4 through sieve mesh # 20 to form granules.

6. Sieve the following ingredients through mesh # 40: CLA, the remaining HPMC-K100, NaHCO3 and EC. And Sieve citric acid and aerosil through sieve mesh # 20.

7. Mix ingredients in step 6 for *5 min.*

8. Mix ingredients in steps 7 and 5 using double-cone ERWEKA mixer for *15 min.*

9. Add Magnesium stearate to mixture in step 8 and mix for additional *10 min.*

2nd-layer:

1. Sieve Avicel PH-102 using sieve mesh #40.

2. Mix the esomeprazole pellets with Avicel PH-102 for *5 min*.

3. Add 1% magnesium stearate to mixture in step 2 and mix for additional *5min*.

Tablet compression:

1. Fill the IR-disc hopper manually with accurate weight of the first layer and compress at low pressure.

2. Then add the accurate weight of the second layer above the first layer & compress at high pressure ***(2.5tons***).

5.5. Quality control tests of the selected formula.

In-vitro evaluation of physical and chemical parameters is very important in controlling the quality of any dosage form. The floating tests were applied to all formulas. Those which passed the floating test will be further undergoing In-vitro dissolution testing. And the

selected formula (formula of choice) based on dissolution results will undergo all the tests described below.

5.5.1. Pre-compression tests / In process control (IPC) (209):

5.5.1.1. Angle of repose (θ):

The frictional forces and hence the flowability of powders and granules can be detected by measuring this angle.

- **Procedure**: It is measured by putting 20 g of the powder as a single heap on a flat surface (a glass surface 30 cm X 30 cm), then raising the surface to the point at which the powder heap starts to fall apart (powder starts to flow). At this point the angle made between the flat surface and ground surface is measured as the angle of repose(210).

- Then the flowability of the powder is then evaluated as follow (**Table 5.10**).

Table 5.10 : The relationship between angle of repose and powder flowability(211).

Flow Property	Angle of Repose (degrees)
Excellent	25–30
Good	31–35
Fair—aid not needed	36–40
Passable—may hang up	41–45
Poor—must agitate, vibrate	46–55
Very poor	56–65
Very, very poor	>66

5.5.1.2. Carr's index(Compressibility Index):

The compressibility is the ability of powder to decrease in volume under pressure. Carr`s index is frequently used in pharmaceutics as an indication of the flowability of a powder.

- **Procedure**: 20 g of the selected formula blend are poured gently through a glass funnel into a granulated cylinder and the volume of unstirred granules is measured. Then, the

cylinder will be tapped100 times from a height of 2.0cm and the volume of granules after stirring is measured.

- **Calculations:**

Compressibility index is calculated using the formula:

$$\textit{Compressibility Index} = (\rho t - \rho 0) / \rho t \times 100\%$$

Where, ρt = Tapped density and ρo = Bulk density

- And the flow property of the powder/granules is evaluated as follow (**Table 5.11**):

Table 5.11: The relationship between Carr`s index and powder flowability(211).

Compressibility Index (%)	Flow Character
≤10	Excellent
11–15	Good
16–20	Fair
21–25	Passable
26–31	Poor
32–37	Very poor
>38	Very, very poor

5.5.2. Post-compression tests:

5.5.2.1. **Description of the tablet:**

The color, odor & taste of the tablet have to be described briefly, they can be evaluated organoleptically.

Thickness and diameter are measured using a calibrated varniear caliper.

5.5.2.2. **Hardness (212).**

The hardness indicates the ability of a tablet to withstand mechanical shocks.

Procedure: measure the hardness of 10 tablets using a Hardness tester "Pharma Test-PTB 311E".

Mean and standard deviation will be computed and reported. It is expressed in kilopascal (kp) or neuton (N).

5.5.2.3. **Weight (mass) variation test (BP 2013) (213).**

This test indicates the uniformity of weight for the tablets.

- **Procedure**: Twenty tablets selected at random and are weighed individually and then the average weight is determined.
- **Acceptance criteria**: Not more than 2 of the individual weights deviate from the average weight by more than the percentage deviation shown in (**Table 5.12**) and none deviates by more than twice that percentage.

Table 5.12: Acceptance criteria for weight variation test.

Pharmaceutical Form	Average Mass	Percentage deviation
Tablets (uncoated and film-coated)	80 mg or less	10
	More than 80 mg and less than 250 mg	7.5
	250 mg or more	5

5.5.2.4. ***Friability test*** **(173).**

This test applied to compressed, uncoated tablets. Measurement of tablet friability supplements other physical strength measurements, such as tablet breaking force.

- **Procedure**: The tablet is performed on 10 tablets. The tablets should be dedusted prior testing, and then they are accurately weighed (Wo) and then placed on the drum of the friability tester. Rotate the drum 100 times, and remove the tablets. Remove any dust on the tablets and accurately weigh again (W).
- **Calculation**: The percent of friability is calculated as described in the following equation:

$$\% \text{ Friability} = (Wo-W)/ Wo \times 100\%$$

- **Acceptance criteria**: A maximum mean weight loss from the samples (% friability) doesn`t exceed 1.0%.

5.5.2.5. **Assay test**

This test is carried out to measure the content of the drug substance in the dosage form units (drug product). In this test two solutions are prepared; standard solution that contains a known concentration of the drug substance alone, and sample solution prepared by taking 10 tablets and grinding them into fine powder, then a known weight of this powder is taken and dissolved in suitable diluent (usually from the mobile phase), small portion of this sample solution is taken and diluted to have a known concentration of the drug substance. Then both of these solutions are analyzed using a suitable method and peak response is considered **(173).**

5.5.2.5.1. Assay of CLA and MTZ:

- ***Procedure***: By HPLC Validated Method (In-House).

Chromatographic conditions:

Mode: LC

Detector: UV; λ=210nm.

Column: C18, 4.6-mm * 15-cm; particle size 5µm.

Flow rate: 1 ml/ min (1000µl/min).

Injection volume: 20µl.

Diluent: sodium acetate buffer (pH=5), 5% methanol can be used in dissolving the sample.

Temperature: 50ºC

Mobile phase: (Acetonitrile and Buffer*)

Program: gradient.

Time	Acetonitrile	Buffer
Zero	10	90
3	10	90
17	65	35
18	65	35
19	10	90
20	10	90

* **Buffer 0.017M** KH_2PO_4, Adjust the **pH to 4** by phosphoric acid.

Standard solution.

Dissolve 166.5 mg of Metronidazole and 138.5 mg of Clarithromycin RS in the 50ml of the diluent (dissolution media), sonicate for 10minutes and then complete the volume to 100 ml with diluent (Standard Stock solution). Transfer 10.0 mL of this stock solution to a 50-mL volumetric flask, dilute with diluent to volume, and mix. Pass through a filter having a 0.45-µm or finer porosity, and use the filtrate as the Standard preparation. (C_S CLA= 277µg/ml, and C_S MTZ= 333µg/ml).

Sample Solution:

Weigh and powder not less than 10 tablets, transfer accurately weighed portion of powder equivalent to 277 mg CLA and 333 mg MTZ to 200ml volumetric flask, add 20 ml of methanol and 50 ml diluent and sonicate for 15 minutes. Dilute with diluent to volume, mix, and allow any insoluble matter to settle. Transfer 10 ml of the supernatant liquid to 50ml volumetric flask, dilute with diluent to volume, and mix. Filter a portion of this solution through a filter having a porosity of 0.45µm or finer and use the filtrate as the assay preparation. (C_U CLA= 277µg/ml, and C_U MTZ= 333µg/ml).

- **Calculations**: Calculate the percentage of the labeled amount of CLA and MTZ in the tablets as follow:

$$\textbf{Assay} = (R_U / R_S) \times (C_S / C_U) \times 100\%$$

Where;

R_U = peak response from the Sample solution

R_S = peak response from the Standard solution

C_S = concentration of drug substance in the Standard solution (µg/mL)

C_U =nominal concentration of the drug substance in the Sample solution (µg/mL)

- **Acceptance criteria:** 90.0% -110.0% (for both CLA and MTZ) (173).

5.5.2.5.2. Assay of Esomeprazole (EZO) (173).

• ***Procedure***: By HPLC Method (USP-NF 36)

Buffer: Prepare a pH 7.3 phosphate buffer by mixing 10.5 mL of 1.0 M monobasic sodium phosphate buffer and 60 mL of 0.5 M dibasic sodium phosphate buffer, and diluting with water to 1000 ml.

Diluent: Prepare a pH 11.0 diluent as follows. Dissolve 5.24 g of tribasic sodium phosphate dodecahydrate in water. Add 110 mL of 0.5 M dibasic sodium phosphate solution, and dilute with water to 1000 ml.

Mobile phase: Mix 350 mL of acetonitrile and 500 mL of the Buffer. Dilute with water to 1000 ml.

Standard solution: Transfer 10 mg of USP Omeprazole RS to a 250-mL volumetric flask, and dissolve in about 10 mL of methanol. Add 40 mL of Diluent, and dilute with water to volume. This solution contains 0.04 mg/mL of USP Omeprazole RS.

Sample stock solution: grind the contents of NLT 10 tablets. Transfer a portion of the powder, equivalent to 20 mg of esomeprazole, to a 100-mL volumetric flask, add 60 mL of Diluent, and shake for 20 min to dissolve the pellets. Sonicate for a few min, if needed, to completely dissolve. Add 20 mL of alcohol, and sonicate for a few min. Cool, and dilute with Diluent to volume. Pass a portion of the solution through a filter of 1-µm pore size.

Sample solution: 0.04 mg/mL of esomeprazole from the Sample stock solution in water. Store this solution protected from light.

Chromatographic system

Mode: LC

Detector: UV 302 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

- **Calculations**: Calculate the percentage of the labeled amount of EZO in the tablets as follow:

$$\textbf{Assay} = (R_U / R_S) \times (C_S / C_U) \times 100\%$$

Where;

R_U = peak response from the Sample solution

R_S = peak response from the Standard solution

C_S = concentration of drug substance in the Standard solution (mg/mL)

C_U =nominal concentration of the drug substance in the Sample solution (mg/mL)

- **Acceptance criteria:** 90.0% -110.0%.

5.5.2.6. **In-vitro dissolution test:**

Dissolution test is performed to determine the compliance of drug release from the dosage with its requirements for such dosage form. In this test the percent of the drug released from the dosage form is calculated versus time. This is usually carried out in suitable dissolution media, maintained at 37 °C, using the USP dissolution apparatus II (paddle). Samples are withdrawn periodically from the dissolution medium with replacement to their size with fresh dissolution media and then their content of the drug substance is analyze using developed HPLC method**. (173),(214).**

5.5.2.6.1. Dissolution test for MTZ and CLA:

- **Procedure**: By HPLC Validated Method (In-House).

Test Conditions.

Media: 0.1M sodium acetate buffer, pH 5.

Apparatus: apparatus II (Paddle).

Volume: 900 ml.

Rotational Speed: 50 RPM.

Dissolution media preparation.

- Prepare 0.1M dissolution media by dissolving 8.2 of sodium acetate anhydrous in each 1L of distilled water. (M.Wt of sodium acetate anhydrous = 82g/mole).
- Adjust the pH to 5 by addition of glacial acetic acid.

Blank Solution:

Take a sample of the freshly prepared dissolution media, filter then inject to the HPLC.

Standard Solution preparation.

Dissolve 27.7mg of clarithromycin W.S and 33.3mg of Metronidazole W.S in 100ml dissolution media.

Sample preparation.

Place the stated volume of the dissolution medium (900mL±1%) in each vessel of the apparatus, assemble the apparatus, equilibrate the dissolution medium temperature at ($37^oC\pm 0.5^o$), and the rotational speed. Place one tablet in each vessel in the apparatus, wait till the tablet floats and then immediately operate the apparatus. At specified time intervals withdraw a specimen of 5ml from each vessel and replace with fresh dissolution media. Filter the sample through 0.45µm filter, fill in HPLC-vial and then inject to the HPLC.

- **Calculations:** Calculate the percentage of CLA and MTZ released from each tablet using the same equation used to calculate the assay.
- **Acceptance criteria:**

Sustained, synchronous release of both MTZ and CLA is required.

5.5.2.6.2. Dissolution of Esomeprazole (173):

- Buffer, Diluent, Mobile phase, System suitability, and Chromatographic system: Proceed as directed in the Assay.
- **Method**: HPLC method according to USP-NF36

Medium: 0.1 N hydrochloric acid; 300 ml. After 2 h, continue with a pH 6.8 phosphate buffer as follows. To the vessel, add 700 ml of 0.086 M dibasic sodium phosphate, and adjust with 2N hydrochloric acid or 2N sodium hydroxide, if necessary, to a pH of 6.8 ± 0.05.

Apparatus 2: 100 rpm

Time: 2 h in 0.1N HCl, and 30 min in a pH 6.8 phosphate buffer

Standard solution: Prepare a solution containing 2 mg/mL of USP Omeprazole RS in alcohol. Dilute this solution with pH 6.8 phosphate buffer to obtain a solution containing (L/1000) mg/mL, where L is the label claim, in mg/tablet (0.02mg/ml). Immediately add 2.0 mL of 0.25 M sodium hydroxide to 10.0 mL of this solution, and mix. {Note— Do not allow the solution to stand before adding the sodium hydroxide solution}

Sample solution: After 30 min in pH 6.8 phosphate buffer, pass a portion of the solution under test through a suitable filter. Transfer 5.0 mL of the filtrate to a suitable glassware containing 1.0 mL of 0.25 M sodium hydroxide. Mix well. Protect from light.

- **Calculations:**

Samples: Standard solution and Sample solution

Calculate the percentage of esomeprazole (C17H19N3O3S) dissolved:

$$\textbf{Result} = (R_U / R_S) \times (C_S / L) \times V \times 100$$

R_U = peak response from the Sample solution

R_S = peak response from the Standard solution

C_S = concentration of the Standard solution (mg/mL)

L= label claim (mg/tablet)

V= volume of Medium, 1000 mL

- **Acceptance criteria**: NLT 75% of the labeled amount of esomeprazole (C17H19N3O3S) is dissolved.

5.5.2.7. **In-vitro floating testing:(208)**

5.5.2.7.1. Floating lag-time:

The time between the introduction of the tablet into the dissolution medium and its floating on the surface of the dissolution medium is termed as floating lag time.

- **Procedure**: introduce one tablet into the dissolution media and estimate the time needed for the tablet to float.
- **Acceptance limit**: NMT 2 min (215).

5.5.2.7.2. Total floating time:

The entire time during which the tablet remains afloat on the surface of the dissolution media is termed as the floating time.

- **Procedure:** The test is usually performed by immersing the tablet in 900ml of the dissolution media maintained at 37 °C, using USP dissolution apparatus II (paddle). Then estimate the time since the tablet floats until it sinks in the dissolution media.
- **Acceptance limit**: NLT 24 h (216)

5.5.2.8. **Swelling-index test (water uptake) (105):**

Swelling test or water uptake (WU) test is important for evaluating the swelling behavior of the swollen polymer in the floating dosage form, and so dissolution behavior could be explained.

- **Procedure:** The test is done by immersing the tablets in 900 ml dissolution media at 37 °C, and determining the weight change at regular time intervals until 24h.
- **Calculations**: WU (swelling index) at each time interval is measured in the terms of percentage of weight gain, as given by the following equation:

$$\% \mathbf{WU = (Wt - Wo) / Wo \times 100 \%}$$

in which: (Wo) is the initial weight of the tablet.

(Wt) is the average weight of the dosage form at time t.

5.6. Kinetic modeling of the selected formula.

To determine the mechanism by which CLA and MTZ are released from the matrix of the floating sustained release layer of our novel bilayer tablet, dissolution data are fitted to different mathematical models (***refer to section 1.6***) using DDSOLVER program, the best model is then selected according to the value of R^2 or $R^2_{Adjusted.}$

Part six.

6. Results and Discussion

6.1. Development of analytical procedure for concurrent determination of CLA and MTZ (for assay and dissolution).

6.1.1. Selection of suitable HPLC conditions and reagents

6.1.1.1. **Selection of suitable wave-length for detection of both CLA and MTZ:**

Measuring the absorbance of CLA and MTZ, proceeding exactly as directed in section 5.1.3.1, gave the following results:

- The maximum absorbance points for ***metronidazole*** were as follow:

  330nm ⟹ A=1.246

  228nm ⟹ A=0.597

  210nm ⟹ A=0.78

- The maximum absorbance points for ***clarithromycin*** were as follow:

  205nm ⟹ A=0.417

  210nm ⟹ A=0.343

So the choice was for λ=210 for HPLC-analysis of both CLA and MTZ, as both substances have a maximum absorbance at this wavelength.

6.1.1.2. ***Selection of HPLC-column* (173) (217):**

High resolution is typically required when separating samples with many components. Column internal diameter is chosen depending on analytical requirements and system limitations. Usually 4.6-mm internal diameter columns are used when working with traditional HPLC systems. The particle size of the stationary phase affects the efficiency of a separation; sure high efficiency is needed especially when separating few components. Smaller particle size gives higher efficiency; but flow rate needs to be adjusted downward. The most widely used particle size is 5 µm in diameter. Select hydrophobic stationary phases (C18, C8) when differences in analyte hydrophobicity are large and can be exploited to affect a separation.

Depending on the columns in the single monographs of USP, and on the fact that we have two different hydrophobicity analytes (CLA and MTZ), and different hydrophobicity matrix components, the choice was for ***C18, 4.6-mm × 15-cm; 5-µm (packing L1).*** As in this column the hydrophilic analyte (MTZ) will be eluted early and away from the hydrophobic analyte (CLA) which elution would be later; so better separation will be obtained.

6.1.1.3. **Selection of suitable mobile phase (program, components and percentages), and suitable injection volume, column temperature and flow rate:**

Different components were used, taking into consideration to use organic and inorganic solvents (buffer), as different polarity analytes have to be separated.

Different concentrations of the mobile phase components were used, and each time the chromatograms were evaluated based on the separation (resolution), shape (almost sharp and symmetrical) and purity of peaks resulted.

Different programs of mobile phase were used, isocratic and gradient.

In parallel to changing in the mobile phase, changing in injection volume (10, 20 and 50µm), changing in column temperature (40 and 50ºC) and changing in the flow rate (1000 to 2000) µl/min took place.

After many trials and many changes applied to the HPLC conditions, good shape, well separated peaks, definite retention times for each substrate in the dosage form (MTZ, CLA and EZO) were obtained. Samples of chromatograms obtained during the development and after the final approved development for the analytical method are shown in (Figure 6.1) and (Figure 6.2) respectively. The optimal HPLC conditions are listed below:

- Selected chromatographic conditions:

Mode: LC

Flow rate: 1 ml/ min (1000µl/min).

Injection volume: 20µl.

Temperature: 50ºC.

Buffer 0.017M, Adjust the pH to 4 by phosphoric acid.

Mobile phase gradient is described in (Table 6.1):

Table 6.1: Gradient of the mobile phase in the analytical procedure of CLA and MTZ.

Time	Acetonitrile	Buffer
Zero	10	90
3	10	90
17	65	35
18	65	35
19	10	90
20	10	90

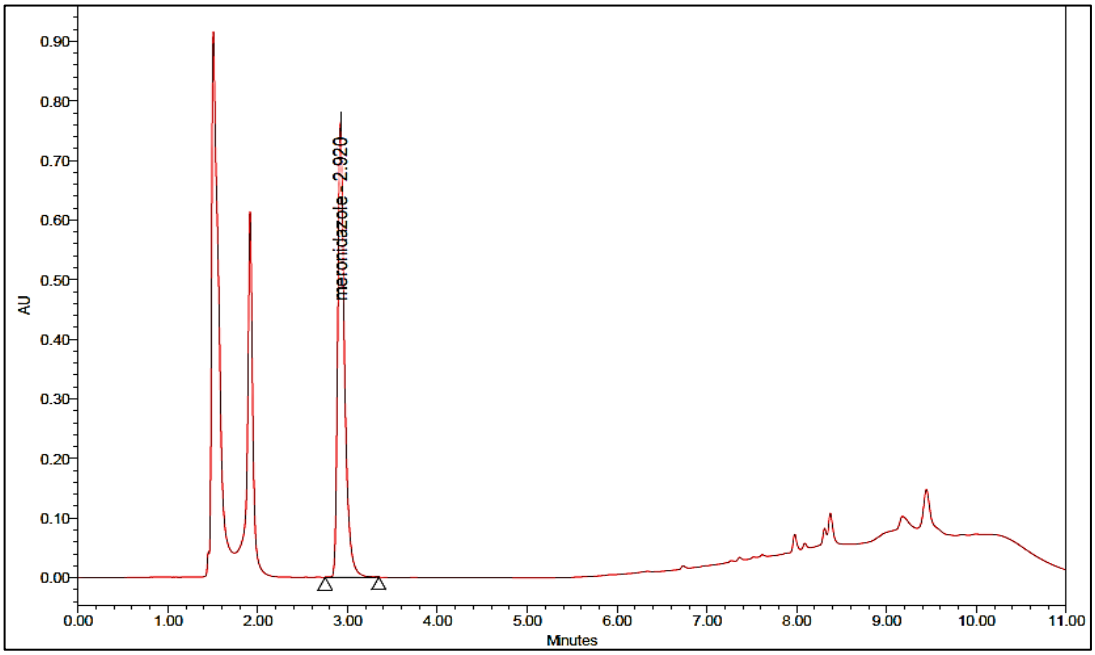


Figure 6.1: Sample of chromatogram **during** development of the analytical method.

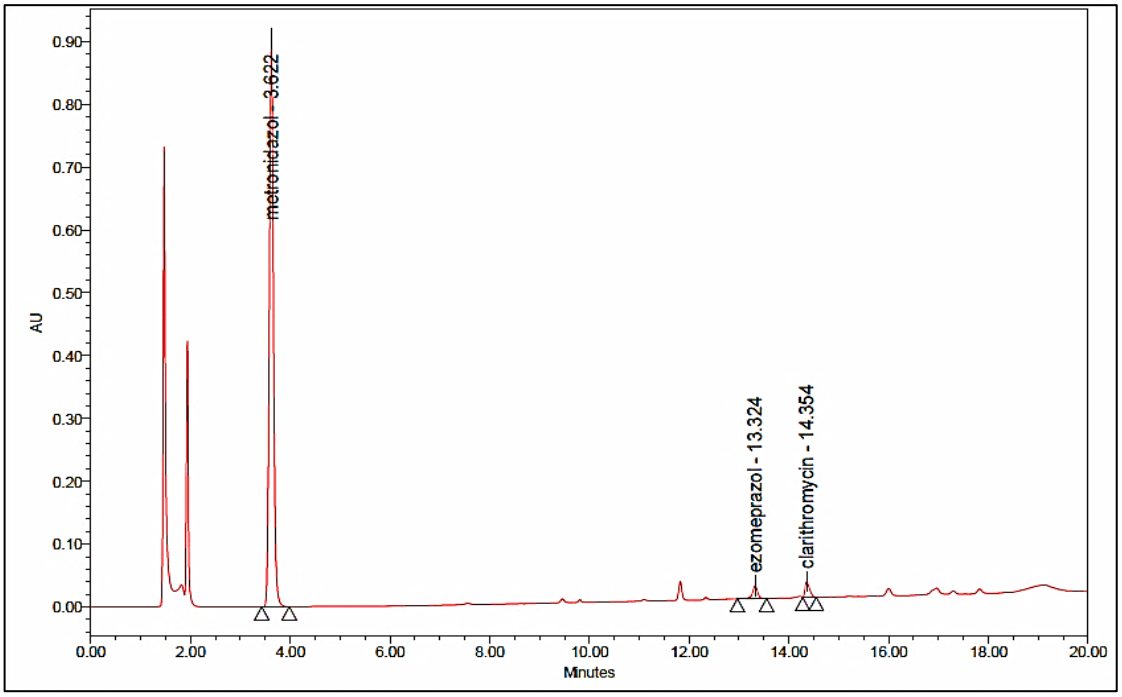


Figure 6.2: The chromatogram obtained after the **final approved** development of the HPLC method.

6.1.2. Dissolution media:

***Selection of dissolution media was based on**:*

1. Stability of the active ingredients (CLA and MTZ):

After comparison the peak response of freshly prepared samples and stability samples (stored 3 hours) that were prepared in different dissolution media, the following results were observed:

- MTZ was not stable at low pH media (0.1N HCL), but was more stable at higher pH HCL media. Furthermore it was *very stable* at sodium acetate media, either at pH 4 or pH 5.
- CLA had poor stability at HCL media with different pH-range (1-5).

  On the other hand, its stability was poor at sodium acetate media with pH 4, but an excellent stability at sodium acetate media with pH 5.

So the dissolution media selection regarding the stability of the components was (*sodium acetate media with pH 5*), due to the high stability of both CLA and MTZ in it. This result

was consistent with the aim of incorporating EZO within the tablet, which was for increasing the pH of the stomach to 5 inorder to protect CLA from degradation at low pH.

2. Solubility of the active ingredients (CLA and MTZ):

This test was applied to the dissolution media that passes the stability test. After dissolving the maximum dose of the drug components in 200ml dissolution media (stock solution), and dilute a sample from this stock to have a concentration of 0.25mg/ml CLA and 0.3mg, then compared the response of this sample to standard solution having the same concentrations (no stock here), we found the following results:

% Recovery = (Peak response of diluted sample/Peak response of standard) x100%

% recovery CLA = (215678/222267) X 100%= 97%

% Recovery MTZ= (8382762/8069070) X 100%=103%

The percentage of recovery from both diluted solutions indicated their complete dissolution in the media. The slight difference in their recovery is due to the higher solubility of MTZ.

As a result, sodium acetate media with pH 5 has been approved to be the dissolution media for the release of MTZ and CLA based on stability and solubility studies.

Furthermore, the dissolution media could be used as a diluent for the assay and dissolution tests, based on the stability and solubility tests, and after comparing the results of standard solutions prepared by dissolving in mobile phase, and another dissolved in dissolution media, they were very close and the alternative use of both diluents was accepted.

6.2. Validation of analytical procedure

The method was validated according to USP-NF 36 and ICH Q2 (R1) guidelines for the quantitation of drug substance in dosage forms. The tests were assessed in our work were: the linearity, accuracy, precision, range, specificity and selectivity, in order to ensure that the method is reliable.

6.2.1. Linearity

Linearity was assessed by analyzing seven standard solutions of different concentrations covering the range 10% -160% of the nominal standard concentration in the assay and dissolution methods. The results are listed in (Tables 6.2 and 6.3**).** The calibration curves were plotted and shown in (Figures 6.3 and 6.4).

The linearity of the method was established from the correlation coefficient (R^2) of the best fit least squares linear regression curve, which was obtained by plotting peak areas versus known concentrations of MTZ and CLA. For these studies, an R^2 value of > 0.990 was considered appropriate to demonstrate the linearity of the analytical method.

Clarithromycin curve: The calibration curve was found to be linear over the concentration range stated, with an **R^2 of 0.9998** and the equation for the line was (**y = 964.98x – 1361.6).**

Metronidazole curve: The calibration curve was found to be linear over the concentration range stated, with an **R^2 of 0.9998** and the equation for the line was (**y = 25621x—19720).**

Table 6.2: Linearity results of HPLC method validation (Clarithromycin).

Concentration %	St. Conc. µg/ml Clarithromycin	Peak area- 1	Peak area-2	Average
160%	443.2	423786	424385	424085.5
120%	332.4	321802	323733	322767.5
100%	277	266824	266621	266722.5
80%	221.6	214582	210862	212722
50%	138.5	129431	128375	128903
25%	69.25	66239	66249	66244
10%	27.7	25845	25765	25805

Table 6.3: Linearity results of HPLC method validation (Metronidazole).

Concentration %	St. Conc. µg/ml Metronidazole	Peak area-1	Peak area-2	Average
160%	532.8	13536480	13565188	13550834
120%	399.6	10307923	10287017	10297470
100%	333	8591386	8551652	8571519
80%	266.4	6852399	6794230	6823315
50%	166.5	4177941	4162606	4170274
25%	83.25	2110005	2100523	2105264
10%	33.3	843358	841235	842296.5

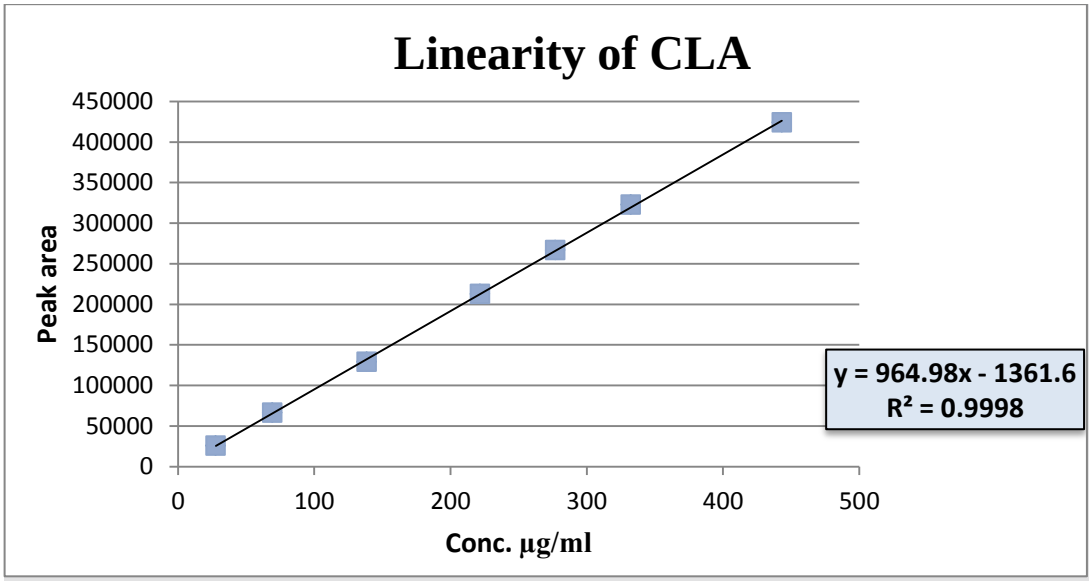


Figure 6.3: Linearity graph of Clarithromycin for HPLC method validation.

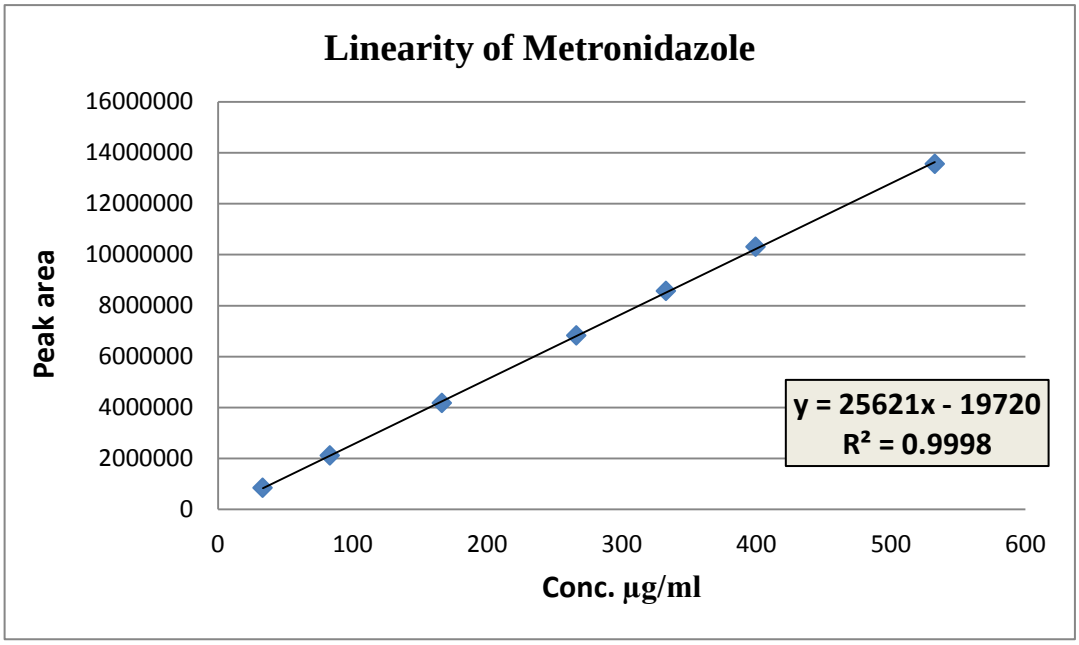


Figure 6.4: Linearity graph of Metronidazole for HPLC method validation.

6.2.2. Accuracy

The percentages recovered from spiked samples for five concentrations covering the range 10%-160% were calculated.

An acceptance criterion for accuracy was considered to have a recovery of 100±2.0% (assay) or 100±5% (dissolution).

The resultant values for recovery complied with the acceptance criteria. Results are illustrated in (**Tables 6.4 and 6.5**) and shown in **(Figures 6.5 and 6.6)** with an $\mathbf{R^2}$ value of **0.9998** for both CLA and MTZ.

Table 6.4: Accuracy results of HPLC method validation (Clarithromycin).

Target Conc. **(%)**	**Theo. Conc. (µg / ml) Clarithromycin**	**Conc. after Spiking (µ/ml) Clarithromycin**	**Name of sample**	**Spiked Sample Response**	**Recovery (%)**
160	443.2	425.6	1.1	411563	101.1
			1.2	414819	101.8
			1.3	413920	101.6
100	277	266	2.1	257317	100.4
			2.2	263824	103
			2.3	253014	98.8
50	138.5	133	3.1	127278	102.8
			3.2	129416	104
			3.3	128991	103
25	69.25	66.5	4.1	63423	99.7
			4.2	65204	102.5
			4.3	66165	104
10	27.7	26.6	5.1	26063	104.8
			5.2	25404	102.5
			5.3	25939	104.6

Table 6.5: Accuracy results of HPLC method validation (Metronidazole).

Target Conc. **(%)**	**Theo. Conc. (µg / ml) Metronidazole**	**Conc. after Spiking (µ/ml) Metronidazole**	**Name of sample**	**Spiked Sample Response**	**Recovery (%)**
160	532.8	480	1.1	12225715	99.9
			1.2	12312165	100.8
			1.3	12287052	100.6
100	333	300	2.1	7627495	98.8
			2.2	7810864	101.1
			2.3	7559596	97.9
50	166.5	150	3.1	3878555	103
			3.2	3866475	102.9
			3.3	3866654	102.9
25	83.25	75	4.1	1940494	102
			4.2	1926180	101.2
			4.3	1940620	102
10	33.3	30	5.1	769989	101.5
			5.2	775767	102.2
			5.3	757297	99.8

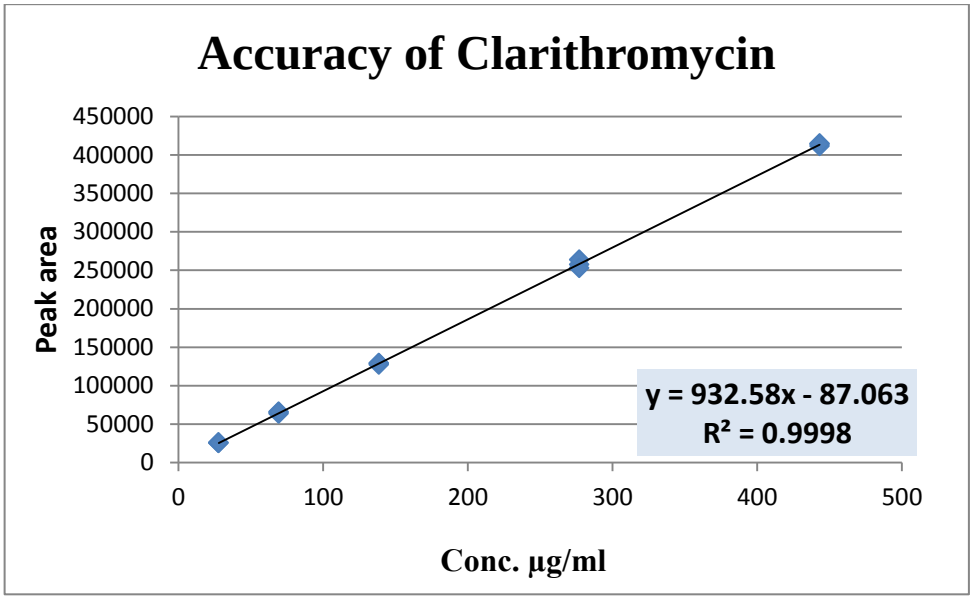


Figure 6.5: HPLC method validation accuracy regression line (Clarithromycin).

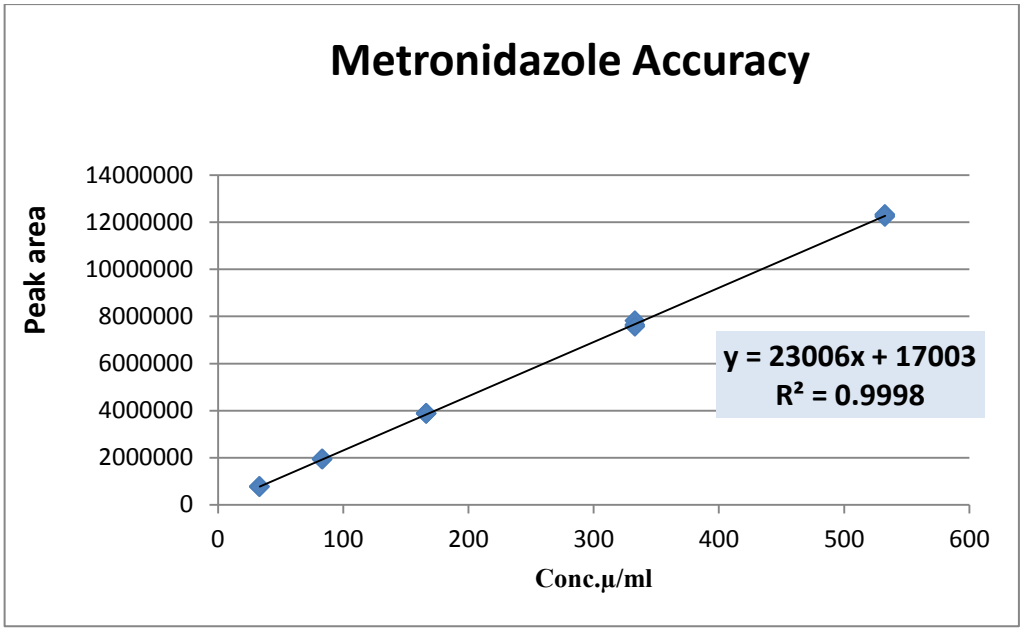


Figure 6.6: HPLC method validation accuracy regression line (Metronidazole).

6.2.3. Precision:

The precision is the ability of a method to produce precise analytical results from a series of measurements of the same homogenous sample under prescribed assay conditions. The relative standard deviation (% RSD) of a series of measurements is usually used to assess

the precision of an analytical method. The % RSD is calculated using the following equation.

$$\% RSD = \frac{\sigma}{X} * 100\%$$

Where,

σ = Standard deviation around the mean of a set number of samples (calculated using nonbiased or n-1 method).

X = Mean of the peak height ratio responses for a set number of samples

The precision of our method was assessed by repeatability. The repeatability was determined by analysis of ***15*** determinations at ***5*** concentrations of the test concentration. The repeatability results obtained are shown in (Tables 6.6 and 6.7**).** The results revealed that % RSD values were within the acceptable limits thus the method is repeatable for the analysis of CLA and MTZ together.

Acceptance criteria:

A value for % RSD < 2 % was set as an acceptable limit.

Table 6.6: Precision results of HPLC method validation (Clarithromycin)

Name of sample	No. of injection	Conc. µg/ml	Response	Average Response	SD of response	% RSD
Sample-1	1	425.6	411563	413920	1681	0.4
	2		414819			
	3		413920			
Sample-2	1	266	257317	258051	5442	2
	2		263824			
	3		253014			
Sample-3	1	133	127278	128561	1131	0.88
	2		129416			
	3		128991			
Sample-4	1	66.5	63423	64930	1391	2
	2		65204			
	3		66165			
Sample-5	1	26.6	26063	25802	350	1.3
	2		25404			
	3		25939			

Table 6.7: Precision results of HPLC method validation (Metronidazole)

Name of sample	No. of injection	Conc. µg/ml	Response	Average Response	SD of response	% RSD
Sample-1	1	480	12225715	12274977	44471	0.36
	2		12312165			
	3		12287052			
Sample-2	1	300	7627495	7665985	129980	1.69
	2		7810864			
	3		7559596			
Sample-3	1	150	3878555	3870561	6923	0.17
	2		3866475			
	3		3866654			
Sample-4	1	75	1940494	1935764	8300	0.48
	2		1926180			
	3		1940620			
Sample-5	1	30	769989	767684	9448	1.23
	2		775767			
	3		757297			

6.2.4. Range

The range is the interval between the upper and lower concentrations of analyte in the sample that have been demonstrated to have a suitable level of precision, accuracy, and linearity. It confirms that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within these concentrations (lower and upper limits).

After applying linearity, precision and accuracy tests to the analytical method, it has been approved that the acceptable range the method was from 10%-160% of the nominal concentrations, which are (27.7- 443.2 µg/ml) for CLA, and (33.3- 532.8 µg/ml) for MTZ.

6.2.5. Selectivity Test:

It measures the degree of interference from materials other than active material, such as excipients, impurities, and degradation products and diluents. It should be ensuring that the peak response is due to a single component only. To validate for selectivity, the interference from excipients and the Interference with degradants were determined.

The interference with excipients and diluent was determined by injecting the excipients alone (placebo sample), the diluent alone, standard solution of 100% concentration and a spiked sample. Then the chromatograms were compared, and the %recovery of the spiked sample was calculated. The chromatograms are shown in (Figures 6.7 and 6.8), it is observed that there is no interference with the excipients or the diluent.

$$\% \text{ Recovery} = \frac{\text{Peak area}_{\text{sample}}}{\text{Peak area}_{\text{standard}}} \times \frac{\text{Conc.}_{\text{standard}}}{\text{Conc.}_{\text{sample}}}$$

% Recovery of CLA = (258051/266722) X (277/266) X 100%

= 100.7 %

% Recovery of MTZ = (7665985/8571519) X (333/300) X 100%

= 99.3 %

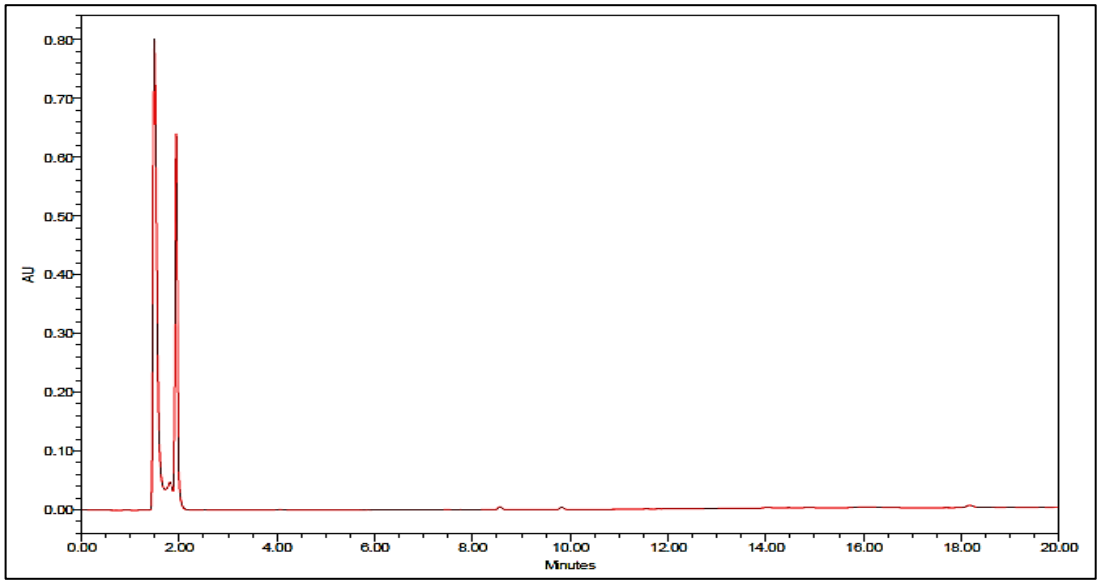


Figure 6.7: Blank (diluent) sample chromatogram/ selectivity.

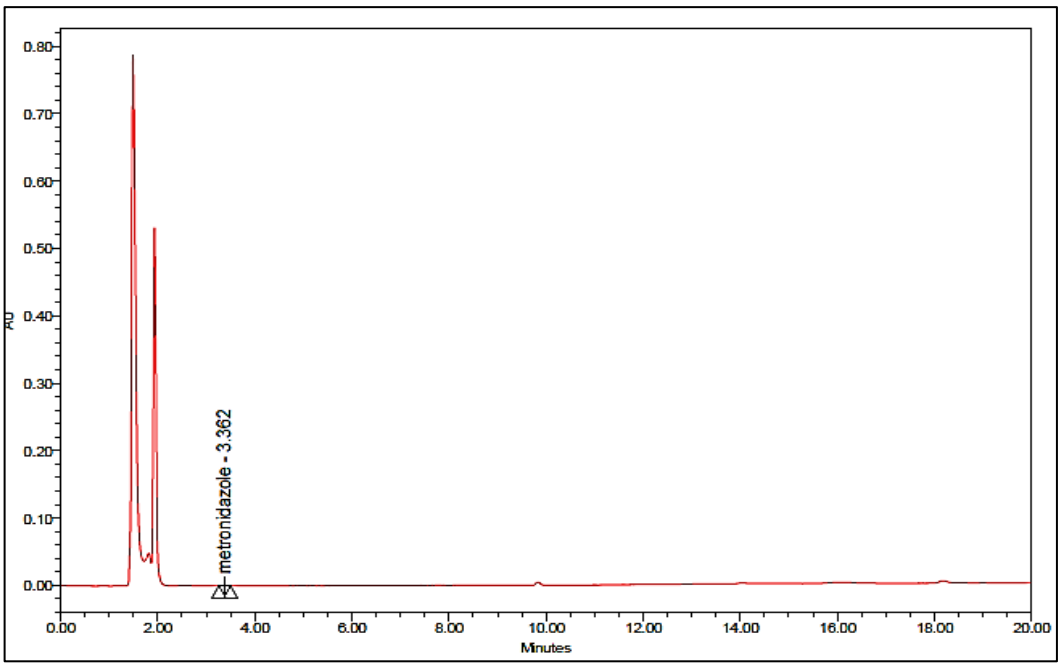


Figure 6.8: Placebo sample/Selectivity test.

6.3. Formulation development:

6.3.1. Selection of the optimal formula and the optimal manufacturing process

The primary objectives of the formulation studies were optimization of the separation between the two layers, optimization of floating lag time and total floating time and optimizing the release of both CLA and MTZ in a synchronous, sustained manner from the dosage form. These objectives were achieved by suitable polymers used and by optimizing the ratios of the gas generating agents to the polymers. In addition different manufacturing procedures were applied in order to achieve the best formula performance.

The results of different formulations generated are shown and discussed below, based on the In-vitro floating performance of the formulations, separation time of the rapidly dissolving layer (RDL) and the integrity of the matrix. These tests are critical steps in the primary acceptance of the formulations. As mentioned before the acceptance criteria for these tests are: Separation time of the rapidly dissolving layer is NMT 2 minutes (187), Floating lag time is NMT 2 minutes (218) (188) and Total floating time (without disintegration) is NLT 24 h.

It is important to mention that in our study we followed the formulation by trial method in which we tried to optimize the floating characteristics of the different formulas and by using the different manufacturing methods (direct compression, wet-granulation and dry granulation). The aim was to obtain a slow synchronous release of both MTZ and CLA. We tried first to include the MTZ and CLA in the same position within the matrix in the direct compression and the wet granulation procedures, and for those formulas that passed the floating test we tested the dissolution.

We found that the release of MTZ was faster than CLA in both direct compression and wet granulation, due to its high solubility. So we thought to include MTZ inside the granules and CLA outside to retard MTZ release. At first we tried the wet granulation

method but we didn`t acquire good release. However changing to dry granulation did provide the acquired release. This could be justified by the presence of PVP-K30 binder within the granules of the wet granulation, which increased the binding between their components and decrease the entry of dissolution media and hence delayed MTZ release to great extent. On the other side in the formulations of dry-granulation the granules had been formulated without using binder solution, and so they decreased the release of MTZ but less than that in the wet granulation.

6.3.1.1. **Wet granulation formulas:**

Table 6.8: Wet granulation results of Floating properties, matrix integrity and separation of RDl.

Formula	Separation of RDL (NMT 2min)	FLT (NMT 2min)	TFT NLT 24 h.	Matrix integrity and behavior.
W1	28 sec.	6.5 min	Intermittent (floated and sank)	Weak, continue to floated and sank then disintegrated after 2 h.
W2	1 min.	1min+7sec.	20 min	Weak, disintegrated (20 min).
W3	35 sec.	1min+40sec.	10min.	Weak, disintegrated (10 min)
W4	1min.	5 min.	15min.	Weak, disintegrated (15 min)
W5	1min+10sec	4.5 min	2min.	Coherent integrity but couldn`t float (sank)
W6	40 sec.	58 sec.	> 24h *	Fairly swollen and coherent matrix.
W7	1+ 20sec.	6min.	1min	Coherent integrity but couldn`t float (sank)
W8	28sec.	42 sec.	> 24h *	Good swollen and coherent matrix
W9	18 sec.	33 sec.	30 min	Weak, disintegrated (30 min)
W10	25 sec.	40 sec.	20 min	Weak, disintegrated (20 min)
W11	22 sec.	30 sec.	5 min	Weak, disintegrated (5 min)
W12	27	34	40 min.	Weak, disintegrated (40 min)
W13	21	26	> 24 h *	Good swollen and coherent matrix.

*: See dissolution (drug release) results and graphs in section 6.4.2.6.

W1-W4:

- Using HPMC-K4M alone as a matrix forming polymer resulted in rapid disintegration of the tablets; this could be explained by failure of the polymer to provide a robust gel layer within suitable time, that could entrap the generated CO_2 for longer times within its

matrix, and hence the gas generated caused erosion and disintegration of the tablet instead of floating(205).

- Distributing the gas forming agent into two portions (W4) within and around the granules accelerated the disintegration time, as gas formation was faster due to its easier contact with the dissolution media when distributed within the matrix outside the granules.

W5-W8:

- Replacement of HPMC-K4M took place with higher viscosity hydrophilic polymer (HPMC-K100M) to get better matrix integrity that could entrap the CO_2 inside for prolonged period and with a hydrophobic polymer (EC) that caused gradual ingress of the media to the system and with its low density it decreased the system density and enabled its floating (153). Also addition of citric acid in a ratio of 1: 4 of sodium bicarbonate to enhance the floating lag time has been done.
- Incorporating of half of the gas generating agents quantity inside the granules (W5) caused failure of the floating of the system , this was because the quantity distributed within the matrix couldn`t generate a sufficient quantity of CO_2 that is required for decreasing the system density and causing its buoyancy.
- Increasing the quantity of HPMC-K100M over 300mg/tablet (W7) causing failure of floating of the system. This could be justified by the insufficient quantity of CO_2 gas generation that could attain the required floating force of the system by decreasing its density below that of the media. So increasing the amounts of gas generating agents took place in W8 in parallel with the hydrophobic polymer EC to prevent tablet disintegration by allowing gradual ingress of fluid media to the system.
- W6 and W8 showed good floating properties. In these formulations immediate generation of CO_2 from gas generating agents occurred upon interaction with the dissolution media, the generated gas entrapped within the swollen polymer matrices. As a result ,expansion of

the polymer matrix occurred causing tablet density to decrease lower than that of the dissolution media and leading to rapid floating time and longer total floating time(188).

W9-W10:

- Additional change was incorporating of lactose within the matrix of the system and within the granules, in order to accelerate the release rate of W6 and W8 (results are detailed in the next section 6.3.2.2.

As it is known lactose could be added to the controlled release dosage forms to enhance the release. Lactose is water soluble channeling agent, when come in contact with fluid, it will dissolve leaving behind channels through which GIT fluid gains access into the tablet matrix causing the active drug to dissolve and diffuse out of the system(219).

- Using lactose with different ratios in the formulas, either 5.5% (W9) or 1.5% (W10) gave undesirable effect of the matrix of the system, caused its rapid disintegration. This could be explained as follow; the dissolving of lactose within the media was much more faster than the gelling forming capacity of the polymers, this cause faster formation of CO_2 inside the matrix that couldn't remain entrapped within a gel layer, so it diffused out quickly causing its disintegration instead of floating.

W11-W13:

- Different grades of HPMC polymers (K4M and k15M) have been incorporated beside HPMC-K100M to enhance the release. These polymers have different relaxation, swelling and gel forming capacities. By suitable tuning for the polymers ratios and grades within the formula, floating properties could be maintained and the release could be optimized(220).
- Using HPMC-K15M to HPMC-K100M in ratio 1: 1 (W11), then using HPMC-K4M to HPMC-K100 in ratio 1:4 (W12), resulted in weak integrity matrices that disintegrated rapidly after introduction to dissolution media (W12 was better than W11). This was

caused by the rapid gas generation occurred in parallel with the replacement of portion of the amount of the high viscosity (and high M.Wt) HPMC-K100M with lower viscosity grades that have lower ability to form a coherent gel-framework matrix that could entrap the generated CO_2 inside, so instead of entrapment of the CO_2, diffusion occurred and cause disintegration instead of remained afloat.

▪ W13: Small modification was applied by decreasing the amounts of gas generating agents, gave good floating , this was because the quantity of CO_2 generated was enough to be entrapped within the swollen matrix leading to decrease the system density and consequently its floating.

6.3.1.2. **Direct compression formulas:**

Table 6.9: Direct compression results of Floating properties, matrix integrity and separation of RDL.

Formula	Separation of RDL. (NMT 2min)	FLT. (NMT 2min)	TFT. (NLT 24 h)	Matrix integrity and behavior
D1	40 sec.	No floating	------	Weak, disintegrated totally (10 min).
D2	1min and 50 sec.	5 min.	>1 h	Coherent integrity but couldn`t remain floating (sank).
D3	42 sec.	1 min+ 40 sec.	30 min.	Weak, disintegrated totally (30 min).
D4	2 min+ 40 min	10 min	30 min	Coherent integrity but couldn`t remain floating (sank).
D5	10 min	7.5 min	17 min	Coherent integrity but couldn`t remain floating (sank).
D6	21 sec	30 sec.	1 h.	Weak, disintegrated totally (1h).
D7	45 sec.	52 sec.	> 24 h. *	Fairly swollen and coherent matrix.

- **D1, D3 and D6:**

The three formulations failed to remain floating and disintegrated rapidly. Among these the faster disintegrating formula was (D1). This was because its matrix involved the low viscosity hydrophilic polymer which is HPMC-K4M, this polymer has lower swelling, gel forming capacities than higher viscosity polymers, so it couldn't form a good matrix gel barrier layer that could entrap the generated CO_2 inside its framework, and hence the generated gas caused rapid disintegration rather than floating. Despite changing the polymer to a higher grade one that is HPMC-K100M in both D3 and D6, and the slight increase in the EC to HPMC-K100M ratio in D6; the parallel addition of citric acid to both formulas caused a disintegration to this system, this could be justified by the rapid formation of CO_2 gas upon interaction of the dissolution media in a faster manner than the formation of the polymer barrier gel layer, the first quantity generated of the gas cause floating to the system, but when this quantity increased before swelling of the polymer, it caused polymer erosion and disintegration to the system.

- **D2, D4 and D5:**

All these formulas failed to remain floating; they floated for some period of time then sank again. Their long floating lag time indicated that the generation of CO_2 inside the systems was slow, this may be caused by the amount of the hydrophobic polymer (EC) that was larger than the hydrophilic polymer (HPMC-K100M), this hydrophobic polymer prevents enough media to ingress to the systems, and so the slow generation of CO_2 inside these systems caused the elongation of the floating lag times, but once being afloat, the polymers swelled more and more by up taking water, their weights increased but no enough CO_2 inside the matrix to maintain low density of the system that is needed for floating , so they sank again after short period of floating time.

Hint: in D2 formula the problem was in the amount of the gas generating agents, as no citric acid was incorporated, in addition to the amount of the hydrophobic polymer.

- **D7:**

This was the most optimized floating formula with the best floating properties among the direct compression formulas; containing 20.5% HPMC-K100M, 16.5% EC, 13.1% NaHCO3 and 3.25% citric acid. This formula passed the in-vitro floating testing and the rapidly dissolving layer separated quickly. So further in vitro dissolution testing was performed, see in-vitro dissolution results.

6.3.1.3. **Dry-granulation:**

Table 6.10: Dry-granulation results of Floating properties, matrix integrity and separation of RDL.

Formula	Separation time of RDL (NMT 2min).	FLT (NMT 2 min).	TFT (NLT 24 h).	Matrix integrity and behavior
DG1	10 sec.	10 sec.	> 24 h.	Good swollen and coherent matrix
DG2	12 sec.	15 sec.	> 24 h.	Good swollen and coherent matrix
DG3	15 sec.	18 sec.	> 24 h.	Good swollen and coherent matrix

- **DG1-DG3:**

The three formulations have excellent floating properties. Having 23.4%HPMC-K100M, 16.8% EC, and 12.5% NaHCO3 with NaHCO3: citric acid ratio (4:1) gave the optimized matrix formulas with optimized floating properties. Which means that the polymers matrix were able to swell in an optimum rate that could entrap the required quantity of the generated CO_2 at the suitable time, leading to floating instead of disintegration, and the gradual swelling in parallel to the gradual CO_2 generation maintain the system afloat for long period.

6.4. Quality control tests of the selected formula (DG2).

6.4.1. Pre-compression tests / In process control (IPC).

6.4.1.1. **Angle of repose (θ):**

The angle of response **was 25º**, which indicates excellent flow properties (211).

6.4.1.2. **Carr's index(Compressibility Index):**

- Compressibility Index = (0.606-0.487) / 0.606 X 100%

  = 19.64 %

This value indicates *Fair-flowability.* (211),

6.4.2. Post-compression tests:

6.4.2.1. **Description of the tablet:**

- *The tablet is:*
  - White.
  - Round-shaped with flat surfaces.
  - Odorless.
  - Bitter taste.
- *Tablets dimensions:*
  - Thickness: 9.3 mm
  - Diameter: 12.96 mm.

6.4.2.2. **Hardness:**

Hardness results (average for 10 tablets) of DG2 tablets are illustrated in (Table 6.11).

Table 6.11: Hardness results of formula DG2.

Hardness (N)	71.97
SD	4.64
% RSD	6.44%

6.4.2.3. **Weight variation test:**

The test was applied to randomly 10selected tablets.

From the results (Table 6.12), it was found that the tablets complied with the requirements for weight variation test of tablets as described in the BP, which recommends that NMT 2 tablets deviates from the average weight of more than 5%, and none deviates by more than twice that percentage.

Table 6.12: Weight variation results of formula DG2.

Tablet nominal weight	1487 mg
Average tablets` weight	1494 mg
% Deviation (min-max)	1.2% (0.27 % - 2.28 %)

6.4.2.4. **Friability test:**

The test was performed on 10 tablets selected at random, and preceded as described under section 5.2.3.4.

% Friability = (Wo-W)/ Wo X 100%

= (14.962g – 14.847) / 14.962 X 100%

= **0.77 %**

The result complied with the USP acceptance criteria mentioned for the test (< 1%).

6.4.2.5. **Assay test.**

The assay was calculated using the following equation:

$$\text{Assay} = (R_U / R_S) \times (C_S / C_U) \times 100\%$$

As the concentrations of standards and samples were equal, then we can omit C_S/C_U.

6.4.2.5.1. Assay of CLA and MTZ:

The assays of both CLA and the MTZ were obtained using the developed HPL method using the following equation:

$$\text{Assay} = (R_U / R_S) \times 100\%$$

- Assay (MTZ) = (7810864 / 8551652) X 100%

  = **91.34 %**

  This result complied with the USP-36 acceptance criteria (90 – 110) %.

- Assay (CLA) = (258051 / 266722) X 100%

  = **96.75 %**

  The result complied with the USP-36 acceptance criteria (90-110) %.

6.4.2.5.2. Assay of EZO:

Omeprazole was used as RS for both tests assay and dissolution of EZO, because both tests measure the quantity of EZO and not the chirality of its structure, and both of omeprazole and EZO are isomers having the same chemical structure.

The assay of EZO was according to the USP-method, and the result was as follow:

- Assay = (1115042 / 1015660) X 100%

  = **109.78 %**

  The result complied with the USP-36 acceptance criteria of the test (90-110)%.

6.4.2.6. **In-vitro dissolution test**

6.4.2.6.1. Dissolution of MTZ and CLA from the SR-floating layer:

The drug release rate from floating gastroretentive systems , and from conventional SR systems of swellable matrix, is usually controlled by transport of the solvent into the polymer matrix, polymer swelling, , drug diffusion through the swollen polymer, the gel-layer thickness dynamics and polymer (matrix) erosion (187). Dissolution of hydrophilic

polymers occurs via two major processes, swelling and erosion. Initially rapid water uptake by the surface polymer will lead to dissolution of the surface incorporated drugs, so some burst release can take place. Upon gradual entry of the water into the matrix, swelling of the hydrophilic polymer and formation of gel layer, through which the drug will be released gradually usually by diffusion, will occur. Once the polymer reaches a disentanglement threshold, its dissolution occur and the release of the remaining drug will be controlled by erosion (another burst release could happen) (221).

When the swellable matrix system is in the dissolution media, three boundaries (fronts) control drug dissolution and release are formed, (Figure 6.9); The swelling boundary in which the rate of water uptake is the major factor affecting polymer swelling and drug dissolution, the diffusion boundary , in which the rate of drug release depends on diffusion from the swollen polymer, and the erosion boundary in which the rate of matrix erosion is the release controlling step(222).

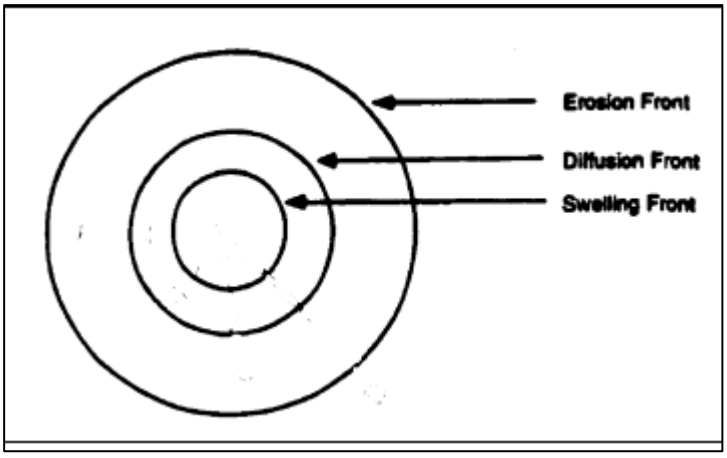


Figure 6.9: Different fronts of a swellable matrix tablet(222)

6.4.2.6.1.1. Drug release from wet-granulation formulas:

- **W6**: as shown in (Figure 6.10), the release from this formula was almost fast and couldn`t match the desired sustained release for both MTA and CLA. This could be justified by: (1) insufficient quantity of the high molecular weight HPMC-K100M that

forms the gel barrier that controls the drug release by gradual diffusion, (2) or insufficient EC quantity incorporated within the matrix; leading to rapid entry of dissolution media to the matrix, as a result weak swollen framework formed, with erosion rate faster than swelling, as a result the release from the eroded polymer instead of gradual diffusion through the swollen polymer controlled the release rate, leading to faster drug release.

(*Retardation for both MTZ and CLA is required*).

- **W8**: As shown in (Figure 6.11), the enhancement has been made to the formula again didn`t match the desired release. As increasing the quantity of the hydrophobic polymer in the matrix, prevented enough quantity of the dissolution media to contact with the MTZ inside the granules, and MTZ has to cross two barriers, the hydrophilic gel layer of the granules and the hydrophobic layer of the matrix, hence its released has been decreased more than desired.

On the other hand, the parallel increase in the quantity of the gas generating agents (NaHCO3 and citric acid) within the matrix increased the neutralization reaction between them, and lead to increase in the matrix porosity and pore diameter(223), and so more media was in contact with CLA and thus its release was greater than MTZ.

(*Accelerating for MTZ release is required*).

- **W13:** As shown in (Figure 6.12), incorporation of the low molecular weight polymer HPMC-K4M within the granules has been done, to enhance the release of MTZ. In parallel, a small decreasing has been done to the amounts of the gas generating reactants to slightly decrease the gas generation, and decrease the porosity formed inside the matrix, thus slightly decreasing the entry of dissolution media that would be in contact with CLA; and hence we could obtain a sustained-synchronous release of both MTZ and CLA.

We have noticed that minor enhancement for MTZ release was obtained, and minor decrease to CLA release was achieved.

(Further enhancement for MTZ release is still required).

Table 6.13: Percentages cumulative release of MTZ and CLA from wet-granulation formulations.

Time (h)	% cumulative drug release					
	W6		W8		W13	
	%MTZ	%CLA	%MTZ	%CLA	%MTZ	%CLA
0.5	40	35	11	13	-----	----
1	51	49	16	32	24	30
2	73	68	20	46	28	45
3	80	75	23	55	33	50
4	-----	-----	27	61	36	53
5	-----	-----	30	65	39	61

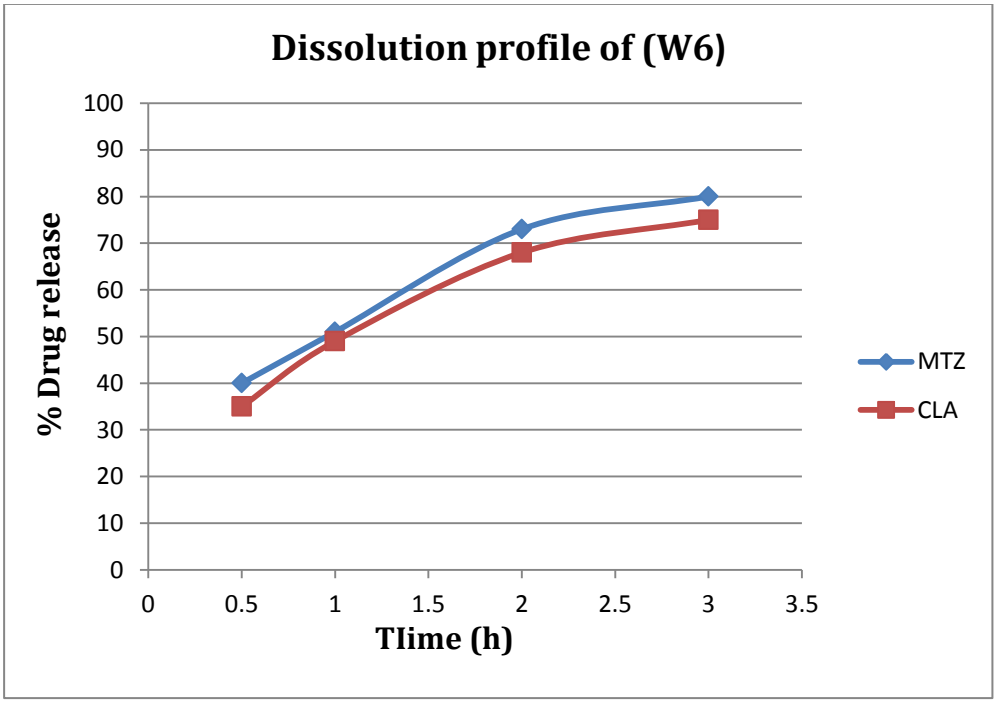


Figure 6.10: Dissolution profile of formula W6.

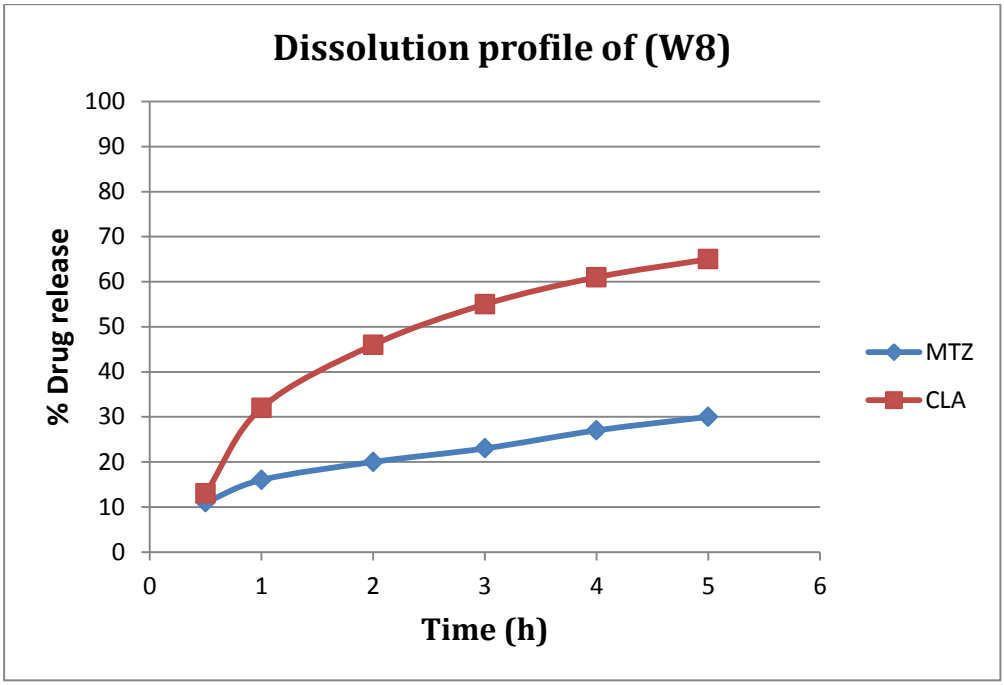


Figure 6.11: Dissolution profile of formula W8.

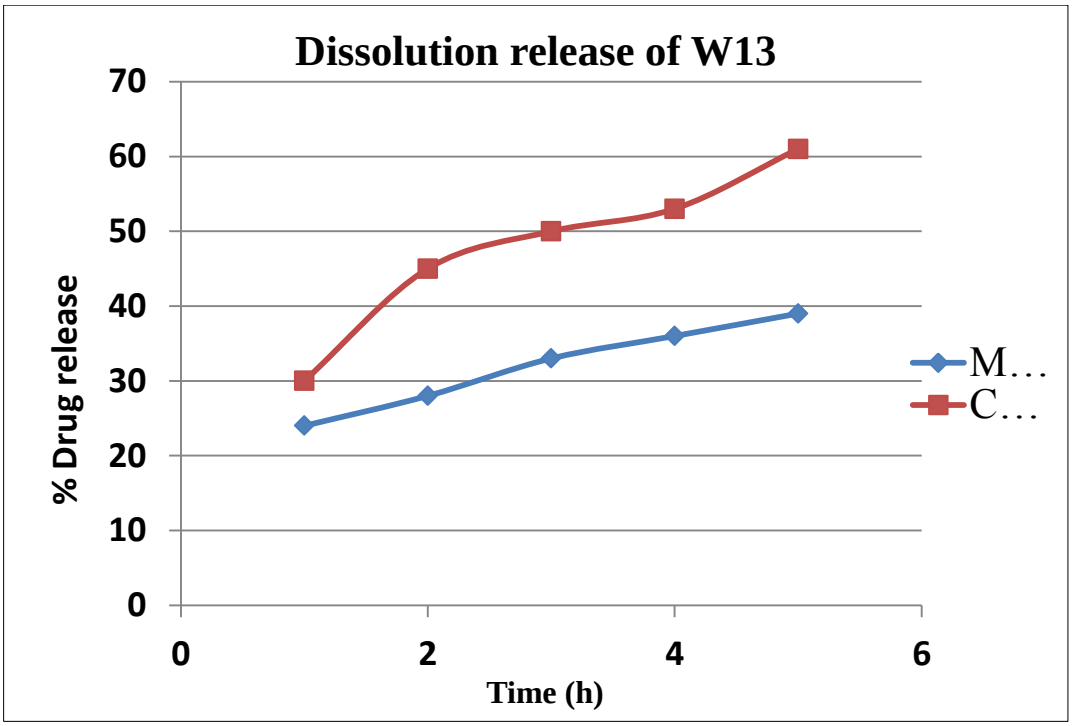


Figure 6.12: Dissolution profile of W13.

6.4.2.6.1.2. Drug release from direct compression formula

In our work, many formulas have been manufactured using direct compression technique, nearly all of them failed in the in-vitro floating tests and the integrity of matrix, except D7 which show good floating properties with acceptable matrix integrity.so further in-vitro drug release was done to this formula (D7), but the results were bad, as rapid drug release of both CLA and MTZ was achieved within the first hour! This could be justified by the absence of PVP within the matrix, as it act as a strong binder between matrix components and hence decrease the release.

On the other hand the release of MTZ was greater than the release of CLA; this could be justified by the higher solubility of MTZ, and its incorporation without granulation in the matrix together with CLA, so its dissolution upon contact with the dissolution media was faster than that of the less soluble CLA, as seen in (Figure 6.13 and Table 6.14).

We concluded from these trials that the direct compression technique was not suitable for designing a single synchronous- SR system for two drugs with different solubility.

Table 6.14: Percentage accumulative release of MTZ and CLA from Direct compression formula D7.

Time (min)	% cumulative drug release from D7	
	% MTZ	%CLA
15	40	35
30	65	51
60	95	64

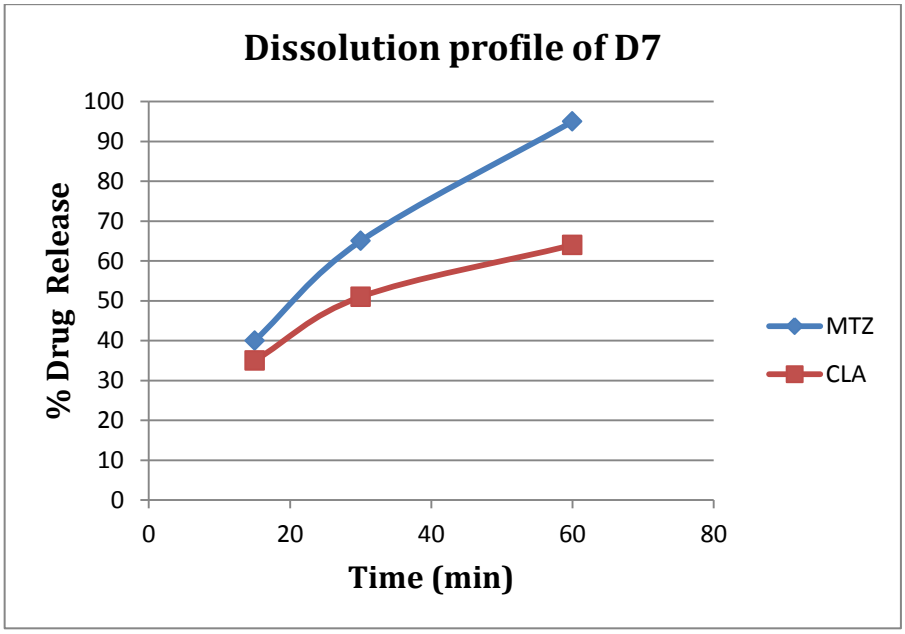


Figure: 6.13: Dissolution profile of formula D7.

6.4.2.6.1.3. Drug release from dry-granulation formulas:

The drug release from the three formulas DG1, DG2 and DG3 was almost synchronous for both MTZ and CLA; this goal was one of the most difficult goals to be met with such difference in the solubility of the drugs. Results are shown in (**Table 6.15**) and (**Figures 6.14, 6.15 and 6.16**).

Regarding the drug release rate, it was very slow for formula DG1, after the burst effect during the first 0.5 h, the rate was nearly 1-2% / h .

Comparing the % of drug released between DG2 and DG3, it could be noted that DG2 has slightly better release rate, so it would be our best selected formula, hence it met all the specific objectives in this research.

The rate of dissolution from formula DG2, exactly matched that of the desired SR systems. At the beginning the % released of both drugs have slightly some burst effect due to rapid water uptake, so dissolution of the surface incorporated drugs occurred. After small period

of time and due to the gradual water uptake and swelling of the hydrophilic polymers, a gel layer (boundary) was formed through which the released was mainly controlled by diffusion. Till the end of testing period, there was a balance between surface polymers erosion and the swelling of the internal polymers leading to regular and gradual drug release rate.

These results of drug release mechanism from the matrix could be exactly explained by the swelling test performed (section 6.4.2.8.), the largest swelling % occurred during the first 0.5 h, the same thing occurred when talking about the drug release.

Thereafter, gradual and nearly regular swelling continued till the end of dissolution test, this was very correspondent with the results of drug release.

So, we concluded that the release rate in this system was mainly controlled by the diffusion through the swellable polymer.

This mechanism of drug release was further explained by applying the kinetic models to release data obtained (Section 6.5).

Table 6.15: Percentage cumulative release of MTZ and CLA from dry-granulation formulations.

Time (h)	% cumulative drug release					
	DG 1		DG 2		DG 3	
	%MTZ	%CLA	%MTZ	%CLA	%MTZ	%CLA
0.5	35	38	29	28	27	23
1	41	45	33	33	30	29
2	44	47	38	34	35	32
3	48	49	----	-----	-----	------
4	51	51	44	38	41	35
5	54	53	-----	-----	-----	------
6	------	------	47	41	46	38
8	-------	-------	55	47	51	43
10	-------	-------	60	52	48	47
20	------	-------	85	76	80	69

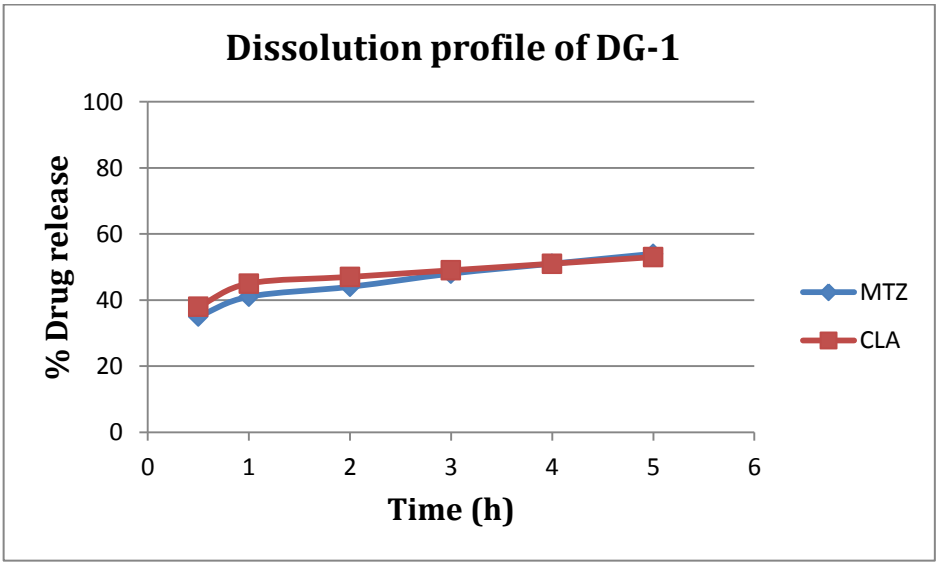


Figure 6.14: Dissolution profile of formula DG1.

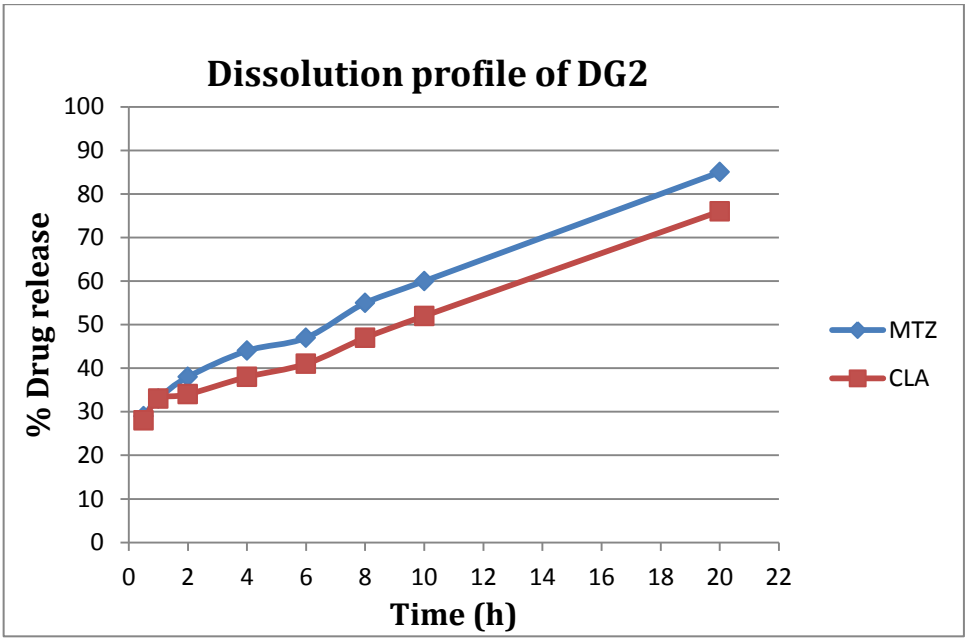


Figure 6.15: Dissolution profile of formula DG2.

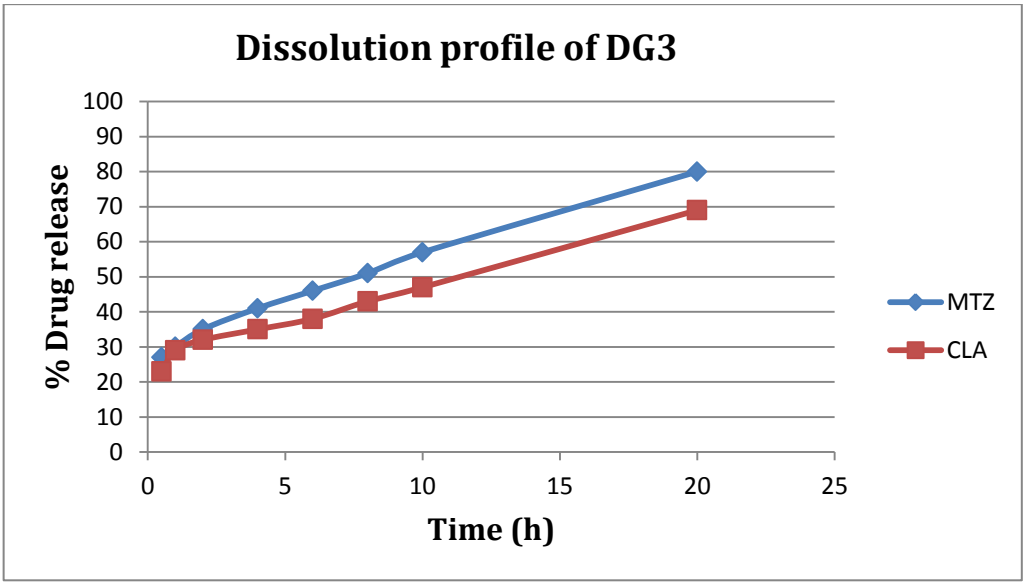


Figure 6.16: Dissolution profile of formula DG3.

6.4.2.6.2. Dissolution of esomeprazole pellets:

After proceeding as directed by the USP method for dissolution of esomeprazole, the % of drug released from the pellets, taking the overage of (R_U) for 6 tablets was as follow:

% Released= (R_U / R_S) × (C_S /L) × V × 100%

= (177952/186644) X (0.02/20) X 1000X100%

=**95.34%**

The result is acceptable according to the criteria given by the USP-36 for this test.

This result has proved that the compression of EZO enteric coated pellets into tablet using Avicel as pressure absorping matrix, has preserved the coat from destruction and thus protect EZO from acid-degradation.

6.4.2.7. **Swelling test (water uptake):**

The test was performed for the **SR-layer** as directed under section 5.4.3.8, and the results were shown in (**Table 6.16**) and (**Figure 6.17** and **6.18**)

Table 6.16: Swelling index (water uptake) results of formula DG2

	Tablet		
	1	2	3
Initial Wt (Wo)	1.3	1.3	1.3
Wt after 0.5 h	1.8	1.8	1.82
Average Wt	1.806		
Swelling index	38.9 %		
Wt after 1 h	1.92	1.82	1.85
Average Wt	1.863		
Swelling index	43.3%		
Wt after 2 h	1.863	1.95	1.93
Average Wt	1.913		
Swelling index	47.2%		
Wt after 4 h	2.04	1.97	2.00
Average Wt	2.003		
Swelling index	54.1%		
Wt after 6 h	2.05	2.3	2.21
Average Wt	2.12		
Swelling index	63.076%		
Wt after 8 h	2.17	2.21	2.02
Average Wt	2.133		
Swelling index	64.1%		
Wt after 24 h	2.25	2.25	2.25
Average Wt	2.25		
Swelling index	73.1%		

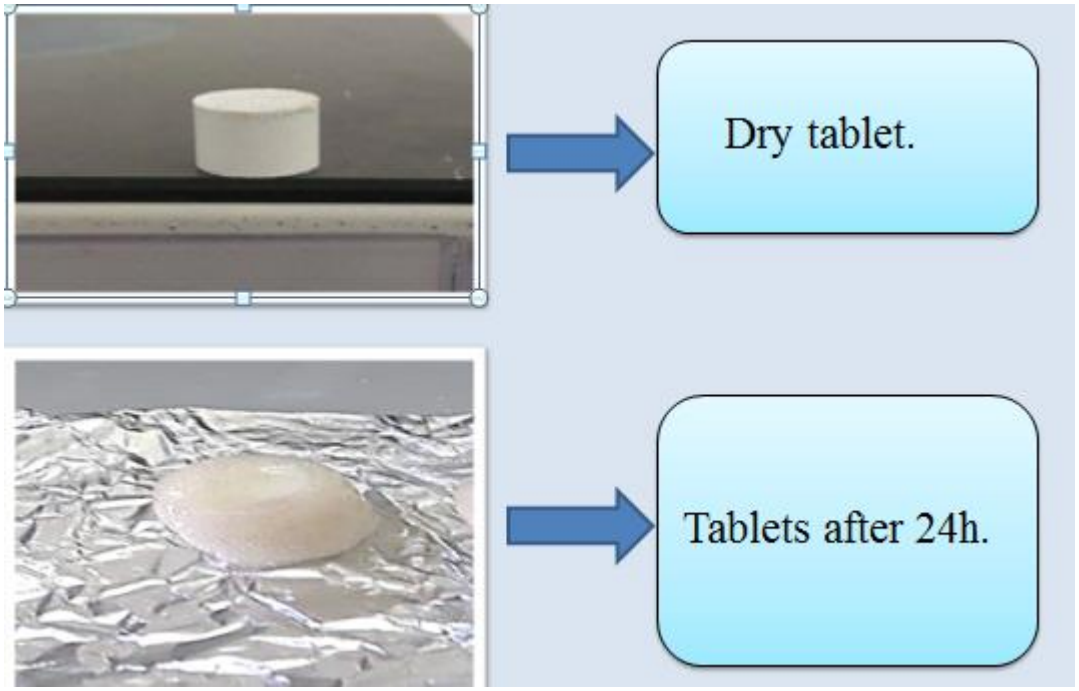


Figure 6.17: Tablet of DG2 formula, before and after swelling test.

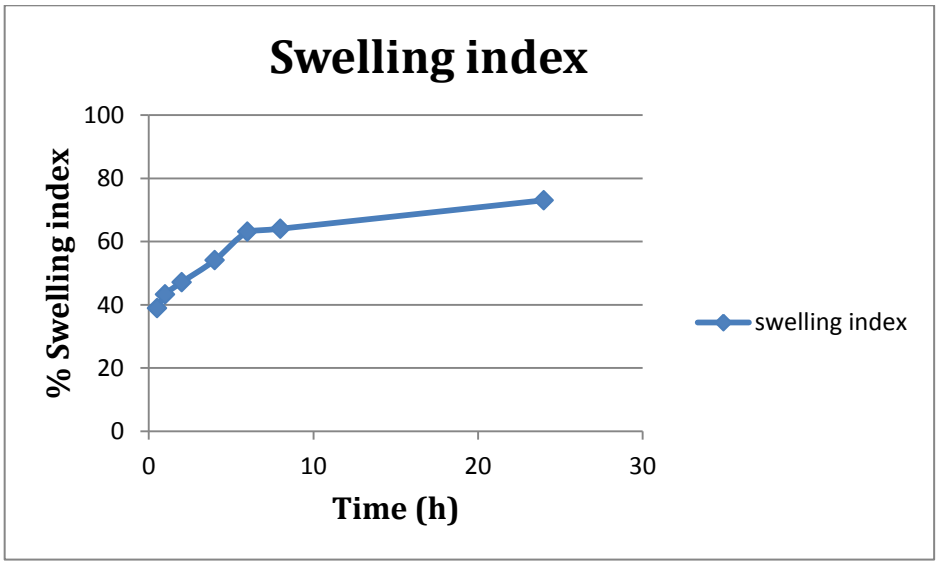


Figure 6.18: % Swelling index of formula DG2.

6.5. Kinetic modeling of the selected formula

To describe the mechanism by which the drug is released from the dosage form in a comprehensive way, dissolution data generated during the study were fitted to various drug

release kinetic models, including the Zero order, First order, Higuchi, Korsmeyer-Peppas and Hixson-Crowe using DDSOLVER program.

The selection criterion for the best-fit model was based on the adjusted coefficient of determination, R2 adjusted. The R2 adjusted value was used to compare the results of fitting data to kinetic models with different numbers of parameters. The results of fitting the dissolution data to selected mathematical models are summarized in **Table 6.17**, and shown in **Figure 6.19** and **Figure 6.20.**

By applying different kinetic models to the release data obtained from the best formula (DG2) of both MTZ and CLA, we concluded that the best fit model that could describe the release of both of MTZ and CLA was **Korsmeyer-Peppas**, depending on the values of R^2 and R^2- adj. This model used to describe the drug release from different modified release dosage forms, and usually the release is controlled by diffusion using swellable polymer like HPMC, and to exactly describe the mechanism of release from the system, we depend on (n) value as mentioned in part one, **Section 1.6**.

Table 6.17: Results of kinetic models parameters obtained following fitting dissolution data of MTZ and CLA.

Kinetic model.	**MTZ**			**CLA**		
	R^2	R^2- adj.	(n)	R^2	R^2- adj.	(n)
Zero-order	0.5984	0.5984	-----	0.8751	0.8751	-----
First-order	0.3288	0.3288	------	0.0177	0.0177	-----
Higuchi	0.7626	0.7626	------	0.6282	0.6282	-----
Korsmeyer -Peppas	0.9363	0.9363	0.325	0.8696	0.8696	0.301
Hixson-Crowell	0.1819	0.1819	-------	0.1930	0.1930	------

For our system (n) value was less than 0.5, this may be caused by the burst effect occurred during the first 0.5 h. A modification for Korsmeyer –Peppas model was prescribed by Kim and Fassihi (170) and by Xiao Huang and Christopher S Brazel (224), when burst

effect release is present, for better explanation of the release this modification was applied to our system, and the results were summarized in **Table 6.18 and Table 6.19**, and shown in **Figure 6.19** and **Figure 6.20** According to this modification, the release at each time will be modified according to the following equation:

$$\frac{Mt}{M\infty} = Kt^n + \mathrm{b}$$

M_t & $M\infty$: are the absolute cumulative amounts of drug released at time t and infinity.

K: is a constant incorporating structural and geometrical characteristics of the device, the k value is experimentally determined.

n: is the exponent, indicative of the mechanism of drug release.

b: the amount of drug released by burst effect.

Table 6.18: drug release from formula DG2 after modification according to Krosmeyer-Peppas model

Time (h)	% cumulative drug release (DG2)			
	Actual		Modified	
	%MTZ	%CLA	%MTZ	%CLA
0.5	29	28	0	0
1	33	33	5.6	6.9
2	38	34	12.7	8.3
3	----	-----	------	------
4	44	38	21.1	13.9
5	-----	-----	------	------
6	47	41	25.4	18.1
8	55	47	36.6	26.4
10	60	52	43.7	33.3
20	85	76	78.9	66.7

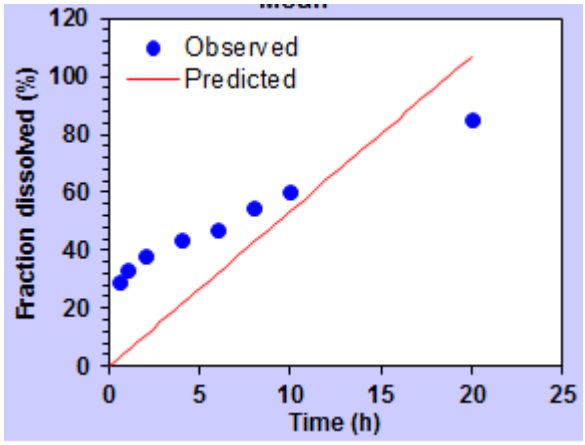
Table 6.19: Kinetic parameters after modification of release according to Krosmeyer-Peppas model:

Kinetic parameter	MTZ	CLA
R^2_{adj}	0.9939	0.9918
kKp	5.929	3.405
N	0.864	0.991

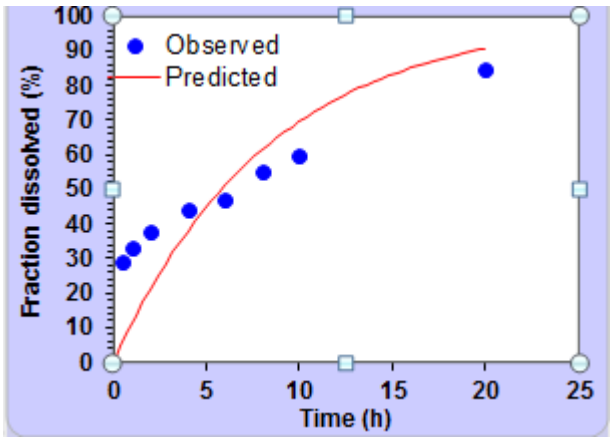
- The (n) value for MTZ from Krosmeyer-Peppas kinetic model after modification was (0.86), and since our system had a cylindrical geometry, this value explained that the mechanism of drug release for MTZ followed Anomalous transport (non-Fickian release), which is in between Case-I (Fickian diffusion) and Case-II transport behaviors. In Case-I transport (diffusion-controlled), the time-scale of drug diffusion, is the rate-limiting step,while in Case-II transport (swelling-controlled), the time-scale for polymer relaxation is the ratelimiting step (225), (226). So, in case of Non-Fickian behavior (Anomalous transport) as MTZ behavior in our system, the release is controlled by both, the diffusion of the drug from the swollen region and the relaxation of the polymers in the matrix. This

diversity of release mechanisms is consistent with the fact that MTZ was incorporated in the formulation in two forms; the free form within the matrix and the granulated form.
These types of release were briefly described by Chien-Chi Lin and Andrew T. Metters(227).

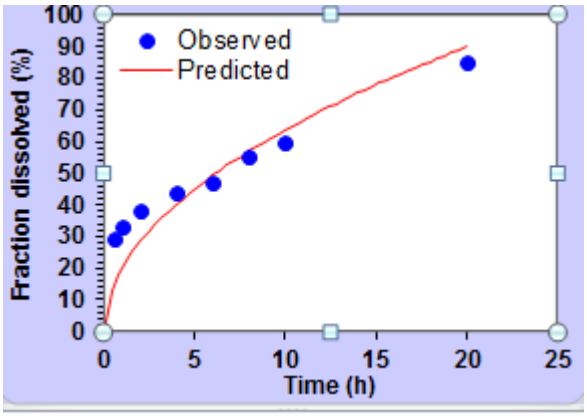
- The (n) value for CLAwhich is very close to 1 indicated that the release followed a zero order release. This type of release usually fits the releaseof low soluble drugs in coated forms and osmotic systems. But in swollen-hydrophilic matrices it was explained that the mechanism of release is controlled by diffusion coupled with erosion. This was occurred because the rates of swelling front into the glassy polymer (core) and the erosion of the rubbery state polymer (gel at tablet periphery) were nearly equal, so that the release rate for the drug remains nearly constant.
This phenomena was described by S.K. Baveja, K.V. Ranga Rao and K. Padmalatha Devi (228).



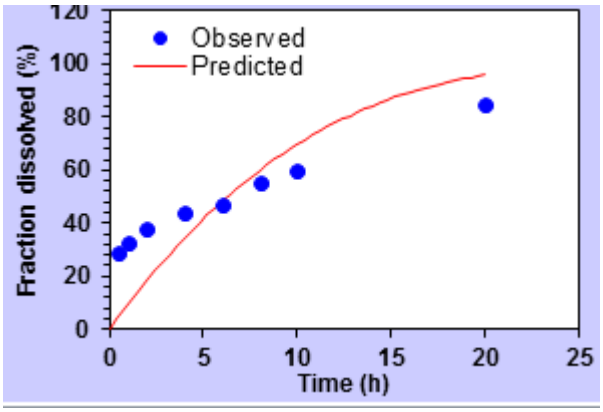
- Zero order.



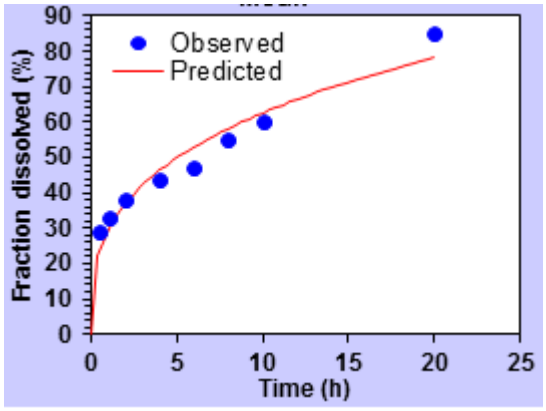
– First line order.



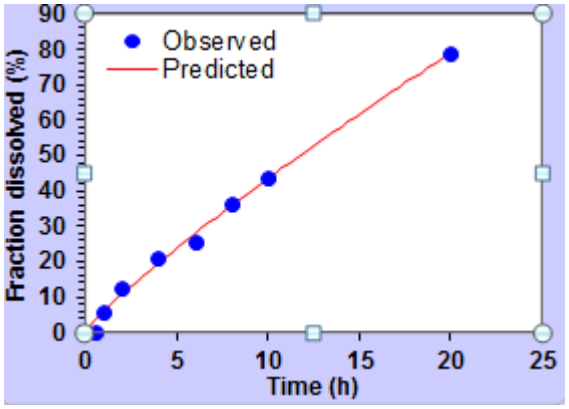
- Higuchi model.



- Hixson-Crowell model.

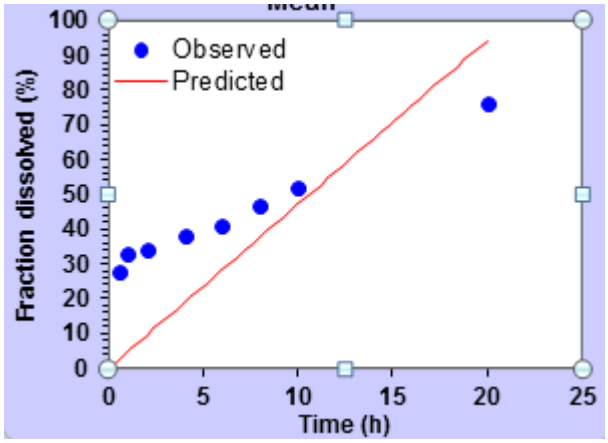


-Krosmeyer-Peppas (before modifying).

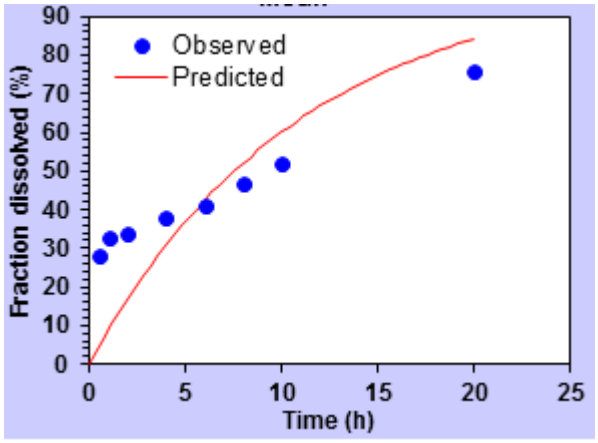


–Krosmeyer –Peppas (after modifying)

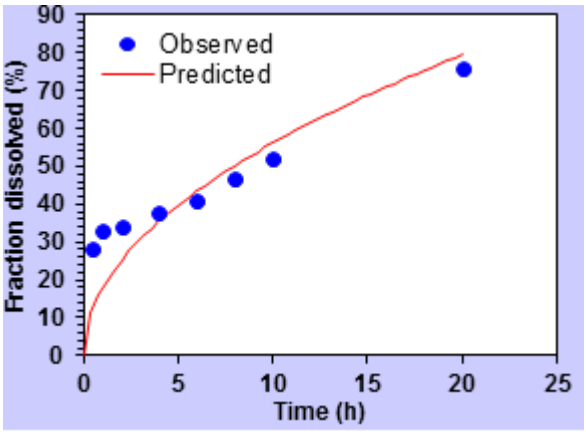
Figure 6.19: kinetic models of MTZ from DG2 formula.



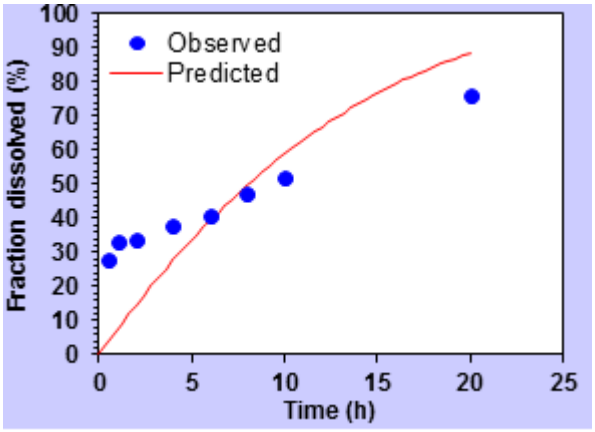
- Zero-order kinetic



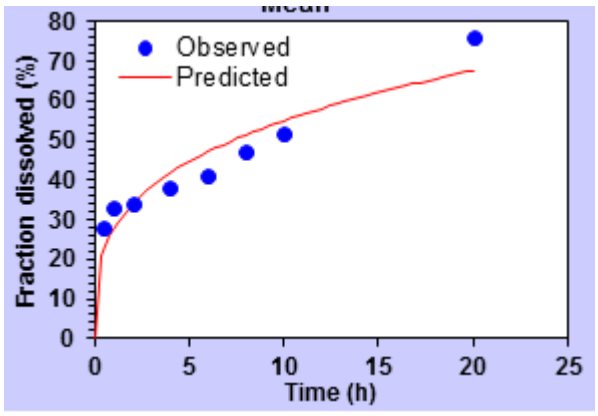
- First-order kinetic



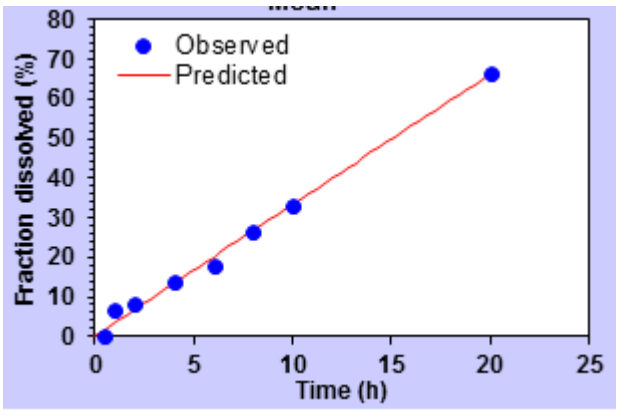
- Higuchi model.



- Hixson-Crowell model.



-Krosmeyer-Peppas (before modifying).



-Krosmeyer –Peppas (after modifying)

Figure 6.20: kinetic models of CLA from DG2 formula.

Part Seven:

7.Conclusion and Future work.

7.1. Conclusion.

We have shown in this study that we have been able to formulate a tablet that would combine a triple therapy for the treatment of H. pylori induced ulcer. This tablet had two layers. The first layer is a fast disintegrating layer that will provide the enteric coated pellets of EZO upon contact with water. We proved that this layer will rapidly deattach and will preserve the enteric coating after compression applied. The second layer containing both MTZ and CLA, proved to exhibit excellent floating properties, having short floating lag time and long duration of floating (up to 24 h). The tablet exhibited a synchronous sustained release of both antibiotics which is advantageous in providing a continuous input of the two antibiotics in the vicinity of the bacteria. This is important since a local treatment of the bacteria is much more benefitial than the systemic one. The analysis of the release kinetics and swelling studies showed that the mechanism of the release was mainly controlled by diffusion coupled with erosion. Moreover, a validated system was developed in this study to analyze both MTZ and CLA in the same run of the HPLC.

So in conclusion, this tablet may potentially a better treatment for H. pylori induced ulcer.

7.2. Future work:

Despite the objectives that have been achieved from this research, further work have to be done, this may include:

- Put the product on stability study program, those include accelerated (40 °C ± 2 °C / 75% RH ± 5% RH) and long term conditions (30 °C ± 2 °C / 65% RH ± 5% RH or).
- Change the shape of the tablet to oblonged- biconvex to be more convenient for administration and easily swallowed.
- Coating the tablet with poly vinyl alcohol (PVA) coat, to protect from humidity, and to easily swallow such large tablet.
- Perform in-vivo studies which include:
  - Radiography (X-ray), on healthy volunteers, to evaluate the floating properties.
  - Histological examination for suitable animal model inoculated and infected with H .pylori, to assess the actual (in-vivo) efficacy of this dosage form in the clearance and eradication of the microorganism.

Part Eight

8. Appendices

8.1. Inactive ingredients monographs.

8.1.1. Hydroxypropyl methyl cellulose (HPMC, Hypromellose):

- Description:

HPMC is an odorless and tasteless, white or creamy-white fibrous or granular powder.

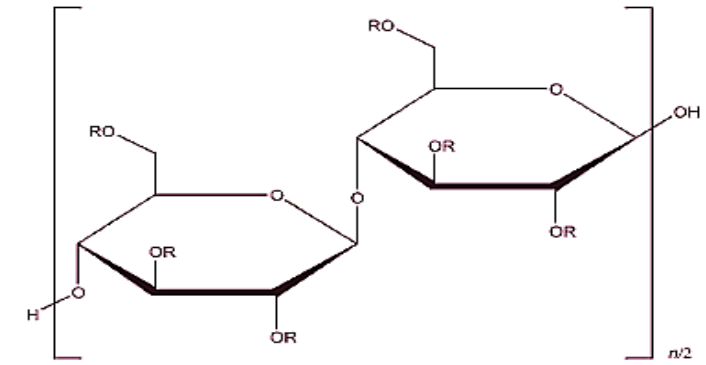
- Chemical Name and CAS Registry Number:

Cellulose hydroxypropyl methyl ether, {9004-65-3}

- Empirical Formula:

HPMC is partly O-methylated and O-(2-hydroxypropylated) cellulose. It is available in several grades that vary in viscosity and extent of substitution. Grades may be distinguished by appending a number indicative of the apparent viscosity, in mPa s, of a 2% w/w aqueous solution at 20ºC.

- Structural formula:



where R is H, CH_3, or $CH_3CH(OH)CH_2$

- Applications in Pharmaceutical Formulation or Technology:

  Hypromellose is widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations.

  In oral-solid dosage forms, hypromellose is primarily used as a tablet binder, in film-coating and as a matrix for use in sustained release tablet formulations.

Concentrations between (2 -5) % w/w may be used as a binder in either wet- or dry-granulation processes. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of (10–80) % w/w in tablets and capsules. Depending upon the viscosity grade (usually low viscosity grades are used), concentrations of (2–20) % w/w are used for film-forming solutions.

Incompatibilities:

Hypromellose is incompatible with some oxidizing agents.

Safety:

Hypromellose is generally regarded as a nontoxic and nonirritating material, although excessive oral consumption may have laxative effect. The WHO has not specified an acceptable daily intake for hypromellose since the levels consumed were not considered to represent a hazard to health.

8.1.2. Ethyl cellulose (EC).

- Description:

  Ethyl cellulose is a tasteless, free-flowing, and white to light tan-colored powder.

- Chemical Name and CAS Registry Number :

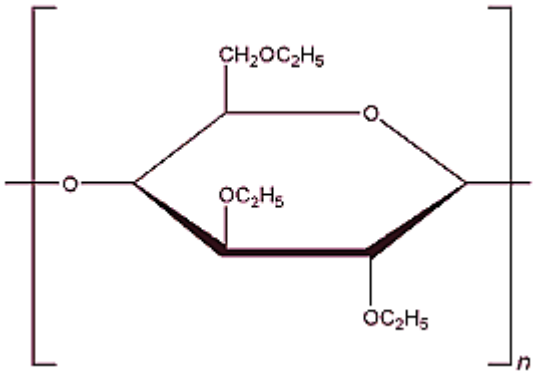
  Cellulose ethyl ether,{9004-57-3}.

- Empirical Formula:

  Ethyl cellulose is partially ethoxylated. Ethyl cellulose with complete ethoxyl substitution

  (DS = 3) is: C12H23O6 (C12H22O5)n C12H23O5 where n can vary to provide a wide variety of molecular weights.

- Structural formula:



- Applications in Pharmaceutical Formulation or Technology.

The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation. Modified-release tablet formulations may also be produced using ethyl cellulose as a matrix former. In tablet formulations, ethyl cellulose may additionally be employed as a binder.

- Incompatibilities:

Incompatible with paraffin wax and microcrystalline wax.

- Safety:

Ethyl cellulose is not metabolized following oral consumption and is therefore a non-calorific substance. Because ethyl cellulose is not metabolized it is not recommended for parenteral products; parenteral use may be harmful to the kidneys. Ethyl cellulose is generally regarded as a nontoxic, non-allergenic, and non-irritating material. As ethyl cellulose is not considered to be a health hazard, the WHO has not specified an acceptable daily intake. The highest reported level used in an oral product is 308.8 mg in an oral SR tablet.

8.1.3. Sodium bicarbonate.

- Description:

Sodium bicarbonate occurs as an odorless, white, crystalline powder with a saline, slightly alkaline taste. Grades with different particle sizes, from a fine powder to free-flowing uniform granules, are commercially available.

- Chemical Name and CAS Registry Number:

Carbonic acid monosodium salt {144-55-8}.

- Empirical Formula and Molecular Weight:

NaHCO3 is 84.01 g / mole.

- Applications in Pharmaceutical Formulation or Technology:

Sodium bicarbonate is generally used in pharmaceutical formulations as a source of carbon dioxide (e.g. in effervescent tablets). It is also widely used to produce or maintain an alkaline pH in a preparation. In effervescent tablets and granules, sodium bicarbonate is usually formulated with citric and/or tartaric acid. Tablets may also be prepared with sodium bicarbonate alone since the acid of gastric fluid is sufficient to cause effervescence.

Recently, sodium bicarbonate has been used as a gas-forming agent in alginate raft systems and in floating, controlled release oral dosage forms.

- Incompatibility:

In powder mixtures, atmospheric moisture or water content from another ingredient is sufficient for sodium bicarbonate to react with some acids. In liquid mixtures containing bismuth subnitrate, sodium bicarbonate reacts with the acid formed by hydrolysis of the bismuth salt. In solution, sodium bicarbonate has been reported to be incompatible with many drug substances such as ciprofloxacin, amiodarone, nicardipine and levofloxacin.

- Safety:

  Sodium bicarbonate is metabolized to the sodium cation, which is eliminated from the body by renal excretion, and the bicarbonate anion, which becomes part of the body's bicarbonate store. Any carbon dioxide formed is eliminated via the lungs. Administration of excessive amounts of sodium bicarbonate may thus disturb the body's electrolyte balance, leading to metabolic alkalosis. Orally ingested sodium

bicarbonate neutralizes gastric acid with the evolution of carbon dioxide and may cause stomach cramps and flatulence.

Sodium bicarbonate is generally regarded as an essentially nontoxic and nonirritant material.

LD50 (mouse, oral): 3.36 g/kg.

LD50 (rat, oral): 4.22 g/kg.

8.1.4. Citric acid anhydrous:

- Description:

It is white, crystalline powder, colorless crystals or granules, very soluble in water, freely soluble in alcohol. It melts at about 153 °C with decomposition.

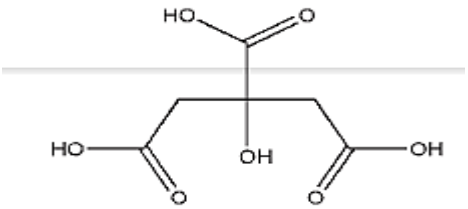
- Chemical Name and CAS Registry Number:

2-hydroxypropane-1,2,3-tricarboxylic acid, { 77-92-9}

- Empirical formula and molecular weight:

$C_6H_8O_7$, (192.1 g/mole).

- Chemical structure:



- Applications in Pharmaceutical Formulation or Technology:

Citric acid is widely used in pharmaceutical formulations and food products, primarily to adjust the pH of solutions. It has also been used experimentally to adjust the pH of tablet matrices in enteric-coated formulations for colon-specific drug delivery. Citric acid anhydrous citric acid is widely used in the preparation of effervescent tablets.

- Incompatibilities

Citric acid is incompatible with potassium tartrate, alkaline earth carbonates and bicarbonates, acetates, and sulfides. Incompatibilities also include oxidizing agents, bases, reducing agents, and nitrates. It is potentially explosive in combination with metal nitrates.

- Safety:

Citric acid is found naturally in the body, mainly in the bones, and is commonly consumed as part of a normal diet. Orally ingested citric acid is absorbed and is generally regarded as a nontoxic material when used as an excipient. However, excessive or frequent consumption of citric acid has been associated with erosion of the teeth. Citric acid and citrates also enhance intestinal aluminum absorption in renal patients, which may lead to increased, harmful serum aluminum levels.

8.1.5. Microcrystalline cellulose (Avicel-PH102)

- Description:

It is white or almost white, fine or granular powder.
Typical mean particle size is 20–200 mm. Different grades may have a different nominal mean particle size and consequently different surface aera.

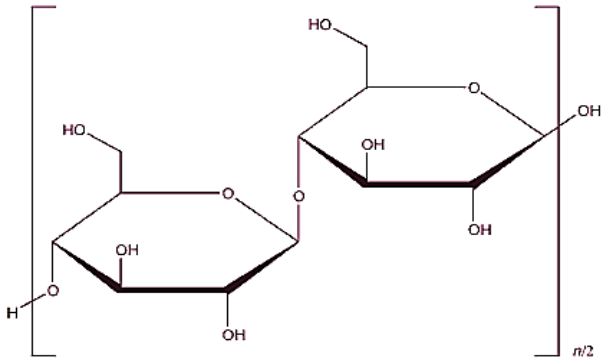
- Chemical Name and CAS Registry Number:

Cellulose, {9004-34-6}

- Empirical Formula and Molecular Weight:

(C6H10O5)n (36 000 g/mole where n 220).

- Chemical structure:



- Applications in Pharmaceutical Formulation or Technology:

Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluent in oral tablet and capsule formulations where it is used in both wet-granulation and direct-compression processes. In addition to its use as a binder/diluent, microcrystalline cellulose also has some lubricant and disintegrant properties that make it useful in tableting

- Incompatibility:

Microcrystalline cellulose is incompatible with strong oxidizing agents.

- Safety:

Avicel is widely used in oral pharmaceutical formulations and food products and is generally regarded as a relatively nontoxic and nonirritant material. Avicel is not absorbed systemically following oral administration and thus has little toxic potential. Consumption of large quantities of cellulose may have a laxative effect, although this is unlikely to be a problem when cellulose is used as an excipient in pharmaceutical formulations.

8.1.6. Polyvinylpyrrolidone (PVP).

- Description:

Povidone occurs as a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder

- Chemical Name and CAS Registry Number:

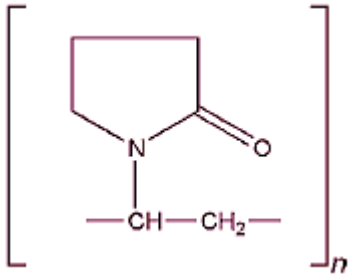
1-Ethenyl-2-pyrrolidinone homopolymer, (9003-39-8)

- Empirical Formula and Molecular Weight:

  (C6H9NO)n , (2500–3 000 000)

  PVP-K30 has M.Wt of 50000 g/mole.

- Chemical structure:



- Applications in Pharmaceutical Formulation or Technology:

Povidone is primarily used in solid-dosage forms. In tableting, povidone solutions are used as binders in wet-granulation processes. Povidone is also added to powder blends in the dry form and granulated in situ by the addition of water, alcohol, or hydro-alcoholic solutions. Povidone solutions may also be used as coating agents or as binders when coating active pharmaceutical ingredients on a support such as sugar beads.

- Incompatibility:

Povidone is compatible in solution with a wide range of inorganic salts, natural and synthetic resins, and other chemicals. It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital and tannin. The efficacy of some preservatives, e.g. thimerosal, may be adversely affected by the formation of complexes with povidone.

- Safety:

Povidone is widely used as an excipient, particularly in oral tablets and solutions. When consumed orally, povidone may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes. Povidone additionally has no irritant effect on the skin and causes no sensitization.

An acceptable daily intake for povidone has been set by the WHO at up to 25 mg/kg body-weight.

LD50 (mouse, IP): 12 g/kg.

8.1.7. Magnesium stearate:

- Description:
  It is white, very fine, light powder, greasy to the touch, practically insoluble in water and in ethanol.

- Chemical Name and CAS Registry Number:

Octadecanoic acid- magnesium salt, (557-04-0).

- Empirical Formula and Molecular Weight:

C36H70MgO4, (591.24 g / mole).

- Structural formula:
  (CH3 (CH2)16COO)2 Mg.

- Applications in Pharmaceutical Formulation or Technology:

Magnesium stearate is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0% w/w. It is also used in barrier creams.

- Incompatibility:

It is incompatible with strong acids, alkalis, and iron salts. Avoid mixing with strong oxidizing materials. Magnesium stearate cannot be used in products containing aspirin, some vitamins, and most alkaloidal salts.

- Safety:

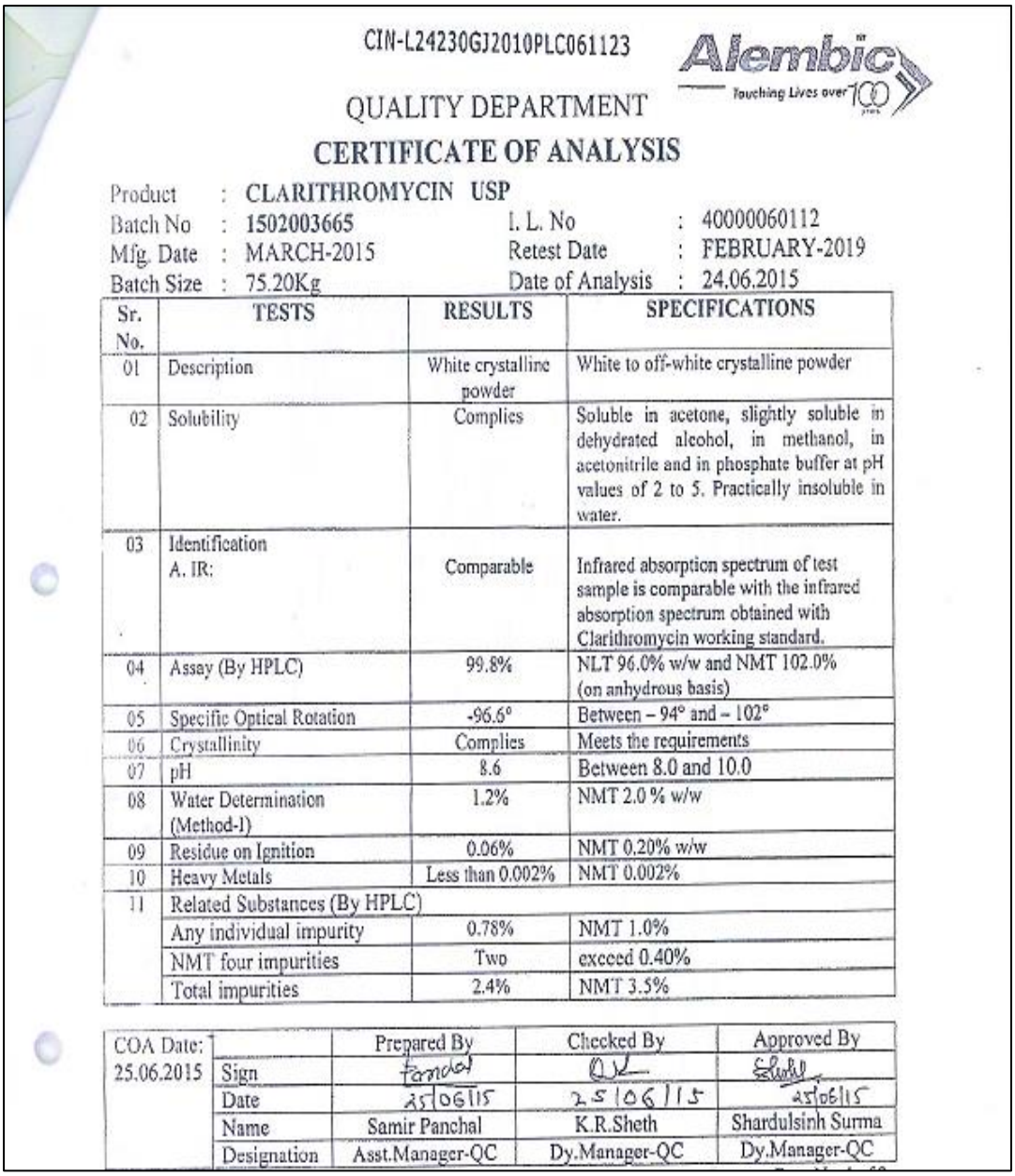
Magnesium stearate is generally regarded as being nontoxic following oral administration. However, oral consumption of large quantities may produce a laxative effect or mucosal irritation. Toxicity assessments of magnesium stearate in rats have indicated that it is not irritating to the skin, and is nontoxic when administered orally or inhaled. Magnesium stearate has not been shown to be carcinogenic when implanted into the bladder of mice.

LD50 (rat, inhalation) : >2 mg/L

LD50 (rat, oral) : >10 g/kg.

8.2. Certificates of analysis (C.O.A) for the active and inactive materials

8.2.1. C.O.A. of Clarithromycin



CIN-L24230GJ2010PLC061123

Alembic — Touching Lives over 100 years

QUALITY DEPARTMENT

CERTIFICATE OF ANALYSIS

Product : CLARITHROMYCIN USP
Batch No : 1502003665 I. L. No : 40000060112
Mfg. Date : MARCH-2015 Retest Date : FEBRUARY-2019
Batch Size : 75.20Kg Date of Analysis : 24.06.2015

Sr. No.	TESTS	RESULTS	SPECIFICATIONS
01	Description	White crystalline powder	White to off-white crystalline powder
02	Solubility	Complies	Soluble in acetone, slightly soluble in dehydrated alcohol, in methanol, in acetonitrile and in phosphate buffer at pH values of 2 to 5. Practically insoluble in water.
03	Identification A. IR:	Comparable	Infrared absorption spectrum of test sample is comparable with the infrared absorption spectrum obtained with Clarithromycin working standard.
04	Assay (By HPLC)	99.8%	NLT 96.0% w/w and NMT 102.0% (on anhydrous basis)
05	Specific Optical Rotation	-96.6°	Between – 94° and – 102°
06	Crystallinity	Complies	Meets the requirements
07	pH	8.6	Between 8.0 and 10.0
08	Water Determination (Method-I)	1.2%	NMT 2.0 % w/w
09	Residue on Ignition	0.06%	NMT 0.20% w/w
10	Heavy Metals	Less than 0.002%	NMT 0.002%
11	Related Substances (By HPLC)		
	Any individual impurity	0.78%	NMT 1.0%
	NMT four impurities	Two	exceed 0.40%
	Total impurities	2.4%	NMT 3.5%

COA Date:		Prepared By	Checked By	Approved By
25.06.2015	Sign	Panchal		
	Date	25/06/15	25/06/15	25/06/15
	Name	Samir Panchal	K.R.Sheth	Shardulsinh Surma
	Designation	Asst.Manager-QC	Dy.Manager-QC	Dy.Manager-QC

8.2.2. C.O.A. of Metronidazole.

Aarti Drugs Limited
Manufacturers of : Bulk Drugs & Chemicals

Ground Floor, Road No. 29, Sion (East), Mumbai-400 022. (India)
Tel. : 91 22 2407 2249 • Fax : 91 22 2407 0144 / 2407 3462
E-mail : sales@aartidrugs.com • Website : www.aartidrugs.com
MANUFACTURING SITE :Plot No. 2902-2904, G.I.D.C., Sarigam,
Dist. Valsad - 396 155. • Tel. : (0260) 2780269 • Fax : (0260) 27802

QUALITY CONTROL
CERTIFICATE OF ANALYSIS

PRODUCT NAME : METRONIDAZOLE USP

Chemical Name **2-Methyl-5-Nitroimidazole-1-ethanol** CAS No :- **[443-48-1]**
Empirical Formula :- C6 H9 N3 O3 Mol wt :- 171.160 Retest Period :- One year

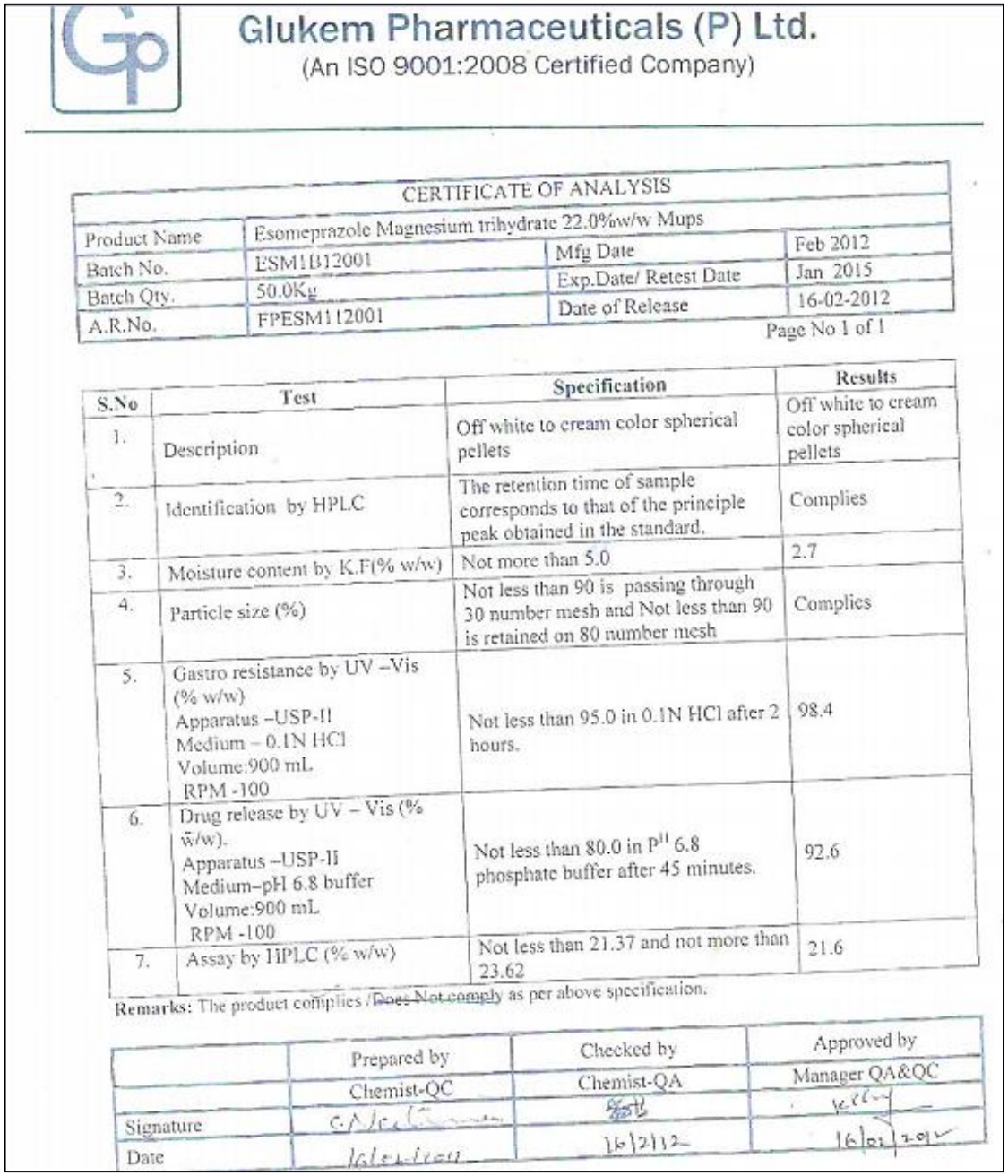
Batch No :- MTZ/ 2100790 Mfg Date :- 30/09/2012 Batch Size:- 1000 Kg
AR NO:- MTZ/ 20790 Expiry Date:- 29/09/2017 Release date :- 08/10/2012

Customer Name: **Birzeit Pharmaceutical Company**
Dispatch date : 09/10/2012 Dispatch Qty :- 500 Kg Dispatch Packing :-20 X 25 Kg

ANALYSIS REPORT

No	Particulars	Standard Limits	Observation	Status
01	Description	White to pale yellow odourless crystalline powder, is stable in air, but darkens on exposure	White to pale yellow odourless crystalline powder, is stable in air, but darkens on exposure	Pass
02	Solubility	Sparingly soluble in water and in alcohol; slightly soluble in chloroform	Sparingly soluble in water and in alcohol; slightly soluble in chloroform	Pass
03	Identification A	IR should be concordent with reference standard (Ref: USP 197 K)	IR Spectrum of sample is concordent with reference standard	Pass
	B	The refernce solution and test solution in the range 200 to 400 nm of a 20 ug per ML solution in sulphuric acid in methanol (1 in 350) ,recorded and compared. (Ref. 197 U)	The spectra concomitantly obtained for the test solution and Standard solution are compared and found the requirements are met	Pass
04	Melting Point	Melts Between 159°C and 163 °C	160-162 °C	Pass
05	Loss on Drying	Not More than 0.5% w/w	0.1959 % w/w	Pass
06	Residue on Ignition	Not More than 0.1% w/w	0.0284 % w/w	Pass
07	Heavy Metals	Not More than 0.005% w/w	Less Than 20 ppm	Pass
08	Non Basic Substances	A 1g portion dissolves completely in 10 ml of dilute HCL (1 in 2)	Resulting solution is Clear	Pass
09	Chromatographic Purity	Related Substance Not More than 0.3% w/w	Less than 0.3 % w/w	Pass
10	Assay	Not less than 99.0% w/w and Not more than 101.0% w/w of C6H9N3O3 calculated with reference to the dried substance	99.62 % w/w	Pass

8.2.3. C.O.A. of Esomeprazole



Glukem Pharmaceuticals (P) Ltd.
(An ISO 9001:2008 Certified Company)

CERTIFICATE OF ANALYSIS

Product Name	Esomeprazole Magnesium trihydrate 22.0%w/w Mups		
Batch No.	ESM1B12001	Mfg Date	Feb 2012
Batch Qty.	50.0Kg	Exp.Date/ Retest Date	Jan 2015
A.R.No.	FPESM112001	Date of Release	16-02-2012

Page No 1 of 1

S.No	Test	Specification	Results
1.	Description	Off white to cream color spherical pellets	Off white to cream color spherical pellets
2.	Identification by HPLC	The retention time of sample corresponds to that of the principle peak obtained in the standard.	Complies
3.	Moisture content by K.F(% w/w)	Not more than 5.0	2.7
4.	Particle size (%)	Not less than 90 is passing through 30 number mesh and Not less than 90 is retained on 80 number mesh	Complies
5.	Gastro resistance by UV –Vis (% w/w) Apparatus –USP-II Medium – 0.1N HCl Volume:900 mL RPM -100	Not less than 95.0 in 0.1N HCl after 2 hours.	98.4
6.	Drug release by UV – Vis (% w/w). Apparatus –USP-II Medium–pH 6.8 buffer Volume:900 mL RPM -100	Not less than 80.0 in P^{H} 6.8 phosphate buffer after 45 minutes.	92.6
7.	Assay by HPLC (% w/w)	Not less than 21.37 and not more than 23.62	21.6

Remarks: The product complies / ~~Does Not comply~~ as per above specification.

	Prepared by	Checked by	Approved by
	Chemist-QC	Chemist-QA	Manager QA&QC
Signature			
Date		16/2/12	16/02/2012

8.2.4. C.O.A of HPMC-K4M

ORISON CHEMICALS LIMITED

CERTIFICATE OF ANALYSIS

Name: Hydroxypropyl Methyl Cellulose (HPMC)

Grade: E4 USP31 Conforming Microbiological Test

Quantity: 400 KGS **Date of Manufacture: 2013-8-4**

Lot Number: 1308-183 **Date of Expiry: 2016-8-3**

-Specification

Test Item	Specification	Test Result
Identification A to C	Conforms	Conforms
Methoxyl Content (wt%)	28.0-30.0	28.3
Hydrooxypropoxyl Content (wt%)	7.0-12.0	9.0
PH (25℃)	5.5-8.0	5.9
Loss on drying (wt%)	5.0 max	3.1
Heavy metal (ppm)	10 max	Less than 10
Arsenic (ppm)	3 max	Less than 2
Apparent Viscosity (cp)	3.0-6.0	4.6
Residue On Ignition (wt%)	1.5 max	0.74
Total Bacterium	1000/gram max	233
Mold	100/gram max	33
Appearance	White Powder	Conform

This materialm eets all requirement of USP31 for the monograph Hydroxypropyl Methyl cellulose 2910.T he EP third edition and JP 13

This material passes USP identification test A, B and C.

This product has been manufactured in accordance with FDA's GMP

8.2.5. C.O.A of HPMC-K15M

ORISON CHEMICALS LIMITED
Add: 701-1,Haowei Bldg A, The 3rd Street, TEDA, Tianjin, China. Post Code: 300457
Tel: +86-22-59886076
Fax+ 86-22-59886075
E-mail:info@orison.com.cn
web site: www.orisonchem.com

CERTIFICATE OF ANALYSIS

Name: Hydroxypropyl Methyl Cellulose (HPMC)
Grade: E15 USP36 Conforming Microbiological Test
Quantity: 100 KGS
Date of Manufacture: 2013-09-20
Lot Number: 1309-235
Date of Expiry:2016-09-19
-Specification

Test Item	Specification	Test Result
Identification A to C	Conforms	Conforms
Methoxyl Content (wt%)	28.0-30.0	29.0
Hydrooxypropoxyl Content (wt%)	7.0-12.0	8.7
PH (25℃)	5.5-8.0	6.68
Loss on drying (wt%)	5.0 max	3.0
Heavy metal (ppm)	10 max	Less than 10
Arsenic (ppm)	2 max	Less than 2
Apparent Viscosity (cp)	12-18	14.5
Residue On Ignition (wt%)	1.5 max	1.04
Total Bacterium	1000/gram max	200
Mold	100/gram max	33
Appearance	White Powder	Conform

This material meets all requirement of USP36 for the monograph Hydroxypropyl Methyl cellulose 2910. The EP third edition and JP 13
This material passes USP identification test A, B and C.
This product has been manufactured in accordance with FDA's GMP

8.2.6. C.O.A of HPMC-K100M

Shin-Etsu

2015/06/02 (1/1)

Certificate of Analysis

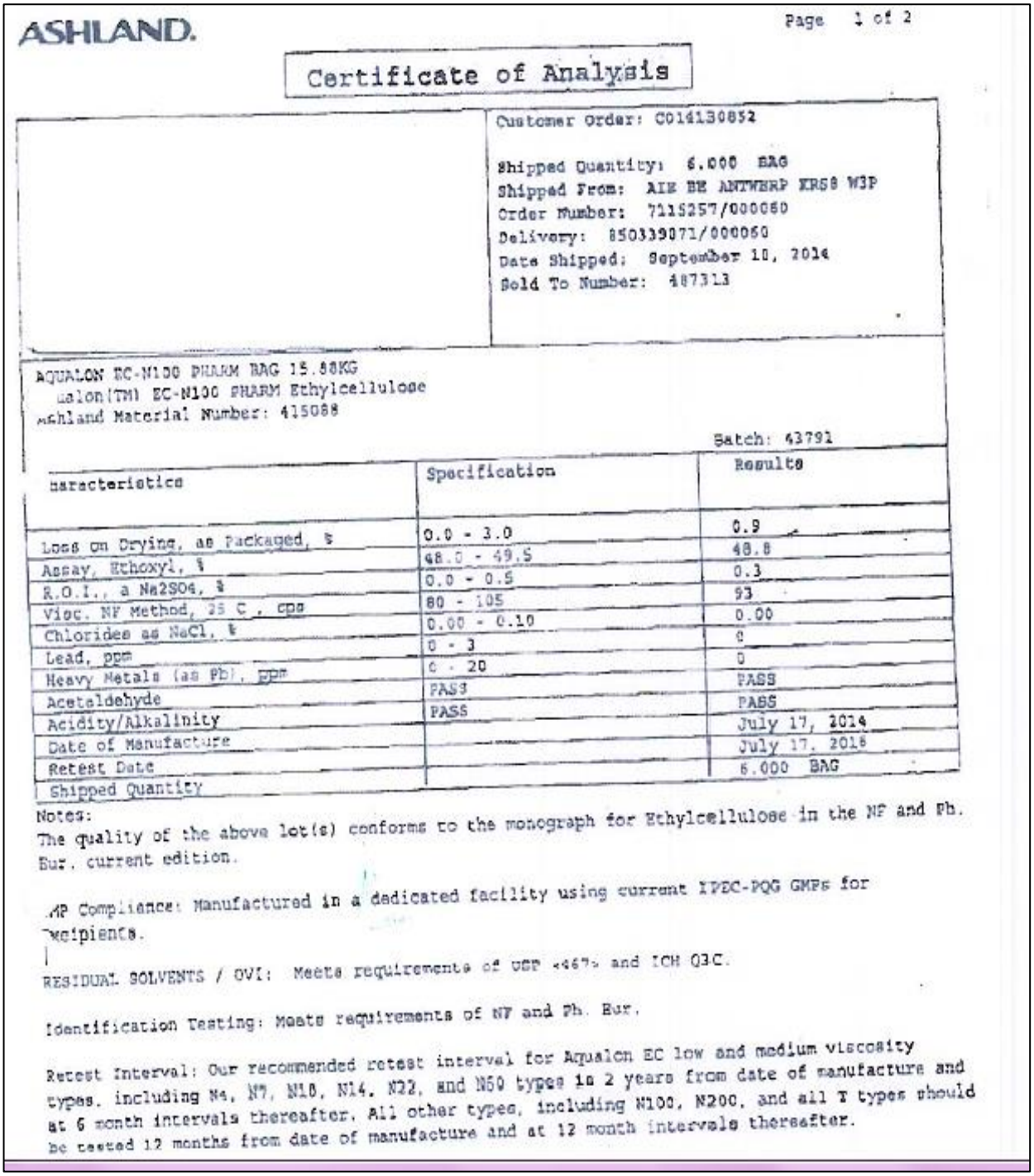
Shin-Etsu Chemical Co., Ltd.
Naoetsu Plant [illegible] ance Department
28-1, Nishifuku[illegible]-ku,
Joetsu-shi, Ni[illegible]

Product Name	METOLOSE (Hypromellose, USP)
Grade	90SH-100000SR
Substitution Type	2208
Viscosity Type	100000 mPa·s
Lot Number	5055289
Quantity	40kg
Manufacture Date	2015/05/13
Recommended Re-Evaluation Date *	2018/05/12
Analysis Date	2015/05/15
Issue No.	D5120150507400002-1-01
Remark	P.O. 1500890

This product complies with the specifications described in the current USP, EP and JP.
This product is manufactured in accordance with GMP.
* Shin-Etsu Chemical recommends that the customer's quality control unit may re-evaluate the quality of this material on its own responsibility prior to use after the Recommended Re-Evaluation date.
Storage Conditions: Store containers sealed and in a dry place. Keep away from heat or sunlight.

Test Item	Unit	Test Result	Specification	Method
Description		Conforms	Conforms	JP/USP
Characters		Conforms	Conforms	EP
Identification (1)/A		Conforms	Conforms	JP/USP/EP
Identification (2)/B		Conforms	Conforms	JP/USP/EP
Identification (3)/C		Conforms	Conforms	JP/USP/EP
Identification (4)/D		Conforms	Conforms	JP/USP/EP
Identification (5)/E		Conforms	Conforms	JP/USP/EP
Appearance of solution		Conforms	Conforms	EP
Viscosity	mPa·s	105000	75000 - 140000	JP/USP/EP
pH		6.7	5.0 - 8.0	JP/USP/EP
Heavy metals	ppm	Not more than 20	Not more than 20	JP/USP/EP
Loss on drying	%	1.0	Not more than 5.0	JP/USP/EP
Residue on ignition	%	0.09	Not more than 1.5	JP/USP/EP
Methoxy content	%	23.5	22.0 - 24.0	JP/USP
Hydroxypropoxy content	%	9.6	8.5 - 10.5	JP/USP
Particle size: 20% cumulation (D20)	μm	32.2	20 - 40	SEC
Particle size: Average (D50)	μm	64.4	50 - 80	SEC
Particle size: 80% cumulation (D80)	μm	127.0	100 - 160	SEC

8.2.7. C.O.A. of Ethyl cellulose



ASHLAND.

Page 1 of 2

Certificate of Analysis

Customer Order: C014130852

Shipped Quantity: 6.000 BAG
Shipped From: AIE BE ANTWERP KRS8 W3P
Order Number: 7115257/000060
Delivery: 850339071/000060
Date Shipped: September 10, 2014
Sold To Number: 487313

AQUALON EC-N100 PHARM BAG 15.88KG
Aqualon(TM) EC-N100 PHARM Ethylcellulose
Ashland Material Number: 415088

Batch: 43791

Characteristics	Specification	Results
Loss on Drying, as Packaged, %	0.0 - 3.0	0.9
Assay, Ethoxyl, %	48.0 - 49.5	48.8
R.O.I., a Na2SO4, %	0.0 - 0.5	0.3
Visc. NF Method, 25 C, cps	80 - 105	93
Chlorides as NaCl, %	0.00 - 0.10	0.00
Lead, ppm	0 - 3	0
Heavy Metals (as Pb), ppm	0 - 20	0
Aceteldehyde	PASS	PASS
Acidity/Alkalinity	PASS	PASS
Date of Manufacture		July 17, 2014
Retest Date		July 17, 2018
Shipped Quantity		6.000 BAG

Notes:
The quality of the above lot(s) conforms to the monograph for Ethylcellulose in the NF and Ph. Eur. current edition.

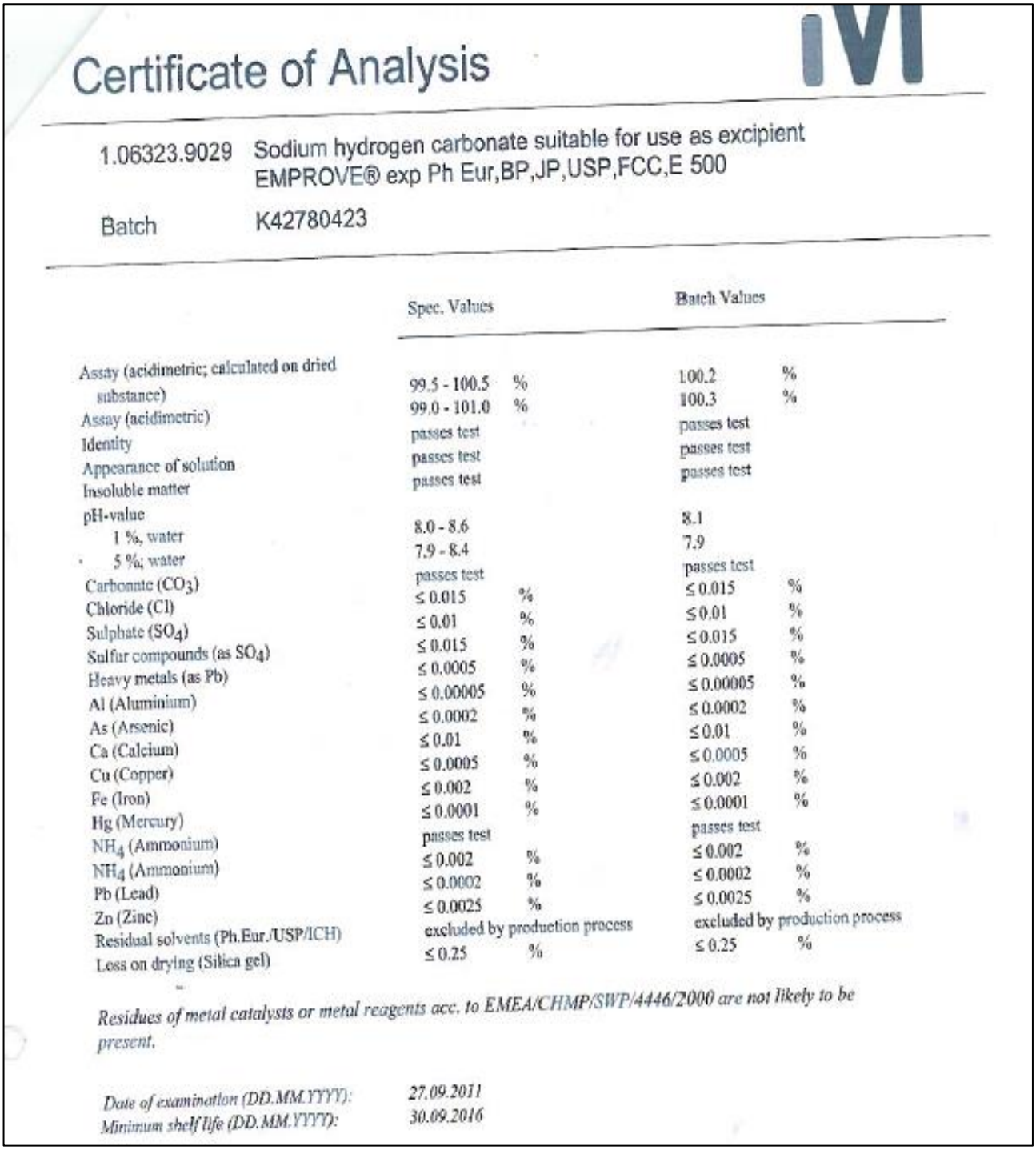
GMP Compliance: Manufactured in a dedicated facility using current IPEC-PQG GMPs for excipients.

RESIDUAL SOLVENTS / OVI: Meets requirements of USP <467> and ICH Q3C.

Identification Testing: Meets requirements of NF and Ph. Eur.

Retest Interval: Our recommended retest interval for Aqualon EC low and medium viscosity types, including N4, N7, N10, N14, N22, and N50 types is 2 years from date of manufacture and at 6 month intervals thereafter. All other types, including N100, N200, and all T types should be tested 12 months from date of manufacture and at 12 month intervals thereafter.

8.2.8. C.O.A. of Sodium Bicarbonate



Certificate of Analysis

1.06323.9029 Sodium hydrogen carbonate suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP,FCC,E 500

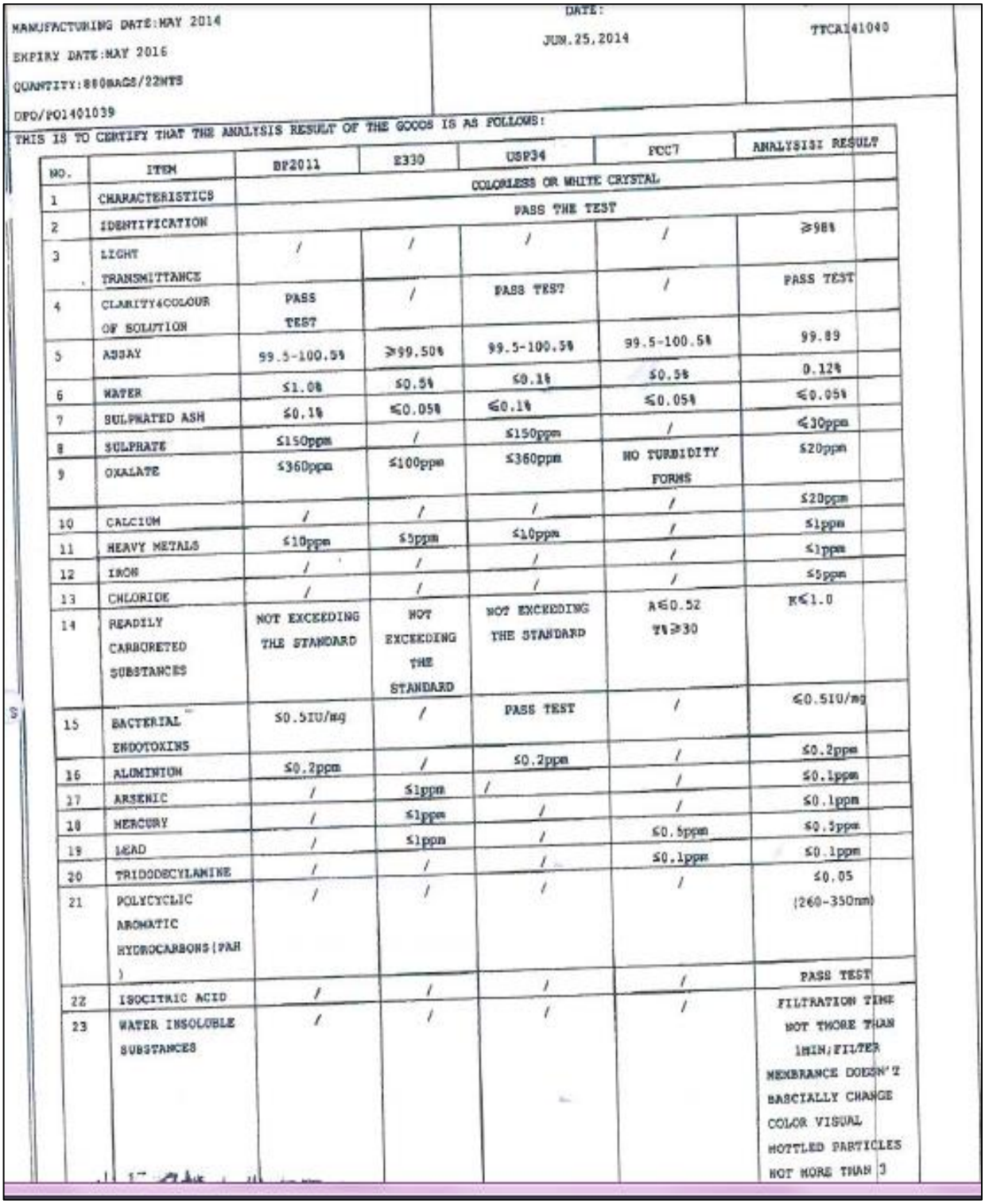
Batch K42780423

	Spec. Values		Batch Values	
Assay (acidimetric; calculated on dried substance)	99.5 - 100.5	%	100.2	%
Assay (acidimetric)	99.0 - 101.0	%	100.3	%
Identity	passes test		passes test	
Appearance of solution	passes test		passes test	
Insoluble matter	passes test		passes test	
pH-value				
1 %, water	8.0 - 8.6		8.1	
5 %; water	7.9 - 8.4		7.9	
Carbonate (CO_3)	passes test		passes test	
Chloride (Cl)	≤ 0.015	%	≤ 0.015	%
Sulphate (SO_4)	≤ 0.01	%	≤ 0.01	%
Sulfur compounds (as SO_4)	≤ 0.015	%	≤ 0.015	%
Heavy metals (as Pb)	≤ 0.0005	%	≤ 0.0005	%
Al (Aluminium)	≤ 0.00005	%	≤ 0.00005	%
As (Arsenic)	≤ 0.0002	%	≤ 0.0002	%
Ca (Calcium)	≤ 0.01	%	≤ 0.01	%
Cu (Copper)	≤ 0.0005	%	≤ 0.0005	%
Fe (Iron)	≤ 0.002	%	≤ 0.002	%
Hg (Mercury)	≤ 0.0001	%	≤ 0.0001	%
NH_4 (Ammonium)	passes test		passes test	
NH_4 (Ammonium)	≤ 0.002	%	≤ 0.002	%
Pb (Lead)	≤ 0.0002	%	≤ 0.0002	%
Zn (Zinc)	≤ 0.0025	%	≤ 0.0025	%
Residual solvents (Ph.Eur./USP/ICH)	excluded by production process		excluded by production process	
Loss on drying (Silica gel)	≤ 0.25	%	≤ 0.25	%

Residues of metal catalysts or metal reagents acc. to EMEA/CHMP/SWP/4446/2000 are not likely to be present.

Date of examination (DD.MM.YYYY): 27.09.2011
Minimum shelf life (DD.MM.YYYY): 30.09.2016

8.2.9. C.O.A. of Citric acid



MANUFACTURING DATE:MAY 2014
EXPIRY DATE:MAY 2016
QUANTITY:880BAGS/22MTS
DFD/PO1401039

DATE:
JUN.25,2014

TTCA141040

THIS IS TO CERTIFY THAT THE ANALYSIS RESULT OF THE GOODS IS AS FOLLOWS:

NO.	ITEM	BP2011	E330	USP34	FCC7	ANALYSIS RESULT
1	CHARACTERISTICS	COLORLESS OR WHITE CRYSTAL				
2	IDENTIFICATION	PASS THE TEST				
3	LIGHT TRANSMITTANCE	/	/	/	/	≥98%
4	CLARITY&COLOUR OF SOLUTION	PASS TEST	/	PASS TEST	/	PASS TEST
5	ASSAY	99.5-100.5%	≥99.50%	99.5-100.5%	99.5-100.5%	99.89
6	WATER	≤1.0%	≤0.5%	≤0.1%	≤0.5%	0.12%
7	SULPHATED ASH	≤0.1%	≤0.05%	≤0.1%	≤0.05%	≤0.05%
8	SULPHATE	≤150ppm	/	≤150ppm	/	≤30ppm
9	OXALATE	≤360ppm	≤100ppm	≤360ppm	NO TURBIDITY FORMS	≤20ppm
10	CALCIUM	/	/	/	/	≤20ppm
11	HEAVY METALS	≤10ppm	≤5ppm	≤10ppm	/	≤1ppm
12	IRON	/	/	/	/	≤1ppm
13	CHLORIDE	/	/	/	/	≤5ppm
14	READILY CARBORETED SUBSTANCES	NOT EXCEEDING THE STANDARD	NOT EXCEEDING THE STANDARD	NOT EXCEEDING THE STANDARD	A≤0.52 T%≥30	K≤1.0
15	BACTERIAL ENDOTOXINS	≤0.5IU/mg	/	PASS TEST	/	≤0.5IU/mg
16	ALUMINIUM	≤0.2ppm	/	≤0.2ppm	/	≤0.2ppm
17	ARSENIC	/	≤1ppm	/	/	≤0.1ppm
18	MERCURY	/	≤1ppm	/	/	≤0.1ppm
19	LEAD	/	≤1ppm	/	≤0.5ppm	≤0.5ppm
20	TRIDODECYLAMINE	/	/	/	≤0.1ppm	≤0.1ppm
21	POLYCYCLIC AROMATIC HYDROCARBONS(PAH)	/	/	/	/	≤0.05 (260-350nm)
22	ISOCITRIC ACID	/	/	/	/	PASS TEST
23	WATER INSOLUBLE SUBSTANCES	/	/	/	/	FILTRATION TIME NOT MORE THAN 1MIN;FILTER MEMBRANCE DOESN'T BASCIALLY CHANGE COLOR VISUAL MOTTLED PARTICLES NOT MORE THAN 3

8.2.10. C.O.A. of Lactose

DFE pharma

ORIGINAL

Certificate of analysis

Issue date
14.01.2014
Purchase order
3769
Delivery item
80540048 000010
Order item
408476 000010
Total Quantity Item
4.000 KG

Zifroni Chemicals Suppliers Ltd.
New Industrial Zone
4 Homa Street
75653 Rishon Le-Zion
Israel

Material:
Pharmatose 200M
Lactose Monohydrate USP/NF,Ph.Eur,JP
In multi layer paper bag with a poly-
ethylene innerbag contents 25 kg net.
(EU)
Production site : FrieslandCampina DMV BV, Veghel, The Netherlands
Product name : Pharmatose 200M
Conforms to USP/NF, Ph.Eur.,JP, Lactose monohydrate monograph, current at time of manufacture.
Product description: A white or almost white, crystalline powder freely but slowly soluble in water, practically insoluble in ethanol
Residual solvents
(CPMP/ICH/283/95) : No class 1,2,3 solvents are used during production
Identification : Complies with Pharmacopoeia when tested

Lot: 10747587 **Quantity:** 4.000 KG
Manufacture date: 12.2013 **Expiry date:** 11.2016

Characteristic	SPECIFICATION Unit	Lower Limit	Upper Limit	Value
Water (KF)	%	4,5	5,5	5,0
Loss on drying	%	0,0	0,5	0,1
Specific rotation 20 °C anhydr	NON	54,4	55,9	55,3
Residue on ignition/Sulph.Ash	%	0,00	0,10	0,03
Absorb.1% , 1cm at 270-300 nm	NON	0,00	0,07	0,01
Absorb.1% , 1cm at 210-220 nm	NON	0,00	0,25	0,03
Absorb.10% , 1cm at 400 nm	NON	0,00	0,04	0,02
Appearance of solution (Ph.Eur Clear and not more coloured than ref.BY7			Passes test	Passes test
Clarity and Colour of Solution Clear and colourless			Passes test	Passes test

8.2.11. C.O.A. of PVP-K30

检 验 报 告

Co., Ltd.
地址：河南省温县工业集聚区鑫源路东段 115 号
Add: No.115, East Xinyuan Road, Wen Town, 454800, Henan, China.
Tel: 0391-6109928
Fax: 0391-6109918

CERTIFICATE OF ANALYSIS

品名/PRODUCT:	PVP K30 POVIDONE	规格/SPECIFICATION:	PHARMA GRADE
批号/LOT NO.:	20131210	数量/AMOUNT :	1500 KGS
生 产 日 期 /MFG DATE:	2013-12-10	有效期/EXPIRY DATE:	2016-12-09

测定项目 TEST ITEMS	指 标 SPECIFICATION	实测值 TEST VALUE
Items	specification	Test results
First Identification: A, E.	Positive	Complies
second Identification: B, C, D, E.	Positive	Complies
Appearance of solution	Clear	Complies
water	5% max	3.06%
Viscosity, as K-Value	27-32.4	30.57
PH	3.0-5.0	3.5
Impurity A(1-Vinylpyrrolidin-2-one)	10 ppm max	4.6ppm
Impurity B (2-Pyrrolidone)	3.0% max	1.98%
sulphated ash	0.1% max	0.05%
Assay, Nitrogen Content	11.5-12.8%	12.17%
Aldehydes, as Acetaldehyde	500 ppm max	190ppm
Heavy metals	10 ppm max	Complies
Hydrazine	1 ppm max	Complies
Peroxides, as H2O2	400 ppm max	40ppm
Appearance	White or yellowish-white powder	White powder

贮藏条件/Storage Conditions : Keep in dry place, store in tight closed container.
分析者意见/OPINION OF THE ANALYSER:

8.2.12. C.O.A. of Avicel PH-102

FMC International
Wallingstown, Little Island
Co. Cork, Ireland
Customer Service: +353-21-435-4133
Fax: +353-21-451-7210

FMC BioPolymer

Certificate of Analysis

Avicel® Microcrystalline Cellulose, NF, Ph. Eur, JP

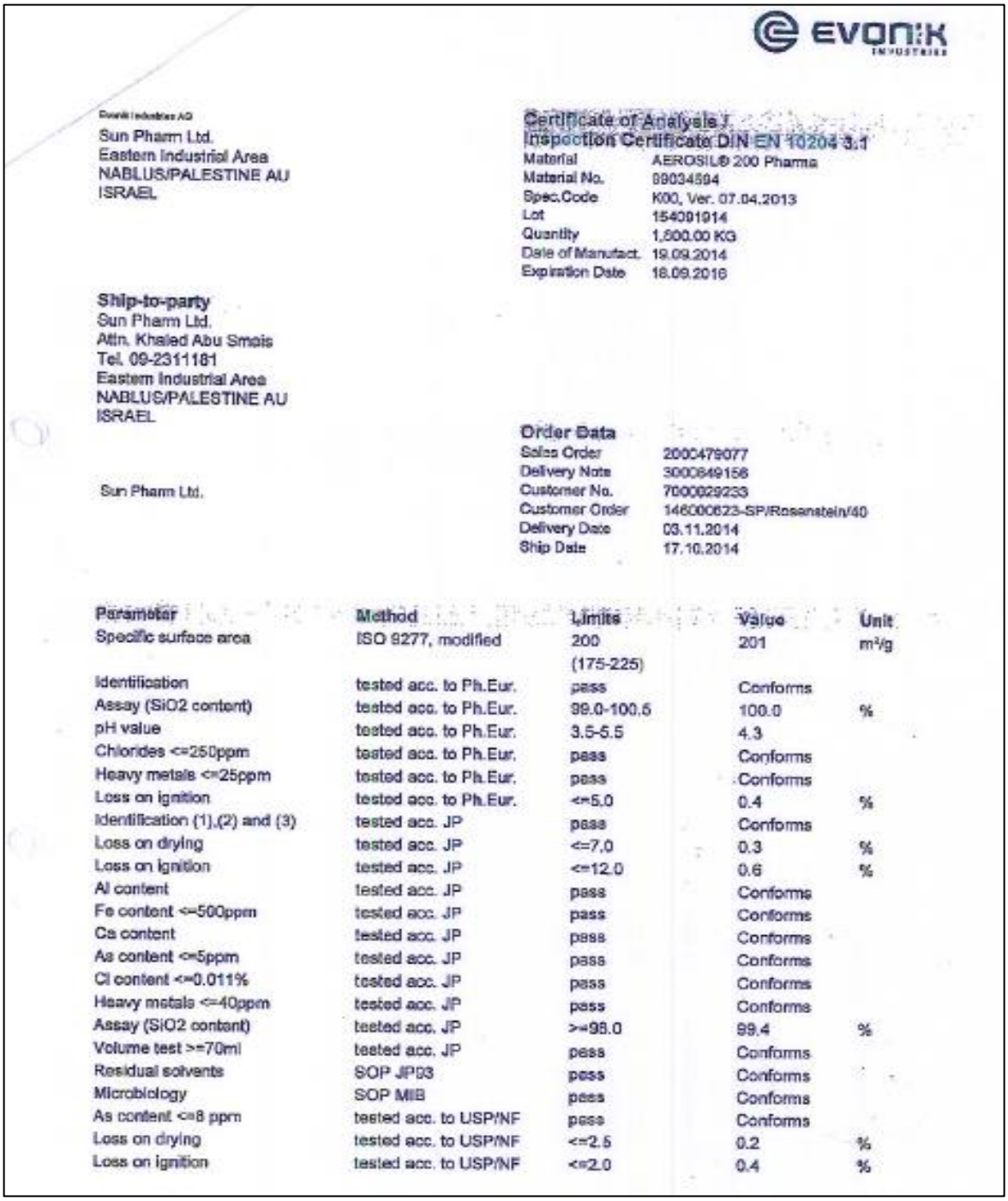
Type : PH-102
Lot No : 71217C
Manufacturing Date: 24-Apr-2012
Reevaluation Date: 23-Apr-2016

Customer Purchase Order : 14/00000282
Delivery Number : 80602839

Standard	Specification	Lot Analysis
Loss on Drying, %	3.0 - 5.0	3.5
Loose Bulk Density, g/cc	0.28 - 0.33	0.30
DP ,units (ID B USP,EP)(ID 3 JP)	NMT 350	211
P.S.D.,Malvern LD, µm ,d10 (FRC, Ph.eur)	15 - 55	31
P.S.D.,Malvern LD, µm ,d50 (FRC, Ph.eur)	80 - 140	110
P.S.D.,Malvern LD, µm ,d90 (FRC, Ph.eur)	170 - 283	229
Identification A(USP,EP, JP 1)	PASS	Pass
Identification 2 (JP)	PASS	Pass
pH	5.5 - 7.0	6.0
Conductivity,µS/cm	NMT 75	44
Residue on Ignition, %	NMT 0.050	0.000
Water Soluble Substances, mg/5g	NMT 12.5	7.4
Water soluble substances, %	NMT 0.25	0.15
Heavy Metals, %	NMT 0.001	Pass
Sol.in Cu Tetramine Hydroxide	Soluble	Pass
Ether Soluble Substances,mg/10g	NMT 5.0	0.5
Air Jet Particle Size, wt. % +60Mesh	NMT 8.0	0.9
Air Jet Particle Size, wt. % +200Mesh	NLT 45.0	63.0
Total Aerobic Microbial Count, cfu/gram	NMT 100	Pass
Total Yeast and Mold Count, cfu/gram	NMT 20	Pass
Salmonella Species	Absent in a 10g sample	Pass
Escherichia coli	Absent in a 10g sample	Pass
Staphylococcus aureus	Absent in a 10g sample	Pass
Pseudomonas aeruginosa	Absent in a 10g sample	Pass
Coliform species	Absent in a 10g sample	Pass

Storage Conditions: Store at ambient conditions, keep containers sealed, material is hygroscopic.

8.2.13. C.O.A. of Silicone dioxide



EVONIK INDUSTRIES

Evonik Industries AG
Sun Pharm Ltd.
Eastern Industrial Area
NABLUS/PALESTINE AU
ISRAEL

Certificate of Analysis /
Inspection Certificate DIN EN 10204 3.1

Material	AEROSIL® 200 Pharma
Material No.	89034594
Spec.Code	K00, Ver. 07.04.2013
Lot	154091914
Quantity	1,600.00 KG
Date of Manufact.	19.09.2014
Expiration Date	18.09.2016

Ship-to-party
Sun Pharm Ltd.
Attn. Khaled Abu Smais
Tel. 09-2311161
Eastern Industrial Area
NABLUS/PALESTINE AU
ISRAEL

Sun Pharm Ltd.

Order Data

Sales Order	2000479077
Delivery Note	3000849156
Customer No.	7000029233
Customer Order	146000623-SP/Rosenstein/40
Delivery Date	03.11.2014
Ship Date	17.10.2014

Parameter	Method	Limits	Value	Unit
Specific surface area	ISO 9277, modified	200 (175-225)	201	m^2/g
Identification	tested acc. to Ph.Eur.	pass	Conforms	
Assay (SiO2 content)	tested acc. to Ph.Eur.	99.0-100.5	100.0	%
pH value	tested acc. to Ph.Eur.	3.5-5.5	4.3	
Chlorides <=250ppm	tested acc. to Ph.Eur.	pass	Conforms	
Heavy metals <=25ppm	tested acc. to Ph.Eur.	pass	Conforms	
Loss on ignition	tested acc. to Ph.Eur.	<=5.0	0.4	%
Identification (1),(2) and (3)	tested acc. JP	pass	Conforms	
Loss on drying	tested acc. JP	<=7.0	0.3	%
Loss on ignition	tested acc. JP	<=12.0	0.6	%
Al content	tested acc. JP	pass	Conforms	
Fe content <=500ppm	tested acc. JP	pass	Conforms	
Ca content	tested acc. JP	pass	Conforms	
As content <=5ppm	tested acc. JP	pass	Conforms	
Cl content <=0.011%	tested acc. JP	pass	Conforms	
Heavy metals <=40ppm	tested acc. JP	pass	Conforms	
Assay (SiO2 content)	tested acc. JP	>=98.0	99.4	%
Volume test >=70ml	tested acc. JP	pass	Conforms	
Residual solvents	SOP JP03	pass	Conforms	
Microbiology	SOP MIB	pass	Conforms	
As content <=8 ppm	tested acc. to USP/NF	pass	Conforms	
Loss on drying	tested acc. to USP/NF	<=2.5	0.2	%
Loss on ignition	tested acc. to USP/NF	<=2.0	0.4	%

EVONIK INDUSTRIES

Parameter	Method	Limits	Value	Unit
Identification, A and B	tested acc. to USP/NF	pass	Conforms	
pH value	tested acc. to USP/NF	3.5-5.5	4.2	
Assay (SiO2 content)	tested acc. to USP/NF	99.0-100.5	99.9	%

AEROSIL® 200 Pharma:
Colloidal Silicon Dioxide tested according to Ph.Eur., USP/NF and JP (current Version)
Manufactured and packaged in a dedicated closed production system according to GMP guidelines established for bulk pharmaceutical excipients by the International Pharmaceutical Excipients Council (IPEC/GMP).

White, fine, amorphous powder.

Residual solvents:
No organic solvents are used in the manufacture of above mentioned product. For this reason, constitutionally no residual solvents as cited in recent versions of the European Pharmacopoeia, (class 1, 2 and 3 or other solvents, USP chapter 467), 2006 and amendments are present in concentration about the control limits quoted in USP. For above mentioned product class 1 residual solvents are tested on a regular basis according to USP/NF: Carbon tetrachloride, 1,2 Dichlorethane, 1,1 Dichlorethene, 1,1,1 Trichlorethane and Benzene.

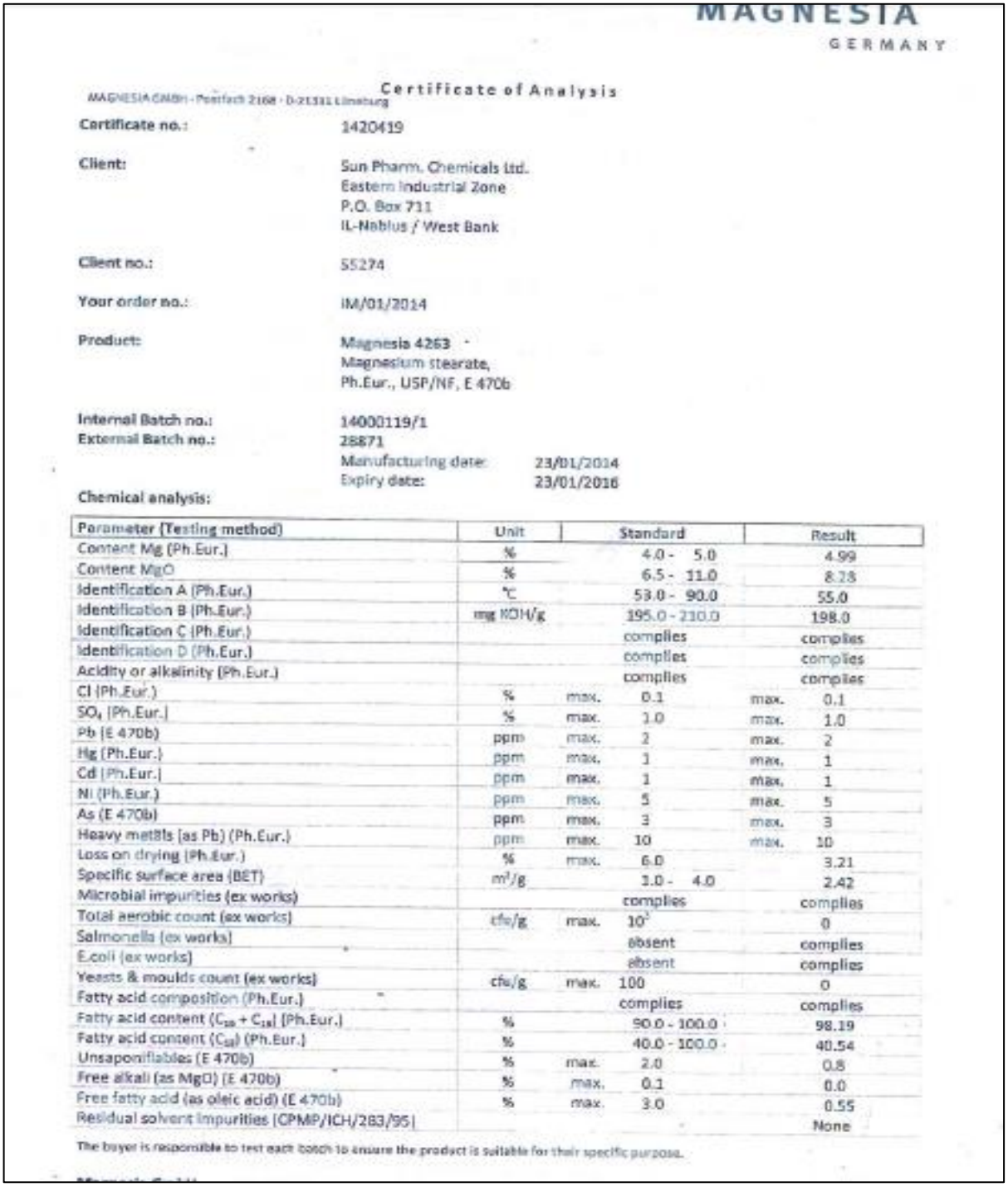
TSE/BSE and materials of plant origin:
No raw materials of animal or plant origin (as mentioned in EMEA/410/01, current version) are used in the production process of AEROSIL® Pharma products. AEROSIL® Pharma products have not been in contact with and constitutionally do not include any material of animal or plant origin. We generally do not use any material of animal or plant origin in our production facilities. AEROSIL® Pharma products are not contaminated with material of animal or plant origin when they leave our production and warehouses.

This product is manufactured in Site Rheinfelden, Untere Kanalstrasse 3, D-79618 Rheinfelden, Germany.

Print Date: 17.10.2014
Dr. Peer Plambeck-Fischer
Inspector, Rheinfelden site

Phone: +49 7623 917235
Mail: peer.plambeck-fischer@evonik.com

8.2.14. C.O.A. of magnesium stearate:



MAGNESIA
GERMANY

MAGNESIA GMBH · Postfach 2168 · D-21331 Lüneburg

Certificate of Analysis

Certificate no.: 1420419

Client: Sun Pharm. Chemicals Ltd.
Eastern Industrial Zone
P.O. Box 711
IL-Nablus / West Bank

Client no.: 55274

Your order no.: IM/01/2014

Product: Magnesia 4263
Magnesium stearate,
Ph.Eur., USP/NF, E 470b

Internal Batch no.: 14000119/1
External Batch no.: 28871
Manufacturing date: 23/01/2014
Expiry date: 23/01/2016

Chemical analysis:

Parameter (Testing method)	Unit	Standard	Result
Content Mg (Ph.Eur.)	%	4.0 - 5.0	4.99
Content MgO	%	6.5 - 11.0	8.28
Identification A (Ph.Eur.)	°C	53.0 - 90.0	55.0
Identification B (Ph.Eur.)	mg KOH/g	195.0 - 210.0	198.0
Identification C (Ph.Eur.)		complies	complies
Identification D (Ph.Eur.)		complies	complies
Acidity or alkalinity (Ph.Eur.)		complies	complies
Cl (Ph.Eur.)	%	max. 0.1	max. 0.1
SO_4 (Ph.Eur.)	%	max. 1.0	max. 1.0
Pb (E 470b)	ppm	max. 2	max. 2
Hg (Ph.Eur.)	ppm	max. 1	max. 1
Cd (Ph.Eur.)	ppm	max. 1	max. 1
Ni (Ph.Eur.)	ppm	max. 5	max. 5
As (E 470b)	ppm	max. 3	max. 3
Heavy metals (as Pb) (Ph.Eur.)	ppm	max. 10	max. 10
Loss on drying (Ph.Eur.)	%	max. 6.0	3.21
Specific surface area (BET)	m^2/g	1.0 - 4.0	2.42
Microbial impurities (ex works)		complies	complies
Total aerobic count (ex works)	cfu/g	max. 10^3	0
Salmonella (ex works)		absent	complies
E.coli (ex works)		absent	complies
Yeasts & moulds count (ex works)	cfu/g	max. 100	0
Fatty acid composition (Ph.Eur.)		complies	complies
Fatty acid content (C_{16} + C_{18}) (Ph.Eur.)	%	90.0 - 100.0	98.19
Fatty acid content (C_{18}) (Ph.Eur.)	%	40.0 - 100.0	40.54
Unsaponifiables (E 470b)	%	max. 2.0	0.8
Free alkali (as MgO) (E 470b)	%	max. 0.1	0.0
Free fatty acid (as oleic acid) (E 470b)	%	max. 3.0	0.55
Residual solvent impurities (CPMP/ICH/283/95)			None

The buyer is responsible to test each batch to ensure the product is suitable for their specific purpose.

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تطوير وتقييم مخبري لمستحضر صيدلاني جديد يحتوي على الايزوميبرازول والكلاريثرومايسين والميترونيدازول معا في حبة واحدة مكونة من طبقتين، احداهما سريعة التفكك والأخرى تبقى طافية في المعدة لفترة طويلة، لعلاج القرحة الهضمية الناتجة عن البكتيريا الملوية البوابية .

إعداد: سائدة محمد عبدالقادر

المشرف: د. طارق الجعبة

الملخص

تعد الاصابة بالبكتيريا البوابية واحدة من أكثر الأمراض المعدية انتشارا والتي تصيب حوالي نصف سكان العالم.و يبقى القضاء على هذه البكتيريا هاما في علاج الامراض المصاحبة لها مثل القرحة الهضمية، والمضاعفات طويلة المدى مثل سرطان المعدة.

الصف العلاجي الأول للبكتيريا ومرض القرحة الهضمية المصاحبة لها هو العلاج الثلاثي المكون من اثنين من المضادات الحيوية(كلاريثرومايسين، ميترونيدازول) ومثبط للمضخة البروتونية (مثل ايزوميبرازول). علاج القرحة الهضمية بالأشكال الصيدلانية التقليدية ممكن ان يفشل، وذلك لقصر الوقت الذي يبقى فيه الدواء في المعدة (المكان الرئيسي لاستعمار البكتيريا) بسبب التفريغ المعدي المستمر. وواحدة من أهم مساوىء هذه العلاجات التقليدية هو الحاجة لجرعات متعددة منها يوميا، ممايزيد من عدم انتظام المريض على أخذ العلاج و ازدياد احتمالية حدوث الأعراض الجانبية.

من طرق تحسين علاج القرحة الهضمية هو وجود علاجات بديلة لها القدرة على البقاء لفترات طويلة في المعدة بحيث تعمل على تحرير الدواء بشكل متاني يضمن بقاءه بشكل دائم وبتركيز عالي في مكان استعمار البكتيريا، مما يؤدي لتحسين فعالية العلاج وتقليل عدد الجرعات اليومية وبالتالي تحسين انتظام المريض على العلاج وتقليل الأعراض الجانبية. المستحضرات الصيدلانية طويلة

الوجود في المعدة لعلاج البكتيريا البوابية لاقت اهتماما كبيرا من قبل الباحثين حول العالم وذلك لقدرتها على توفير االمزايا المذكورة آنفا.

في هذا البحث تم تطوير شكل صيدلاني جديد يحتوي على أدوية الصف العلاجي الأول (كلاريثرومايسين و ميترونيدازول و ايزوميبرازول) لعلاج القرحة الهضمية. اعتمد تصميم المستحضر على ان يكون حبة واحدة مكونة من طبقتين، لها القدرة على الانتفاخ والطفو وتحرير الدواء بشكل متأني. واحدة من الطبقات هي سريعة التفكك وتحتوي عل حبيبات الايزوميبرازول، والطبقة الثانية تحتوي على المضادات البكتيرية (الكلاريثرومايسين والميترونيدازول) بحيث تبقى طافية في المعدة وتحرر الأدوية بشكل متأني.

اعتمد تشكيل الحبوب على استخدام بوليمرات الهيدروكسي بروبيل ميثل السليولوز بدرجات لزوجة مختلفة واستخدام بوليمر الايثيل سيليولوز كمواد غير فعالة مسؤولة عن انتفاخ الحبات ومنظمة لتحرير الدواء. كما تم استخدام مادتي بايكربونات الصوديوم وحمض السيتريك كمواد مسؤولة عن تكوين غاز ثاني أوكسيد الكربون داخل الحبة. تم استخدام تقنيات تصنيع مختلفة وهي الضغط المباشر للحبوب والتحبيب الرطب والتحبيب الجاف ، لاختيار الأفضل منه.

اعتمد اختيار افضل تركيبة أولا على فحوصات خصائص الطفو للحبات بحيث تم اختيار الحبات التي استغرقت أقل وقت لتطفو واستمرت بالطفو لأطول فترة ممكنة، ثانيا على فحوصات خصائص تحرير الدواء من الحبة بحيث تم اختيار الحبات التي تعمل على تحرير كلا المضادات الحيوية المستخدمة بشكل متأني ومتزامن.

في التركيبة الأمثل تم استخدام طريقة التصنيع بالتحبيب الجاف. حيث تم تحبيب مادة الميترونيدازول في الطبقة متأنية التحرير بينما تم دمج مارة الكلاريثرومايسين دون تحبيب مع باقي مكونات الطبقة.

وفي الطبقة سريعة التفكك تم دمج حبيبات الايزوميبرازول مع باقي المكونات بحيث تنفصل عن الطبقة متأنية التحرير بوقت قصير لا يتجاوز الدقيقتين. وقد تم فحص الخصائص الفيزيائية والكيميائية للحبوب والتي تشمل فحوصات الصلابة والتفتيت و التباين في الوزن والفحص الكمي ، وقد وجدت جميعها مطابقة لمتطلبات دساتير الادوية العالمية، كما تم فحص خصائص الخليط قبل كبسه الى حبوب

كم تم فحص قدرة الحبة على الانتفاخ و تقييم آلية تحرر المواد الفعالة باستخدام نماذج رياضية عدة ، وقد وجد أن تحرر المواد الفعالة (الميترونيدازول والكلاريثرومايسين) يتناسب مع نموذج كورس ماير– بيباس ، واعتمادا على قيمة (n) لهذا الموذج يتضح ان التحرر كان مختلطا عن طريق الانتشار من خلال طبقة الجل المنتفخة وعن طريق تآكل البوليمر على سطح الحبة.

تم احتساب كميات مادتي الكلاريثرومايسين والميترونيدازول بشكل متزامن في نفس العينة لكلا الفحصين، الفحص الكمي وفحص تحرر الدواء من الحبة، وذلك باستخدام طريقة لاجراء الفحوصات تم تطويرها والتثبت من صحتها ودقتها في هذا البحث والتي اعتمدت على استخدام جهاز الاستشراب السائل عالي الانجاز (HPLC).

التركيبة الأمثل التي تم تصنيعها باستخدام طريقة التحبيب الجاف احتوت على مانسبته 23% من بوليمر الهيدروكسي بروبيل ميثيل سيليولوز عالي اللزوجة (K-100M) و 17% من بوليمر الايثيل سيليولوز و 12% بايكربونات الصوديوم و 3% من حمض السيتريك في الطبقة المتانية التحرير. في هذه التركيبة استغرق انفصال الطبقتين سريعة التفكك و متأنية التحرير وقتا لم يتجاو20 ثانية واستغرق وقت حدوث الطفو وقتا لم يتجاوز 30 ثانية، فيما استمرت الحبة بالطفو لمدة تجاوزت 24 ساعة. وبلغت كمية الدواء التي تم تحريرها من الطبقة المتانية التحرير 85% لمادة المترونيدازول

و76% لمادة الكلاريثرومايسين. هذه النتائج حققت الاهداف من هذه الدراسة بتصنيع شكل صيدلاني جديد له القدرة على البقاء لوقت طويل في المعدة مع تحرير الدواء بشكل متاني، آملين ان يؤدي ذلك لتحسين الفعالية الموضعية لهذه المضادات الحيوية في القضاء على البكتيريا البوابية.