Development of Formulations of Penicillin G for the Local Treatment of *Helicobacter pylori*

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Dedicated to my mother

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Abbreviations

API	Active pharmaceutical ingredient
ARAs	Acid-reducing agents
BP	British Pharmacopeia
CAEMs	Chitosan–alginate–ethyl-cellulose microparticles
CagA	Cytotoxin-associated gene A
CFR	Constant flow rate
C_{max}	Maximum salivary concentration
CO_2	Carbon dioxide
CT	Computerized tomography
DAD	Diode array detector
DSC	Differential scanning calorimetry
DSTD	Dissolution stress test device
EFSA	European Food Safety Authority
EMA	European Medicines Agency
E-test	Epsilometer test
FDA	Food and Drug Administration
FSM	Fed Stomach Model
FTM	Flow-through model
GCV	Gastric content volume
GERD	Gastroesophageal reflux disease
GET	Gastric emptying time
GI	Gastrointestinal

GIT	Gastrointestinal tract
GRAS	Generally Recognized As Safe
GRT	Gastric residence time
HCl	Hydrochloric acid
HME	Hot-melt extrusion
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
I.D.	Internal diameter
IMMC	Interdigestive migrating motor complex
ICH	International Conference on Harmonization
IgG	Immunoglobulin G
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
LVF	Levofloxacin
MIC	Minimum inhibitory concentration
MMC	Migrating motor complex
MMM	Magnetic marker monitoring
MRI	Magnetic resonance imaging
MS	Mass spectrometry
NAB	Nocturnal Acid Breakthrough
n	Number
PCB	Phosphate-citrate buffer

PGS	Penicillin G sodium
PBPs	Penicillin-binding proteins
PBS	Phosphate buffer solution
pH_M	Microenvironmental pH
PPIs	Proton pump inhibitors
PVC	Polyvinyl chloride
PVP	Polyvinyl pyrrolidone
PUD	Peptic ulcer disease
RMC	Residual moisture content
rpm	Revolution per minute (unit)
RSD	Relative standard deviation
SGF	Simulated gastric fluid
SGF_{sp}	Simulated gastric fluid *sine pepsin*
SR	Swelling ratio
TIGR	The Institute for Genomic Research
T_{max}	Time at maximum concentration
USP	United States Pharmacopeia
VacA	Vacuolating cytotoxin A
VFR	Variable flow rate
UBT	Urea breathe test
WHO	World Health Organization

1 Introduction

1.1 *Helicobacter pylori*

Helicobacter pylori is one of the gram-negative bacteria that colonizes the human stomach. It has a spiral shape and grows under microaerophilic conditions. The bacterium was first successfully isolated from gastric biopsies of patients with gastritis and cultivated *in vitro* in 1983 by Marshall and Warren.[1,2] It is 2.5 to 3.5 µm long and 0.5 to 1.0 µm in diameter with a smooth surface, and one to six polar-sheathed flagella emerge from one end of its rounded ends. This organism colonizes primarily the gastric antrum and the cardia, where parietal cells are rare.[3] It produces a urease enzyme that converts urea to ammonia and carbon dioxide. It can be cultured in an environment within a pH range of 4.5–9 and a temperature of 30–37 °C.[4]

1.2 Prevalence of *Helicobacter pylori* infection

Helicobacter pylori infection continues to be a significant and worldwide public health concern. About half of the world's population and the majority of people in developing countries are infected with this pathogen. Figure 1.1 shows the global prevalence of the infection. The highest incidence has been reported in Africa, Latin America, the Caribbean, and Asia, with the prevalence rate range 50–80 %. In contrast, *H. pylori* prevalence is the lowest in Northern America and Oceania (25–37 %). The prevalence among European populations is in the region of 20–40 %.[5–9] Moreover, the incidence is lower in developed than in developing countries. Factors such as older age, lower socioeconomic status, and poor hygiene are generally associated with high prevalence rates.[9–11]

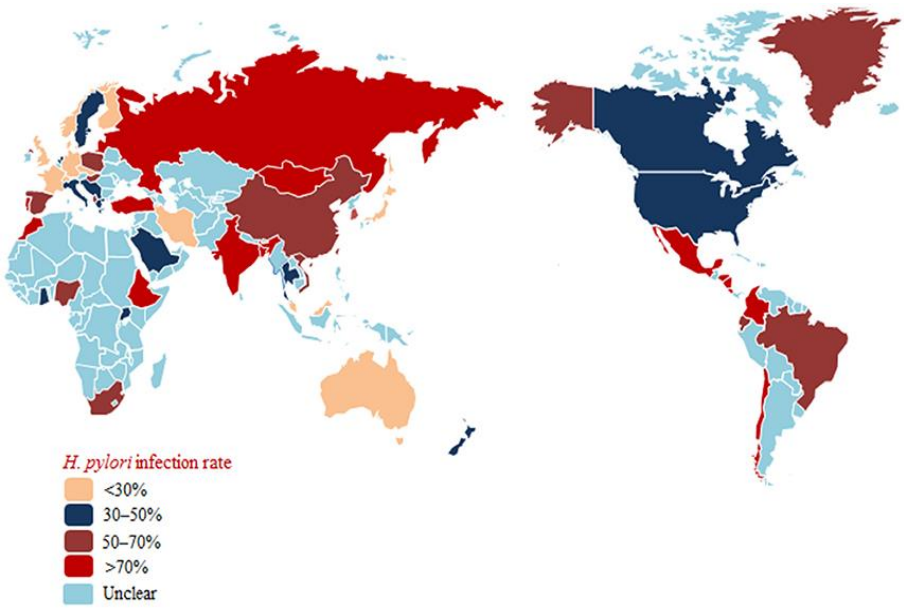


Figure 1.1 *Helicobacter pylori* prevalence in populations worldwide reported in 2017.[12]

1.3 Pathophysiology of *Helicobacter pylori* infection

H. pylori is a pathogen because its presence is associated with chronic gastritis, peptic ulceration, and stomach cancer.[13] Several virulent factors are involved in the pathogenesis of *H. pylori* infections (Figure 1.2). It produces an abundant urease enzyme that hydrolyzes urea to ammonia, thereby permitting the bacterium to survive in an acidic milieu. Motility is essential for colonization, and *H. pylori* flagella have adapted to the gastric niche.[14] A large amount of mucus produced by epithelial cells also contributes to protecting the bacterium from gastric acid.[15]

The bacterium can be found close to the epithelial surface and attaches to the gastric epithelium via specialized adhesins such as Blood group antigen-binding adhesin A (BabA) and Sialic acid-binding adhesin (SabA), which leads to successful colonization and persistent infection.[16] It also releases several effector proteins, including Cytotoxin-associated gene A (CagA) and Vacuolating cytotoxin A (VacA). The CagA is a highly immunogenic protein and causes higher inflammatory response.[17] The VacA protein plays a significant role in the pathogenesis of both peptic ulceration and gastric cancer. Its effect is via membrane channel formation, disruption of

endosomal and lysosomal activity, effects on integrin receptor-induced cell signaling, interference with cytoskeleton-dependent cell functions, induction of apoptosis, and immune modulation.[18,19]

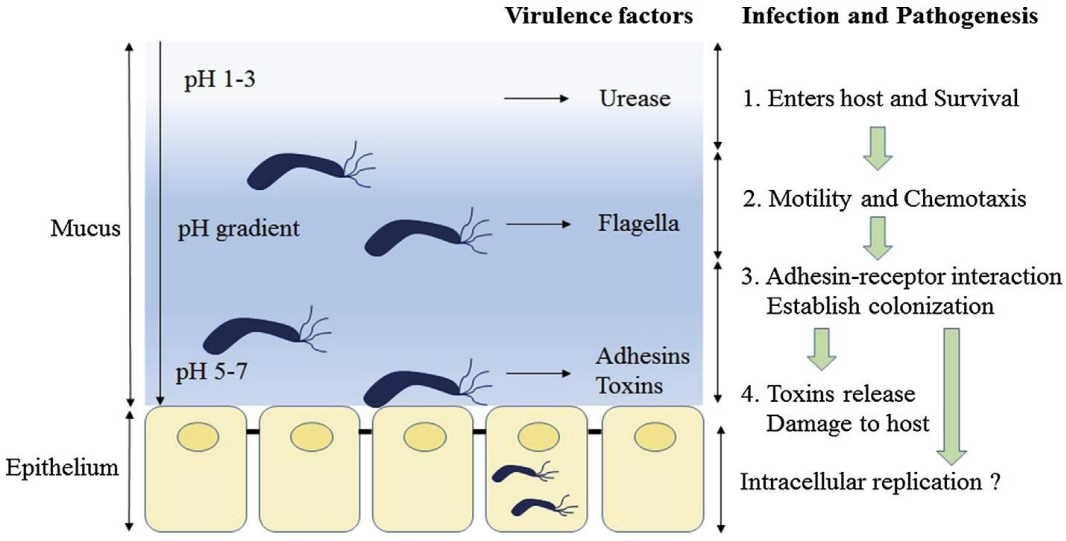


Figure 1.2 Schematic representation of *Helicobacter pylori* infection and pathogenesis.[19]

1.4 Diagnosis of *Helicobacter pylori* infection

H. pylori infection can be diagnosed using either non-invasive or invasive methods. These methods include urea breathe test (UBT), serologic tests, stool antigen assays, and urease tests on endoscopic biopsies. The use of a specific test mainly depends on clinical settings.[20,21] Serological tests identify circulating IgG antibodies specific to *H. pylori*. There are three main methods for these tests: the enzyme-linked immunosorbent assay test, latex agglutination tests, and Western blotting. Stool antigen tests use monoclonal and polyclonal antibodies to detect the presence of *H. pylori* antigens in stools.[20,22] Use of the UBT method involves drinking ^{13}C or ^{14}C-labeled urea, which is converted to labeled carbon dioxide by the urease in the presence of *H. pylori*. Then, the labeled gas in a breath sample is measured. UBT has a sensitivity and a specificity of more than 95 %.[23] The invasive methods involve endoscopic detection of biopsy of the gastric mucosa using histology, rapid urease test, and culture investigation techniques.[24]

1.5 Pharmacotherapy of *Helicobacter pylori* infection

1.5.1 Antibiotics

Economic models suggest that *H. pylori* eradication is cost-effective compared to offering no treatment.[25] *H. pylori* eradication proved to decrease gastric ulcer recurrence and the prevention of bleeding. Indeed, failure to eradicate causes more complications, including gastric cancer.[26] Thus, antibiotics have become the central part of any *H. pylori* eradication therapy, and a useful tool in the fight against *H. pylori*-associated diseases.[27] Several medicines are part of the various *H. pylori* treatment regimens. Amoxicillin, clarithromycin, metronidazole, tetracycline, fluoroquinolones, furazolidone, rifabutin, and bismuth are the most commonly used antibiotics in *H. pylori* eradication therapies.[28,29]

1.5.2 Acid-reducing agents

Acid-reducing agents (ARAs) are prescribed to reduce the acidity of gastric contents and include proton pump inhibitors (PPIs), H2 receptor blockers, and others.[30] Among them, PPIs are the most commonly used drugs in the current treatment of acid-related gastrointestinal disorders, including gastroesophageal reflux disease (GERD) and peptic ulcer disease (PUD).[31] PPIs achieve a higher degree and longer duration of gastric acid suppression and better healing rates. They act by blocking the gastric H^+/K^+-ATPase enzyme (the proton pump) in the parietal cells, which is the final step in gastric acid secretion.[30,32] The ultimate goal of PPI therapy is to promote ulcer healing while the etiology of the ulcer is addressed simultaneously. However, the length of PPI therapy depends on the ulcer location, etiology, and associated complications.[33] Omeprazole, esomeprazole, rabeprazole, lansoprazole, and pantoprazole are among commonly used PPIs.[34]

The second class of acid-reducing agents is H2 receptor blockers. They are selective and potent competitive antagonists of the H2 receptors and cause a marked reduction in H^+ output, pepsin

activity, and the total volume of gastric secretions.[35] The four available drugs in this class are cimetidine, ranitidine, famotidine, and nizatidine. Some studies demonstrated that PPIs do not sufficiently suppress acid secretion overnight, and the pH can be at a potentially harmful level due to Nocturnal Acid Breakthrough (NAB). The NAB is described as a phase where the intragastric pH is continuously less than pH 4 for more than 1 h overnight. It occurs in 75 % of GERD patients and healthy subjects and is usually accompanied by oesophageal reflux.[36] Therefore, adding a bedtime H2 blocker to the treatment enhances nocturnal gastric pH control and decreases NAB compared to PPIs based on a twice per day regimen.[37]

1.5.3 Treatment regimens

The goal of *Helicobacter pylori* eradication therapy is to cure PUD and reduce the lifetime risk of gastric cancer. Various treatment regimens have been investigated and supported by numerous scientific panels and consensus groups around the globe to achieve this goal.[29,38] The Toronto consensus in 2016, for instance, has proposed several treatment regimens for *H. pylori* infection. The focus of the scientific agreement was to recommend appropriate treatment alternatives. In this regard, both the resistance rate of *H. pylori* to antibiotics and the regional variabilities in resistance rates were considered.[38] The summary of the treatment regimens and their definitions based on the consensus are described below (Table 1.1).

Table 1.1 Recommended *Helicobacter pylori* eradication regimens based on the Toronto consensus 2016.

Recommendation	Regimen	Definition
First-line		
Recommended option	Bismuth quadruple	PPI + bismuth + metronidazole [a] + tetracycline
Recommended option	Concomitant nonbismuth quadruple	PPI +amoxicillin + metronidazole [a] + clarithromycin
Restricted option [b]	PPI triple	PPI + amoxicillin + clarithromycin PPI + amoxicillin + metronidazole [a] PPI + metronidazole [a] + clarithromycin
Not recommended	Levofloxacin triple	PPI + amoxicillin + levofloxacin
Not recommended	Sequential non-bismuth quadruple	PPI + amoxicillin followed by PPI + metronidazole [a] + clarithromycin
Prior treatment failure		
Recommended option	Bismuth quadruple	PPI + bismuth+ metronidazole [a] + tetracycline
Recommended option	Levofloxacin-containing therapy	PPI + amoxicillin + levofloxacin [c]
Restricted option [d]	Rifabutin-containing therapy	PPI + amoxicillin + rifabutin
Not recommended	Sequential non-bismuth quadruple therapy	PPI + amoxicillin followed by PPI + metronidazole [a] + clarithromycin
Undetermined	Concomitant non-bismuth quadruple therapy	PPI + amoxicillin + metronidazole [a] + clarithromycin

[a] Tinidazole substituted for metronidazole

[b] Restricted to areas of low clarithromycin resistance (< 15 %) or proven high local eradication rates (> 85 %)

[c] There is some evidence that adding bismuth to this combination may improve outcomes

[d] Restricted to cases in which at least three recommended options have failed

H. pylori eradication rates have been declining while the prevalence of antibiotics resistance rates has been rising. Metronidazole and clarithromycin resistance rates are alarming, although they vary among populations.[9,39] Recently, the Maastricht V/Florence Consensus report 2017 recommended different treatment regimens.[40] Most of the recommendations are similar to The

Toronto Consensus 2016, but more emphasis is given to the requirements of susceptibility guided treatment and also to regions of high clarithromycin resistance (> 15 %).

The primary clarithromycin resistance of *H. pylori* in Germany in the period 2015–2018 was reported to be low (11.3 %).[41] The primary treatment can be a standard triple therapy containing clarithromycin or bismuth-containing quadruple therapy. In the triple therapy, clarithromycin can be used either with amoxicillin or metronidazole. Moreover, a treatment that lasts 10 to 14 days is more likely to eradicate the pathogen better than therapy for seven days.[42]

Despite several efforts to treat *H. pylori* infections, the eradication rates from many of the treatment regimens have been declining at an alarming rate, mainly due to increased antibiotic resistance.[9,39,43,44] Consequently, in 2017 WHO has categorized clarithromycin-resistant *H. pylori* on a high priority list of antibiotic-resistant bacteria which need urgent direction on research, discovery, and development of new antibiotics.[45]

1.6 Physiological considerations in the local treatment of gastric diseases

On top of the physicochemical properties of the drug and the dosage form, the physiological factors such as buffer species, pH, gastric emptying rate, GI motility, and volume of secretion play essential roles in drug dissolution and absorption.[46,47] In addition to the factors mentioned above, the effect of intragastric pressure on drug release is discussed in the dissolution stress test device (DSTD) part. In this section, the anatomy of the stomach and the primary physiological factors that could affect the performances of gastric-specific drug delivery systems are discussed.

1.6.1 Anatomy of the stomach

The stomach is the most dilated part of the GIT with a capacity of 1.0–1.5 L in the adult. It is composed of anatomical regions: cardia, fundus, corpus, antrum, and pylorus (Figure 1.3). The

fundus and body expand greatly to accommodate ingested food and also to regulate the emptying of liquids. The antrum grinds the components into smaller particles (< 2 mm) that can be emptied into the duodenum. The gastroesophageal sphincter prevents reflux of gastric fluid into the esophagus, and the pyloric sphincter controls emptying into the small intestine.[48,49]

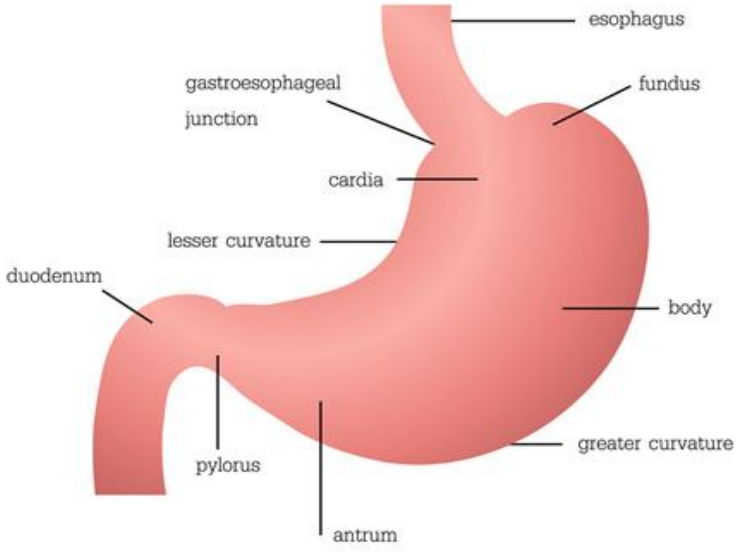


Figure 1.3 Anatomy of the stomach.[49]

1.6.2 Gastric motility and emptying

Gastric motility is the result of the interaction of smooth muscles with neural and hormonal stimuli.[50,51] Depending on the feeding conditions, the gastric motility is variable.[52] In the fasted state, the upper GI motility is dominated by a distinct cyclic pattern called the inter-digestive migrating motor complex (IMMC). This recurrent event moves from the stomach to the terminal ileum for 1.5–2 h.[53,54] IMMC has four phases: Phase I is the quiescent phase with no contractions lasting from 30 to 60 min. Phase II is characterized by random contractions that gradually increase in intensity, and lasts about 20 to 40 min. Later in this phase, the discharge of fluid and tiny particles begins. Phase III has a sudden onset and ends with a burst of 4–5 contractions per min with maximal amplitude and lasting about 10 to 20 min. These contractions, also known as

"house-keeper waves," sweep gastric contents down the small intestine irrespective of size. Phase IV causes a rapid decrease in contractions and lasts 0 to 5 min. It is followed again by phase I.[46,55] An important consideration is the effect of motility on the fate of co-administered water during medication intake. *In vivo* studies revealed fast emptying of the co-administered water within 10 to 50 min in the fasted state.[56,57]

In the postprandial state, feeding disrupts IMMC activity, and this effect can last for several hours. Therefore, gastric residence times (GRT) for large and non-disintegrating tablets are typically longer. Thus, the frequency and the caloric content of the meals strongly affect the GRT of oral dosage forms.[46,58] Most food effect studies are performed according to FDA and EMA guidelines for bioequivalence and bioavailability studies. Based on the guidelines, medications are recommended to be administered 30 min after beginning the intake of a high-caloric (800–1000 kcal), high-fat standard meal. Besides, 240 mL of tap water should be co-administered with the medication.[59,60] In many *in vivo* studies, however, the co-administered water is emptied from the stomach in less than 1 h despite the presence of food. Koziolek *et al*. have confirmed that quick gastric water emptying is due to a phenomenon called "Magenstrasse" literally to mean "stomach road". A recent MRI study by Grimm and colleagues has also confirmed similar fast water emptying rates for other types of solid meals.[54,61]

1.6.3 Gastric volume and pH

Gastric fluid volume and pH are major physiological factors affecting the success of gastric drug delivery. The gastric fluid is made up of saliva, gastric secretions, dietary food, and liquid, and refluxed liquid from the duodenum.[47,62] Therefore, the volume and pH of gastric contents can be variable depending on several factors, such as co-administered food and fluid intake. In the fasted state, the resting gastric content volume ranges from 10 to 50 mL.[54] Following the ingestion of a

glass of water (usually 240 mL), the gastric content volume initially rises. Subsequently, it declines rapidly, and the mean value typically returns to the baseline in less than 1 h after a drink.[63,64] On the other hand, food intake increases the gastric content volume (GCV) as a result of ingested food and increased gastric secretion, while the rate of gastric emptying is typically reduced.[65,66] Depending on the type of food, the GCV can rise to 1700 mL. After the intake of high-calorie meals together with 240 mL of water, a GCV of up to 800 mL has been reported in several studies.[61,65,67]

Similarly, the intragastric pH is variable, mainly depending on the food and fluid intake. In the fasted state, the median pH value of pH 1.7–1.8 with high inter-subject variation was reported.[68] Furthermore, co-administration of 240 mL of water with the dosage form typically results in an initial increase in pH values up to pH 4.6. However, it drops rapidly following faster water emptying.[57] After taking food, there is an increase in the intragastric pH, and the values are variable among individuals. After the high-calorie, high-fat standard meal used for food-effect studies, the median pH value 5 min after capsule intake in the range of pH 3.3–5.3 was reported. However, the pH value drops to baseline values of around pH 1 within 4 h.[64,69]

1.7 Monolithic and multiparticulate systems

Oral solid dosage forms are the most common and cheap drug delivery systems. Based on the number of units in the dosage form, these systems are classified into two classes. Conventional tablets represent a single unit and are therefore known as single unit (monolithic) systems. Those composed of more than one unit are known as multiple unit (multiparticulate) systems.[70]

In a solid monolithic matrix system, the drug is dispersed or dissolved, and its release is modulated through the incorporation of a suitable polymeric agent(s). The use of capsules as single controlled release systems requires the selection of appropriate excipients.[71] In the case of

floating formulations, for example, monolithic matrix tablets can be prepared by mixing gas-generating agents, the drug, and the matrix-forming agent such as HPMC within the matrix.[72,73]

Multiparticulate dosage forms consist of small, single, and individual units (e.g. pellets, granules, and mini-tablets). A single unit filling can be accomplished through their encapsulation in hard gelatin capsules. In the case of tablets, both individual units and excipients compressed to produce the final multiparticulate system.[74] These systems are used to administer pharmaceutical forms that could contain different drugs, dosages, and release profile. They have additional advantages of lower toxicity due to a lower risk of dose dumping, reduced dependency on gastric emptying and avoidance of the all-or-none effect and minimal local irritation.[75–77]

1.8 Gastric-specific drug delivery systems

Complete eradication of *H. pylori* requires a high concentration of antibiotics maintained within gastric mucosa for a prolonged time. In this regard, gastroretentive dosage forms have been explored as a treatment strategy to achieve this objective.[78–80] In the last two decades, various formulation strategies have been reported to prolong the GRT and the drug release. Out of these trials, some products have been developed successfully and even marketed.[81,82]

In this part, the general principles of major formulation approaches to achieve gastric specific drug delivery are discussed. Moreover, some specific examples of formulations that were developed to target *H. pylori* within the stomach are included. The approaches can be categorized in one of the following major categories: floating systems, bio-/mucoadhesive systems, floating-bioadhesive systems, expanding/swelling systems, and high density/sinking systems (Figure 1.4).

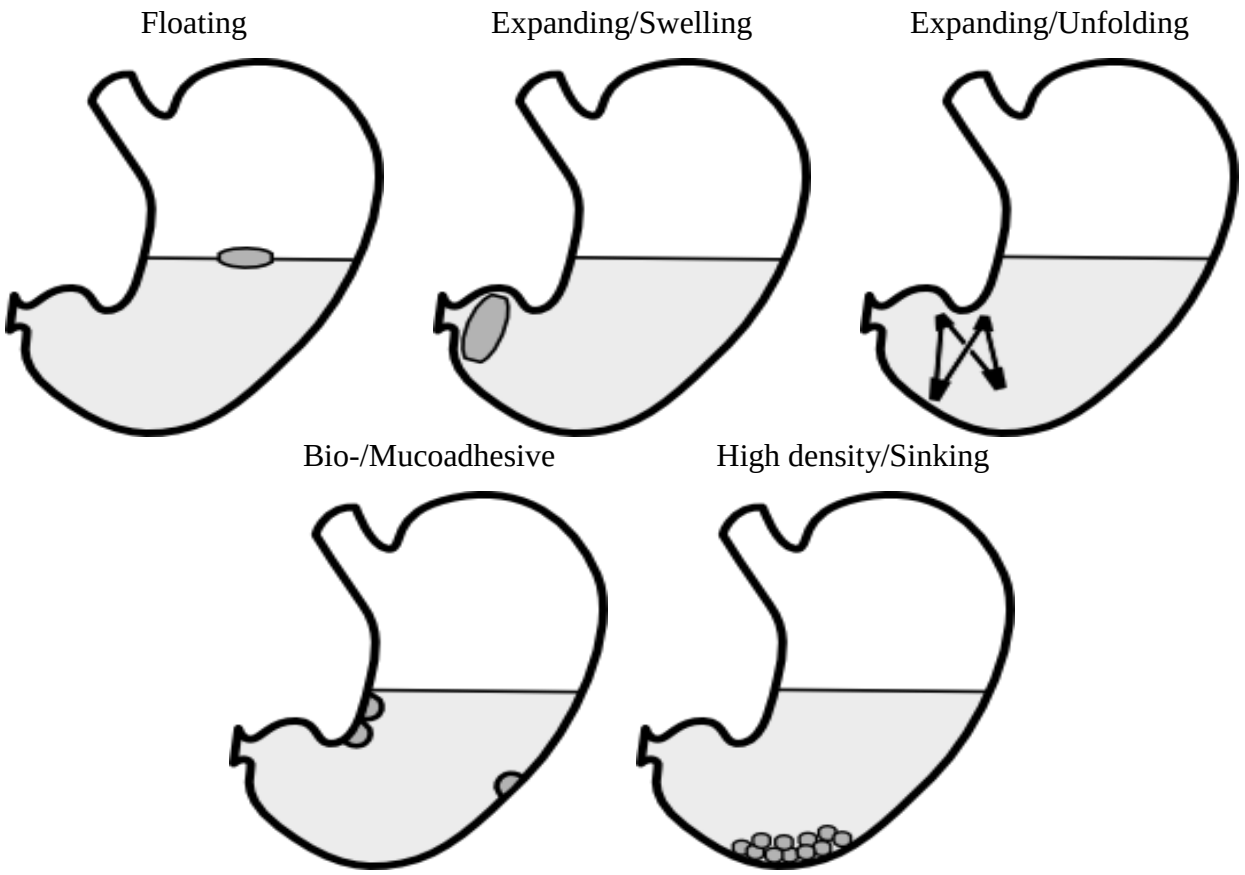


Figure 1.4 Schematic representation of the most common formulation strategies to achieve gastric-specific drug delivery.[62]

1.8.1 Floating systems

Floating systems are the first and most frequently reported approaches to formulate gastric-specific drug delivery systems.[83] They can float in and on top of the gastric contents based on the principles of buoyancy.[84] Buoyancy can be achieved when the bulk density of the dosage form is less than that of the gastric fluid. Usually, a dosage form with a bulk density of 1.004 to 1.010 g/ml and below floats after a particular lag time.[73,85] Floating of the dosage form enables the system to release the drug at the desired rate during its GRT.[86]

Floating systems can be either effervescent systems or non-effervescent systems. The effervescent systems can be either gas-generating or volatile liquid containing systems. In gas-generating systems, matrices are prepared with swellable polymers (e.g. HPMC and chitosan),

and effervescent mixtures. Sodium bicarbonate and either citric acid or tartaric acid are standard components of most effervescent mixtures that produce carbon dioxide (CO_2) in the presence of (gastric) acid. The gas generated is retained in the gel hydrocolloid matrix and influences drug release from the system.[46,87] Volatile liquid systems contain a volatile liquid (e.g. ether or cyclopentane) introduced into an inflatable chamber. The gas volatilizes at body temperature allowing for inflation of the chamber in the stomach. Similar to non-effervescent systems, hydrophilic polymers, such as alginate and different types of HPMC, are often used to form matrices and control drug release.[46,88]

The non-effervescent systems swell unrestrained via the imbibition of gastric fluid to the extent that it prevents their exit from the stomach. Floating of the system can be achieved based on two approaches. The first approach is mixing the drug with a gel-forming agent during formulation. In contact with gastric fluid, the gelling agent swells and maintains the shape integrity while the bulk density of the system within the outer gelatinous barrier becomes less than 1.[81,89] Moreover, the gel layer around the system core controls drug release while the entrapped air in the gel layer confers buoyancy to the system. Excipients used most commonly in such systems include HPMC, polyacrylate polymers, polyvinyl alcohol, polyacrylic acid, agar, sodium alginate, calcium chloride, polyethylene oxide, and polycarbonates.[90] The second approach is using a microporous compartment containing a drug reservoir inside and pores along its top and bottom walls. In the stomach, the entrapped air causes the system to float over the gastric content.[91]

There are several reports of floating dosage forms containing single or combination of antibiotics against *H. pylori*. Nama *et al*. prepared HPMC-based clarithromycin floating matrix tablets containing various concentrations of sodium bicarbonate. The *in vitro* evaluation demonstrated a promising result from one optimized formulation with a floating lag time of less than 3 min and a

total floating time of 12 h. Additional x-ray studies on human subjects showed the enhanced GRT of the tablet to 220 ± 30 min.[92] A bilayer floating matrix tablet of amoxicillin and aloe vera gel powder for the treatment of gastric ulcers was described in another publication. One formulation containing sodium bicarbonate and citric acid in 1:4 ratio gave floating lag time of less than 1 min and a total floating time of more than 8 h with 97.0 % drug released in 8 h.[93] Yang *et al*. proposed a floating triple-drug therapy based on the swellable asymmetric triple-layer tablet approach. The core layer contains rate-controlling polymers, tetracycline, and metronidazole for controlled drug delivery. The outer layer contains bismuth salt for an immediate release. The *in vitro* study confirmed that the tablet was floated with a lag time of 17–28 min with sustained delivery of tetracycline and metronidazole over 6–8 h.[94]

1.8.2 Bio-/Mucoadhesive systems

Bio-/Mucoadhesive drug delivery systems are used as a delivery device within the lumen to enhance site-specific drug absorption. This approach increases the intimacy and duration of drug contact with biological membranes.[95,96] Several theories have been applied to explain bio-/mucoadhesion. The first theory is known as electronic theory and states that bioadhesion is the result of the attractive electrostatic forces between the glycoprotein mucin network and the bioadhesive polymeric material. The second theory is known as the adsorption theory and proposes that bioadhesion is due to the presence of secondary forces such as Van der Waals forces and hydrogen bonding. The third theory is known as the wetting theory, which explains bioadhesion based on the ability of bioadhesive polymers to spread and develop intimate contact with the mucous layers. The last theory is also known as the diffusion theory and involves the interpenetration of the polymer chains in the interfacial region.[80,97]

Bioadhesive polymers may be natural or synthetic and defined by their ability to adhere to biological tissues. They can be either cytoadhesive or mucoadhesive polymers. The cytoadhesive polymers bind to the epithelial cell layer through interactions with cell-specific receptors, while the mucoadhesive polymers bind to the mucus layer.[98] Carbopol®, chitosan, Gantrez®, cholestyramine, tragacanth, sodium alginate, HPMC, Sephadex, sucralfate, PEG, dextran, cyanoacrylate, and polylactic acid are examples of such polymers.[80,99] Achieving an effective bioadhesion is challenging due to the rapid turnover of mucus in the GIT. Furthermore, the bioadhesive properties of the polymers can be affected by the highly hydrated stomach content.[80,100]

Despite hurdles of success, several study groups have developed mucoadhesive systems to target *H. pylori* locally within the stomach. One group, for example, has developed amoxicillin mucoadhesive microspheres containing carbopol-934P and ethyl cellulose. An *in vitro* mucoadhesive test demonstrated strong adhesion to the gastric mucous layer for an extended time. One optimized formulation showed a high drug entrapment efficiency (56 %), mucoadhesion after an hour (80 %), a particle size of 109 mm, and sustained drug release for more than 12 h. After an oral single and multiple-dose administration to *H. pylori*-infected Wistar rats under fed conditions, a better *H. pylori* clearance was obtained from mucoadhesive microspheres than the powder.[101] In 2008, Higo *et al*. formulated an acidic slow-release tetracycline-sucralfate complex. The adhesive paste formed from the acidic compound displayed a longer sustained release profile of tetracycline using a flow-through cell method mimicking the fasted state of the stomach. Furthermore, the paste formulation protected tetracycline from decomposition under an acidic condition. The authors suggested that this antibiotic delivery mechanism requires only a minimum dosage, and could be an alternative for effective eradication of *H. pylori*.[102]

1.8.3 Floating-bioadhesive systems

Some of the challenges of floating systems include lack of site-specific drug release and gastric emptying, which may reduce the buoyancy of the floating systems in the stomach. Similarly, the bioadhesive capacity can be affected by rapid gastric mucosa turnover and gastric emptying. Therefore, the formulation with dual floating and bioadhesive functions could reduce the limitations of individual systems and would improve the therapeutic outcomes.[78]

Thombre and Gide prepared floating-bioadhesive beads of amoxicillin trihydrate by the ionotropic gelation method. The developed beads demonstrated 79–92 % drug release, 65–89 % drug entrapment efficiency, and 61–89 % mucoadhesion. *In vivo* mucoadhesion study on Wistar rats showed more than 85 % mucoadhesion of beads even after 7 h. *In vitro-in vivo* growth inhibition study showed complete eradication of *H. pylori*.[103] Zheng *et al*. proposed floating-bioadhesive microparticles containing clarithromycin for the eradication of *H. pylori* within the stomach. *In vitro* investigations have demonstrated that 74 % of the chitosan–alginate–ethyl-cellulose microparticles (CAEMs) were floated in an acetate buffer for 8 h. Similarly, 90 % of clarithromycin was released in a sustained manner within 8 h. Further testing on rats showed that 61 % of the CAEM retained in the stomach for 4 h. In one PPI treated group, higher gastric clarithromycin concentration was found from the CAEM group than the solution group.[104]

1.8.4 Expanding systems

Another approach to achieve gastric retention is through an *in situ* expanding or swelling systems. The swelling of the system to dimensions greater the pyloric sphincter prevents emptying from the stomach. The cut-off size depends on food intake. Some studies have speculated the minimum size of the swelling system based on the orifice of the resting pylorus (12.8 ± 7 mm). Thus, swelling of the dosage forms more than 15–16 and 12–13 mm are required

to prevent gastric emptying in fasted and fed state, respectively. In addition to size, strength in at least two dimensions is an additional requirement for GR systems to prevent premature rupture of the dosage form and to empty with the housekeeper wave.[105,106] Once such systems are in the stomach, the polymers within the system swell to a size that prevents the passage of the system through the pyloric sphincter. Therefore, polymeric materials with extensive swelling behavior can produce such systems. However, a balance between the swelling and erosion behavior of the polymer is crucial to achieve optimum benefits and to avoid unwanted side effects.[46,107,108]

The gastric residence time of such systems can be prolonged by either swelling or unfolding processes.[83,109] Swellable systems are retained in the stomach due to increased size after coming into contact with gastric fluids, i.e., after hydration. Hydrophilic polymers such as HPMC, polyethylene oxide, and polyacrylic acid polymers are commonly used to formulate such systems.[110] Unfoldable systems are composed of polymers that can be folded and encapsulated in a carrier and degraded in the stomach. Carrier degradation allows the drug release from the system as it unfolds and reacquires its initial geometrical form.[111]

There are several reports of expandable formulations targeting *H. pylori*. Park and Kim have prepared amoxicillin-containing glycol chitosan Superporous hydrogels (SPHs) for an efficient drug-delivery system to eradicate *H. pylori*. The hydrogels were prepared by either physical dispersion or chemical conjugation of the drug with chitosan. The result demonstrated that the drug-conjugated system had much slower drug release patterns than the drug-dispersed system, thus prolonging the drug-delivery effect.[112] In 2014, El-Zahaby *et al*. have developed expanding gastro-retentive tablets of levofloxacin (LVF) hemihydrate. The tablets were prepared using *in situ* gel-forming polymers (gellan gum, sodium alginate, pectin, xanthan gum) and cross-linkers (calcium and aluminum chloride). The evaluation of the tablets revealed that the addition of a

cross-linker to gellan gum formulations significantly decreased drug release. The most sustained LVF released from a formula containing xanthan gum without any cross-linker and demonstrated increased in diameter with time, thus acting as a plug-type dosage form. Further infrared spectra and DSC thermograms confirmed drug stability.[113]

1.8.5 High density/sinking systems

High-density/sinking systems are intended to lodge in the rugae of the stomach withstanding the peristaltic movements. Such systems have a higher density than that of gastric fluids that allow them to settle down to the bottom of the stomach, where they remain located.[80,114] Pellets with a bulk density of 2.6–2.8 g/mL reported settling in rugae or the folds near the pyloric region. The GI transit time can be extended to up to 25 h, which depends mainly on density rather than the diameter of pellets.[115] Incorporation of substances such as barium sulfate, iron powder, titanium oxide, and zinc oxide can increase the density of the dosage form.[115,116]

Another approach to formulate sinking systems is the one proposed by Hao and colleagues. They have formulated Fe_3O_4-based sinking magnetic nanoparticles using the electrospray method. The nanoparticles displayed strong magnetism and a density of 3.52 g/cm^3 and retained in the stomach for over 8 h.[79] Due to the complexity of the gastric environment, increasing the density of the oral dosage form alone, however, may not be sufficient to cause a significant change in the GRT.[83]

1.9 *In vitro* tools for the simulation of gastric residence and transit

In vitro drug release is the primary parameter in predicting the *in vivo* performance of gastric-specific drug delivery systems. Dosage forms are intended to remain in the stomach and release the drug either immediately or over an extended time. These studies are performed commonly in USP I or II in 0.1 N HCl or simulated gastric fluid (SGF) and maintained at 37 ^{o}C.[117] These

methods, however, associated with several drawbacks such as adherence of the dosage form to the paddle shaft, incomplete exposure to the dissolution medium, and failure to mimic the *in vivo* conditions.[118] For floating systems, for example, floating causes partial exposure of the dosage form to the dissolution medium and affects the drug release. One strategy to solve this challenge is the use of helical wire sinkers where the dosage form placed.[119,120] However, if the size of the sinker is small, it may affect the three-dimensional swelling behavior of the dosage form and, consequently, the drug release.[118] The use of either a single or double ring mesh assembly is also recommended to prevent floating and sticking of dosage forms.[120,121] USP dissolution apparatus I (basket apparatus) has been suggested to study the drug release of the swellable-floating type tablets. However, swelling of the tablets constricts the radius of the basket and thus impedes further swelling and drug release.[121] Use of shaker incubator or "custom-built stomach model" to reduce the limitations of USP apparatus are also reported in various studies.[122,123]

Although the multiple modifications mentioned above improve the dissolution profile of dosage forms, they do not mimic the *in vivo* conditions of the stomach. Several attempts have been made to mimic the realistic conditions in the human stomach. These include simulation of physiological parameters such as gastric volume, gastric emptying, gastric secretion, and intragastric pressure.[62,118] A promising development in this regard is a Fed Stomach Model (FSM), which was developed by Koziolek and colleagues. FSM is a modified version of the USP II paddle apparatus designed to simulate various mechanical aspects such as pressure and fluid hydrodynamics in the GIT in a fed state.[124] Another bio-relevant device is the one developed by Garbacz and Weitschies called dissolution stress test device (DSTD). This device has become an essential tool to simulate various intragastric pressure scenarios a dosage form could face under fasted and fed states.[125] Recently, Schneider *et al*. have tested two marketed gastroretentive

dosage forms under different pressure scenarios using this device. For both dosage forms, a highly pressure-sensitive drug release behavior was demonstrated in all three pressure scenarios. Moreover, a higher amount of drug was released compared to compendial testing.[126]

Garbacz *et al*. have also applied a dynamic open flow-through model to test oral dosage forms. This model is part of the already developed DSTD and designed to simulate various physiological conditions of the GIT such as media volume, hydrodynamics, temperature, motility, and transport in a fasted state. This setup was further tested using various types of hard capsules by applying different test programs of flow rate, pressure, and test media temperature.[127]

A new development in bio-predictive dissolution testing is a GastroDuo model. This model was developed by Schick *et al.* and designed to simulate various physiological conditions of the stomach and duodenum. The model was constructed by combining both the dynamic open flow-through model and FSM setups. Furthermore, the model was applied to explain the *in vivo* variations in the onset of plasma concentrations of two IR formulations in a fed state condition.[128] Recently, Sager *et al.* has successfully applied GastroDuo model to study the disintegration and drug release behavior of four IR formulations in comparison with an *in vivo* result from healthy volunteers using a salivary tracer technique.[129]

1.10 *In vivo* tools for the characterization of gastric emptying and transit

Although various *in vitro* techniques are available to evaluate the gastro-retentive performance of dosage forms, *in vivo* evaluation techniques remained most valuable. Many sophisticated *in vivo* visualization and tracking techniques are helpful in this regard. In this part, some of the most common and recently used *in vivo* techniques, namely; γ-scintigraphy, magnetic resonance imaging, magnetic marker monitoring, and salivary tracer techniques, are discussed.

1.10.1 γ-scintigraphy

In scintigraphy, gamma-emitting materials are incorporated into a dosage form to capture images of the dosage form in the GI tract with the aid of a gamma camera. Widely used γ-emitting materials are ^{111}In (Indium) and 99m Tc (Technetium). The main advantage of this technique is that it allows visualization overtime of the entire transit of a formulation through the GI tract.[118,130] One of the most significant drawbacks of γ-scintigraphy is the ethical problem that healthy volunteers become exposed to radiation without having any personal benefit as well as the limited temporal and spatial resolution.[131,132]

1.10.2 Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a non-invasive and safe imaging technique in the *in vivo* imaging of the human GI tract. MRI provides a very high spatial resolution in combination with excellent contrast resolution. This technique uses magnetic fields and radio waves to view the complete anatomical structure along with the location of the ingested dosage form.[133] The limitation of using MRI is a short acquisition time, and motion derived artifacts in the images as a result of even small body movements during the imaging procedure. These artifacts can be minimized if subjects held their breaths during imaging. Moreover, compounds with superparamagnetic properties (e.g. ferrous oxide) incorporated into the dosage form can also be used for better visualization and identification.[134]

This technique helps to investigate the various physiological conditions in the GIT, including the determination of gastric volume and GET in fasted and fed state conditions. Steingoetter *et al*. have investigated the gastric location and residence time of gadolinium chelates (Gd-DOTA) floating tablets containing black iron oxide in human volunteers.[135] Recently, Grimm *et al*. have investigated the gastrointestinal transit and disintegration behavior of floating and sinking acid-

resistant capsules using the MRI. The investigations were conducted on human subjects under fasted state conditions. Based on this investigation, the GET of both floating and sinking capsules was not different (45 ± 35 and 36 ± 18 min, respectively). Moreover, the mean disintegration times of 139 ± 35 and 163 ± 55 min were recorded for floating and sinking capsules, respectively.[136]

1.10.3 Magnetic marker monitoring

Magnetic Marker Monitoring (MMM), also known as Magnetic Moment Imaging (MMI), is another imaging technique to investigate the behavior of solid oral dosage forms within the GIT. An appropriate magnetic sensing device is used to measure the magnetic field generated from a magnetically tagged solid dosage form while in the GIT. The three-dimension localization and orientation, as well as the strength of the magnetic dipole, are reconstructed from the data.[132,137] Magnetic labeling of solid dosage forms usually achieved by the incorporation of small amounts of either magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). The iron oxide can be directly included in the formulation or introduced later, e.g., into drilled holes.[131,133,138] Weitschies *et al*. have successfully applied MMM as an essential tool to investigate the location and disintegration behavior of magnetically marked tablets and capsules *in vivo*.[139,140] The technique does not involve the use of any radiation; thus, it is less hazardous. The drawbacks are the need to incorporate iron oxide, which may affect the performance of oral drug delivery systems and also limits the broader application of this technique.

1.10.4 Salivary tracer technique

The imaging and diagnostic techniques employed in investigating the performance of dosage forms in the GIT have some limitations and disadvantages. MRI investigations, for example, are non-invasive but expensive. Blood sampling is an invasive technique and can be costly.

Therefore, investigational techniques which are relatively non-invasive and cheap are preferred. One such technique is the salivary tracer technique. Saliva sampling offers a simple, non-invasive, and affordable method with no contamination risk compared with plasma sampling. The salivary excretion data of several drugs have been used for drug bioavailability, therapeutic drug monitoring, pharmacokinetics and drug abuse studies.[141–143]

The concentration of certain drugs in saliva has been shown to provide a reliable index of their plasma concentration.[144] Miles and colleagues have found a strong correlation between serum and saliva topiramate concentrations in epileptic patients ($r^2 = 0.97$).[145] A similar strong relationship was reported for lacosamide, an antiepileptic drug ($r^2 = 0.835$).[146] In another study, a strong correlation between serum and saliva concentrations of metformin, tolterodine, rosuvastatin, and paracetamol with saliva to plasma concentrations ratios ranged from 0.17–1.5 was reported.[147]

Recently, Sager and colleagues have developed a novel salivary tracer technique using a low dose of caffeine to predict the gastric emptying of water under fasted and fed state conditions. Simultaneous MRI studies confirmed further validation of the technique. Accordingly, strong correlation between normalized salivary and MRI data with correlation coefficients of 0.912 ± 0.055 and 0.887 ± 0.047 established in fasted and fed state, respectively.[56] Similarly, the *in vivo* disintegration time of immediate release hard gelatin capsules containing 50 mg caffeine and 5 mg iron oxide was investigated using a combined salivary tracer and MRI investigations. The mean disintegration times of the capsules were 8.8 and 12.5 min for MRI and saliva method, respectively.[148]

1.11 Objectives

Many epidemiological studies confirmed the high global prevalence of *Helicobacter pylori* infections. As a result, several medication concepts are in use to treat infections associated with this pathogen. However, the antibiotics in the eradication therapies are limited in number due to the emergence of *H. pylori* strains that are resistant to major antibiotics, including clarithromycin. Therefore, the search for a new, effective medicine, as well as devising a novel *H. pylori* treatment strategy, continues to address the problem.

The main aim of this work was the development and optimization of novel approaches for the local treatment of *H. pylori* within the stomach. To achieve this aim, the following four tasks had to be accomplished:

1. Screening of antibiotics suitable for local eradication of *H. pylori*
2. Design of *in vitro* tools for the characterization of gastric transit and emptying
3. Development and *in vitro* characterization of the formulation concepts
4. *In vivo* study to identify the most suitable concept

The first task was aimed to identify antibiotics, which are effective against *H. pylori* through *in vitro* susceptibility investigations.

The local eradication of *H. pylori* requires intragastric concentrations of the antibiotic above the MIC for a sufficiently long time. Various physiological factors such as gastric content volume, gastric pH and gastric emptying can affect the performance of dosage forms acting locally in the stomach. Therefore, the second objective of this work was to develop an *in vitro* tool that can simulate certain physiological conditions of the stomach at different fasted and fed conditions.

In the next step, two different formulation concepts had to be developed to enable the local eradication of *H. pylori* by achieving high intragastric drug concentrations for a longer time. The first strategy was based on effervescent granules containing the antibiotic and gas-generating excipients. The latter should provide rapid disintegration, and uniform mixing with the gastric contents by inducing motility. The second formulation strategy was to develop a monolithic extended-release formulation based on a hydrogel matrix. Since drug release should be maintained over several hours, suitable excipients had to be identified, which guarantees prolonged drug release and stability of the drug within the formulation during gastric transit. Besides, the *in vitro* performance of the formulation concepts had to be performed using standard testings and also in bio-relevant test models.

In the last part of the thesis, the different formulation approaches for local drug delivery to the stomach should be investigated *in vivo* by using caffeine as a salivary marker. Moreover, additional magnetic resonance imaging (MRI) studies should be conducted to investigate the localization and disintegration behavior of the matrix tablets under fed conditions.

2 Materials and Methods

2.1 Materials

In this section, the major active pharmaceutical ingredients (APIs) and excipients necessary for formulation development are described in detail. The other chemicals, devices, and buffers that were used in the *in vitro* and *in vivo* characterization are also listed.

2.1.1 Penicillin G sodium (PGS)

Penicillin G sodium (Figure 2.1), also known as benzylpenicillin sodium, is a semi-synthetic, broad-spectrum β-lactam antibiotic and widely used to treat several bacterial infections. It is effective against most gram-positive bacteria. However, some gram-negative organisms are also susceptible to benzylpenicillin.[149]

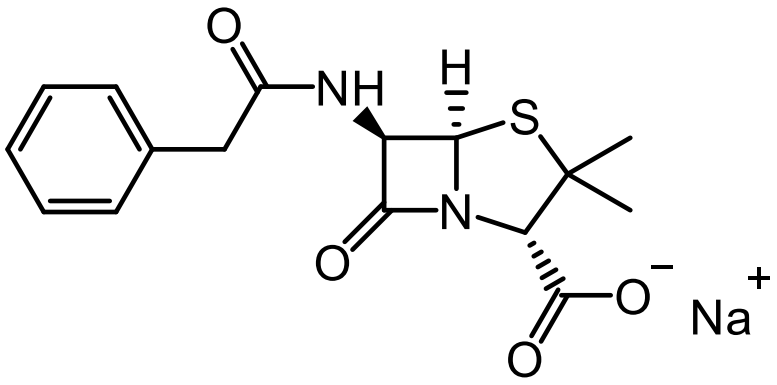


Figure 2.1 Chemical structure of penicillin G sodium.

Penicillin G, like any beta-lactam antibiotics, produces its effect through inhibition of the bacterial cell wall synthesis. The bacterial cell wall is responsible for its cell strength and rigidity. A bacterial cell consists of penicillin-binding proteins (PBPs), which play a significant role in the cell-wall synthesis process. However, antibiotics such as PGS molecules bind to PBPs and interfere with the cross-linkage of peptidoglycan chains. Consequently, the cell wall weakens,

which results in bacterial cell lysis and death. The principal determinant of the efficacy of β-lactams is the time above the minimum inhibitory concentration (MIC), equivalent to the time for which the drug level at the site of infection exceeds the MIC. This type of effect termed as time-dependent killing and the pharmacodynamic parameter is simplified to the time that serum concentrations remain above the MIC during the dosing interval (t > MIC).[150] With the use of *in vitro* killing-curve studies, maximum killing is usually achieved at 3–4 times the MIC.[151] In this study, penicillin G sodium salt was purchased from Glentham Life Sciences (Corsham, Wiltshire, UK). Table 2.1 shows a summary of some of the typical characteristics of PGS.

Table 2.1 Some physicochemical characteristics of penicillin G sodium salt.[152]

Parameter	Specifications
Form	White to off-white crystalline powder
Molecular formula	$C_{16}H_{17}N_2NaO_4S$
Molecular weight	356.37 g/mol
Solubility	Very soluble in water but insoluble in fatty oils and paraffin
Assay	96.0 – 102.0 %
pH	5.5 – 7.5 (2 g/20 mL in water)
pKa	2.74
Absorbance	264 nm: 0.80 – 0.88, 280 nm: ≤ 0.1, 325 nm: ≤ 0.10
Individual impurity	≤ 1%
Bacterial endotoxins	≤ 0.16 IU/mg

The percentage of dose absorbed after oral administration of 500 mg of penicillin G is reported in the range of 15–30 %.[153] It is commercially available as solutions or suspensions for intravenous or intramuscular administration. Oral formulations are not available in the market owing to the rapid degradation of penicillin G in the gastro-intestinal fluids, especially in gastric fluid. The instability is due to the sensitivity of the β-lactam ring to cleavage in the presence of acidic and basic agents, nucleophiles, heavy metals, oxidizing agents and penicillinases.[154–156]

Penicillin G degradation is pH and temperature-dependent. It is more stable at the pH range of 5.0–8.0 and lower temperatures. Moreover, its stability decreases by an increase in temperature in all pH values.[157,158] Therefore, PGS should be stored under strict pH and temperature conditions. A temperature range usually not above 4 °C is recommended. Penicillin G undergoes several intermediate and final degradation processes. The primary degradation product is penicillenic acid, which is an intermediate product and converted rapidly to a range of different degradation products.[158,159]

2.1.2 Caffeine

Caffeine is found mainly in liquid foods such as coffee, tea, cocoa, and soft drinks, but also smaller quantities in solid foods such as chocolate. It is also found in food and nutritional supplements, either as original content or additive. Structurally, caffeine belongs to the group of xanthines and classified under alkaloids. Pharmacologically, caffeine is grouped under stimulants. The dose of caffeine as a medicine is in the ranges of 50–200 mg. In a study in 2015, the European Food Safety Authority (EFSA) determined the average caffeine content in various foods (Table 2.2). As part of a risk assessment, EFSA concluded that a single dose up to 200 mg of caffeine poses no risk to healthy volunteers.[160]

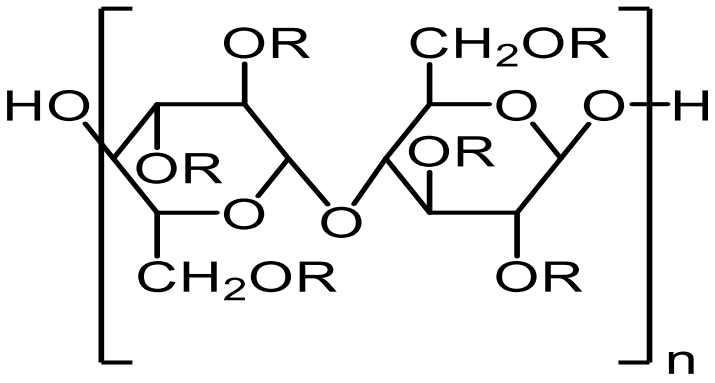
Table 2.2 Caffeine content of various foods modified according to EFSA 2015.

Drink	Portion unit	Caffeine per portion (mg)
Filter coffee	one cup (200 mL)	90
Energy drinks	one can (250 mL)	80
Espresso coffee	one cup (60 mL)	80
Black tea	one cup (200 mL)	45
Coca beverage	one can (330 mL)	35
Cocoa beverage	one cup (200 mL)	8–35
Green tea	one cup (200 mL)	30
Dark chocolate	half a bar (50 g)	25
Milk chocolate	half a bar (50 g)	10

The pharmacokinetics of caffeine in the blood and saliva are already well studied. In one *in vivo* study after oral administration of 50 mg of caffeine, the plasma and salivary caffeine elimination constant (Ke) and half-life ($t_{1/2}$) values were determined. Accordingly, a comparable Ke of 0.163 and 0.189 h^{-1} were found in plasma and saliva, respectively. Similarly, the elimination half-lives were 5.7 and 3.7 h in plasma and saliva, respectively.[161] Zylber-Katz *et al*. also described comparable concentration-time profiles with saliva to plasma ratio of 0.75 to 1.[162]

2.1.3 Hydroxypropyl methylcellulose (HPMC)

HPMC, also known as hypromellose, is one of the most commonly used hydrophilic matrix-forming agents to formulate swellable matrix systems (Figure 2.2). It has a wide range of applications because of its comparative advantages. These include rapid hydration, gelling characteristics, cost-effectiveness, compressibility, and less toxicity. Moreover, HPMC-based matrix systems provide a desirable drug release and a wide range of regulatory acceptance.[163–165]



R = -H, -CH3, or -$CH_2CH(CH_3)OH$

Figure 2.2 Chemical structure of hydroxypropyl methylcellulose.

The rate and kinetics of drug release from swellable matrix tablets can be affected by the concentration, the grade and the viscosity of the polymer.[166–169] Various HPMC grades are marketed under different trade names. In this study, Methocel K4M premium was used in the preparation of extended-release matrix tablets. Table 2.3 shows some of the physicochemical properties of K4M in comparison with a highly viscous grade (K100M).

Table 2.3 Comparison of two hydroxypropyl methylcellulose grades.[170]

Specification	K4M Premium	K100M Premium
Methoxyl (%)	19 – 24	19 – 24
Hydroxypropoxyl (%)	7 – 12	7 – 12
Chlorides, max. (%)	0.5	0.5
Apparent viscosity, 2 % in water at 20 °C, cP	3000 – 5600	80000 – 120000
pH, 1 % in water	5.5 – 8.0	5.5 – 8.0

2.1.4 Other chemicals and devices

In this study, several chemicals and devices were used and are described in Table 2.4 and Table 2.5, respectively.

Table 2.4 List of chemicals used in this study.

Material	Producer/Supplier
Acetic acid	Carl Roth GmbH + Co.KG, Karlsruhe, Germany
Acetonitril (HPLC grade)	VWR International, Fontenay-sous-Bois, France
Ammonium acetate	AppliChem GmbH, Darmstadt, Germany
Anhydrous citric acid	Fagron GmbH & Co. KG, Barsbüttel, Germany
Caffeine	Caesar & Loretz GmbH, Hilden, Germany
Colloidal anhydrous silica	Fagron GmbH & Co. KG, Barsbüttel, Germany
HPMC / MethocelK4M	Dow Pharma and Food solutions, Dartford, UK
Hydrochloric acid	AppliChem GmbH, Darmstadt, Germany
Lactose monohydrate	DFE Pharma, Nörten-Hardenberg, Germany
L-Arginine	Carl Roth GmbH + Co.KG, Karlsruhe, Germany
L-Lysine	Glentham Life Sciences, Corsham, Wiltshire, UK
Light magnesium oxide	Fagron GmbH & Co. KG, Barsbüttel, Germany
Mannitol, spray-dried	SPI Pharma, Septèmes-les-Vallons, France
Meglumine	Glentham Life Sciences, Corsham, Wiltshire, UK
Methanol (HPLC grade)	VWR International, Fontenay-sous-Bois, France
MCC/Pharmacel 101	DFE Pharma, Nörten-Hardenberg, Germany
Penicillin G sodium	Glentham Life Sciences, Corsham, Wiltshire, UK
Polyethylene glycol 4000	Fagron GmbH & Co. KG, Barsbüttel, Germany
Potassium phosphate monobasic	Merck KGaA, Darmstadt, Germany
PVP (Kollidon® 90 F)	BASF, Ludwigshafen, Germany
Sodium bicarbonate/Effersoda 12	SPI Pharma, Septèmes-les-Vallons, France
Sodium carbonate	Merck KGaA, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck KGaA, Darmstadt, Germany
Sodium hydroxide	Honeywell research chemicals, Seelze, Germany

Table 2.5 List of devices used in this study.

Device	Manufacturer
Analytical balance	Sartorius AG, Göttingen, Germany
Analytical column	LiChrospher 100 RP-18, Merck KGaA, Darmstadt, Germany
Centrifuge	SIGMA §-30 KH, Sigma Zentrifugen GmbH, Germany
Cubic mixer	ERWEKA AR 400-FGS, Heusenstamm, Germany
Dissolution stress test device	ERWEKA Type BRD 700, Heusenstamm, Germany
Erweka friabilator	TA 120, Langen, Germany
ERWEKA Granulator	ERWEKA GmbH, Langen, Germany
Erweka hardness tester	TBH 225 D, Langen, Germany
Hot-melt extruder	Three-Tec GmbH, Seon, Switzerland
HPLC system	Shimadzu Corporation, Kyoto, Japan
Micro Balance	Sartorius SE2, Satorius AG, Göttingen, Germany
MRT Scanner	MAGNETOM Aera 1.5T, Siemens Healthcare, Germany
Ohaus MB35 moisture analyzer	OHAUS Europe GmbH, Nänikon, Switzerland
pH meter	Mettler-Toledo, Schwerzenbach, Switzerland
Riva Piccola rotary press	RIVA EUROPE, Hampshire, UK
Sieve analyzer	Retsch AS 200 basic, Retsch GmbH, Haan, Germany
Surface pH electrode	E-202 Planar pH composite electrode, Shanghai, China
Tray dryer	FDL 115 – 230 V, Fa.BINDER GmbH, Tuttlingen, Germany
TURBULA Mixer	Willy A. Bachofen AG, Muttenz, Switzerland
USP dissolution test apparatus II	PharmaTest DT70, Hainburg, Germany
Vortexer	IKA-Werke GmbH & CO.KG, Staufen, Germany
Water purification system	Merck Milli-Q® reference, Darmstadt, Germany

2.1.5 Buffer solutions and test media

In this study, various buffers and test media were utilized in the characterization of the formulations. These solutions were prepared based on European Pharmacopoeia 5.17.1 and are described in Table 2.6.

Table 2.6 Buffer solutions and test media used in various *in vitro* studies.

Buffer solutions and test media	Content per Liter
Ammonium acetate buffer (50 mM, pH 5.0)	2.446 g of ammonium acetate 9.131 mL of 2 M acetic acid dilute to 1000 mL with water R
HCl media pH 1.8	250 mL of 0.2 M NaCl 102 mL of 0.2 M HCl, dilute to 1000 mL with water R
Phosphate buffer solution (PBS) USP pH 6.8	250 mL of 0.2 M potassium dihydrogen phosphate 112 mL of 0.2 M sodium hydroxide dilute to 1000 mL with water R
Phosphate-citrate buffer (PCB) (25:25 mM)	4.8 g of citric acid, anhydrate 3.9 g of sodium dihydrogen phosphate dihydrate 1 M sodium hydroxide of 10, 37 and 84 mL for pH 3.0, 4.5 and 6.8, respectively dilute to 1000 mL with water R
Simulated gastric fluid sine pepsin (SGF*sp*) pH 1.2	2 g of sodium chloride 80 mL of 1 M HCl dilute to 1000 mL with water R

2.2 Screening antibiotics against *Helicobacter pylori*

Many literature data have confirmed the *in vitro* susceptibility of several active substances, including antibiotics and crude extracts against *H. pylori*. Among them, four antibiotics having high activity against *H. pylori* were selected based on literature data. Moreover, these antibiotics have been used for many years in the treatment of several clinical indications with a known safety record. Furthermore, the current minimum inhibitory concentration (MIC) of the antibiotics was demonstrated using a method adapted from Cederbrant *et al*. 1993.[171] The tests were conducted against a known clinical isolate of *H. pylori* species in which GC agar plates were flooded with 500 µL Bone Broth (BB) containing 10^6 colony-forming units (*Helicobacter pylori* strain 26695, TIGR). E-test strips were applied on dried plates, incubated at 37 °C under

microaerobic conditions, and the MIC values were determined after 3, 4, 5, 6, and 7 days. The other method was based on the determination of MIC by the growth monitoring of liquid cultures overnight. In all testes, the data were derived from five independent experiments.

2.3 HPLC method development and validation

2.3.1 HPLC system

In all studies, the quantitative determination of penicillin G sodium was performed using a Shimadzu high-performance liquid chromatography (HPLC) system. The system consists of two pumps, a DAD, an autosampler, a column oven, a system controller, and an inline degasser. Data acquisition and integration were performed using a CLASS-VP™ series version 6.1 software.

2.3.2 Chromatographic conditions

Separation and quantification of analytes were performed using an end-capped LiChrospher® 100 RP-18 column with a dimension of 125 x 4 mm and 5 µm particle size. Furthermore, the column was fitted with a LiChrospher® 100 RP-18 guard column of 4 x 4 mm, having the same particle size. The mobile was prepared by mixing methanol, acetonitrile, and ammonium acetate buffer (50 mM, pH 5) in a ratio of 20:10:70 (v/v). Prior to use, the mobile phase was degassed by ultrasonication at 30 °C for 40 min and pumped with an isocratic elution mode at a flow rate of 0.7 mL/min while the column temperature was kept at 40 °C. Samples were filtered through a 0.45 µm polyethersulfone filter (Merck KGaA, Germany), which avoids any interference from the impurities. The injection volume was 40 µL. In all analyses, the detector was set at 230 nm.

2.3.3 Preparation of stock and working solutions

A stock solution of PGS (100 µg/mL) was prepared by dissolving PGS API powder in a phosphate-citrate buffer (PCB, 50 mM, pH 6.8). 5 mg PGS powder was weighed in a

microbalance and transferred to a 50 mL volumetric flask, the buffer was added and shaken to facilitate complete dissolution and the final volume was adjusted. Out of the stock solution, a second working solution (10 µg/mL) was prepared by transferring 1 mL of the stock solution to a 10 mL volumetric flask and diluted to volume by adding the buffer. A series of 9 working solutions of 1–10 and 20–100 µg/mL were prepared from 10 and 100 µg/mL solutions, respectively. All the working solutions were stored in refrigerator temperature below 4 °C protected from light and analyzed within 3 h of preparation.

2.3.4 Method validation

The performance of the newly developed HPLC method was validated against standards based on the International Conference on Harmonization (ICH) and the United States Pharmacopoeia (USP) validation guidelines.[172,173] The validation parameters include linearity and range, method precision, accuracy, the limit of detection (LOD), and limit of quantification (LOQ).

2.3.4.1 Linearity and range

The method linearity and effective range were validated over the PGS concentration range of 1–100 µg/mL. Nine analytical standards at concentration levels of 1, 2, 5, 10, 20, 40, 60, 80, and 100 µg/mL were injected. Standard curves were constructed by drawing the chromatographic responses versus the concentrations. Linearity was assessed by a regression analysis method for the average points of three calibration curves at each concentration level.

2.3.4.2 Precision

The intra-day (repeatability) and inter-day (intermediate) precisions were determined based on 6 determinations at three concentration levels. The intra-day precision was determined by repeatedly injecting PGS concentration of 1, 50, and 100 µg/mL. Intermediate precision was

assessed on two different days by repeated injections of PGS concentrations of 1, 50, and 100 µg/mL. The precision of the method was predicted from the percent relative standard deviation (RSD %) of the mean values.

2.3.4.3 Accuracy

The method accuracy was determined by calculating the percent recovery at three concentrations of 1, 50, and 100 µg/mL in both days.

2.3.4.4 Limit of detection and limit of quantification

The LOD and LOQ values of the HPLC method were determined based on standard deviation (σ) of the response and slope (S) of the three calibration curves by applying 3.3 σ /S and 10 σ /S for LOD and LOQ, respectively.

2.4 *In vitro* stability studies

The *in vitro* stability studies were conducted in various acidic pH for 12 h. The test media were SGFsp (pH 1.2) and PCB (50 mM, pH 3.0–6.8). In each experiment, 2 g of PGS were transferred to a brown glass vial containing 4 mL of the test medium. The vials were placed in a water bath at 37 °C. In each vial, a magnetic stirrer was placed and set to 700 rpm. After specified time intervals, 0.3 mL of samples were withdrawn and immediately diluted by adding 1.2 mL of PCB (pH 6.8) to stop further degradation. The diluted samples were stored at 4 °C and analyzed by HPLC at a wavelength of 230 nm. The measurements were performed in triplicate.

2.5 Development and evaluation of a flow-through model

2.5.1 Model setup

The gastric environment is very variable and dynamic. The physiological factors such as gastric emptying rate and motility, pH, volume, the buffer capacity, viscosity, and osmolality of the

gastric fluid are variable depending on the feeding state.[54] These conditions could affect the disintegration, drug release, and the GRT of dosage forms.[64,174]

In this study, an *in vitro* model called a flow-through model (FTM) was developed. The layout of the FTM is described in Figure 2.3. The model was a modified version of the USP II and designed to predict the intragastric drug concentration versus time profile under simulated conditions. The effects of some physiological variables such as flow rates (gastric secretion and gastric emptying rate) and gastric content volume (GCV) on the intragastric concentration of the drug were investigated.

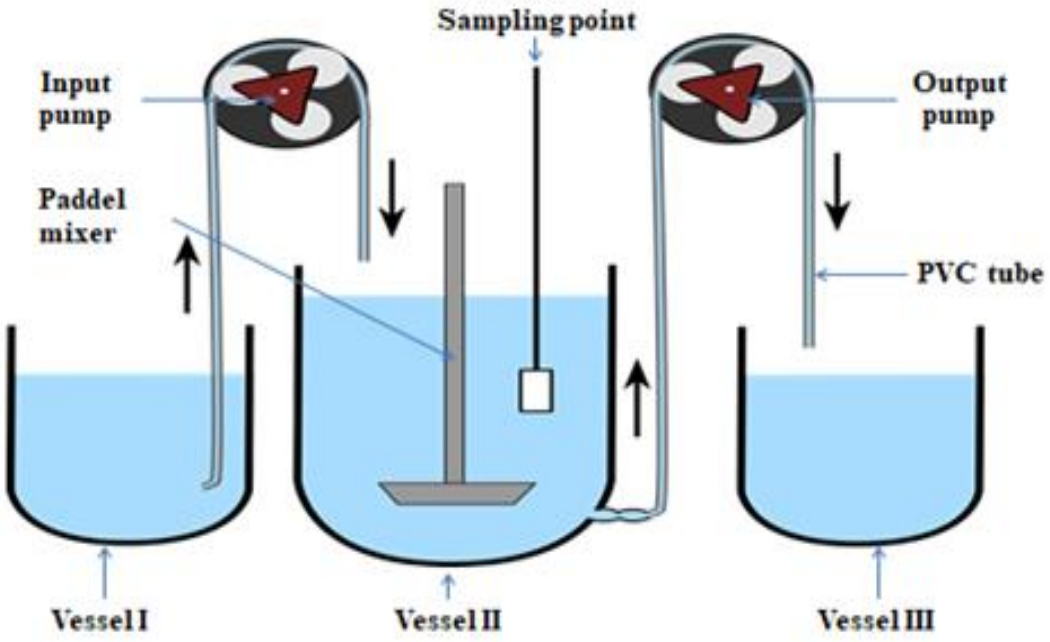


Figure 2.3 Schematic representation of a flow-through model (FTM).

The model consists of three vessels: vessel I (donor), vessel II (dilution), and Vessel III (receiver) vessels. Vessel I was used in simulating the gastric secretion and connected to an input pump by PVC-tube (I.D. 2.29 mm). An input pump was used to control the rate of fluid flow into the dilution vessel. Vessel II was a dilution vessel where dissolution, dilution, mixing, and sampling takes place. The dilution vessels were the standard USP dissolution vessels modified on the semi-

spherical end to permit fluid outflow (Figure 2.4). This vessel was placed in a water bath with a thermostat set to 37 ± 0.5 °C. The USP II paddle was used to achieve mixing, and its rate was adjusted to account for the GI motility in the stomach. Samples were taken by tube fitted with a filter and a syringe. Similarly, the rate of fluid discharge from the dilution vessel was controlled by an output pump. Vessel III was the receiver vessel, which was used to collect the fluid that came out from the dilution vessel.

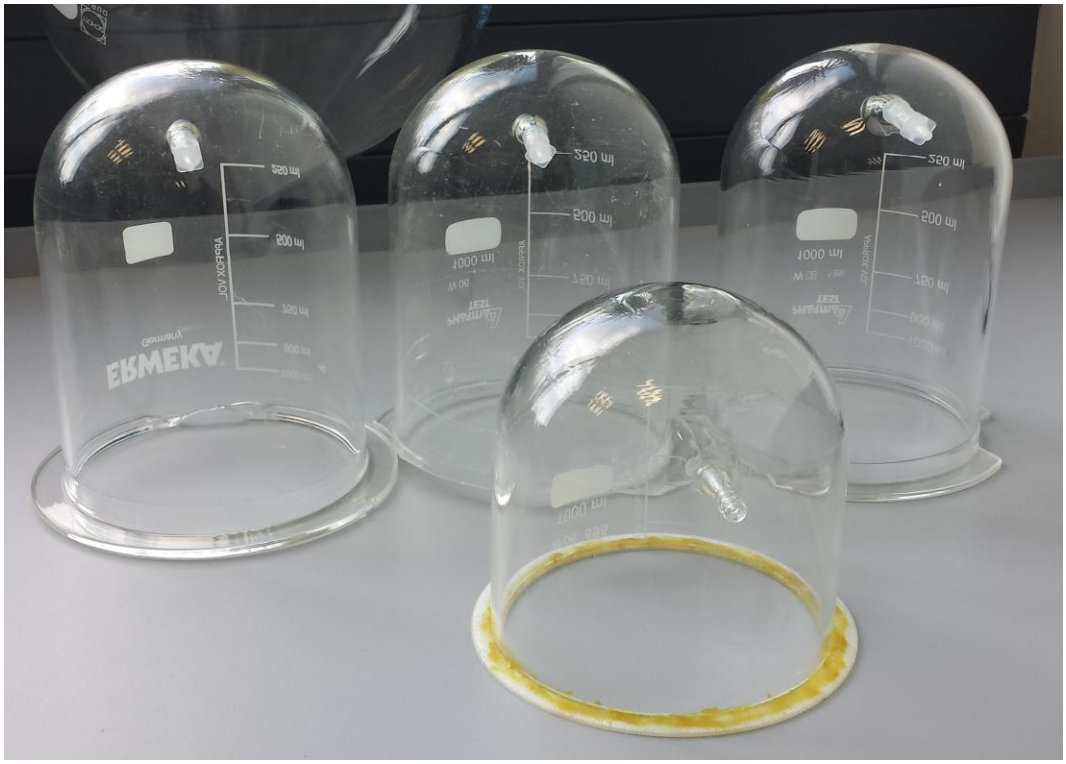


Figure 2.4 A modified version of the USP dissolution vessel for use as a dilution vessel in FTM (modified by the workshop at the faculty of mathematics and natural sciences).

The performance of the FTM was evaluated by conducting dilution studies at constant and variable flow rates. The in- and outflow rates were designed to represent gastric secretion and gastric emptying rates, respectively. In both modes, 500 mg of penicillin G sodium powder was used as API.

2.5.2 Dilution studies at a constant flow rate (CFR)

The *in vitro* dilution studies were performed in the FTM at a temperature of 37 ± 0.5 °C and a stirring speed of 25 rpm. The initial volume in the dilution vessel was 500 mL of phosphate buffer solution (PBS, 50 mM, pH 6.8), while the donor vessel was filled with a sufficient amount of the same buffer. The experiments were conducted at various constant in- and outflow rates of 3, 6, and 9 mL/min. In all tests, 1.5 mL of sample was withdrawn from the dilution vessels at 0, 15, 30, 60, 120, 240, 300, and 360 min. Samples were transferred into vials and analyzed by HPLC after appropriate dilution with PCB of pH 6.8. The concentration of the drug left in the dissolution vessel over time was calculated using the equation obtained from a standard curve.

2.5.3 Dilution studies at a variable flow rate (VFR)

The dilution studies with a variable in- and outflow rates were conducted using the same protocol employed for CFR studies while the initial volume was set to 780 mL. This volume represents the average initial gastric content volume (GCV), which was determined recently by MRI after the intake of a high-calorie, high-fat FDA breakfast by healthy human subjects.[65] Based on this assumption, the GCV change over time 30 min after FDA breakfast was simulated for 6 h using an excel sheet (Figure 2.5).

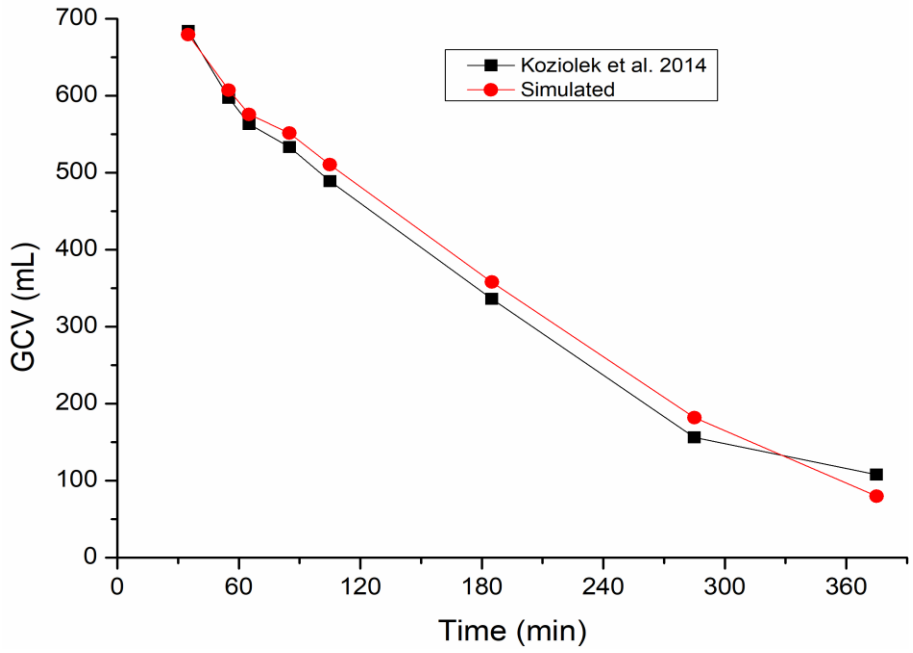


Figure 2.5 Simulation of the gastric content volume change over 6 h in comparison with published data from Koziolek *et al.* 2014 in a fed state.

After the GCV simulation, the in- and outflow rates were adjusted to reflect the GCV decrease over time due to the gastric secretion and gastric emptying rates, respectively (Figure 2.6).

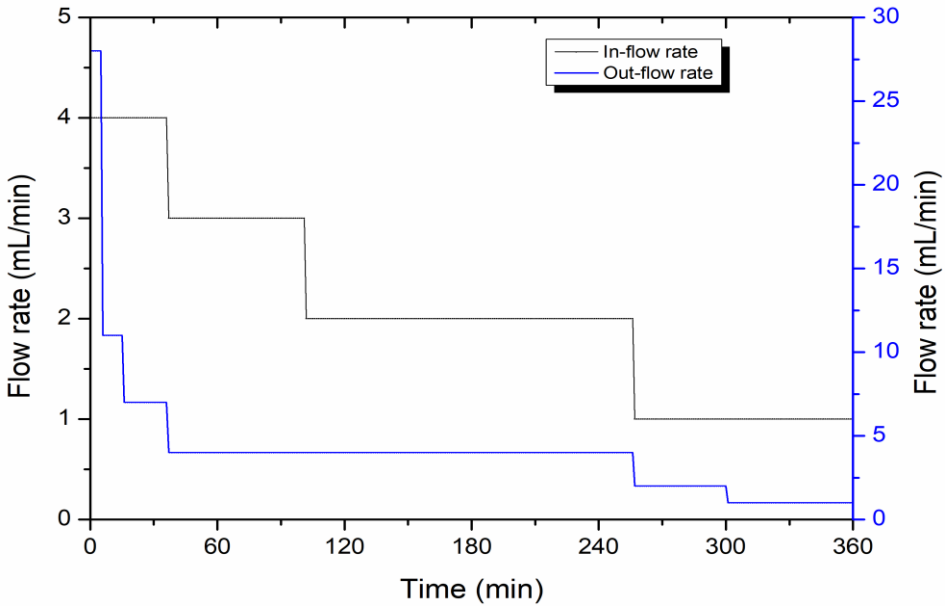


Figure 2.6 Inflow and outflow rate simulations applied to study PGS dilution under variable flow rate (VFR) condition in a flow-through model.

2.6 Formulation development

2.6.1 Formulation of PGS effervescent granules

In this study, three batches of effervescent (F1–F3) and one batch of non-effervescent (F4) granules were prepared by wet granulation. The effervescent mixtures containing various molar ratios of sodium bicarbonate and citric acid accounted for 36 % (w/w) of the total formulation (Table 2.7).

Table 2.7 Composition of the effervescent mixtures in effervescent granule formulations.

Formulation code	Molar ratio	Mass (mg)		Mass ratio
	$NaHCO_3$: CA	$NaHCO_3$	Citric acid	$NaHCO_3$: CA
F1	1.5:1.0	126.01	192.12	0.66:1
F2	3.0:1.0	252.02	192.12	1.31:1
F3	6.0:1.0	504.04	192.12	2.62:1

Table 2.8 shows the composition of the granule formulations. Before granulation, sodium bicarbonate, citric acid, and mannitol were pulverized by passing through a 500 µm sieve. Microcrystalline cellulose and effervescent agents were dried individually in a tray dryer at 45 °C for 45 min. After drying, sodium bicarbonate and citric acid were blended in a cubic mixer for 5 min. Subsequently, PGS, PVP, MCC, and mannitol were added to the effervescent mixture and mixed for 10 min. Pure isopropyl alcohol was slowly added to the powder mixture until a white, pasty mass was formed. The wet mass was granulated in a granulator through a 1 mm sieve at a granulating speed of 100 rpm and dried in a tray dryer at 50 °C for 1 h. Finally, the dried granules were re-sieved through 1mm sieve, wrapped in aluminum foil and packaged in plastic tubes.

Table 2.8 Composition of granule formulations with and without effervescent mixtures.

Composition (% w/w)	F1	F2	F3	F4
Penicillin G Sodium	33.33	33.33	33.33	33.33
Sodium bicarbonate	14.26	20.43	26.07	-
Citric acid anhydrate	21.74	15.57	9.93	-
Microcrystalline cellulose	20.00	20.00	20.00	20.00
Mannitol, spray-dried	8.67	8.67	8.67	44.67
Polyvinyl pyrrolidone	2.00	2.00	2.00	2.00

Based on the initial drug content of effervescent granules, further investigation of the possible causes of PGS degradation was necessary. For this purpose, one granule batch containing 1.5:1 molar ratio of sodium bicarbonate and citric acid (F11) and one similar batch without citric (F12) were formulated by the same granulation process. Granule drying time, moisture content, and the presence of citric acid were the parameters to be investigated. For the drying time study, both batches of granules were dried at 50 °C for 0.5, 1, and 2 h. In both batches, dry powder mixture samples were taken before granulation. In all studies, the drug contents of the samples were determined in the phosphate buffer of pH 6.8. The compositions of the formulations are described in Table 2.9.

Table 2.9 Composition of PGS effervescent granules with and without citric acid for use in the degradation study.

Composition (% w/w)	F11	F12
Penicillin G Sodium	33.33	33.33
Sodium bicarbonate	14.26	14.26
Citric acid anhydrate	21.74	-
Microcrystalline cellulose	20.00	20.00
Mannitol, spray-dried	8.67	30.41
Polyvinyl pyrrolidone	2.00	2.00

2.6.2 Formulation of matrix tablets

PGS granule batches (F1–F4) containing 10, 15, 20, and 30 % (w/w) HPMC were prepared by wet granulation. Wet granulation was preferred to achieve a uniform dispersion of HPMC throughout the matrix system. The compositions of the formulations are depicted in Table 2.10. Before the granulation process, the ingredients were blended in a cubic mixer for 10 min. Water was slowly added to the powder mixture until a white, pasty mass was formed. The wet mass was granulated in an ERWEKA granulator through a 1 mm sieve at a granulating speed of 100 rpm and dried in a tray dryer at 50 °C for 2 h. Before tableting, the dried granules were placed in a cubic mixer, and 1 % (w/w) colloidal anhydrous silica was added and mixed for 3 min. Subsequently, 1 % (w/w) magnesium stearate was added, and the whole mixture mixed for 2 min. Finally, tablets of 800 mg containing 500 mg of PGS were compressed in a Piccola-Classic rotary tablet press by using a 20 x 9 mm biconvex, oblong punch set. A compression force was applied that resulted in a final hardness of the tablets of 100 ± 10 N.

Table 2.10 Composition of matrix tablets containing 10, 15, 20 and 30 % (w/w) HPMC.

Component (% w/w)	F1	F2	F3	F4
Penicillin G Sodium	62.5	62.5	62.5	62.5
HPMC (Methocel K4M)	10.0	15.0	20.0	30.0
Lactose monohydrate	11.5	9.0	6.5	1.5
Microcrystalline cellulose	12.0	9.5	7.0	2.0
Polyvinyl pyrrolidone	2.0	2.0	2.0	2.0
Colloidal anhydrous silica	1.0	1.0	1.0	1.0
Magnesium stearate	1.0	1.0	1.0	1.0

2.6.3 Formulation of alkalizer containing matrix tablets

Due to the instability of PGS in acidic media, stabilization strategies were sought. One such approach was the addition of alkalizers in a monolithic matrix system to increase the microenvironmental pH. In this study, the alkalizing effects of six basic pH modifiers on the pH change of acidic media were screened by titration. The alkalizers under investigation were MgO, Na_2CO_3, $NaHCO_3$, meglumine, L-Lysine, and L-Arginine. 150 mg of alkalizer powder was transferred into an Erlenmeyer flask, which was placed on a magnetic stirrer with a heat plate while the temperature and the stirring speed were set to 37 °C and 700 rpm, respectively. With the aid of a burette filled either with SGFsp (pH 1.2) or PCB (50 mM, pH 3.0), an acidic medium was gradually added into the flask. The pH value of the solution after adding 25, 50, 100, 150, 200, and 400 mL of the medium was noted using a pH meter.

After the alkalizer screening, four batches of PGS granules containing 15 % (w/w) HPMC and 18.5 % (w/w) alkalizers (MgO, L-Lysine, $NaHCO_3$, and Na_2CO_3) were prepared by wet granulation. The compositions of the formulations are shown in Table 2.11. The names of the

formulations were designated based on their respective alkalizer contents. The granulation and tableting processes were similar to matrix formulations containing no alkalizers in part 2.6.2.

Table 2.11 Composition of matrix tablets containing 15 % (w/w) HPMC and 18.5 % (w/w) alkalizers.

Component (% w/w)	MgO	L-Lysine	$NaHCO_3$	Na_2CO_3
Penicillin G Sodium	62.5	62.5	62.5	62.5
HPMC (Methocel K4M)	15.0	15.0	15.0	15.0
Alkalizer	18.5	18.5	18.5	18.5
Polyvinyl pyrrolidone	2.0	2.0	2.0	2.0
Colloidal anhydrous silica	1.0	1.0	1.0	1.0
Magnesium stearate	1.0	1.0	1.0	1.0

2.7 Characterization of PGS formulations

2.7.1 Characterization effervescent granules

2.7.1.1 Drug content determination

Content determination tests are used to confirm the percentage of a drug present in a formulation. In effervescent granules, the experiments were performed in PBS (pH 6.8) by randomly taking 1.5 g of dried granules from each batch and transferring to 1 L volumetric flask. Subsequently, the buffer was added, and the contents were mixed by gentle shaking for 10 min, and the final volume was adjusted. Samples were withdrawn, and after appropriate dilution, the drug concentration in the ultimate solution was determined by HPLC. Drug content determination experiments were performed in triplicate.

2.7.1.2 Residual moisture content determination (RMC)

The residual moisture content (RMC) of effervescent granules were determined based on a method adapted from BP but with modification.[175] 1 g of granules were transferred to an aluminum moisture pan and dried in an Ohaus MB35 moisture analyzer at 50 °C until a constant weight was noted. The final weight of the granules was measured, and the percent residual moisture content (% RMC) was calculated by using the following Eq. (2.1).

$$\% RMC = \frac{W_0 - W_f}{W_0} x\ 100 \quad (2.1)$$

Where W_o and W_f are the initial and final weight of the granules before and after drying, respectively.

2.7.1.3 Measurement of effervescent time

The duration at which granules liberated CO_2 when in contact with water or gastric fluid was determined by using a method described elsewhere.[176] 1.5 g effervescent granules were placed in a beaker containing 200 mL of purified water at 20 °C ± 1 °C. The time at which a clear solution without particle was taken as an effervescent time and was noted using a stopwatch. The measurements were conducted in triplicate.

2.7.1.4 In vitro drug release studies

The *in vitro* drug release profile of PGS from the effervescent granules was determined using the USP II dissolution testing apparatus. The dissolution medium was 900 mL of PBS (50 mM, pH 6.8) at a temperature of 37 ± 0.5 °C and a stirring speed of 75 rpm. In all experiments, 1.5 mL of samples were withdrawn at 2, 5, 10, 15, 20, 30, 40, and 60 min. Samples were transferred into vials and analyzed by HPLC at 230 nm after appropriate dilution with PBS of pH 6.8. A sink

condition was maintained by replacing the volume withdrawn with an equal amount of fresh medium at the same temperature. The drug release studies were performed in triplicate.

2.7.2 Characterization of matrix tablets

2.7.2.1 Physical characteristics

The weight variation, the crushing strength, and the friability of the matrix tablets were determined using compendial and non-compendial methods. Weight uniformity tests were conducted on 20 tablets, which were selected randomly from each batch and assessed gravimetrically on an individual basis using an analytical balance. The weight of each tablet was compared with the ± 5 % deviation values of the mean of 20 tablets.

The tablet crushing strength, which is the load required to break the tablet into two halves at room temperature, was determined using an ERWEKA hardness tester. Moreover, friability was evaluated by randomly taking ten tablets from each batch, dedusted, and weighed before conducting the test. Then the tablets were placed in an ERWEKA friability tester and subjected to its tumbling actions at 25 rpm for 4 min, once again dedusted and reweighed to determine the percentage loss of weight.

2.7.2.2 Drug content determination

Due to the instability of PGS in the low pH ranges, the drug content of the matrix tablets was determined in pH 6.8 PCB. Ten tablets were selected from each batch randomly, crushed individually to a fine powder and transferred to 1 L volumetric flasks containing the buffer. The contents were ultrasonicated for 10 min at 25 °C, and the final volume was adjusted. Samples were withdrawn, diluted, and analyzed by HPLC.

2.7.2.3 Swelling studies

The water uptake and swelling behavior of the matrix tablets were analyzed gravimetrically using a method described elsewhere.[177–179] The studies were performed in USP II with the same conditions which were applied for the drug release studies. For tablets without alkalizer, the swelling studies were conducted in water over 4 h. On the other hand, the swelling studies were performed in water, SGFsp (pH 1.2) and PCB (pH 3.0 and pH 6.8) for tablets containing alkalizers. In all cases, the percent swelling ratios were calculated based on Eq. (2.2).

$$SR\ (\%)\ =\ \frac{W_{Wet}}{W_0} x\ 100 \qquad (2.2)$$

Where *SR* is the swelling ratio, W_0 is the weight of the matrix tablet before exposure, and W_{wet} is the weight of the hydrated matrix tablet at time t. The hydrated tablets were kept at room temperature for 10 min on paper wipes before measuring the tablet weight.

2.7.2.4 In vitro drug release studies

The *in vitro* drug release studies were performed in a USP II in which the stirring speed and temperature were set at 75 rpm and 37 ± 0.5 °C, respectively. Each vessel was filled with 900 mL of dissolution medium. The dissolution media were SGFsp (pH 1.2 and pH 1.8) and PCB (pH 3.0, 4.5 and 6.8). Sinkers were used to prevent the tablets from floating. Samples of 1.5 mL were withdrawn at predefined time points for a total period of 4–12 h, transferred into a vial and diluted immediately by adding PCB (pH 6.8). In all batches, the drug release studies were conducted in triplicate.

2.7.2.5 Drug stability inside the hydrated matrix tablets

The effect of alkalizers on the stability of PGS in acidic media was demonstrated from the amount of drug left undegraded inside the matrix tablets. For these tests, tablets were removed

from the dissolution vessels after exposure for 2 h to the different dissolution media (pH 1.2 SGFsp and pH 3.0 and pH 6.8 PCB). Subsequently, the tablets were transferred to 0.5 L volumetric flasks containing PCB (pH 6.8), ultrasonicated for 10 min at 25 °C and the volume was adjusted using the same buffer. The samples were withdrawn, diluted, and the drug content was determined by the HPLC method.

2.7.2.6 Kinetic analysis of drug release

The drug release profiles of matrix tablets containing various concentrations of HPMC in PCB (pH 6.8) fitted to different mathematical models (Table 2.12). The release kinetic constant (K) and correlation coefficient (R^2) values of the models were determined from the slopes and linear regression graphs, respectively. The best model fitting was identified for each formulation, and the release mechanisms were explained based on n values (Table 2.13).

Table 2.12 Mathematical models used to describe drug release kinetics from HPMC matrix tablets.

Kinetic model	Equation	Plot	References
Zero-order	$Q_t = Q_0 + K_0t$	Cumulative percent drug release versus time	[164,180]
First-order	$LogQ_t = logQ_0\text{-}K_1t/2.303$	Log cumulative percent drug remaining versus time	[164,181]
Higuchi	$Q = K_H \times t^{1/2}$	Cumulative percent drug release versus square root of time	[182]
Hixson-Crowell	$W_0^{1/3} - W_t^{1/3} = K_{HC}t$	Cube root of the drug remaining in matrix versus time	[183]
Korsmeyer-Peppas	$Mt/M\infty=K_{KP}t^n$	Log cumulative percent drug release versus log time	[184,185]

Q_t = amount of drug dissolved in time t, Q_0 = total amount of drug present in the solution at t_0, W_0 = initial dose of the drug in the dosage form, W_t = remaining amount of drug in the dosage form at time t, $Mt/M\infty$ = fraction of drug released at time t; K_0, K_1, K_H, K_{HC} & K_{KP} = release rate constants of respective models; n = release exponent.

Table 2.13 Interpretation of different release mechanisms from polymeric films.[185]

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.45 < n < 1.0$	Non-Fickian diffusion	t^{n-1}
1.0	Case-II transport	Zero-order release
$n > 1$	Super case-II transport	t^{n-1}

2.7.2.7 Macroenvironmental pH determination

The effect of alkalizers on the macroenvironmental pH was demonstrated by measuring the pH of the dissolution media. The pH measurements were performed at the end of the drug release studies as well as after the pH_M investigations using a pH meter.

2.7.2.8 Microenvironmental pH (pH_M) determination

The microenvironmental pH (pHM) of the matrix tablets was determined according to the methods described previously.[186–188] The tablets were exposed to SGFsp (pH 1.2) and PCB (pH 3.0) in USP II. After 30 min, the tablets were carefully removed from the dissolution vessels and frozen immediately at -80 °C for 1 h. Subsequently, the surface pH of the tablets was assessed using a surface pH electrode in triplicate measurements.

2.8 Drug release studies in the dissolution stress test device

2.8.1 DSTD setup

In this study, the influence of intragastric pressure on the drug release behavior of matrix tablets was investigated. The dissolution stress test device (DSTD) was used to expose dosage forms to the different magnitude of pressure events that could occur *in vivo*. The use and specific description of this device are described elsewhere.[125,189,190] Figure 2.7 shows the major components and the setups of DSTD. The central part of the device consists of a central apparatus

axis and a probe chamber attached to it. The probe chamber consists of steel wire netting spheres, and a balloon attached to it. During the dissolution testing, dosage forms are placed inside the chamber. One end of the central axis is connected with a pressure regulation unit and the opposite end with a stepping motor. Pressure waves are generated by pulsatile inflation and deflation of the balloons located inside the chambers. A computer-controlled pressure-reducing device regulates the inflation and deflation of the balloons. A programmable stepping motor drives the central axis, and an impeller achieves mixing.

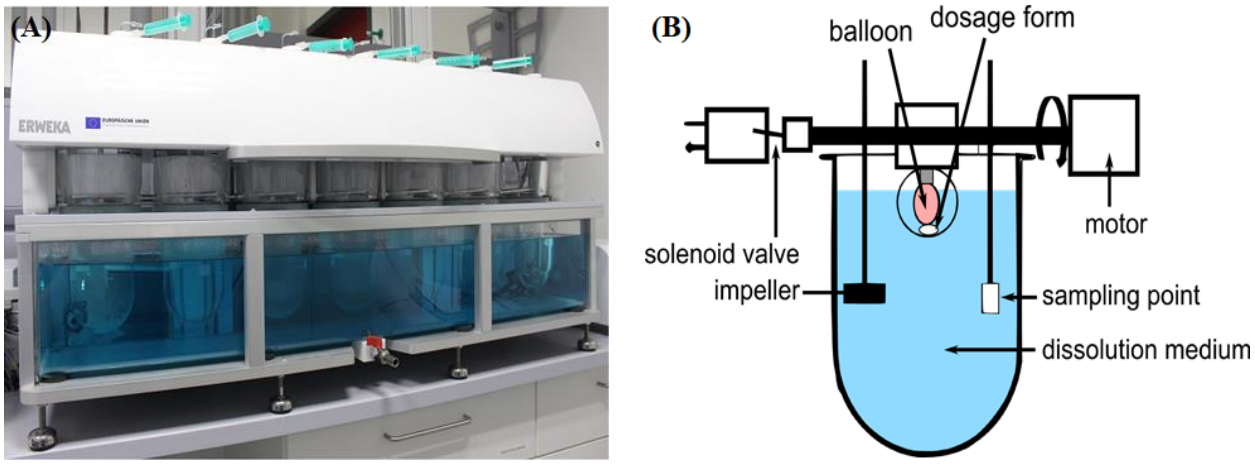


Figure 2.7 Photographic (A) and schematic (B) representation of a dissolution stress test device (SDTD).

2.8.2 Pressure profiles

Recently, Schneider *et al*. have investigated the influence of postprandial intragastric pressure on drug release from matrix tablets by applying realistic pressure profiles in SDTD.[126] Pressure profiles were simulated based on a previous SmartPill® study by Koziolek *et al*. that assessed the intragastric pH and pressure profile after administering FDA breakfast. Accordingly, pressure events of various magnitude ranging from 50–450 mbar was recorded.[69]

In this study, we decided to use 150 and 350 mbar pressures that represent the average pressure events that occur during the normal prandial state in the stomach and gastric emptying,

respectively. Further considerations of the swelling behavior of PGS matrix tablets were taken into account during pressure profiling. Prior swelling studies revealed that maximum swelling of the matrix tablets occurred 1 h after exposure to various test media. Based on these assumptions, three pressure profiles that simulate various pressure events were designed over 8 h (Figure 2.8). Thus, program 1 (P1) was designated as low-stress conditions where no pressure events occur except the gastric emptying. Program 2 (P2) was intended to simulate an intermediate intragastric pressure after the maximum tablet swelling. Program 3 (P3) was regarded as a high-stress program, where pressure events were applied before and after the maximum tablet swelling. At each pressure interval, three pressure events each 6 s duration (a total of 18 s) were applied.

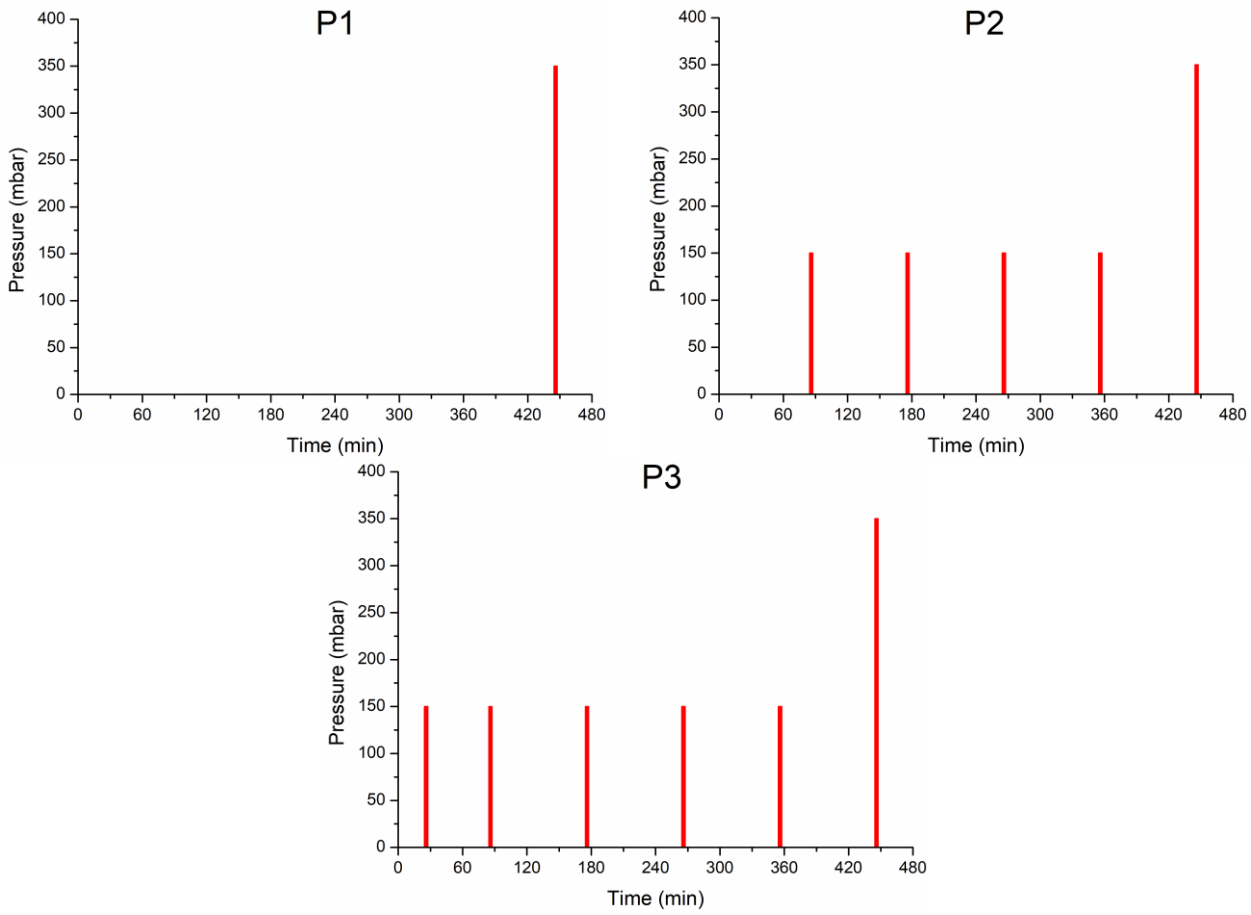


Figure 2.8 Pressure programs applied to study the effect of pressure on drug release from matrix tablets in a dissolution stress test device: program 1 (P1), program 2 (P2), and program 3 (P3).

2.8.3 Experimental conditions

The dissolution stress test investigations were performed in 1100 mL of PCB (pH 4.5). This pH was selected to reflect the actual intragastric pH that could occur after food intake and also considered patients on PPI treatment schedule. The temperature and stirring speed were kept constant at 37 ± 0.5 °C and 100 rpm, respectively. Immediately before running the test, the matrix tablets were placed in the probe chambers and submerged in the standard vessels containing the dissolution medium. In all experiments, 1.5 mL of samples were withdrawn at 5, 15, 30, 45, 60, 90,120, 180, 240, 270, 300, 360, 420, 450 and 480 min. The volume withdrawn was replaced with an equal volume of fresh medium at the same temperature to maintain sink condition. Samples were transferred into vials and analyzed by HPLC after appropriate dilution with pH 6.8 PCB. Similar to USP II, the drug release experiments were conducted in triplicate.

2.9 Testing of the formulations in a flow-through model

The concentration of PGS left inside a dilution vessel containing PCB (pH 4.5) over 6 h was determined using a flow-through model (FTM). The flow rates were simulated similar to the one used during the evaluation of the model under variable flow rate (VFR) studies but with small modifications (Figure 2.9). The in- and outflow rates were designed to reflect the gastric secretion and emptying rates, respectively. In this model, one batch of effervescent granules (F3) and one matrix tablet batch containing sodium bicarbonate were investigated.

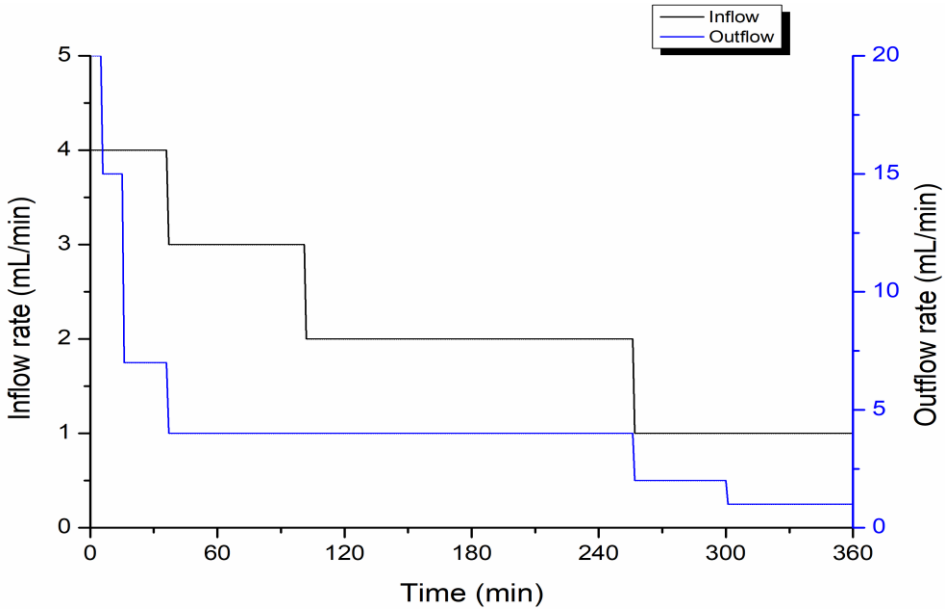


Figure 2.9 Inflow and outflow rate simulations applied to test effervescent granules and matrix tablets under variable flow rate (VFR) condition in a flow-through model.

2.10 *In vivo* evaluation of the formulation concepts

In addition to the *in vitro* studies, *in vivo* investigations were performed to predict the gastroretentive behavior of the formulation concepts. The *in vivo* studies were designed in the partial fulfillment of the diploma thesis of Constantin Foja. Besides, the studies were conducted within the conceptual framework of the formulation of penicillin G for the local treatment of *H. pylori* as part of this doctoral work. The investigations were performed on granules, and matrix tablets, each containing 100 mg of caffeine. The granules were formulated using an effervescent mixture containing sodium bicarbonate and anhydrous citric acid (molar ratio 6:1). The matrix tablets were conventional oblong, biconvex 20 x 9 mm tablets. Both formulations contain approved pharmaceutical excipients that are considered safe and listed in GRAS.

2.10.1 Formulation of caffeine effervescent granules

The first formulation concept in the local treatment of *H. pylori* was to formulate effervescent granules containing PGS. Previously, three batches of PGS effervescent granule formulations

were prepared. The compositions of the baseline PGS effervescent granules (F3) are described in part 2.6.1. Caffeine is one of the model markers in several *in vivo* investigations, including a recent study by Sager *et al.* in predicting gastric emptying of water and drugs.[56] The recommended dose of caffeine for use as an active drug or as co-analgesic is 50–200 mg. Table 2.14 shows the compositions of effervescent granules containing 100 mg of caffeine with a total weight of 1500 mg per dose. The caffeine granules were prepared using a similar wet granulation process employed for PGS effervescent granules.

Table 2.14 Composition of caffeine effervescent granule formulation prepared by the wet granulation.

Composition	Part (% w/w)
Caffeine	6.67
Lactose monohydrate	26.67
Sodium bicarbonate	26.07
Citric acid anhydrate	9.93
Microcrystalline cellulose	20.00
Mannitol, spray-dried	8.67
Polyvinyl pyrrolidone	2.00

Effervescent formulations need strict requirements during preparation and storage to avoid premature effervescence while in contact with moisture. Therefore, precautionary measures such as choosing ingredients with minimum moisture content, prior drying, and use of non-aqueous granulating fluid are essential. In the first formulation, granulation was performed using absolute isopropanol as a granulating fluid. However, the granulation process was challenging, especially in producing granules with the desired size and size distribution. Moreover, the formulation process was not our objective in the *in vivo* investigations. Therefore, the final granule

formulation for the *in vivo* study was prepared by the Hot-melt extrusion (HME) method. HME has the advantage of avoiding the use of water during the melting process. One challenge in the use of HME is the selection of an additional excipient suitable for extrusion. In this formulation, 20 % w/w of high molecular weight of Polyethyleneglycol 4000 was used to produce the desired granule size of 355–500 µm. Table 2.15 depicts the compositions of the effervescent granules prepared by HME.

Table 2.15 Composition of caffeine effervescent granule formulation prepared by the Hot-melt extrusion for use in the *in vivo* study.

Composition	Part (% w/w)
Caffeine	6.67
Lactose monohydrate	6.67
Sodium bicarbonate	26.07
Citric acid anhydrate	9.93
Microcrystalline cellulose	20.00
Mannitol, spray-dried	8.67
Polyvinyl pyrrolidone	2.00
Polyethyleneglycol 4000	20.00

Sodium bicarbonate, citric acid, and microcrystalline cellulose were pulverized by passing through a 500 µm sieve and dried individually in an oven with circulating air at 45 °C for 45 min. Moreover, caffeine-lactose mixture, PVP, and mannitol were sieved by passing through a 500 µm sieve. After drying, the ingredients were blended in a Turbula mixer for 5 min at 49 rpm. Subsequently, the powder mixture was extruded in a hot-melt extruder. The temperature, screw speed, and the feeding rate of the extruder were set at 70 °C, 100 rpm, and 15 %, respectively.

The extruded masses containing agglomerates were further disaggregated and fractionated by a sieve analyzer.

2.10.2 Preparation of granule carrier

In the *in vivo* study, granules were packed in a rounded cylindrical polymer sachet before administering to the volunteers. The sachet was designed to prevent contamination of the oral cavity with granule particles and the subsequent interfere with saliva analysis. It was made of approved pharmaceutical excipients for human use, such as Kollicoat® IR, PVA, glycerol, and coloring agent. The diameter and height of the sachet was approximately 1.5 cm and 2 cm, respectively.

2.10.3 Formulation of caffeine matrix tablets

The second concept of treating *H. pylori* locally in the stomach was using PGS monolithic matrix tablets. Similarly, the GET and the concentration of drug remaining in the stomach was predicted by conducting an *in vivo* study using caffeine as a model drug in a matrix tablet formulation. The caffeine matrix tablets were formulated based on a baseline PGS matrix formulation (F2) containing 15 % (w/w) HPMC, as described in part 2.6.2. For further use of the tablets in the MRI investigations, 1.25 % (w/w) iron oxide was added to help image detection. Table 2.16 shows the compositions of the caffeine matrix tablets. The granulation and tableting processes were similar to the one used for PGS matrix tablets.

Table 2.16 Composition of a matrix tablet formulation containing 100 mg of caffeine for use in the *in vivo* study.

Composition	Part (% w/w)
Caffeine	12.50
HPMC (Methocel K4M)	15.00
Lactose monohydrate	46.50
Microcrystalline cellulose	22.00
Polyvinyl pyrrolidone	2.00
Colloidal anhydrous silica	1.00
Magnesium stearate	1.00
Iron oxide	1.25

2.10.4 Clinical study protocol

The *in vivo* study was a randomized, four-arm cross-over study with one day per arm. Twelve healthy volunteers (6 males, 6 females) aged 20–27 years were participated in this study. The body mass index of the volunteers was between 18 and 30 kg/m^2. Before the study, informed consent was obtained from each participant. Moreover, the study protocol was approved by the ethics committee (die Ethikkommision der Universitätsmedizin Greifswald, No. BB 203/18b).

2.10.5 Randomization and study procedures

The participants were randomized into four-study arms (three fed and one fasted state arms), and randomization determines which day and which formulation tested. In all study arms, blind saliva samples were taken from each participant before either taking the standard breakfast in the fed state or water in a fasted state. Fed arm studies (arms 1–3) conducted in the first three days, and volunteers were set to take the formulations after taking the standard breakfast. Polymer sachets

filled with granules were taken with 20 and 240 mL of tap water on the first and the second days, respectively. Similarly, matrix tablets were taken with 240 mL of water on the third day.

The consumption of caffeine-containing foods (e.g., coffee, tea, soft drinks, and chocolate) was prohibited from 72 h before the trial days. Subjects were not allowed to consume food 6 h before taking the vehicle. A low-calorie breakfast that does not exceed 500 kcal was allowed on this day until 7:00. The ingestion of calorie-containing drinks (e.g. juices, milk, soft drinks) was stopped 3 h before the start of the examination. Non-caloric fluids such as water were allowed up to 90 min before the beginning of the investigation.

The standard meal was a high-calorie, high-fat breakfast recommended by the FDA. This meal composed of two slices of toast with 10 g of butter, two fried eggs in 5 g butter, two strips of fried breakfast ham (bacon), 113 g potato hash browns, and 240 mL whole milk. The products available in German supermarkets were used to prepare the meal (Table 2.17). The total calorie content of the meal was 800–1000 kcal. Moreover, the food was consumed entirely within 15 min. The formulations were taken in an upright position along with water 30 min after the start of the meal, and saliva samples were collected for 6 h. In study arm 3, additional MRI images were taken after 240 min to investigate the disintegration and location of the tablets.

Table 2.17 List of food items purchased from German supermarkets for FDA breakfast.

Ingredient	Product and Producer
Bacon	Tulip Bacon (TULIP FOOD COMPANY, Danemark)
Butter	Meggle Alpenbutter (MEGGLE AG, Germany)
Toast	Sammy's Super Sandwich (HARRY-BROT GmbH, Germany)
Eggs	Freilandeier Größe L (Poseritzer EierHOF, Germany)
Hash browns	Gut & Günstig Rösti-Ecken (Gut & Günstig Eigenmarke der EDEKA Group)
Whole milk	Gut & Günstig H-Vollmilch 3.5% Fett (Gut & Günstig Eigenmarke der EDEKA Group)
Drinking water	Tap water (Stadtwerke Greifswald: Wasserwerke Schönwalde & Hohenmühl)

In a fasted state study (arm 4), the subjects were not allowed to consume food 12 h before taking the vehicle. The ingestion of calorie-containing drinks (juices, milk, soft drinks) was stopped 3 h before the start of the examination. Non-caloric liquids such as water were allowed to be taken up to 90 min before the beginning of the investigation. In this arm, the granules were filled in polymer sachet and taken with 240 mL of tap water, and saliva samples were collected for 12 h. After 4 h, the volunteers were given the standardized lunch of Spaghetti Bolognese (apetito AG, Rheine, Germany) with 240 mL of tap water and similarly consumed within 15 min.

2.10.6 Salivary sample collection, preservation, and analysis

Saliva samples were collected by directly spitting into 2 mL SafeSeal microtubes (Sarstedt, Nübrecht, Germany). A total of 30 and 36 samples were collected for each study arm in arms 1–3 and arm 4, respectively, and stored at -80 °C until analysis. The deep freeze samples in the microtubes were thawed for 1 h at room temperature and centrifuged with 1800 rpm for 15 min. To precipitate the proteins, 200 mL of the supernatant was taken and mixed with 400 µL of a

solution composed of acetonitrile with 6 % formic acid in a 1.5 ml microtube. The resulting mixture was vortexed at maximum speed for 1 min followed by centrifugation at 1300 rpm for 15 min. Subsequently, 150 µL of the supernatant was placed in 300 µL vials and diluted with 150 µL LC-MS grade water containing 4 % of formic acid, vortexed at maximum speed for 1 min and ready for analysis.

The caffeine content in the saliva samples was determined by using an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled with a triple quadrupole mass spectrometer API4000 QTRAP via electrospray ionization source Turbo VTM. Validated Analyst 1.6 software (AB Sciex, Darmstadt, Germany) was used to control the LC-MS/MS system. Caffeine quantification method, validation, and instrumental setups were adopted from a recent study by Sager *et al*.[56]

2.10.7 Magnetic resonance imaging

In study arm 3, the location and disintegration behavior of the matrix tablets in the GIT were studied by additional MRI investigation on subjects 4 h after taking the tablets. The study was conducted at the Institute of Diagnostic Radiology and Neuroradiology of the University Medizin Greifswald using a Siemens MR-scanner with a field strength of 1.5 T. MRI investigations were performed in the supine position with head up and subjects hold their breath for up to 23 s during imaging. The coronal and transversal TRUFI (True Fast Imaging with Steady State Precision) sequences were applied.

2.11 Data analysis and statistics

Statistical analysis was performed by using a one-way analysis of variance (ANOVA) on computer software called GraphPad Prism 7 (GraphPad Software, San Diego, USA). Tukey's multiple comparison tests were performed to demonstrate the similarities and differences among specific formulation parameters. At a 95 % confidence interval, P values less than or equal to 0.05 were considered significant. The figures were drawn by OriginPro 8.5.1 (OriginLab Corporation, Northampton, MA, United States). The data values were presented in the form of the mean and the standard deviation.

3 Results

3.1 Antibiotic screening

The *in vitro* susceptibilities of the investigated antibiotics against known isolates of *Helicobacter pylori* were determined and presented in Table 3.1. Accordingly, amoxicillin and penicillin G were the drugs with the lowest MIC values. Similarly, rifampicin had low MIC and was regarded as an alternative antibiotic for *H. pylori* treatment. Fosfomycin showed high MIC, and several resistant colonies were observed.

Table 3.1 Minimum inhibitory concentration of four antibiotics against known *H. pylori* isolates (n = 5).

Antibiotic	Antibiotic range (µg/mL)	MIC (µg/ml)
Amoxicillin	0.016 – 256	0.023
Fosfomycin	0.016 – 256	3.000*
Penicillin G	0.002 – 32	0.125
Rifampicin	0.002 – 32	1.000

* Lots of resistant colonies

3.2 HPLC method development and validation

A novel and valid HPLC method that could effectively quantify penicillin G sodium in various test media was developed and also validated. The validation parameters were performed against international standard protocols and presented below.

3.2.1 Linearity

The calibration curve of working standards were determined by drawing the chromatographic response versus 9 concentrations in the range of 1–100 µg/mL in a phosphate-citrate buffer (PCB, pH 6.8) at 230 nm (Figure 3.1). The drawing yielded a linear curve with a regression

equation of y = 19632.164x + 1930.503 (where y is the peak area in mAu, and x represents drug concentration in µg/mL). The correlation coefficient (r^2) of the method was 0.99998, and the value demonstrated the high degree of correlation between the peak area and the concentration.

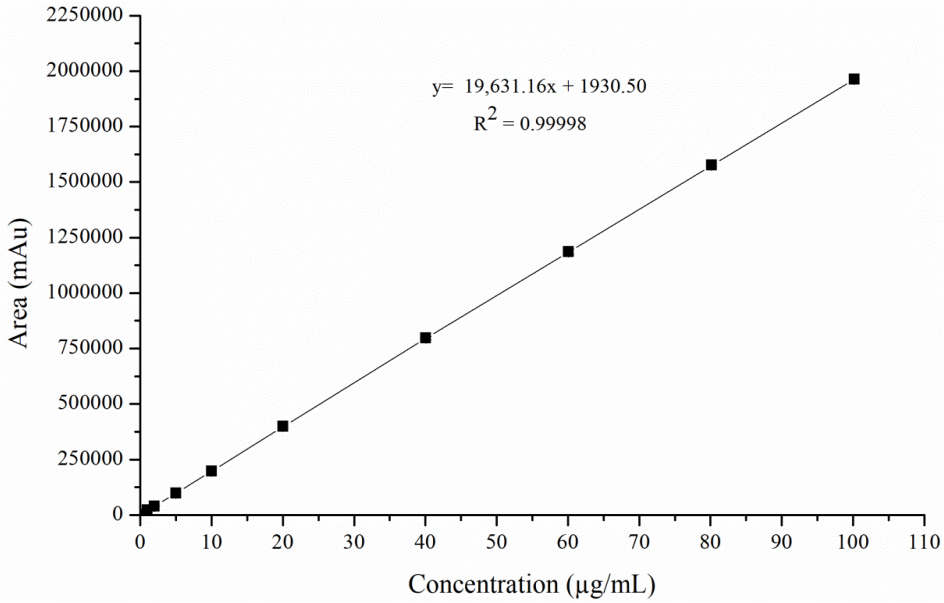


Figure 3.1 Calibration curve of penicillin G sodium working standards in a phosphate-citrate buffer of pH 6.8 at 230 nm with a 95 % confidence interval (r^2 = 0.99998).

3.2.2 Precision and accuracy

The intra-day and inter-day variabilities, as well as the accuracy of the method, are depicted in Table 3.2. The method precision and accuracy values at each level were within the acceptable limit of ± 5 % RSD, and the overall % RSD values were below 2 %. Moreover, the values at the higher concentration levels (50 and 100 µg/mL) were minimal (mean % RSD < 0.2 %), which demonstrated that the method was both accurate and precise.

Table 3.2 Intra-day and inter-day precisions and the accuracy of the HPLC method at 1, 50, and 100 µg/mL of penicillin G sodium (n = 6).

Intra-day (n = 6)			
Theoretical conc. (µg/mL)	Mean conc. ± SD (µg/mL)	RSD (%)	Accuracy (%)
1	0.91 ± 0.044	4.85	91.41
50	50.54 ± 0.144	0.27	100.98
100	100.91 ± 0.120	0.20	100.82
Inter-day (n = 6)			
1	0.96 ± 0.027	2.77	96.32
50	50.13 ± 0.053	0.13	100.13
100	100.50 ± 0.155	0.16	100.38

3.2.3 Limit of detection and limit of quantification

The LOD and LOQ of the method were also found to be 0.0764 and 0.2314 µg/mL, respectively.

3.3 *In vitro* stability studies

The *in vitro* stability and degradation behavior of PGS was studied in various media of pH (pH 1.2, 3.0, 4.5, and 6.8) and shown in Figure 3.2. The pH values were selected to simulate the possible gastric pH scenarios that could happen in various fasted and fed state conditions. Accordingly, PGS was very unstable at pH 1.2 and entirely degraded within 30 min (data not shown). When the pH of the media increased to pH 3.0, there was a sensible amount of PGS for at least 4 h. However, the drug was degraded totally within 6 h. The degradation study in pH 4.5 showed that the amount of PGS decreased over time but with over 30 % available after 12 h. In contrast, PGS was stable at pH 6.8 with more than 97 % of the drug available after 12 h.

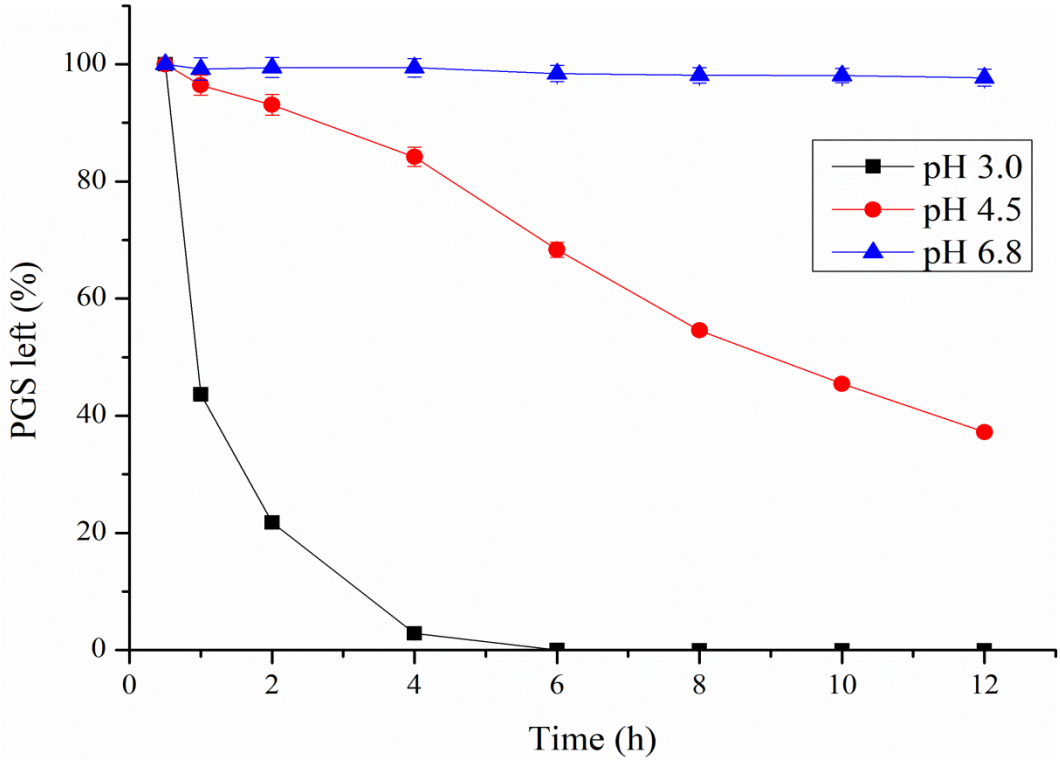


Figure 3.2 Stability and degradation behavior of PGS over 12 h in phosphate-citrate buffers of pH 3.0, 4.5, and 6.8 at 37 °C (n = 3, means ± SD).

3.4 Evaluation of a flow-through model

The performance of the FTM was evaluated by conducting dilution studies at constant and variable flow rates using PGS powder. In a constant flow rate (CFR) studies, the performance of the newly developed model was evaluated at 3, 6, and 9 mL/min flow rates. The result indicated that the experimental data at various CFR were similar to excel generated simulated values (Figure 3.3).

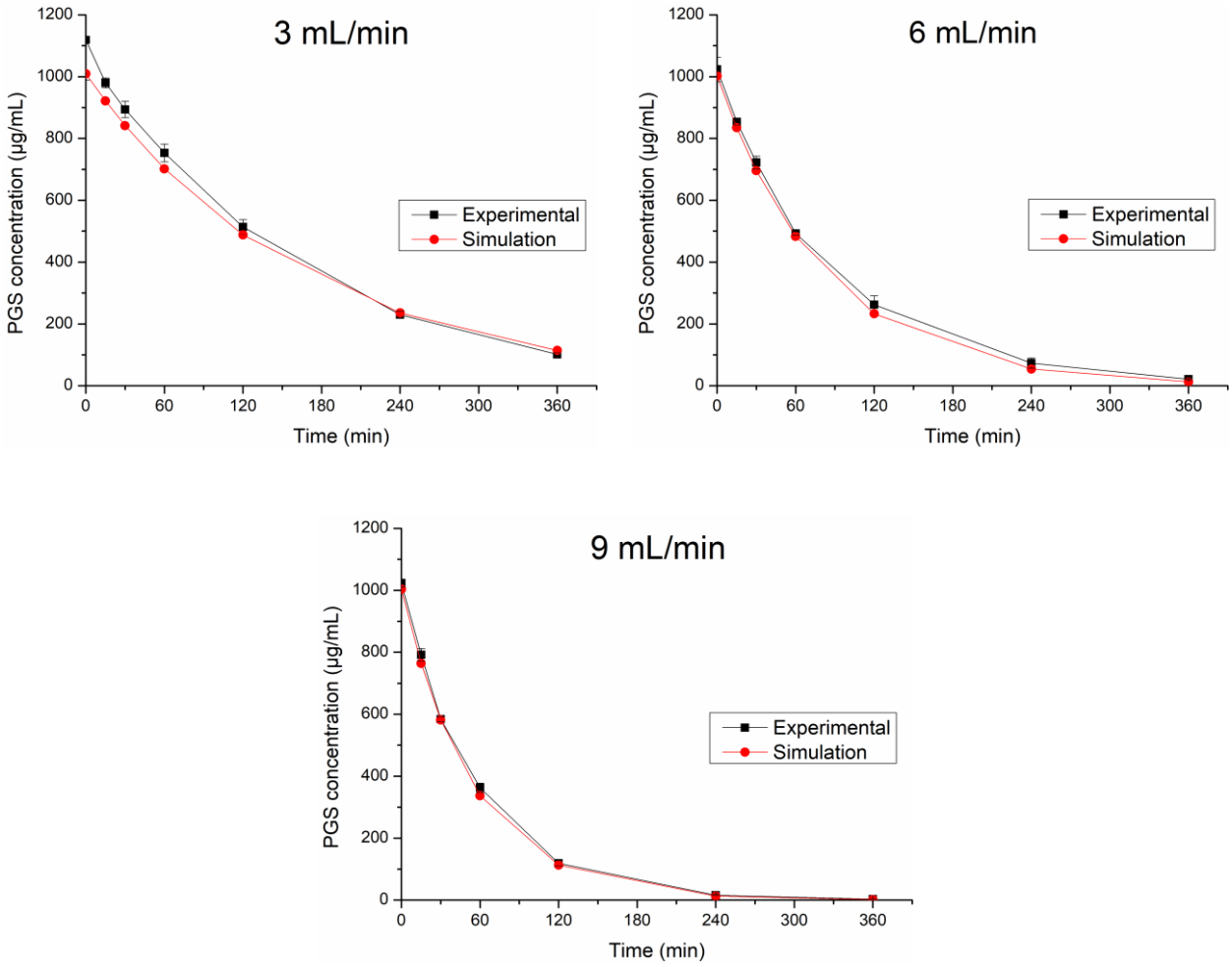


Figure 3.3 PGS concentration left in the dilution vessel over 6 h at constant in- and outflow rates in a flow-through model in phosphate buffer of pH 6.8 (n = 3, means ± SD).

In addition to the CFR, the dilution tests were conducted at a variable flow rate (VFR) conditions. This study was aimed to simulate the actual gastric volume, the gastric secretion, and gastric emptying rates, which are expected in fed conditions. Similar to the CFR studies, the experimental data in VFR were comparable to the simulated values (Figure 3.4).

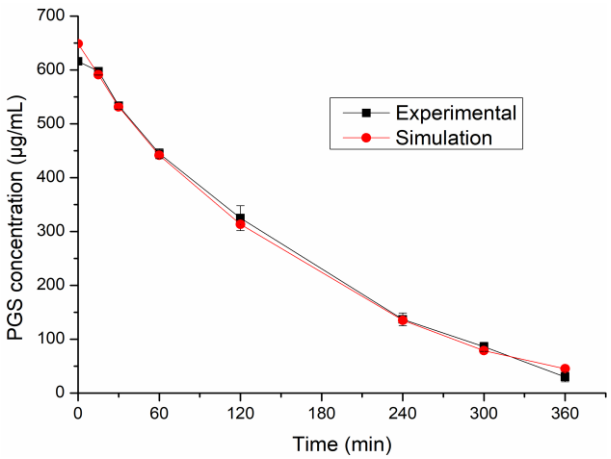


Figure 3.4 PGS concentration left in the dilution vessel over 6 h at a variable in- and outflow rates in a flow-through model in phosphate buffer of pH 6.8 (n = 3, means ± SD).

3.5 Characterization of effervescent granules

3.5.1 Drug content determination

The drug content of effervescent granules containing various molar ratios of sodium bicarbonate and citric acid were determined in pH 6.8 PBS and depicted in Table 3.3. The result revealed that F1 and F2 formulations were with the lowest drug content, and they did not meet the compendial requirements set for content uniformity. In contrast, the drug content of F3 and F4 granules were within the acceptable range.

Table 3.3 PGS content of effervescent granules in phosphate buffer of pH 6.8 (n = 3, means ± SD).

Formulation code	Molar ratio ($NaHCO_3$: Citric acid)	PGS content (%)
F1	1.5:1.0	39.91 ± 1.50
F2	3.0:1.0	57.86 ± 3.03
F3	6.0:1.0	103.18 ± 0.91
F4	-	102.02 ± 3.46

The causes of low drug release were further investigated by formulating F1 granules with and without citric acid at various drying conditions. The results confirmed that citric acid was the major cause of degradation (Table 3.4).

Table 3.4 PGS content of effervescent granules with and without citric acid in phosphate buffer of pH 6.8 in degradation studies (n = 3, means ± SD).

Formulation	Drug content (%)			
	Powder mixture	0.5 h	1 h	2 h
F11 (with citric acid)	106.15 ± 1.99	46.63 ± 1.30	53.21 ± 1.00	54.45 ± 1.11
F12 (without citric acid)	104.29 ± 1.75	101.86 ± 1.41	103.27 ± 1.10	101.09 ± 2.57

3.5.2 Residual moisture content and effervescent time

The relative moisture content (RMC) and effervescent time of the granules were investigated in water. The RMC values from all batches were below 1 % independent of the composition of effervescent mixtures in the formulation (Table 3.5). However, the effervescent action of granules was affected by the molar ratio of sodium bicarbonate and citric acid. As a result, granules containing the highest molar ratio of sodium bicarbonate to citric acid (F3) generated CO_2 for a longer time (> 1 min), while F1 and F2 granules generated CO_2 for less than 1 min.

Table 3.5 Residual moisture content and effervescent time of granules (n = 3, means ± SD).

Formulation code	Molar ratio ($NaHCO_3$: Citric acid)	Residual moisture content (%)	Effervescent time (second)
F1	1.5:1.0	0.82 ± 0.15	38.00 ± 2.65
F2	3.0:1.0	0.76 ± 0.17	47.67 ± 5.03
F3	6.0:1.0	0.94 ± 0.18	63.67 ± 6.11
F4	-	0.98 ± 0.18	-

3.5.3 *In vitro* drug release studies

The *in vitro* drug release profile of granules were performed in PBS (pH 6.8) and depicted in Figure 3.5. Accordingly, only granules containing a high molar ratio of sodium bicarbonate to citric acid (F3) and a blank batch (F4) gave faster and maximal drug release. Moreover, F3 granules released the drug entirely within 5 min. However, granule batches containing less amount of sodium bicarbonate (F1 and F2) released less than 60 % of the drug after 1 h. The degradation of the drug was the cause of low drug release, as presented in part 3.5.1.

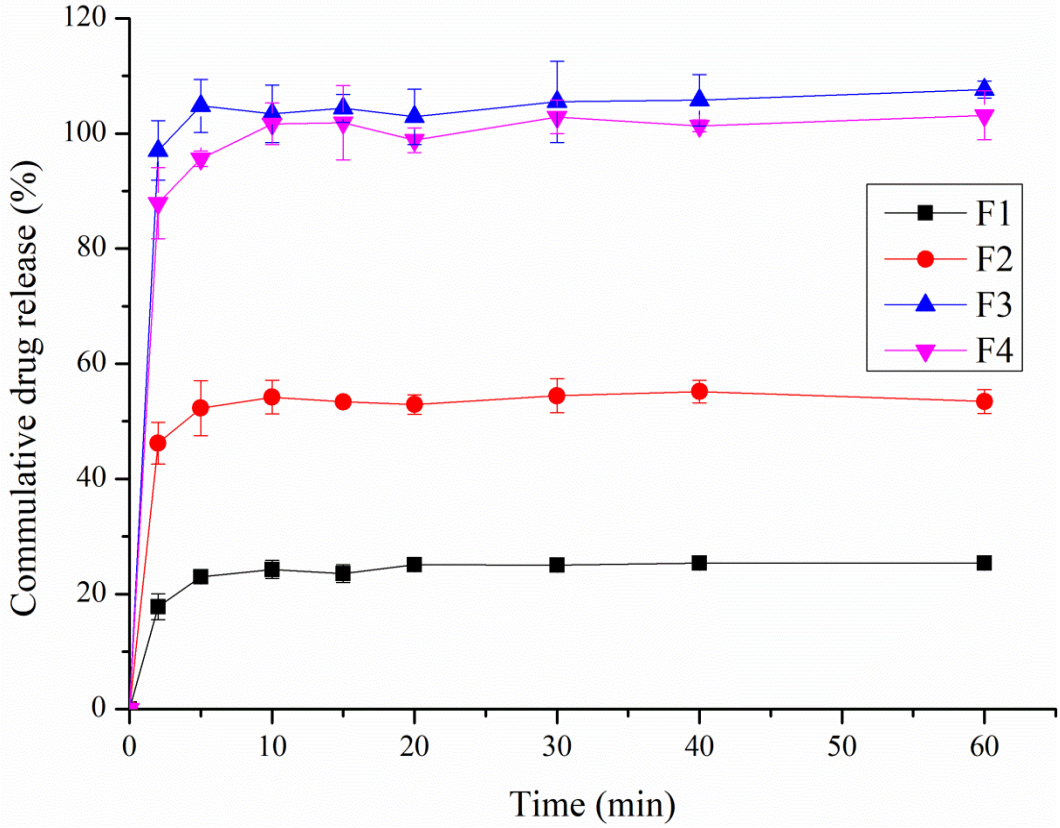


Figure 3.5 Drug release profiles of effervescent granules containing different molar ratios of sodium bicarbonate and citric acid in comparison with non-effervescent granules in a phosphate buffer solution of pH 6.8 (n = 3, means ± SD).

3.6 Characterization of matrix tablets without alkalizer

3.6.1 Physicochemical characteristics

Some physicochemical characteristics of matrix tablets were determined using compendial and non-compendial methods and shown in Table 3.6. Compendial specification for tablets weighing more than 324 mg states that weight of not more than two tablets deviate from the mean weight by more than 5 %. According to this specification, all the tablets met the criteria set for weight variation. Crushing strength shows the ability of tablets to withstand pressure or stress during handling, packaging, and transportation. Friability is important because the tablet subjected to various abrasive motions during production and use and with a compendial specification of not more than 1 %.[173] The result proved that all the tablets met the compendial specifications set for friability with sufficient crushing strength. Moreover, all the tablets demonstrated nearly 100 % of the claimed drug content.

Table 3.6 Some physicochemical characteristics of PGS matrix tablets containing 10, 15, 20 and 30 % (w/w) HPMC.

Formulation	Weight (mg)	Crushing strength (N)	Friability (%)	Drug content (%)
F1	801.80 ± 7.04	104.20 ± 8.24	0.20	99.99 ± 1.78
F2	802.00 ± 4.45	101.50 ± 3.54	0.24	102.21 ± 2.66
F3	801.85 ± 7.01	100.80 ± 7.24	0.28	101.99 ± 2.16
F4	803.75 ± 8.14	99.80 ± 8.24	0.29	100.30 ± 2.47

3.6.2 Swelling studies

The water uptake behaviors of the matrix tablets without alkalizer containing various concentrations of HPMC were conducted in water and depicted in Figure 3.6. The result confirmed that maximum swelling was recorded 1 h after exposure to water and then decreased

with time. Moreover, F1 and F4 were with the minimum and the maximum swelling, respectively. This variation indicated that swelling was dependent on the concentration of HPMC. The percent swelling ratios of the matrix tablets were in the order of F4 > F3 > F2 > F1.

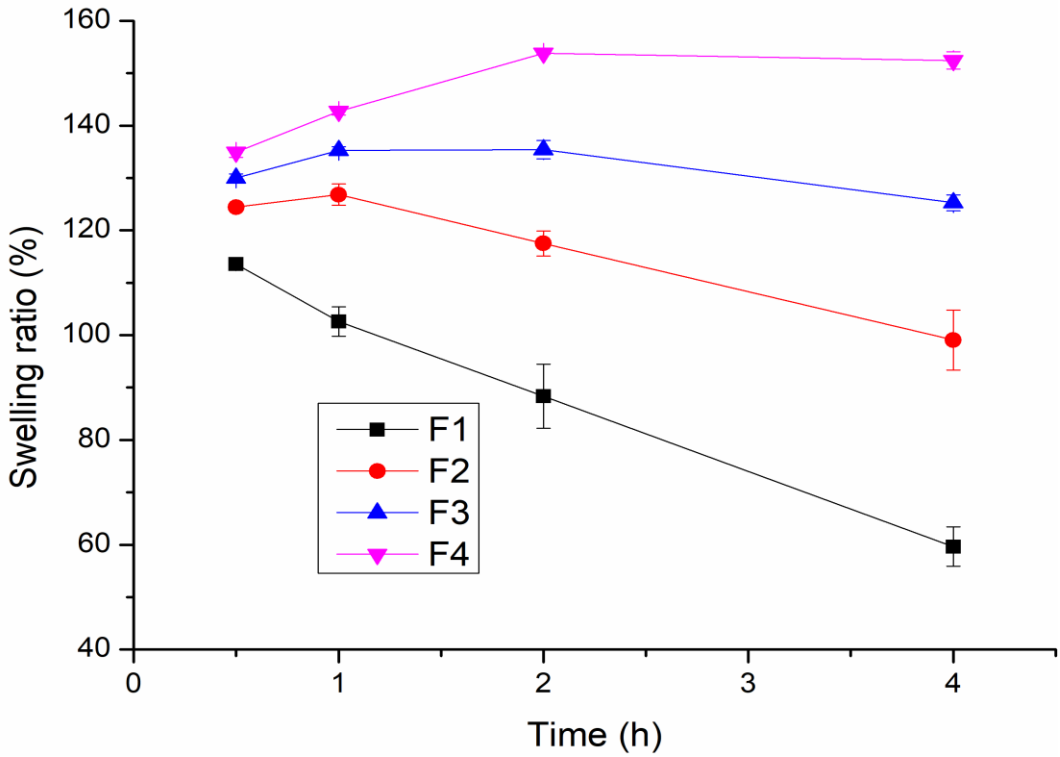


Figure 3.6 The swelling behavior of PGS matrix tablets containing 10, 15, 20, and 30 % (w/w) HPMC in water for 4 h (n = 3).

3.6.3 *In vitro* drug release studies

The *in vitro* drug release profiles of matrix tablets containing various concentrations of HPMC were investigated in pH 6.8 PCB and depicted in Figure 3.7. The result revealed an inverse relationship between the rate of drug release and the percentage of the matrix former. F1 tablets containing 10 % (w/w) of HPMC demonstrated the fastest drug release, while the drug release from F4 tablets containing 30 % (w/w) was with the slowest drug release. All the formulations released more than 95 % of the drug at the end of 12 h. On the contrary to the swelling ratio, the drug release profiles were in the following order: F1 > F2 > F3 > F4.

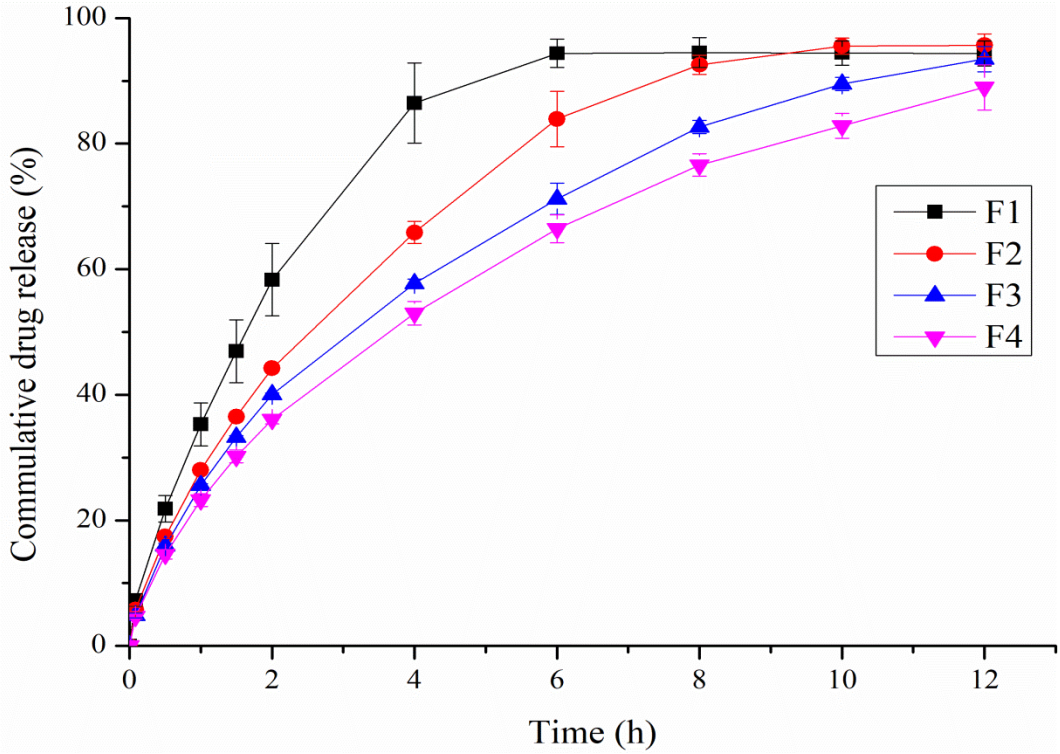


Figure 3.7 PGS release profiles of matrix tablets containing 10, 15, 20 and 30 % (w/w) HPMC without alkalizer in a phosphate-citrate buffer of pH 6.8 (n = 3, means ± SD).

3.6.4 Drug concentration profiles in media of different pH

The pH-dependent degradation of PGS was investigated in various media with pH ranges of pH 1.2–6.8 over 12 h in USP II and shown in Figure 3.8. The PGS concentration profiles in pH 6.8 (Figure 3.8c), which were in the range of 480–522 µg/mL, were utilized for comparison purpose. Variations in the drug concentrations were due to the differences in the HPMC content in the matrix and were arranged in the following order: F1 > F2 > F3 > F4. In pH 4.5 (Figure 3.8b), the drug concentrations were decreased for all formulations compared with profiles in pH 6.8. Further decreases in drug concentrations were observed when the pH of the media dropped to 3.0 (Figure 3.8a). In pH 3.0 and 4.5, the drug concentrations were decreased in the following order: F1 > F2 > F3 > F4. Finally, concentration measurements were conducted in pH 1.2 media over 12 h. However, there was no drug found in the dissolution vessels (data not shown).

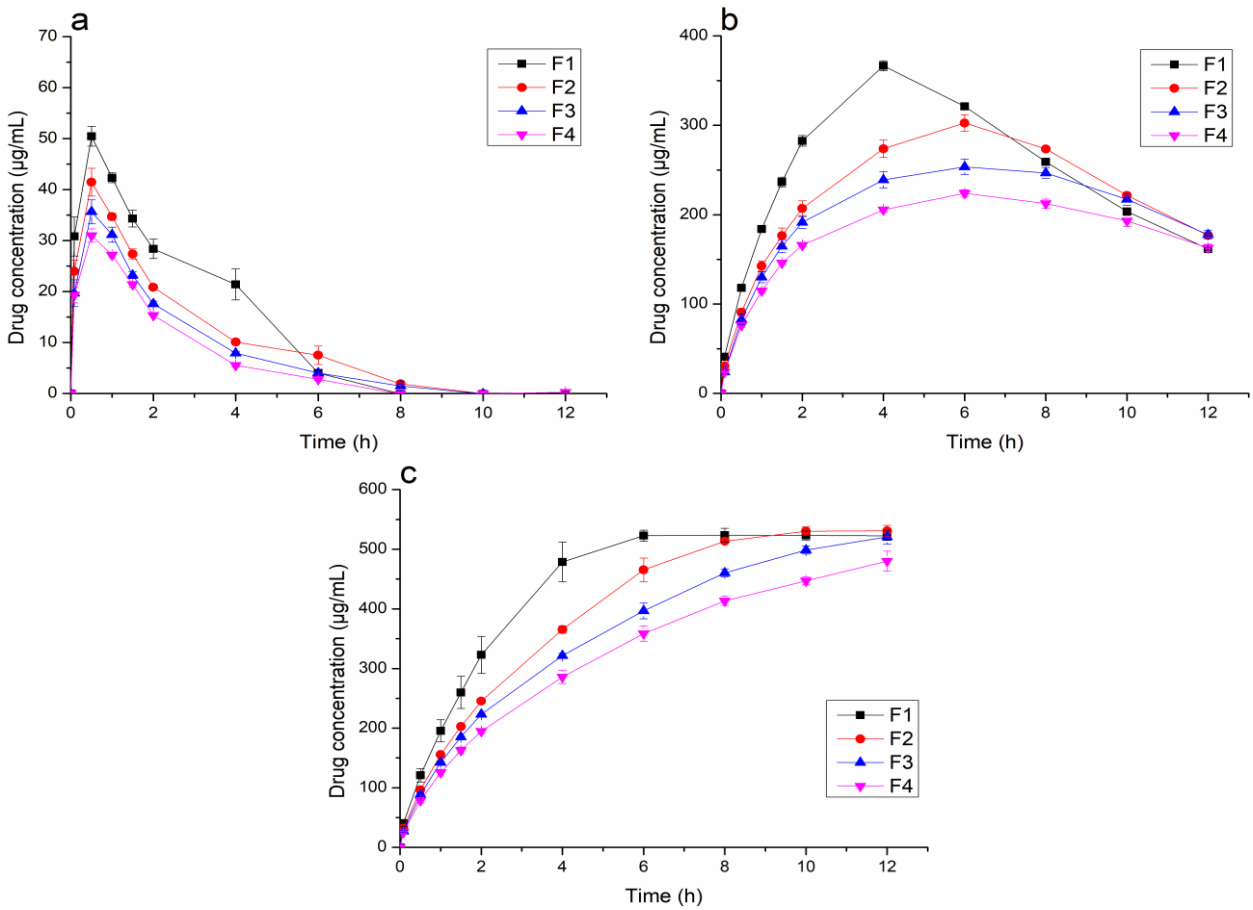


Figure 3.8 PGS concentration profiles of matrix tablets containing 10, 15, 20, and 30 % (w/w) HPMC without alkalizer in a phosphate-citrate buffer. (a) pH 3.0, (b) pH 4.5 and (c) pH 6.8 (n = 3, means ± SD).

3.6.5 Kinetic analysis of drug release

The *in vitro* drug release data in PCB pH 6.8 were analyzed to assess the release kinetics according to zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas kinetic models. The coefficients of determination (r^2) values were calculated to determine a specific model to which the release profile best suited (Table 3.7). Based on the data, the drug release profiles were fitted best to the Korsmeyer-Peppas model, as demonstrated by relatively high r^2 values.

Table 3.7 The coefficient of determination (r^2) values after fitting the drug release profiles of tablets containing 10, 15, 20, and 30 % (w/w) HPMC in a phosphate-citrate buffer of pH 6.8 to kinetic models.

Formulation	Zero-order	First-order	Higuchi	Hixon-Crowell	Korsmeyer-Peppas
F1	0.7459	0.8659	0.9174	0.8377	0.9997
F2	0.8760	0.9837	0.9794	0.9664	1.0000
F3	0.9159	0.9970	0.9952	0.9908	0.9991
F4	0.9266	0.9978	0.9968	0.9886	0.9996

Additionally, the release exponents were calculated using a Korsmeyer-Peppas model (Table 3.8). The n values were between 0.45 and 0.89, which confirmed the mechanism of drug release was based on anomalous (non–Fickian) diffusion.

Table 3.8 Release kinetic constant (k) and release exponent (n) values after fitting the drug release profiles of tablets containing 10, 15, 20, and 30 % (w/w) HPMC in phosphate-citrate buffer of pH 6.8 to kinetic models.

Formulation	Zero-order	First-order	Higuchi	Hixon-Crowell	Korsmeyer-Peppas	
	K_0 (h^{-1})	K_1 (h^{-1})	K_H ($h^{1/2}$)	K_{HC} ($h^{-1/3}$)	K_{KP} (h^{-n})	n
F1	7.5594	0.2760	30.4652	0.2602	2.0597	0.6966
F2	8.0664	0.2869	30.9943	0.2715	2.2096	0.6214
F3	7.6203	0.2196	28.8646	0.2278	2.2171	0.5974
F4	7.1925	0.1753	27.1085	0.1955	2.1114	0.5873

3.6 Characterization of matrix tablets containing alkalizers

The alkalizing effects of six pH modifiers were determined in two different aqueous media at pH 1.2 and pH 3.0 (Figure 3.9). Accordingly, the alkalizing potential was dependent mainly on the

type and concentration of the alkalizer. Besides, the pH of the media had a role in the alkalizing effect. Among the alkalizers, MgO was the most potent alkalizer, followed by Na_2CO_3 and $NaHCO_3$. In contrast, the amino acids (meglumine, L-Lysine, and L-Arginine) were not able to change the pH of the media to the desired level, even at their maximal concentrations.

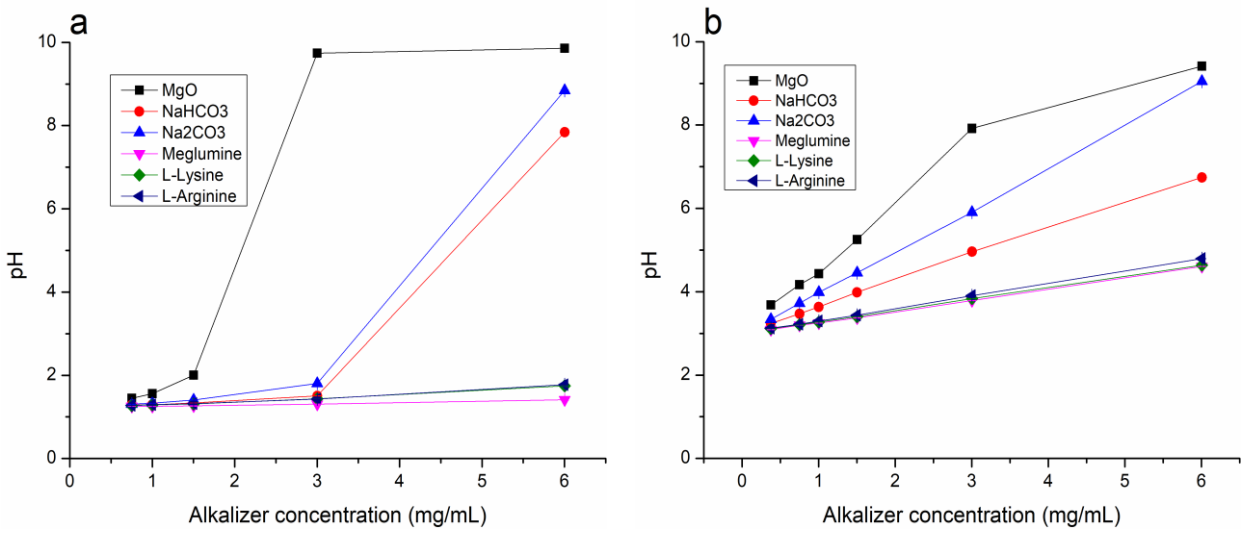


Figure 3.9 Effect of alkalizer concentration on pH increase in various acidic media at 37 °C. (a): SGFsp pH 1.2 and, (b): 50 mM PCB pH 3.0 (n = 3, means ± SD).

3.6.1 Physicochemical characteristics

The weight uniformity, crushing strength, friability, and the drug content of the matrix tablets containing various alkalizers were determined using compendial and non-compendial methods (Table 3.9). The tablets met the requirements for weight uniformity and demonstrated sufficient mechanical strengths concerning crushing strength and friability. In contrast to matrix tablets without alkalizers, the drug contents of alkalizer-containing matrix tablets in PCB (pH 6.8) were variable. Tablets containing $NaHCO_3$ and Na_2CO_3 had comparable drug content (around 100 %). Moreover, MgO containing matrix tablets contained a sufficient amount of drug (≈ 98%). However, matrix tablets containing L-Lysine contained only ≈ 79% of the targeted drug content.

Table 3.9 Some physicochemical characteristics of PGS matrix tablets containing alkalizers.

Formulation	Weight (mg)	Crushing strength (N)	Friability (%)	Drug content (%)
MgO	803.76 ± 5.22	100.08 ± 5.65	0.34	97.60 ± 3.25
L-Lysine	801.10 ± 4.87	102.20 ± 5.75	0.30	79.25 ± 1.30
$NaHCO_3$	803.05 ± 3.52	104.90 ± 2.96	0.31	101.93 ± 1.24
$NaHCO_3$	802.80 ± 3.07	102.40 ± 5.10	0.36	100.00 ± 1.62

3.6.2 Swelling studies

The swelling behaviors of alkalizer-containing matrix tablets were investigated in water and various aqueous media (pH 1.2, 3.0, and 6.8). For comparison purposes, the swelling characteristics of blank matrix tablets were studied under the same conditions (Figure 3.10). In water, the maximum swelling ratios were recorded after 1 h and then decreased over the next 3 h. Besides, matrices containing L-Lysine and MgO demonstrated the minimum and maximum percent swelling in water, respectively. In addition to water, the swelling behaviors of the matrix tablets were investigated after exposing tablets to media of various pH for 1 h. Accordingly, the swelling actions of the matrix tablets were variable depending on the pH and alkalizer type. For instance, MgO containing matrices showed the maximum swelling in all pH ranges. On the other hand, Na_2CO_3 tablets demonstrated low swelling in pH 1.2 but with a subsequent increase in the next pH ranges. On the contrary, L-Lysine and $NaHCO_3$ matrices showed high swelling in pH 1.2, but swelling decreased in the other pH ranges.

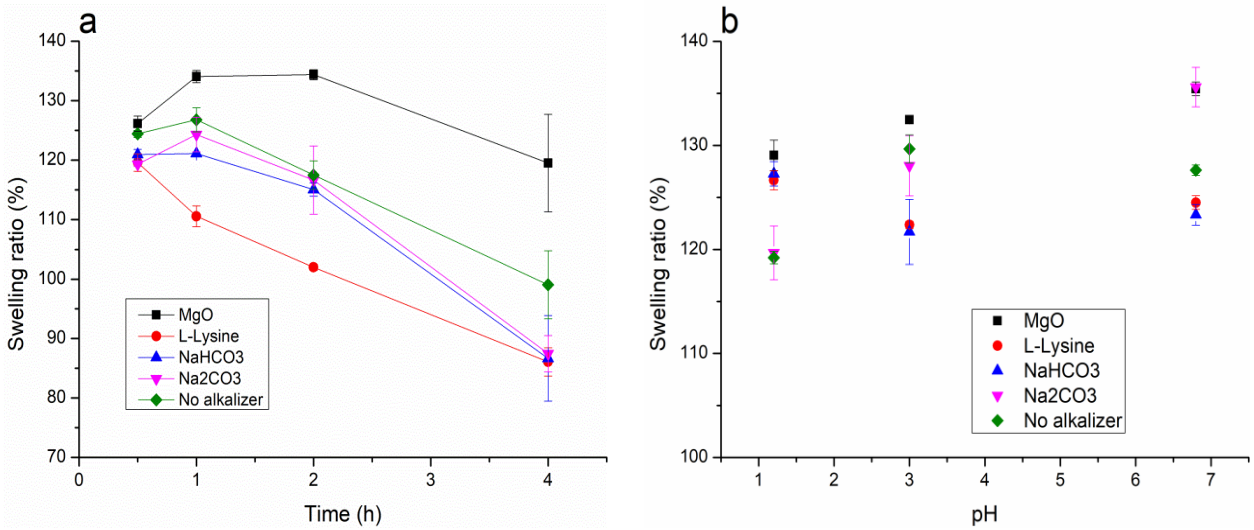


Figure 3.10 Swelling behavior of alkalizer-containing matrix tablets compared to tablets without alkalizer in various media. (a): water, (b) SGFsp (pH 1.2) and PCB (pH 3.0 and pH 6.8) (n = 3, means ± SD).

3.6.3 *In vitro* drug release study

The drug release profiles of matrix tablets containing various alkalizers were determined in PCB (pH 6.8) using the USP paddle apparatus. The percent drug releases at predefined time intervals were calculated from the concentrations determined at each interval and the initial drug concentrations in the tablets. Figure 3.11 shows the drug release profiles of the matrix tablets. Based on the profiles, more than 85 % of the drug was released in 6 h only from formulations containing $NaHCO_3$ and matrixes without alkalizer. Moreover, the drug release profiles between the two formulations were not statistically significant ($p = 0.9534$). However, the drug releases from the two matrix tablets were significantly higher than the other formulations containing MgO, L-Lysine, and Na_2CO_3 ($p < 0.0001$). Among the matrices, MgO and L-Lysine tablets showed the lowest drug release.

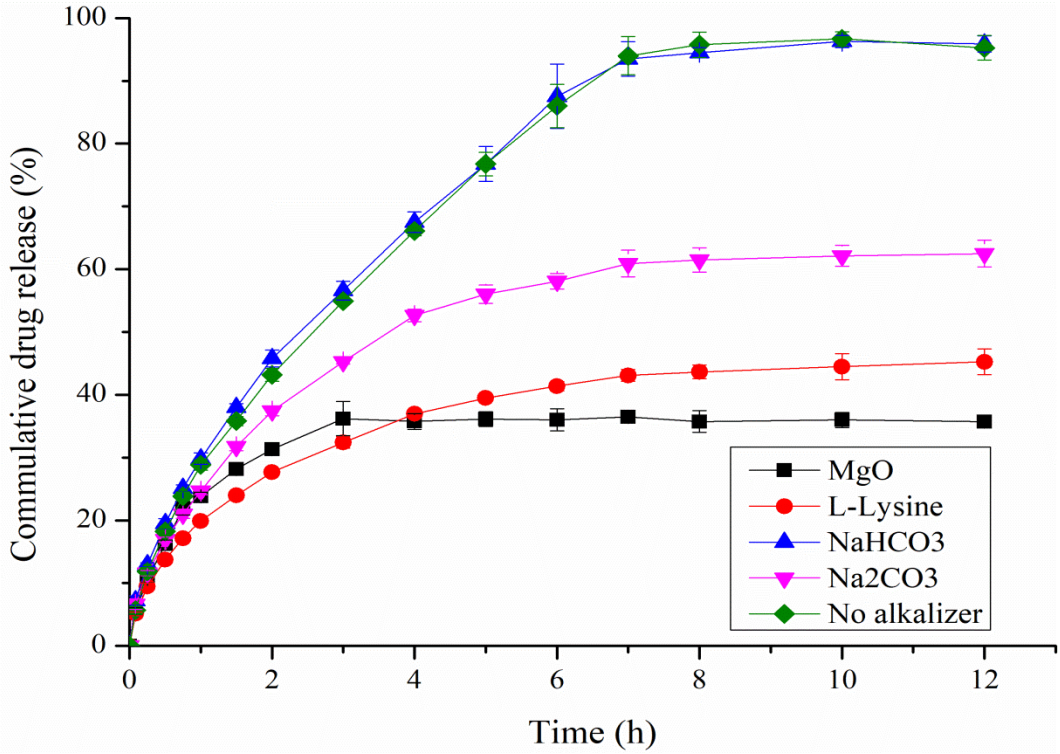


Figure 3.11 Drug release profiles of matrix tablets containing alkalizers compared to one batch without alkalizer in a phosphate-citrate buffer of pH 6.8 (n = 3, means ± SD).

3.6.4 Stability of PGS in various acidic media

The effects of alkalizers on the stability of PGS in the matrix tablets were investigated in acidic media by determining the drug concentrations over time in the dissolution vessels. Moreover, the drug concentration profiles were compared with profiles from blank tablets without alkalizer. The studies were performed in media with a pH range of 1.8–6.8 in the USP II apparatus. PGS was proved to be stable in pH 6.8, and the drug concentration profiles in this pH were considered for comparison purposes. The result demonstrated that more than 500 µg/ml of the drug was found in pH 6.8 in 8 h from tablets containing $NaHCO_3$ and blank tablets (Figure 3.12d). Such high concentration profiles indicated that the drug was released entirely in both formulations without degradation. However, the drug concentrations decreased for all formulation when the pH of the media lowered to pH 4.5 (Figure 3.12c). At this pH, $NaHCO_3$ maintained a statistically higher

drug concentration compared with the remaining alkalizers ($p < 0.0002$). In low acidic pH media (i.e. pH 1.8 and pH 3.0), matrix tablets containing $NaHCO_3$ and Na_2CO_3 exhibited higher drug concentrations than the other formulations (Figure 3.12a and b). In pH 1.8, statistically higher drug concentrations were presented from $NaHCO_3$ and Na_2CO_3 matrices after 2 h than the blank tablets ($p < 0.0001$). Furthermore, $NaHCO_3$ formulations showed a significantly higher drug concentration in pH 3 over the first 6 h than the other formulations ($p < 0.0001$).

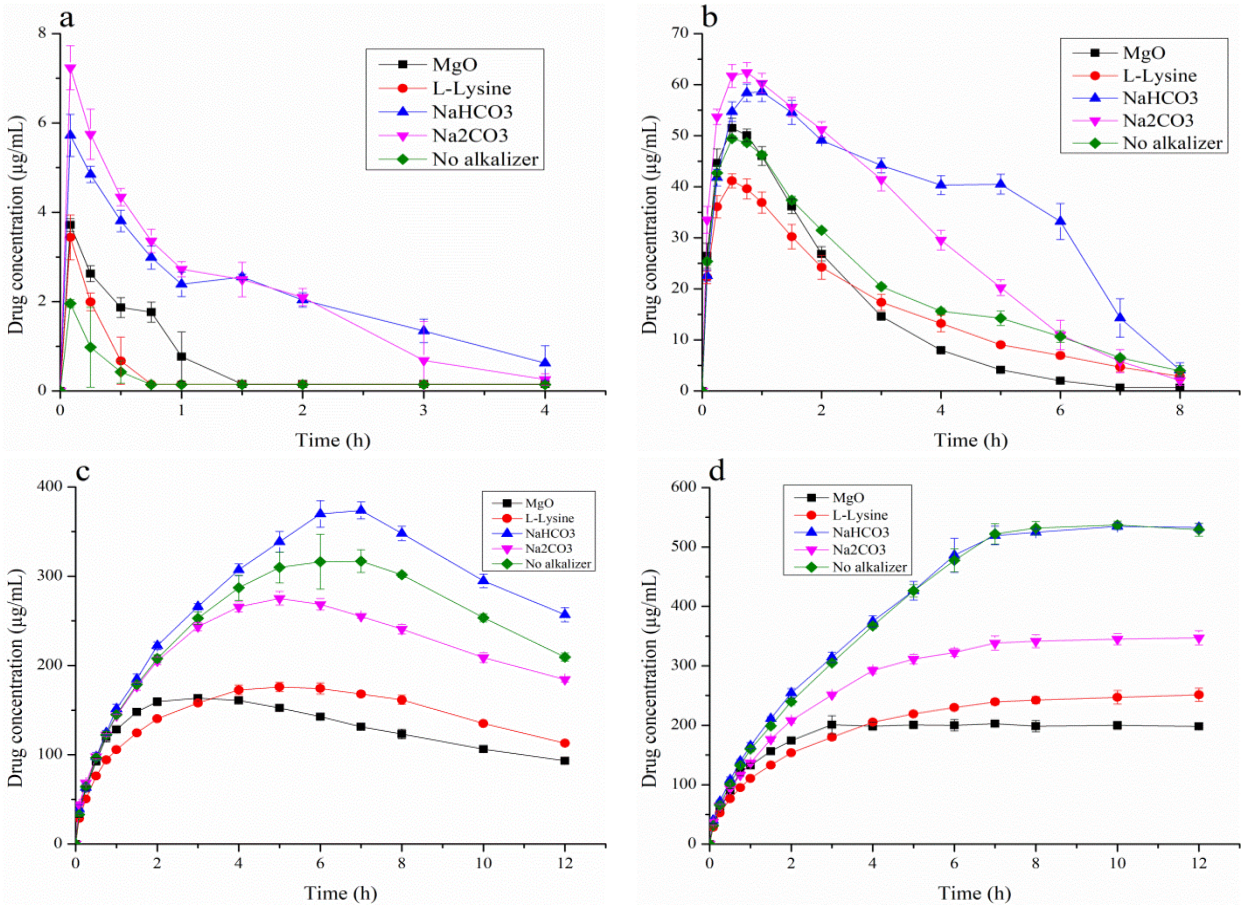


Figure 3.12 Drug concentration profiles of matrix tablets containing alkalizers compared to one batch without alkalizer at various pH. (a) SGFsp pH 1.8, (b) pH 3.0 PCB, (c) pH 4.5 PCB and (d) pH 6.8 PCB (n = 3, means ± SD).

3.6.5 Drug content inside the hydrated matrix tablets

The effects of alkalizers on the stability of the drug inside hydrated tablets were assessed by determining the drug contents after exposing the matrices to test media for 2 h. The studies were

conducted using USP II in media (pH 1.2, 3.0, and 6.8) under similar conditions as utilized during the *in vitro* drug release studies. The percentage of PGS left undegraded inside the hydrated tablets is shown in Figure 3.13. Accordingly, the maximum amount of PGS was found in tablets containing $NaHCO_3$, followed by tablets without alkalizer and Na_2CO_3 in all pH ranges. However, MgO and L-Lysine were accounted for the least stabilization effect, as demonstrated by the lowest amount of drug in the tablets. Moreover, slight differences in the stabilization effect of individual alkalizers were observed within various pH media.

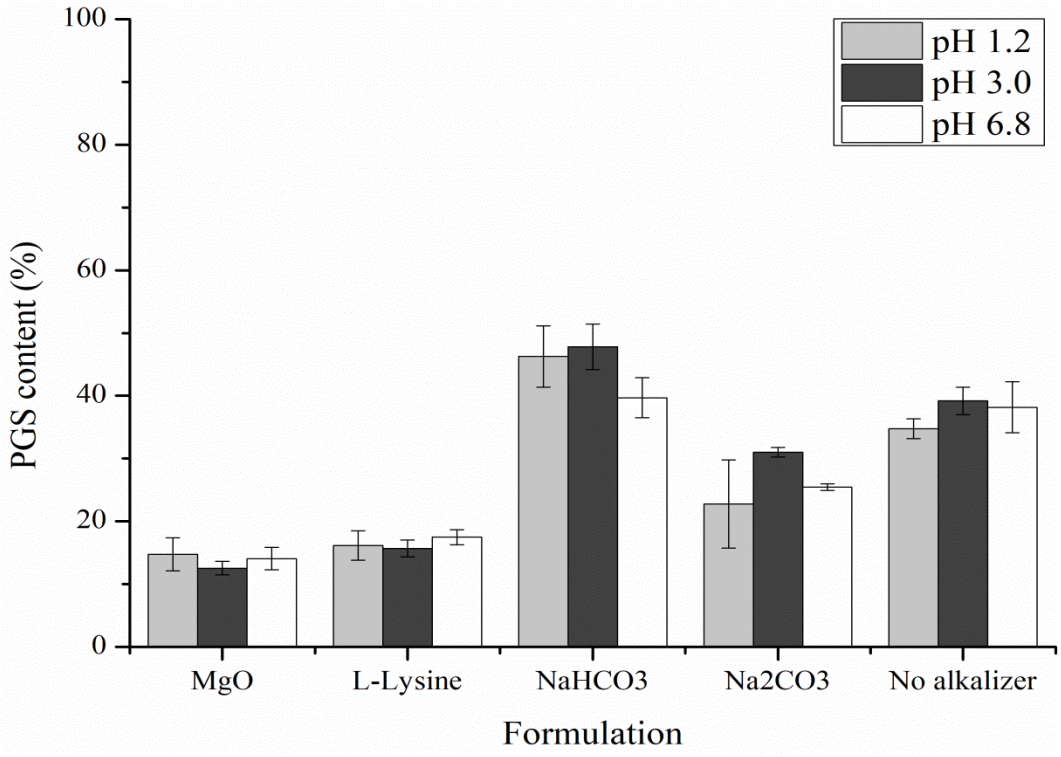


Figure 3.13 Percentage of PGS inside hydrated matrix tablets containing alkalizers compared to one batch without alkalizer after exposure to media of different pH for 2 h at 37 °C (n = 3, means ± SD).

3.6.6 Macroenvironmental pH measurement

The effects of alkalizers on macroenvironmental pH changes in various dissolution media were investigated and given in Table 3.10. Based on the result, the pH values of the dissolution media were only marginally influenced.

Table 3.10 Solution pH change after drug release studies of matrix tablets containing alkalizers compared to one batch without alkalizer in media of various pH (n = 3, means ± SD).

Formulation	Solution pH			
	pH 1.8	pH 3.0	pH 4.5	pH 6.8
I MgO	1.92 ± 0.05	3.37 ± 0.01	4.69 ± 0.00	7.04 ± 0.01
II L-Lysine	1.84 ± 0.00	3.13 ± 0.00	4.53 ± 0.00	6.86 ± 0.00
III $NaHCO_3$	1.86 ± 0.00	3.20 ± 0.00	4.61 ± 0.00	6.93 ± 0.00
IV Na_2CO_3	1.89 ± 0.01	3.26 ± 0.01	4.67 ± 0.01	6.97 ± 0.01
V No alkalizer	1.81 ± 0.00	3.06 ± 0.00	4.51 ± 0.00	6.83 ± 0.00

3.6.7 Microenvironmental pH (pH_M) measurement

The effect of alkalizers on the surface pH_M was investigated in pH 1.2 and 3.0 dissolution media. In addition to alkalizer-containing matrices, further pH_M tests were performed on blank tablets without alkalizers. The pH_M measurements were conducted after exposing the matrix tablets for 30 min to the acid media in the USP paddle apparatus. Figure 3.14 depicts the pH_M of the matrix tablets in various acidic media. The pH_M of alkalizer-containing tablets, except L-Lysine, were significantly higher than tablets containing no alkalizer in both dissolution media ($p < 0.0001$). In pH 1.2 media, Na_2CO_3 demonstrated the highest pH_M and the values were arranged as Na_2CO_3 > MgO > $NaHCO_3$ > L-Lysine > no alkalizer. In pH 3.0, MgO followed by Na_2CO_3 showed the maximum pH_M modulation effect while the effects in the remaining formulations were in a similar order as in pH 1.2.

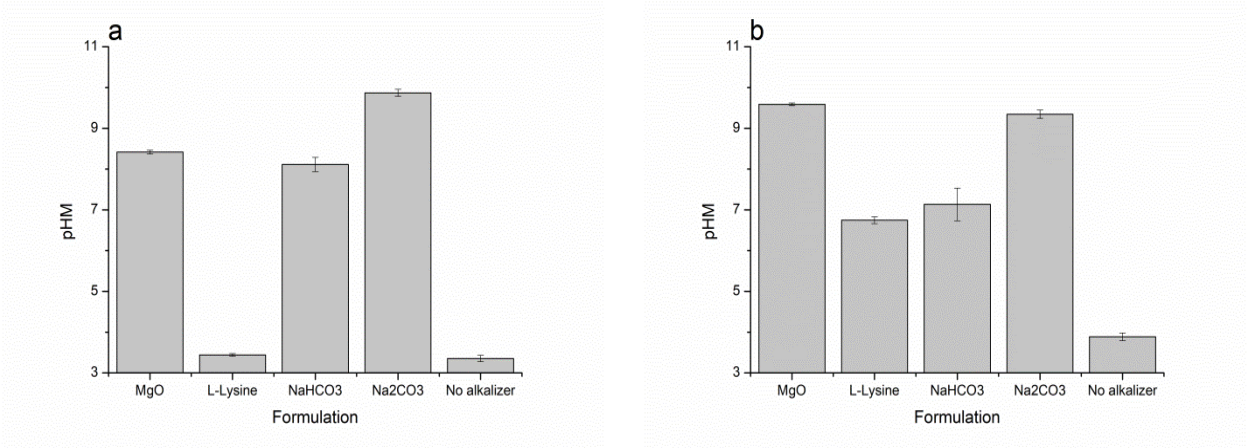


Figure 3.14 Surface pH_M of PGS matrix tablets containing alkalizers compared to one batch without alkalizer after exposure for 30 min to acidic media at 37 °C. (a) SGFsp pH 1.2 and (b) PCB pH 3.0 (n = 3 means ± SD).

3.7 *In vitro* evaluation of the formulations in various models

3.7.1 *In vitro* drug release in a DSTD

In this study, the drug concentration profiles of $NaHCO_3$-containing matrix tablets were determined in PCB (pH 4.5) under various pressure programs in a dissolution stress test device (DSTD). Under a low-stress program (P1), the drug concentration was lower, and about 280 µg/mL of the drug was released in 7 h which was lower compared to 374 µg/mL in the compendial testing (Figure 3.15). Observational investigation of the tablet structure showed that the swollen tablets were intact for at least 4 h. Immediately after applying the maximum pressure event (pressure event during gastric emptying), the drug release in the stress test was stepped up. However, similar drug concentration profiles were exhibited at the end of the experiments both in DSTD and compendial testing.

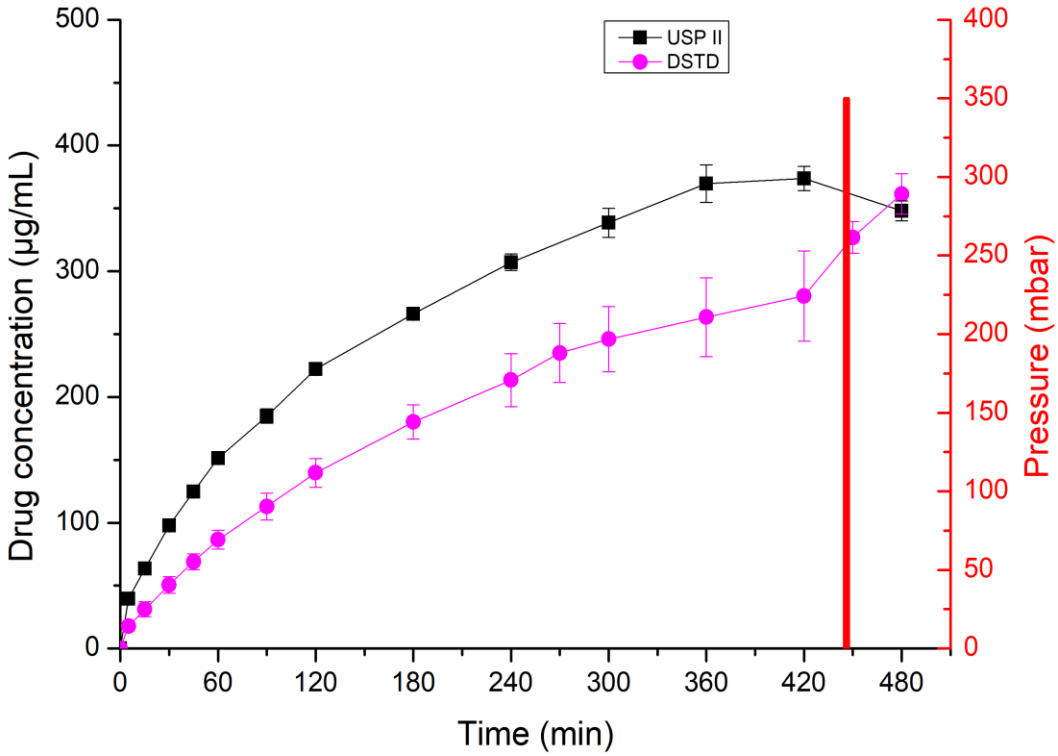


Figure 3.15 PGS concentration released from matrix tablets under a low-stress program in DSTD compared to USP II in a phosphate-citrate buffer of pH 4.5 (n = 3, means ± SD).

Under the intermediate pressure program (P2), the initial drug release in the DSTD was slower compared to the compendial test (Figure 3.16). After applying the first pressure event at 85 min, the drug release was increased but still lower compared to the compendial test. Moreover, significant portions of the tablets were destroyed as a result of the first pressure event. Five min after applying the second pressure event, the concentration of the drug was slightly higher than the compendial test. Afterward, the drug release was continued to increase until the maximum pressure was applied, and then similar drug concentrations exhibited from both test devices.

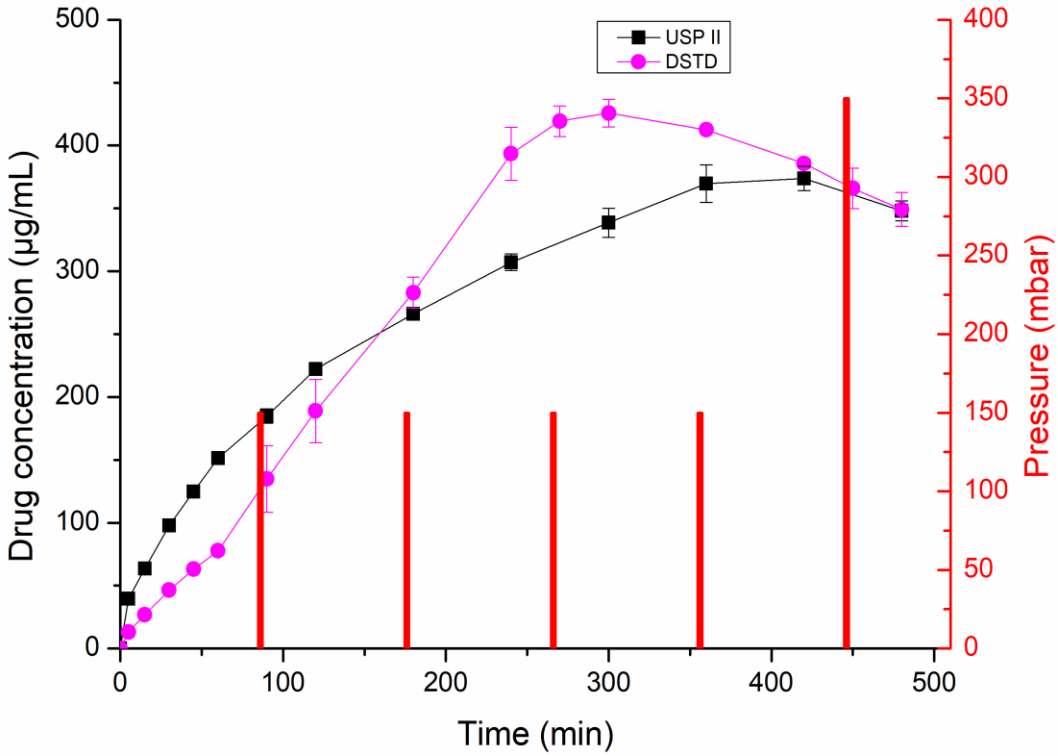


Figure 3.16 PGS concentration released from matrix tablets under an intermediate-stress program in DSTD compared to USP II in a phosphate-citrate buffer of pH 4.5 (n = 3, means ± SD).

Drug release under a high-stress program (P3) showed an overall rapid and maximal drug release compared to the compendial testing (Figure 3.17). Immediately after applying the first pressure event at 25 min, the drug release was started to increase and continued with subsequent pressure events. The maximum drug concentration, which was comparable with program 2, was exhibited 5 h after the beginning of the tests. Similarly, the tablets were destroyed by the first pressure event at 25 min.

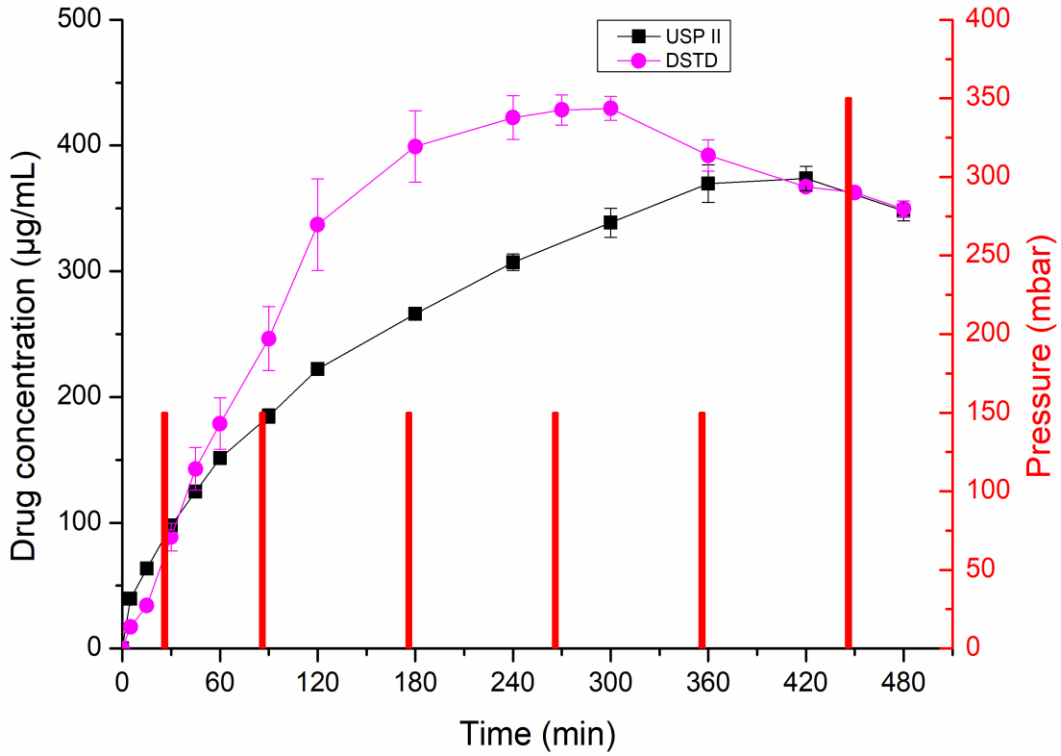


Figure 3.17 PGS concentration released from matrix tablets under a high-stress program in DSTD compared to USP II in a phosphate-citrate buffer of pH 4.5 (n = 3, means ± SD).

3.7.2 Testing of formulations in a flow-through model (FTM)

The drug concentrations left inside the dilution vessels were determined from effervescent granules and matrix tablets over 6 h in PCB (pH 4.5). The tests were performed at a variable in- and outflow rates in the FTM. In the case of effervescent granules, there was faster initial drug release in FTM compared to compendial testing (Figure 3.18). Afterward, the drug concentration was decreased proportionally over time, with nearly 20 µg/mL of drug left inside the dilution vessel at the end of the test.

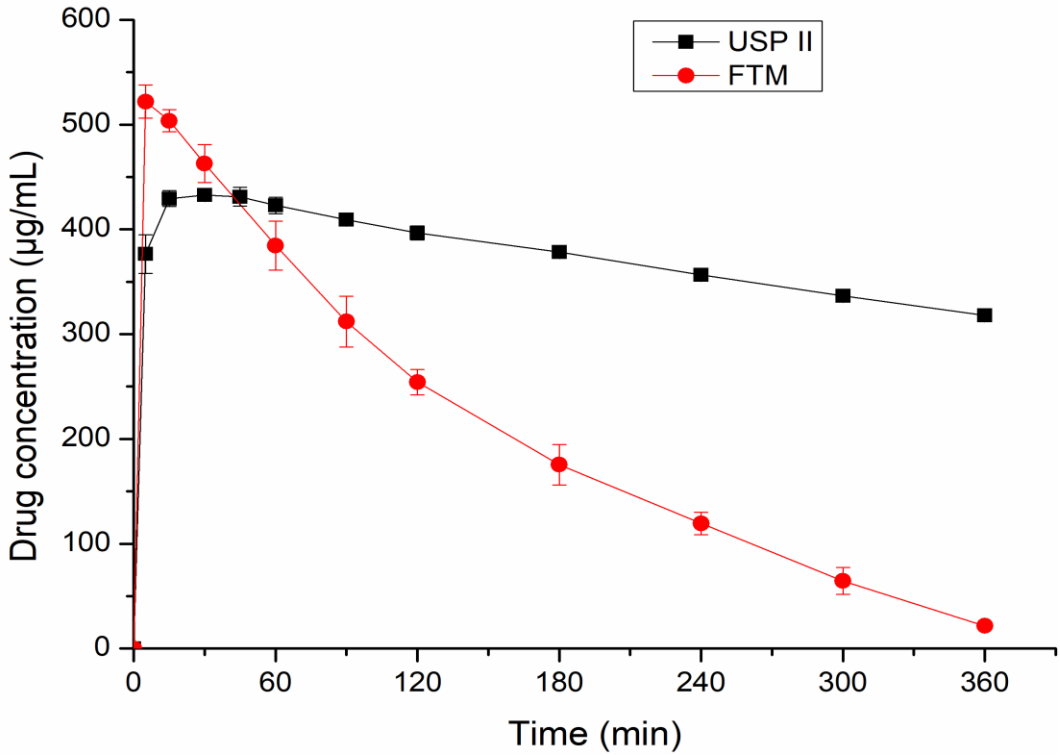


Figure 3.18 PGS concentration in a dissolution vessel from effervescent granules over time in PCB of pH 4.5 in the FTM at a variable in- and outflow rates compared to compendial testing (n = 3, means ± SD).

In the case of matrix tablets, the drug concentrations left inside the dilution vessels over time profiles were different from effervescent granules (Figure 3.19). Despite the fluid outflow and subsequent dilution, the drug concentrations in the dilution vessels were similar to compendial testing for the first 4 h. Afterward, a higher amount of drug was released compared with compendial testing, where the maximum concentration was noted at the end of the test.

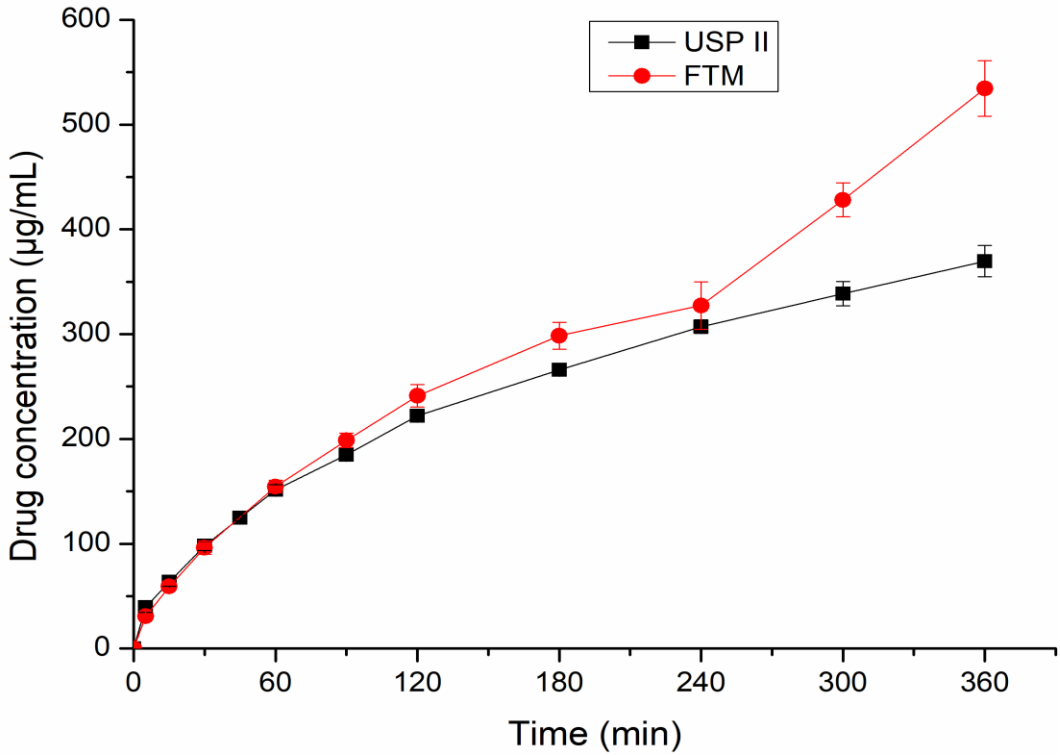


Figure 3.19 PGS concentration in a dissolution vessel from matrix tablets over time in PCB of pH 4.5 in the FTM at a variable in- and outflow rates compared to compendial testing (n = 3, means ± SD).

3.8 *In vivo* evaluation of the formulation concepts

3.8.1 Salivary caffeine concentrations

Figure 3.20 shows the mean salivary caffeine concentrations versus time profile from granules and matrix tablet formulations administered to 11 subjects under fed and fasted states. The study was conducted on 12 volunteers randomized into four study arms (3 fed and 1 fasted state arms). Moreover, the results from subject 1 in the fasted state arm and subject 9 from all fed state arms were excluded due to initial caffeine contamination of the mouth during swallowing.

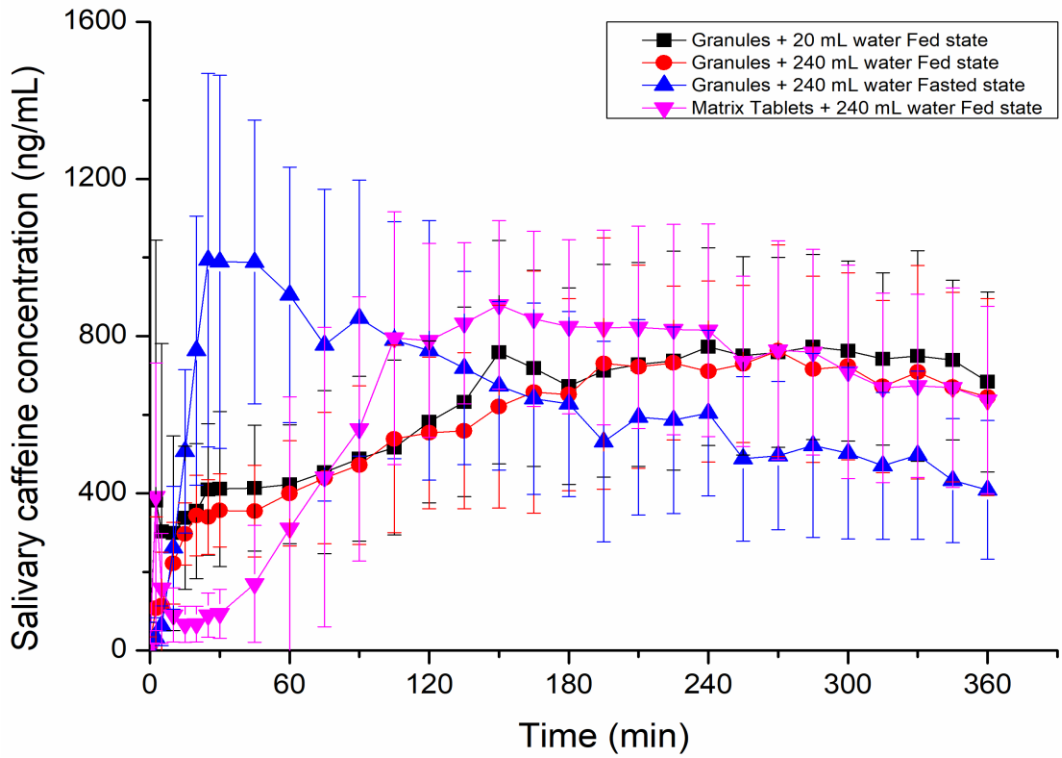
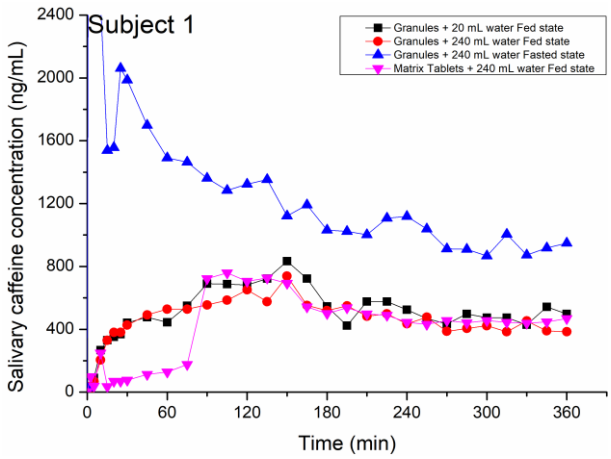
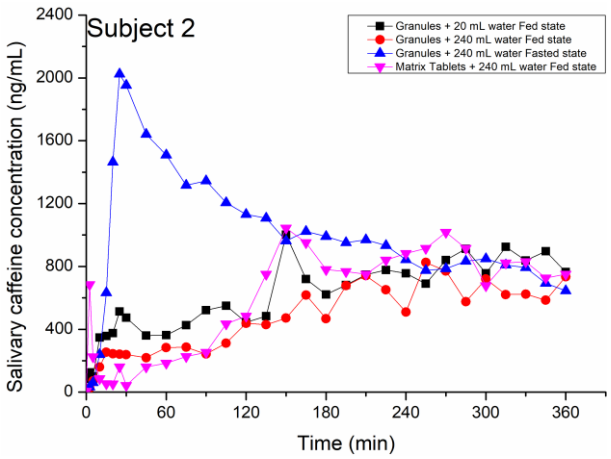
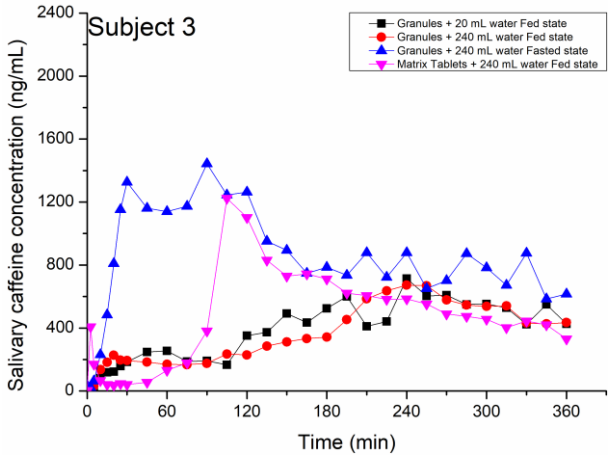


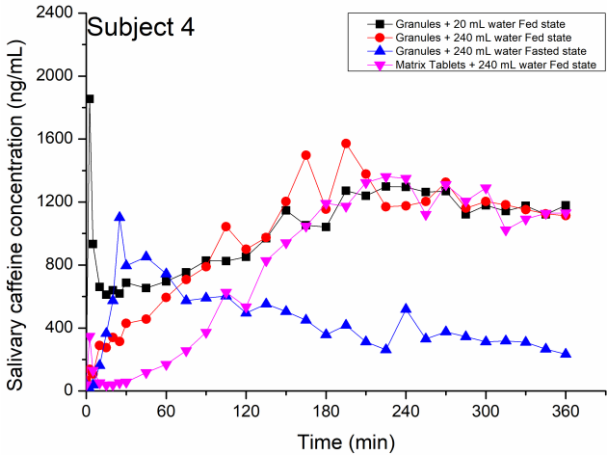
Figure 3.20 The mean salivary caffeine concentrations versus time profile of 11 subjects from granules and matrix tablets, each containing 100 mg of caffeine administered either 30 min after FDA meal or in a fasted state with tap water over 6 h (n = 11, means ± SD).

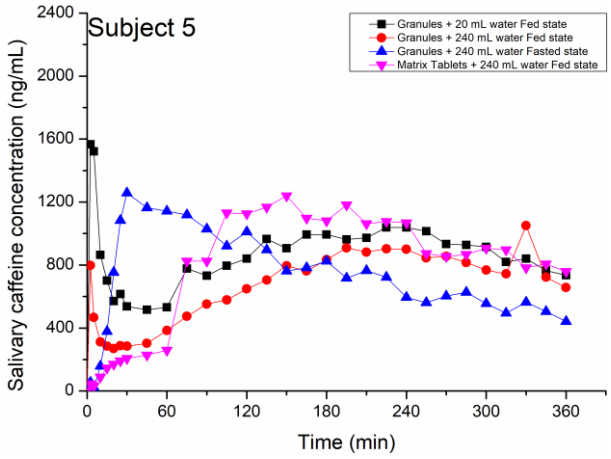
In fed state study arms, there was an initial but significant caffeine concentration after 2.5 min of sampling. This effect was pronounced after taking granules and matrix tablets with 20 and 240 mL of tap water, respectively. Such initial concentrations were due to contamination of the mouth during dosage administration, which was confirmed by lower salivary caffeine concentrations in the subsequent sampling intervals. In comparison, granules and matrix tablets which were taken with 240 mL of tap water presented the lowest and the highest salivary caffeine concentrations, respectively. In a fasted state, mean salivary caffeine concentrations higher than in all the fed state arms were exhibited. In order to understand the individual variations, the salivary caffeine concentration-time profiles of subjects in both fed and fasted states are presented in Figure 3.21.

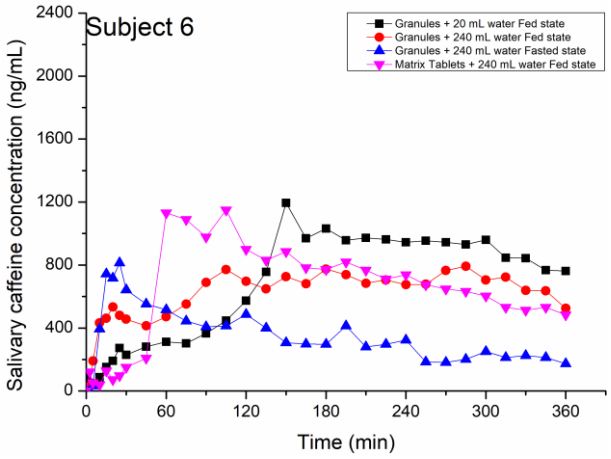

Subject 1
Granules + 20 mL water Fed state
Granules + 240 mL water Fed state
Granules + 240 mL water Fasted state
Matrix Tablets + 240 mL water Fed state
Salivary caffeine concentration (ng/mL)
Time (min)


Subject 2
Granules + 20 mL water Fed state
Granules + 240 mL water Fed state
Granules + 240 mL water Fasted state
Matrix Tablets + 240 mL water Fed state
Salivary caffeine concentration (ng/mL)
Time (min)


Subject 3
Granules + 20 mL water Fed state
Granules + 240 mL water Fed state
Granules + 240 mL water Fasted state
Matrix Tablets + 240 mL water Fed state
Salivary caffeine concentration (ng/mL)
Time (min)


Subject 4
Granules + 20 mL water Fed state
Granules + 240 mL water Fed state
Granules + 240 mL water Fasted state
Matrix Tablets + 240 mL water Fed state
Salivary caffeine concentration (ng/mL)
Time (min)


Subject 5
Granules + 20 mL water Fed state
Granules + 240 mL water Fed state
Granules + 240 mL water Fasted state
Matrix Tablets + 240 mL water Fed state
Salivary caffeine concentration (ng/mL)
Time (min)


Subject 6
Granules + 20 mL water Fed state
Granules + 240 mL water Fed state
Granules + 240 mL water Fasted state
Matrix Tablets + 240 mL water Fed state
Salivary caffeine concentration (ng/mL)
Time (min)

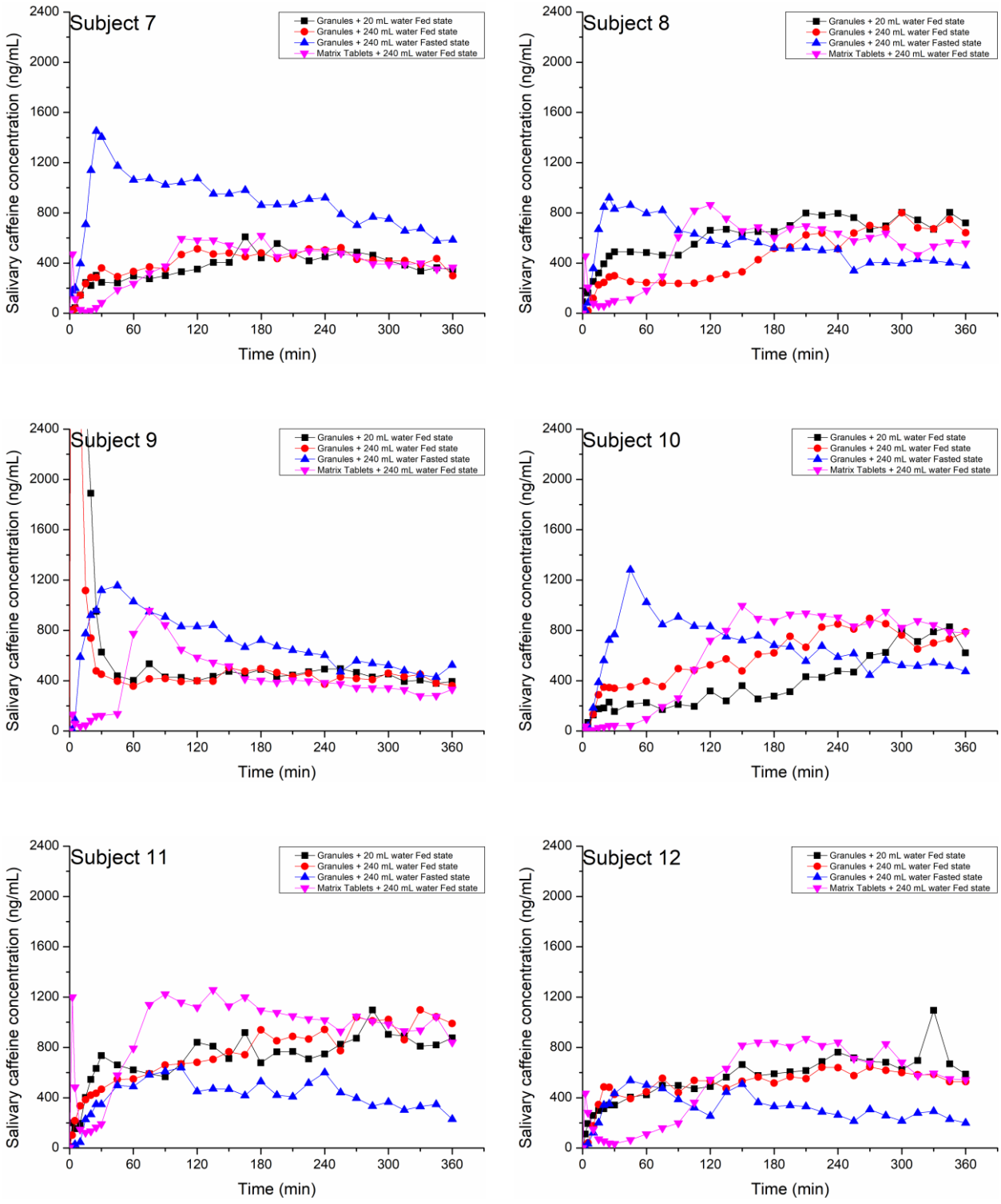


Figure 3.21 Individual mean salivary caffeine concentrations over time profile of subjects from granules and matrix tablets, each containing 100 mg of caffeine administered 30 min after FDA meal or in a fasted state with tap water over 6 h (n = 11, means ± SD).

The C_{max} and T_{max} values were used to understand the salivary caffeine kinetics after the administration of granules and matrix tablets with various volumes of water. The values represent the C_{max} and T_{max} of the mean curve and calculated within the whole study duration (Table 3.11). Accordingly, granules taken with 240 mL of tap water in a fasted state demonstrated the highest C_{max}, which was occurred at 25 min. In a fed state, the matrix tablet showed the highest C_{max} and was exhibited at 2.5 h. The C_{max} values of granules taken with 20 and 240 mL of tap water were differed only by ≈ 10 ng/mL and occurred at 4 and 4.5 h, respectively.

Table 3.11 The C_{max} and T_{max} of the salivary caffeine concentration versus time mean curve after administration of granules and matrix tablets, each containing 100 mg of caffeine to 11 subjects either 30 min after FDA meal or in a fasted state with tap water (n = 11, means ± SD).

Study arm	C_{max} (ng/mL)	T_{max} (min)
Granules + 20 mL water Fed state	773.18 ± 251.36	240
Granules + 240 mL water Fed state	762.82 ± 269.21	270
Granules + 240 mL water Fasted state	993.55 ± 475.45	25
Matrix tablets + 240 water Fed state	879.27 ± 214.42	150

3.8.2 MRI study

In addition to the salivary tracer study, the location and disintegration behavior of the matrix tablets were studied by MRI. The investigations were conducted with all volunteers after 4 h of administration of the matrix tablets with 240 mL of tap water in the fed state. For an effective visualization and localization of the matrix tablets, iron oxide was incorporated into the matrix tablets. Figure 3.22 shows the MRI images taken from two exemplary volunteers. Accordingly, the matrix tablets were intact or mainly intact in some subjects and either broken or both broken and dispersed in the remaining participants. Moreover, the tablets were located either exclusively in the stomach (nearly 60 %) or both in the stomach and intestines.

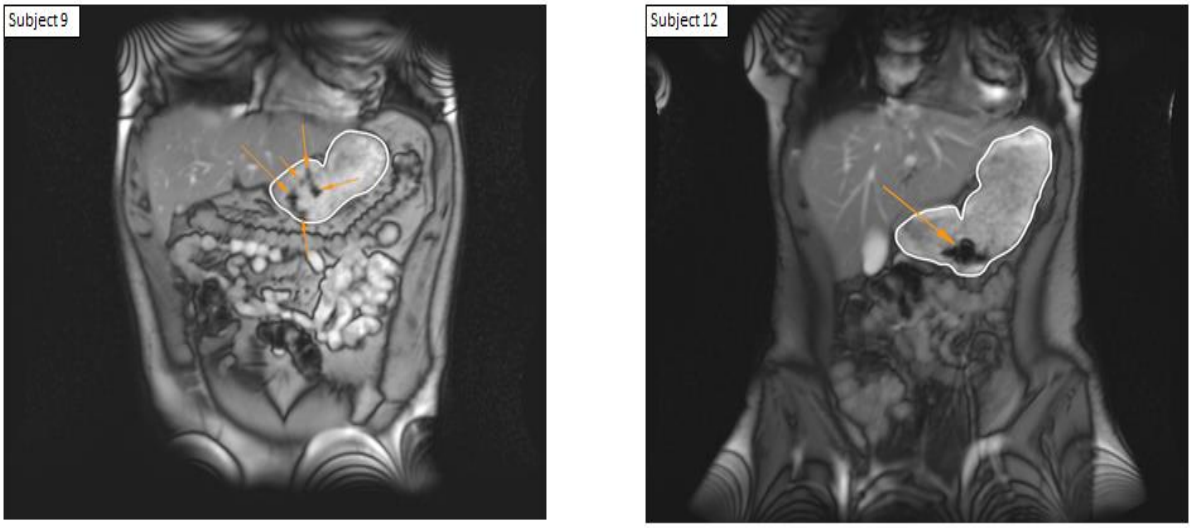


Figure 3.22 Representative MRI pictures showing the location and disintegration behavior of matrix tablets taken 4 h after administration of tablets containing 100 mg of caffeine to subjects 30 min after FDA meal with 240 mL of tap water.

4 Discussion

4.1 Devising a new *Helicobacter pylori* treatment strategy

In the current *H. pylori* treatment regimens, after oral administration, antibiotics are assumed rapidly absorbed in the intestine. Thereafter, they are secreted from the bloodstream to the luminal side of the stomach, where this bacterium is situated.[191] Concomitantly, patients are recommended to use proton pump inhibitors as they raise the intragastric pH and thus, ensure the optimum effects of the antibiotics.[192] However, the effectiveness of PPIs can be affected by metabolism. Lansoprazole, for example, provided an average pH between pH 4.5 and pH 5.5 over 24 h in rapid and slow metabolizers (by CYP2C19), respectively.[193] Therefore, fast metabolizers for PPIs are assumed to have lower *H. pylori* eradication rates.[194]

In our view, the secretion of antibiotics from the systemic circulation into the gastric lumen is less likely. This assumption is valid for common antibiotics in *H. pylori* treatment, including amoxicillin and clarithromycin. Studies in humans showed that 2 h after administration of amoxicillin, no drug could be detected in the stomach.[191] Studies in rats confirmed that PPIs inhibit the gastric secretion of metronidazole and clarithromycin.[195] In this regard, if the secretion of antibiotics into the stomach would occur from the basolateral side, PPIs should instead reduce the eradication rate. From this context, it is likely that eradication of *H. pylori* can be performed in the form of local antibiotic therapy and should not necessarily be based on systemic therapy. This concept is further supported by co-administrating a triple therapy (bismuth + metronidazole + tetracycline) with food. Even though the oral bioavailability of all the three antibiotics is significantly lowered by co-administered food, the eradication rate, however, remained enhanced.[196] Therefore, the aim of the present work was to develop a new and novel strategy for the local eradication of *H. pylori* within the stomach.

A significant challenge in the treatment of *H. pylori* infection is antibiotic resistance. Several studies reported a considerable resistance rate to common antibiotics employed in the *H. pylori* treatment.[9,197] This bacterium has already developed high resistance to many antibiotics, including those used for eradication therapies. Recently, a high resistance rate to metronidazole, clarithromycin, and amoxicillin has been reported in Africa.[198] A similar high resistance to metronidazole, levofloxacin, clarithromycin, amoxicillin, and tetracycline was also reported for China.[199] Therefore, the search for a new antibiotic that is effective against *H. pylori* has become a vital issue.

In this study, various literature data on the *in vitro* susceptibilities of medicines, plant extracts, and peptides to *H. pylori* were explored. Based on the literature data, four antibiotics (amoxicillin, penicillin G, fosfomycin, and rifampicin) have been selected. These antibiotics were further screened based on their current *in vitro* effects against a resistant *H. pylori* strain. The initial *in vitro* experiments revealed that amoxicillin and penicillin G exhibited low MIC against this bacterium. The MIC values were similar to studies conducted on beta-lactam antibiotics, including amoxicillin, ampicillin, and penicillin G.[200,201] Amoxicillin has been the central antibiotic in many of the *H. pylori* treatment regimens and did not account for our objective of identifying a drug which has not been used in any of the treatment regimens. Therefore, penicillin G was selected for further use in the formulation strategy. Apart from its high effectiveness against *H. pylori*, penicillin G has low oral bioavailability. The concomitant administration of different antacids and the intake of food do not affect the bioavailability of penicillin G, which is always in the range of 10–30 %. By this, the risk of systemic side effects caused by antibiotic therapy is expected to be smaller compared to other antibiotics.

The basic requirements for successful local eradication of *H. pylori* in the stomach are (1) to achieve high local concentrations of the antibiotic in the stomach and (2) to maintain the drug concentration above the MIC for a longer time in the stomach. These requirements are ideally achieved by using gastoretentive dosage forms. Unfortunately, none of the formulation concepts for gastric retention have been proven to be successful in humans.[62] A recent *in vivo* study, however, confirmed that formulations such as matrix tablets can be retained in the stomach if they are dosed together with food.[131] Therefore, we decided to develop both effervescent granules and matrix tablets containing 500 mg of PGS, intended for oral intake after a meal. Moreover, the larger volume of gastric contents present after ingestion of food also helps to eradicate *H. pylori* in different parts of the stomach.[65]

In addition to formulation development, the investigation of the effect of gastric physiology on drug release, mixing, and gastric emptying is essential to understand the *in vivo* performance of formulations in the human GI tract. For this purpose, various *in vitro* and *in vivo* tools are available. In this study, a flow-through model (FTM) was developed to predict the effect of GCV, gastric secretion, and gastric emptying rate on the gastric concentration of a drug over time. The performance of this model was evaluated by conducting dilution studies at constant and variable in- and outflow rates using penicillin G sodium powder. In studies performed at constant flow rates, rates of 3, 6, and 9 mL/min, and 500 mL of initial volume in the dilution vessels were applied. In variable flow rate studies, the flow rates were simulated to achieve the actual gastric content volume (GCV) decrease over time profiles in fed conditions. An initial volume of 780 mL was chosen to reflect GCV profile after intake of the high-calorie, high-fat FDA standard meal based on recent *in vivo* studies.[56,65] In both constant and variable flow rate studies, the concentrations over time profiles were comparable to excel generated values. Therefore, the

new *in vitro* model was regarded as an alternative tool to predict the *in vivo* performance of gastric-specific drug delivery systems under realistic physiological conditions.

4.2 Formulation strategies

4.2.1 Effervescent granules

In this study, three batches of effervescent granules were prepared by wet granulation using raw materials containing the least amount of moisture and pure isopropanol as a granulating fluid. After the formulation, some physicochemical characteristics of the granules were investigated. One important parameter was the drug content. Interestingly, only effervescent granules containing a 6:1 molar ratio of sodium bicarbonate and citric acid and non-effervescent granules reached 100 % of the targeted PGS content. The remaining two batches of effervescent granules did not contain the desired amount of drug. Additional investigations confirmed that citric acid was identified as the primary cause of PGS degradation. In the absence of citric acid, neither pure isopropanol nor moisture caused PGS degradation. The duration of drying had also some effect in the presence of citric acid. Therefore, the molar ratios of the components in the effervescent mixtures should be taken into account while formulating acid-labile drugs such as PGS.

The residual moisture content (RMC) of effervescent formulations could lead to premature effervescence during storage and handling. The RMC studies confirmed low moisture content of less than 1 % (w/w), which showed that drying at 50 °C for 2 h was sufficient to avoid high moisture content in the granules. Further investigations showed a longer effervescent time (> 1 min) for granules containing a high molar ratio of sodium bicarbonate (F3). A similar study has demonstrated variations in the effervescent action which was dependent on the molar ratios of components in the effervescent mixture.[202]

Another essential granule parameter was the *in vitro* drug release, which was investigated in PBS (pH 6.8) using the USP paddle apparatus. Similar to drug content, only F3 effervescent and non-effervescent granules released 100 % of the drug within 1 h. Moreover, F3 formulation released 100 % of the drug within 5 min. The remaining two effervescent granules (F1 and F2) demonstrated poor drug release due to degradation.

4.2.2 Matrix tablets

The second formulation approach was based on monolithic hydrogel matrix tablets that, when dosed with food, should release the drug over an extended period in the stomach. For this objective, four batches of matrix tablets containing 10, 15, 20, and 30 % (w/w) HPMC were prepared by wet granulation. HPMC (Methocel K4M) was selected because of its desirable matrix-forming effect. It was defined that a successful formulation should show > 80 % of drug release over 6–8 h.

After the tableting process, the quality of the matrix tablets was assured by measuring some physical characteristics of the matrix tablets, such as weight uniformity, crushing strength, and friability. If the weight of a solid dosage form varies in a batch, there will be variations in the API content, disintegration, and dissolution. The weight variation tests confirmed that all tablets met the compendial requirements. Besides, the tablets had sufficient mechanical strength, as demonstrated by adequate crushing strength and low friability. Further drug content determination tests confirmed the presence of 100 % of the claimed drug content in the tablets.

For hydrogel matrix tablets, the disintegration and drug release from matrix tablets are affected by their swelling properties. It was evident from the water uptake studies that swelling was directly proportional to the HPMC concentration. As a result, matrix tablets containing 10 % and 30 % of HPMC demonstrated the lowest and the highest swelling, respectively.

The *in vitro* drug release profiles of the matrix tablets were determined in a phosphate-citrate buffer of pH 6.8. As expected, with the increasing percentage of HPMC in the matrix tablets, the drug release decreased to time spans of more than 12 h. In the first 6 h, nearly 95 % of the drug was released from a matrix containing 10 % HPMC compared to 83.94 %, 71.21 %, and 66.43 % of the drug for 15, 20, and 30 % HPMC, respectively. It was also evident from the water uptake studies that swelling was proportional to the HPMC content and had an inverse relationship with the drug release. In addition to the drug release studies, the concentrations of the drug over time were studied in a phosphate-citrate buffer of acidic pH (pH 3.0 and pH 4.5). In both media, lower drug concentrations over time were exhibited compared to the profile in pH 6.8 which is mostly due to degradation. The drug concentration profiles were in the order of F1 > F2 > F3 > F4, with the least drug concentration profiles exhibited in pH 3.0.

The kinetics and the mechanism of drug release from the matrix tablets were predicted after fitting the drug release profiles in pH 6.8 to various kinetic models. Based on the result, the drug release profiles were best fitted to the Korsmeyer-Peppas model, as confirmed by the high r^2 values, which were ≈ 1 for all matrices. Further comparison of the n values against the standard confirmed that the drug release mechanism was found to be anomalous (non–Fickian) diffusion.

4.2.3 Enhancing the stability of PGS in matrix tablets

The major challenge of formulating PGS into a matrix tablet was its instability at acidic conditions. Due to a well–established report of significant degradation in acidic media, prior investigations on the stability of PGS were conducted at various pH values. According to the results, PGS was highly unstable at lower pH ranges. It was degraded completely within 30 min at pH 1.2 but showed sufficient stability at pH values above pH 4.5. The stability data are in line with the literature findings, where PGS is unstable at pH values below pH 4. A further study at

pH 6.8 confirmed maximum PGS stability. It should be, however, noted that the degradation behavior of PGS was not studied at pH values above pH 6.8, as such values are not likely to occur in the human stomach. Nonetheless, there are reports that PGS also degrades at pH values above pH 8.[157,158] Therefore, formulation strategies to improve the stability of PGS in the lower pH of the gastric fluid were noteworthy.

Recent SmartPill® data revealed that pH values of pH 1–5 could be expected in the fed stomach. Due to poor mixing and strong secretion of highly acidic fluids, even directly after food intake, pH values of 1–2 can occur during digestion.[69] Such acidic conditions within the stomach probably occur in areas close to the stomach walls and can be prevented by the administration of acid-reducing agents (ARAs) such as PPIs or H2 blockers. In addition to the use of ARAs, another safety line to improve the stability of PGS within the formulation was regarded as advantageous. One such formulation approach can be the modulation of the microenvironmental pH (pH_M) in the matrix system. The microenvironmental pH (pH_M), which is the pH of the saturated solution in the immediate vicinity surrounding the drug particle, can positively affect drug degradation in the formulation.[203,204] In recent years, pH_M modulation has got great attention as a formulation strategy to improve drug stability and dissolution.[186,203,205]

Microenvironmental pH modulation involves the use of either acidic or basic pH modifiers as part of the formulation. For acid-labile drugs such as PGS, the incorporation of basic pH modifiers has been shown to increase the microenvironmental pH inside or surrounding a dissolved or wetted solid matrix. As a result, it creates a suitable pH environment to enhance the stability of the drug in acidic media. In various studies, basic modifiers such as $NaHCO_3$, Na_2CO_3, $CaCO_3$, $Ca(OH)_3$, and MgO have been reported to serve as potent alkalizers for oral dosage forms.[188,206] In our titration studies, the effect of different alkalizers on pH when added to

various acidic media (pH 1.2 and 3.0), suggested that MgO, Na_2CO_3, and $NaHCO_3$ were the most potent alkalizers. However, amino acids, namely meglumine, L-Lysine, and L-Arginine, were not able to change the pH of the media to the desired level.

In this study, four batches of monolithic matrix tablets containing 15 % (w/w) HPMC and 18.5 % (w/w) of alkalizers (MgO, L-Lysine, $NaHCO_3$, and Na_2CO_3) were prepared by wet granulation. The granulation and tableting processes were similar to the preparation processes of matrix tablets without alkalizers. For comparison purposes, one batch of matrix tablets containing 15 % (w/w) of HPMC but without alkalizer was taken from the previous matrix formulations. The tablets were tested for specific quality requirements, and all the tablets demonstrated acceptable properties in terms of weight uniformity and mechanical strength. Another important parameter was drug content, which was determined at pH 6.8. Among alkalizers, only $NaHCO_3$ and Na_2CO_3 containing matrices accounted for nearly 100 % of the targeted drug content. MgO containing tablets accounted for almost 98 % of the drug content. However, L-Lysine matrix tablets contained only around 80 % of the drug. Additional chromatographic peaks confirmed drug degradation during the granulation and drying processes in the presence of L-Lysine.

The investigation of the swelling behavior of the matrix tablets in water and various media (pH 1.2, 3.0, and 6.8) showed that most of the matrices accompanied a large amount of fluid. Moreover, the swelling patterns were different depending on the alkalizer type in the tablets. In all tablet batches, the percent swelling in various media decreased gradually over time. It is evident that erosion or diffusion of the drug and excipients proceed as a function of time, and result in a decrease in the swelling ratio of matrix tablets, and contribute to differences in drug release.[207,208] In water, the swelling ratios decreased in the following order: MgO > Na_2CO_3 > $NaHCO_3$ > L-Lysine. However, the swelling patterns of the matrix tablets in buffers were

variable depending on the pH of the test media. In pH 3.0 and pH 6.8 buffers, MgO and Na_2CO_3 matrix tablets demonstrated the maximum swelling while L-Lysine and $NaHCO_3$ matrix tablets were with the poorest swelling. In pH 1.2 media, Na_2CO_3 and MgO had the lowest and highest swelling, respectively.

The drug release is a critical parameter in predicting the performance of alkalizer containing matrix tablets. Among the alkalizers tested, only formulations containing $NaHCO_3$ showed more than 95 % drug release over 12 h in pH 6.8. A comparable drug release was obtained from blank tablets containing no alkalizer. Further investigations to understand the effect of alkalizers on the stability of PGS in different acidic conditions were performed by determining the concentrations of PGS left undegraded in dissolution media. The tests were performed in various media within a pH range of pH 1.8–6.8 over the study period of 4–12 h. According to the study results, only matrices containing $NaHCO_3$ demonstrated a consistent and proportional increase in drug concentration in various acidic media compared to other alkalizers and without alkalizer. The stabilization effect was similar to studies conducted on clarithromycin formulations containing alkalizers, where $NaHCO_3$, followed by Na_2CO_3, enhanced acidic stability and dissolution of clarithromycin.[206] However, MgO demonstrated a weak stabilization effect despite its high alkalizing potential shown by the titration studies. One reason could be its poor solubility in water.[209]

Whether alkalizers affect the pH of the dissolution media with a subsequent effect on drug stability, was investigated by measuring the macroenvironmental pH change in various dissolution media. The result revealed that the pH of the dissolution media was only marginally influenced by the presence of alkalizers in the matrix. Although MgO and Na_2CO_3 resulted in a

slightly higher media pH as compared to $NaHCO_3$, they were not able to enhance the stability of the drug.

Further literature data also suggested that alkalizers incorporated into matrices increase the pH_M surrounding drug particles, which results in enhanced stability of acid-labile drugs as long as the pH modifiers are maintained inside the formulation.[210] Moreover, the surface pH_M of a solid dosage form is more critical owing to continuous exposure of the tablet surface to the dissolution media, which can affect the stability of the drug inside the formulation.[186,211] In this study, the effect of alkalizers on stability and drug release was studied by assessing the pH_M of the matrix tablets in acidic media (pH 1.2 and pH 3.0). For all formulations containing alkalizers, except for L-Lysine, the pH_M in both media was increased compared to a formulation without alkalizer and clearly explains the effect of alkalizers on pH_M modulation. A similar pH_M modulation effect has been reported for solid dispersions containing various alkalizers.[186] Among the alkalizers, the highest pH_M modulations were exhibited by MgO and Na_2CO_3. However, the pH_M values were within a pH range where PGS is expected to be unstable, which partially explains why they failed to improve the stability of the drug in acidic media despite their high alkalization effect during titration studies. In contrast, $NaHCO_3$ demonstrated an optimum pH_M, which was in a pH range of PGS stability (pH 5–8), which explains the role of $NaHCO_3$ in enhancing drug stability and release in acidic media. The result supports the assumption that if pH_M within the matrix tablet maintained near-neutral pH values, the degradation of PGS within the tablet could be stopped or, at least, slowed down. In addition to the parallel administration of ARAs, pH_M modulation approach would be another safety line for a successful *H. pylori* eradication therapy with PGS locally in the stomach.

4.3 *In vitro* evaluation of the formulations in bio-relevant models

Various physiological factors affect the intragastric performance of a formulation. These include intragastric fluid content (volume, pH, viscosity), GI motility, gastric emptying, and intragastric pressure.[124] Therefore, *in vitro* investigations of such factors could help to predict the *in vivo* performance of the formulation concepts.

Recent studies revealed that intragastric pressure could affect the drug release rates from various dosage forms.[212] In this study, the drug release profile of PGS matrix tablets containing sodium bicarbonate was investigated after exposing to various pressure events. The drug release studies were performed using the dissolution stress test device (DSTD), a specialized test device designed to expose dosage forms to different stress conditions such as pressure and motility.[125] For this purpose, three test programs representing no pressure event (P1), pressure events after maximal tablet swelling (P2), and pressure events before and after the maximal tablet swelling (P3) were employed over 8 h. Besides, a maximum pressure event that represents gastric emptying was applied 35 min before the end of each test program. In all studies, the release profiles from DSTD were determined in a phosphate-citrate buffer of pH 4.5 and compared with compendial testing. Our results demonstrated that the lowest drug release, even lower compared to compendial testing, was observed in P1. In P2, drug release was still low for the first 2 h compared to compendial testing. However, immediately after the first pressure event at 85 min, the tablet was destroyed, and rapid drug release was noted for the next 5 h. In P3, faster drug release was observed after the first pressure event at 25 min with the maximum concentration reached within 5 h. A similar pressure effect has been reported in a recent study where two marketed gastroretentive systems containing metformin (tablets and capsules) were investigated using various stress programs in the same device.[126]

In the case of gastroretentive formulations, their effectiveness depends on their ability to generate sufficient drug concentration over an extended time within the stomach. However, various physiological conditions in the stomach can affect drug concentration profiles. Drug release studies using compendial methods, which are performed in a static environment, do not reflect the physiological conditions. In this study, the hydrodynamic nature of the gastric environment was simulated by testing the formulation concepts under a bio-relevant testing model called a flow-through model (FTM). The model was designed to mimic some physiological factors such as GCV, gastric secretion, and gastric emptying rate. Various inflow (gastric secretion) and outflow (gastric emptying) rates were simulated based on a previous *in vivo* study.[65] The tests help to quantify the drug concentration left inside the dilution vessel under simulated conditions in FTM, which are intended to allow a prediction of the *in vivo* drug concentration profile in the stomach. For further understanding, the drug concentration profiles compared to the compendial testing results were tested in a phosphate-citrate buffer (pH 4.5). The tests were conducted for PGS effervescent granules (F3) and matrix tablets containing sodium bicarbonate. For effervescent granules, the drug concentration was the highest in the first sampling point at 5 min, followed by a proportional decrease over 6 h, with concentrations as low as 20 µg/mL at the end of the experiment (Figure 4.1). The initial high concentration could be the combined effect of CO_2 generation and the multiparticulate nature of the granules, while subsequent dilution and drug removal with outflowing fluid caused the decrease of the PGS concentration over time. Such a concentration decrease over time pattern was similar to the dilution study performed during the evaluation of the model using PGS powder.

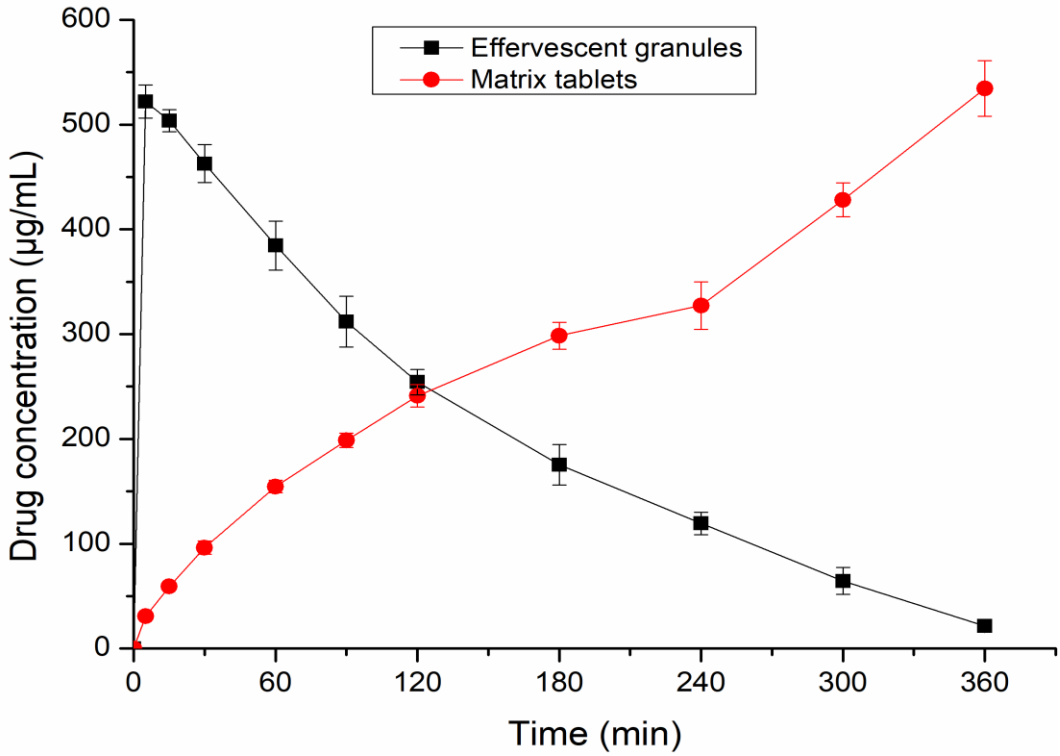


Figure 4.1 Comparison of PGS concentrations in a dissolution vessel from granules and matrix tablets in a phosphate-citrate buffer of pH 4.5 at VFR in FTM over 6 h (n = 3, means ± SD).

In contrast, the concentration profile from matrix tablets was low in the first 4 h. The higher fluid outflow rate in the first 30 min, which decreased the media volume in the dilution vessel, can partially explain this disparity. During this period, no significant amount of drug was released into the media but accumulated in the intact tablets, which caused high drug concentrations in the later sampling intervals. Another reason could be the time-dependent swelling and disintegration behavior of the matrix tablets. The tablets were intact and enlarged in size for the first 4 h. Afterward, the tablets were disintegrated faster, which resulted in higher drug concentrations in the subsequent sampling intervals than the compendial test. It should be, however, noted that testing of matrix tablets in FTM was conducted without pressure events. In dissolution stress tests, it was already confirmed that the disintegration and drug release from matrix tablets were significantly affected by applied pressure events. Therefore, testing of matrix tablets in FTM could be misleading as it does not reflect the realistic pressure conditions *in vivo*.

4.4 *In vivo* evaluation of the formulation concepts

Oral solid formulations are the most widely used dosage forms meant for application to produce either systemic or local effects. These formulations may comprise single units such as coated tablets, capsules, and matrix tablets or multiple units such as granules, microcapsules, and beads.[213,214] The GI passage conditions of solid oral formulations play a crucial role in drug release, and hence drug efficacy. After oral intake of a dosage form, it passes through the oro-esophageal cavity via peristaltic movements and reaches the stomach together with co-administered water. Unless the gastric emptying hindered, the next destination of the dosage form is the small intestine where drug absorption typically occurs. Therefore, gastric emptying, in particular, has a decisive influence on the resulting plasma levels.[215] In the fed state, dissolved drug particles are emptied together with the liquid components of the gastric contents, while larger objects with particle sizes > 1-2 mm (e.g. delayed-release tablets) are initially retained and, if possible, are mechanically, physicochemically and enzymatically comminuted.[216]

The gastric emptying process is affected by various physiological factors as well as by food and drink intake. In fasting conditions, the fluid available to the drug delivery process is primarily composed of the co-administered water (usually 240 mL in clinical trials) and a small amount of approximately 10–50 mL of gastric juice due to gastric secretion.[67,217] Previous *in vivo* studies by Weitschies *et al.*, however, confirmed that the ingested water is emptied quickly with subsequent rapid onset of the drug from fast-releasing dosage forms and readily water-soluble drugs.[218] Therefore, the gastric retention of dosage forms containing active ingredients for local effect in the stomach, such as treatment of *H. pylori* under fasted conditions, in our view, is very unlikely.

In fed state conditions, the gastric emptying of dosage forms can be delayed due to gastric sieving. Only liquids and small particles (< 1–2 mm) are emptied during the digestive phase.

However, large and non-digestible components are retained until the event of the migrating motor complex (MMC) occurs.[219] The duration of the delay is affected by the volume and the caloric content of the food. The co-administration of high-caloric solid foods is known to extend the gastric emptying time to several hours.[220,221] Until recently, various gastric-specific drug delivery systems were designed to achieve longer GRT. In a recent review, Lopez *et al*. have concluded that gastroretentive delivery systems are promising drug delivery strategies with positive results in studies with humans for the local delivery of drugs in the stomach.[83] Unfortunately, *in vivo* studies revealed that only a few formulations were successful and reached to market yet.[108] Therefore, the search for a new and novel approach to achieve effective gastric retention is noteworthy.

In this study, the *in vivo* behavior of the two formulations (effervescent granules and matrix tablets), each containing 100 mg of caffeine were investigated under fasted state and fed conditions using the salivary tracer technique. In all study arms, significant salivary caffeine concentrations were measured throughout the study period. In a fasted state, the salivary caffeine concentration from granules was higher than from both granules and matrix tablets in a fed state. Moreover, the C_{max} value was high, which was reached only at 25 min. This high and fast salivary concentration was expected due to fast water emptying kinetics. Grimm *et al.* have confirmed that much of the initial available gastric volume emptied after 30 min of administration.[222] A similar MRI study also confirmed faster water emptying, which was returned to the baseline volume within 45 min of water intake in a fasted stomach.[63] Moreover, food intake after 4 h had no effect on the salivary caffeine concentration from granules, which was taken in a fasted state. The granules were dissolved and emptied in the first 30 min with the co-administered water. This was further confirmed by a uniform decrease in salivary caffeine concentration after the food intake over 12 h (Figure 4.2).

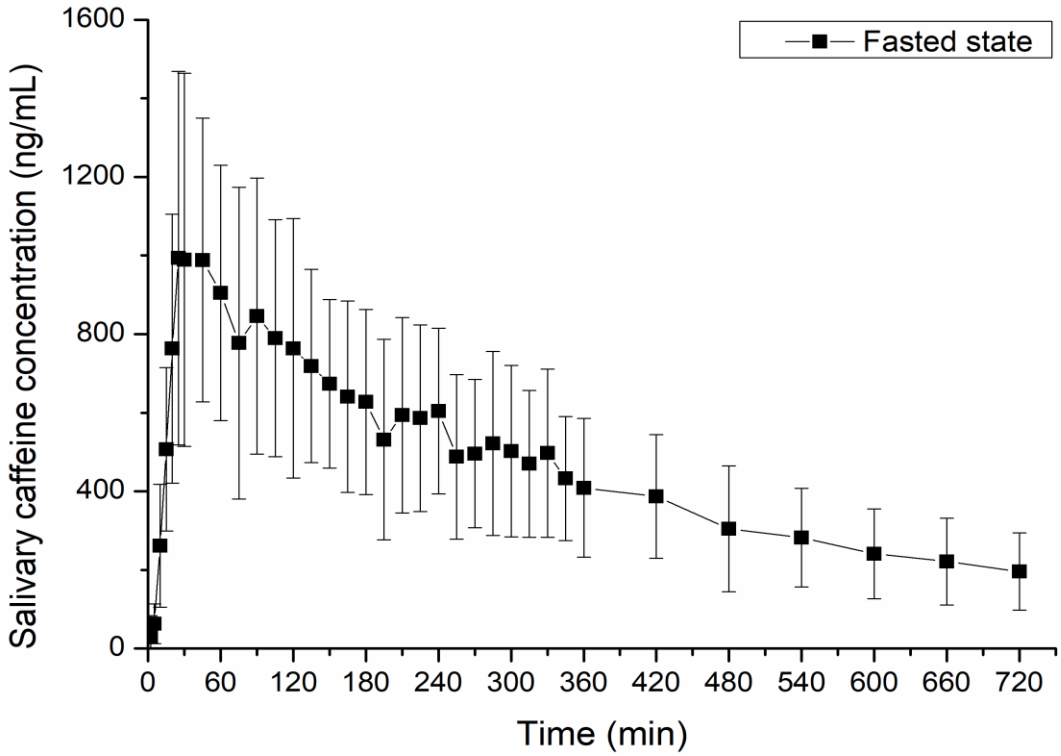


Figure 4.2 The mean salivary caffeine concentration versus time curve of 11 subjects from granules containing 100 mg of caffeine administered in a fasted state with 240 mL of tap water over 12 h (n = 11, means ± SD).

In a fed state, an initial increase in salivary caffeine concentrations was demonstrated from granules for the first 30 min, followed by a slow and even increase in caffeine concentrations over the remaining period. Furthermore, the effect of the volume of water on gastric emptying kinetics was investigated by co-administration of the granules either with 20 or 240 mL of tap water. In various studies, water emptying typically follows first-order kinetics, which could be directly related to the gastric emptying rate of drugs, as demonstrated by the Magenstrasse.[54,63] However, higher salivary caffeine concentrations were observed for the granules administered with 20 mL of water. Figure 4.3 shows representative data from one subject.

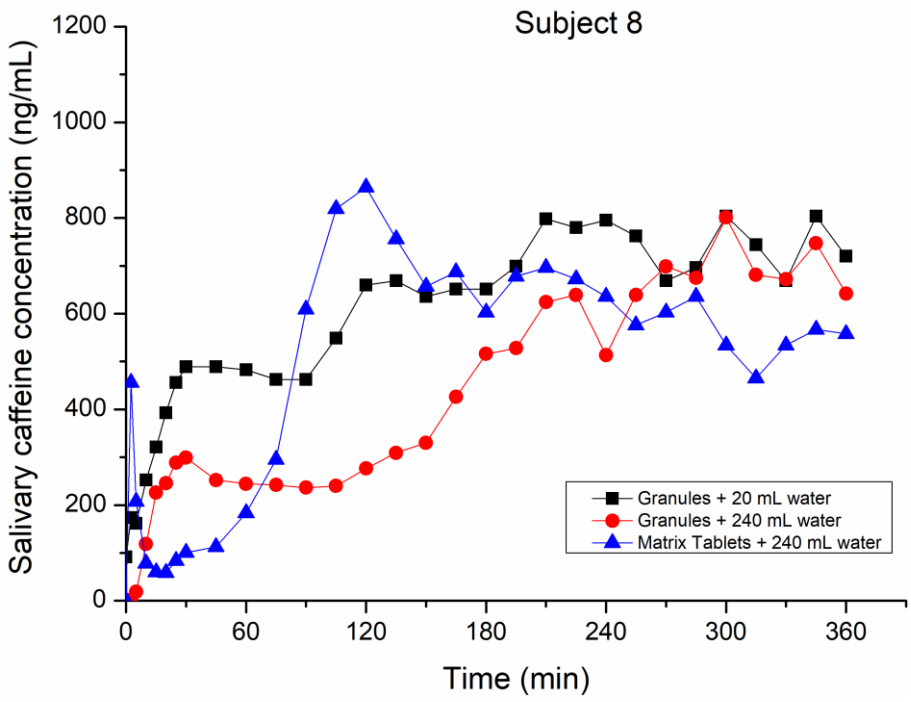


Figure 4.3 The salivary caffeine concentrations over time profile of one subject from granules and matrix tablets, each containing 100 mg of caffeine administered 30 min after FDA meal with tap water over 6 h (n = 11, means ± SD).

Moreover, maximum salivary caffeine concentrations were reached after 4 h and 4.5 h from granules administered with 20 and 240 mL of water, respectively. This difference could be explained by the effervescence activity in the presence of various volumes of water and food. Co-administration of 240 mL of water could have dissolved the granules faster due to the evolution of a large amount of CO_2, which support faster and uniform mixing of caffeine with gastric contents and contribute to the extended gastric residence. Some studies concluded that carbonated water stimulates gastric motility by liberating CO_2.[223] This effect could have contributed to the initial increase in salivary caffeine concentrations. However, a slow and even caffeine release in the later study period suggested that the effect of CO_2 on gastric motility was restricted to the initial phase. In addition to CO_2, the position of the granular carrier, which determines the contact time with the gastric fluid, could affect caffeine release.

Further comparison of the salivary caffeine concentration versus time profile of granules under fed and fasted state conditions confirmed the effect of food on the gastric emptying rate (Figure 4.4). Despite the same volume of co-administered water (240 mL), the gastric emptying rate from granules in a fasted state was by far faster compared to a fed state. By this evidence, gastric-specific delivery of drugs in an empty stomach was unlikely.

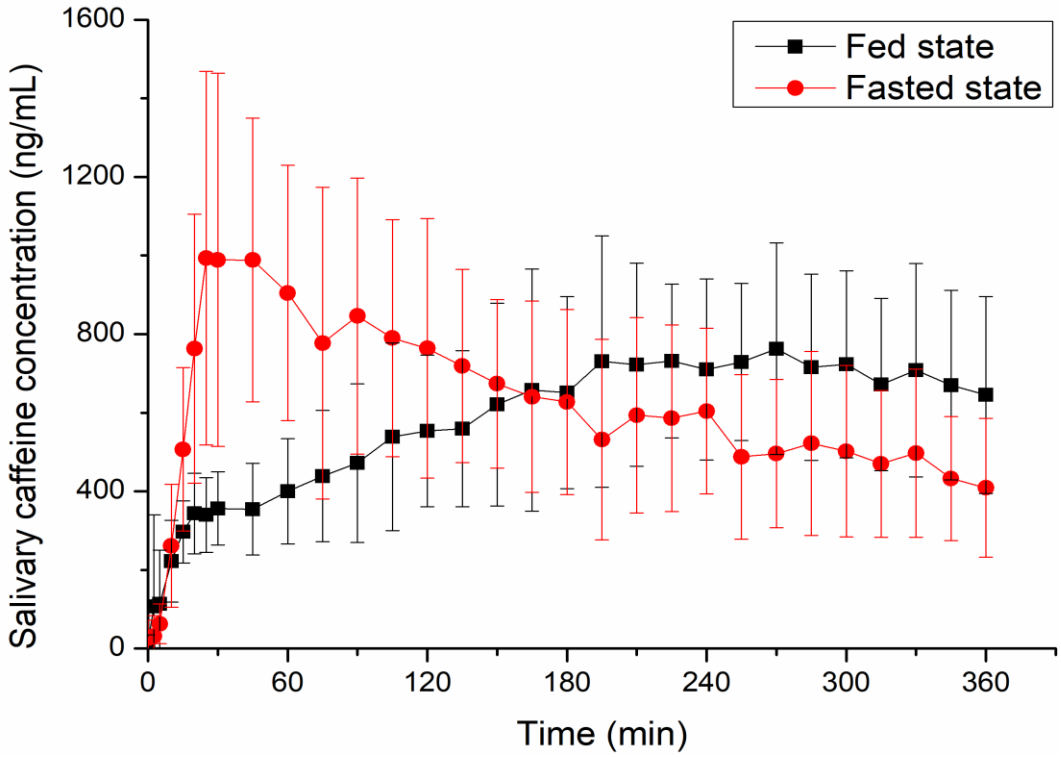


Figure 4.4 Comparison of the mean salivary caffeine concentrations over time profiles of 11 subjects from granules containing 100 mg of caffeine administered in fed and fasted state with 240 mL of tap water over 6 h (n = 11, means ± SD).

Regarding matrix tablets, there was a general slow caffeine release for the first 30 min, followed by faster release over the remaining period. Similar to the granules, high caffeine concentrations were exhibited from some subjects immediately after administration that could be due to contamination of the oral cavity during tablet swallowing. This effect was pronounced on subjects 2 and 11 (Figure 4.5). Rapid dissolution of the caffeine at the surface of the tablet with subsequent emptying with rapidly clearing water might have contributed to this effect. Two

studies by Weitschies *et al.* have suggested that longer gastric residence can be achieved for HPMC matrix tablets when dosed together with food. For instance, drug release from felodipine matrix tablets was delayed for more than 3 h, depending on food intake and the position of the matrix tablet in the stomach.[218,224]

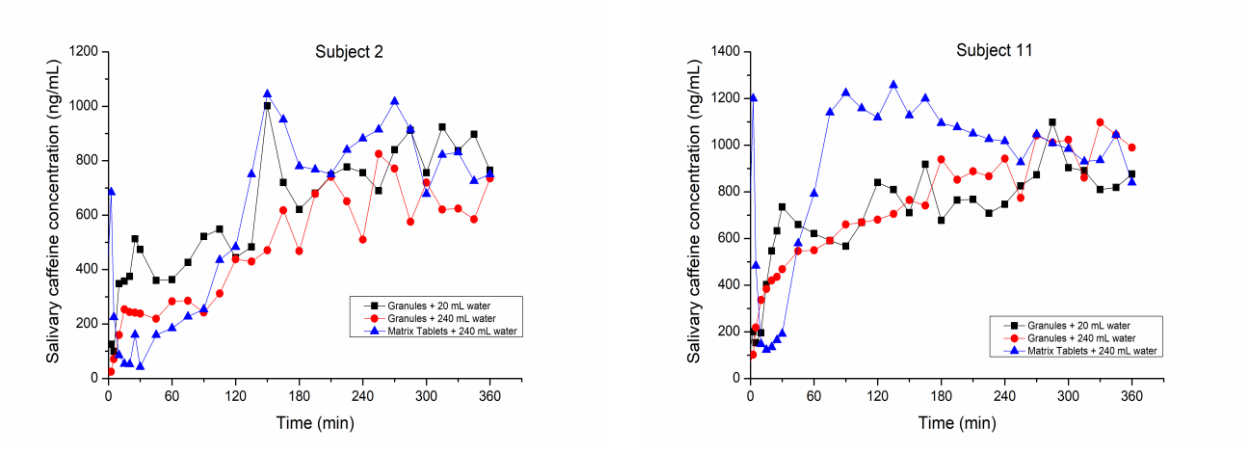


Figure 4.5 The salivary caffeine concentrations over time curve of two subjects from granules and matrix tablets, each containing 100 mg of caffeine administered 30 min after FDA meal with tap water over 6 h (n = 11, means ± SD).

In this study, there was a faster caffeine release from the matrix tablets compared to *in vitro* expectations. On average, the maximum salivary caffeine concentration was reached after only 1.5 h. This effect was caused by physiological parameters such as intragastric pressure and gastric motility, which can cause variations in the plasma level of drugs as was shown in previous studies by Weitschies *et al.* In the worst case, matrix tablets could be broken by peristaltic contractions, which can lead to dose dumping.[125,225] The dissolution stress test confirmed the effect of pressure on the disintegration and drug release behavior of the matrix tablets. In the intermediate and high-stress programs, the first pressure events destroyed the tablets with a subsequent increase in drug release. Studies conducted using the same device have found similar pressure effects for matrix tablets.[126,226]

In addition to the salivary tracer studies, the localization and disintegration behavior of the matrix tablets was studied by MRI studies. These investigations were performed on all 12 subjects 4 h after administering the matrix tablets. According to the MRI data, 10 out of 12 matrix tablets (≈ 83 %) were broken. The other two tablets (≈ 17 %) were either intact or mainly intact and found exclusively in the stomach. An exemplary MR image of one subject is depicted in Figure 4.6.

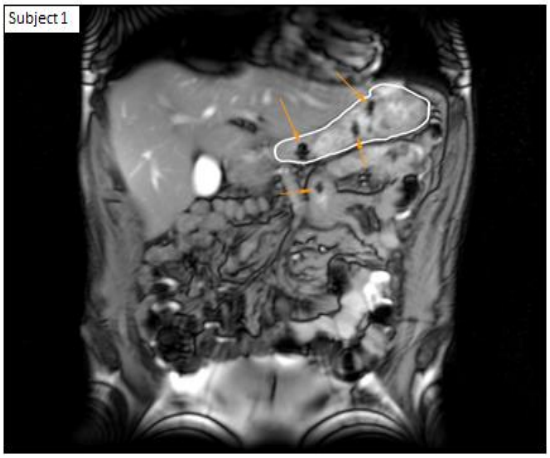


Figure 4.6 MRI picture showing the localization and disintegration behavior of a matrix tablet taken 4 h after administration of the matrix tablet containing 100 mg of caffeine to a healthy subject 30 min after FDA meal with 240 mL of tap water (n = 12).

Regarding the localization, in 7 out of the 12 subjects (58.33%), the tablets were exclusively located in the stomach. In the remaining 5 subjects (41.67%), the tablets were distributed both in the stomach and intestines (Figure 4.7). Although many of the tablets were localized in the stomach, the caffeine in the tablet left the stomach faster than granules, as demonstrated by the salivary tracer studies.

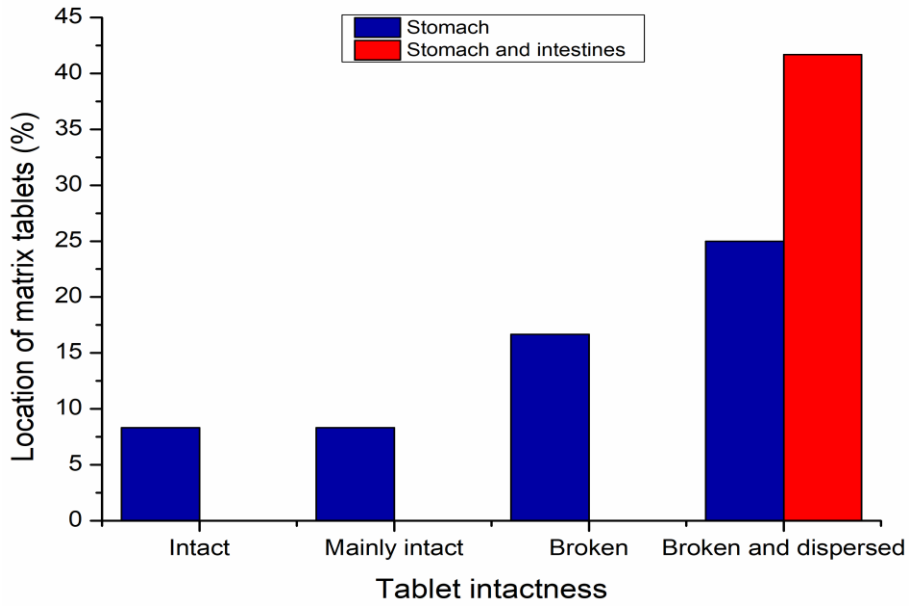


Figure 4.7 The percent frequency of locations of the matrix tablets investigated by MRI 4 h after administration of matrix tablets containing 100 mg of caffeine to 12 subjects 30 min after FDA meal with 240 mL of tap water (n = 12).

In this regard, the objective of gastric retention from matrix tablets containing 15 % (w/w) HPMC was not guaranteed as a result of dose dumping. Studies confirmed that increasing the concentration and viscous grade of HPMC could control the release of the drug over an extended period keeping the tablet either entirely or partially intact.[227] In the previous formulations, matrices containing 20 % (w/w) or more of HPMC were able to decrease the *in vitro* drug release with intact tablets for several hours. Therefore, formulation and optimization of the HPMC type and concentration could control drug release in the stomach over an extended period and reduce the risk of dose dumping.

In all developments, effervescent granules and matrix tablets meant for gastric-specific drug delivery were developed successfully. Among formulations, granules administered in the fed state together with 240 mL of tap water demonstrated superior gastric retention. Therefore, the administration of granule formulations in combination with acid-reducing agents after each food intake could be an alternative approach to eradicate *H. pylori* locally within the stomach.

5 Conclusion

In the present study, a strategy for the local eradication of *Helicobacter pylori* was developed. Due to its high antibacterial activity and poor oral bioavailability, penicillin G represented the most promising antibiotic drug for this purpose. To maintain local drug concentration above MIC over a longer time, effervescent granules and hydrogel matrix tablets that could be dosed after food intake were successfully developed. In order to predict the stability of PGS, and also to determine its concentration in various media, a validated HPLC method was developed.

PGS was found to be unstable in acidic media but stable at pH 6.8. The addition of alkalizers to the matrix tablets exhibited different stabilization effects. Among the alkalizers, $NaHCO_3$ showed the most promising stability enhancement, which was mainly caused by the microenvironmental pH modulation but not due to a pH change in the dissolution media.

A flow-through model (FTM) was developed and applied to simulate the *in vivo* intragastric concentration versus time profiles of the drug from granules and matrix tablets. In the case of granules, the drug concentration was decreased over time. In contrast, an increased concentration versus time profile was exhibited from matrix tablets. A faster drug release from matrix tablets in a dissolution stress test device compared to compendial testing, however, confirmed the absence of pressure events in FTM, which caused the difference.

In the *in vivo* investigations, caffeine-containing formulations were administered to subjects in fed and fasted states. Accordingly, granules that were administered after FDA meal demonstrated longer gastric retention than in the fasted state. The most extended gastric retention was exhibited from granules which were administered with 240 mL of water. Matrix tablets showed poor gastric retention compared to granules in a fed state due to dose dumping as a result of tablet disintegration, which was confirmed by the MRI investigations.

6 Summary

Infections with *Helicobacter pylori* are a global challenge that affects both developed and developing countries. This infection is currently treated using multiple antimicrobials that are mostly absorbed after oral administration and subsequently secreted into the gastric lumen. The eradication rates from the different therapeutic regimens, however, are declining nowadays, primarily due to high antibiotic resistance and possibly the mode of drug delivery. *H. pylori* is commonly found adhering to epithelial cells, and therefore, intragastric drug delivery may be a more direct treatment option. In this work, we developed a new strategy for the local eradication of *H. pylori* within the stomach.

Initial *in vitro* experiments revealed that penicillin G shows promising antibiotic activity against resistant strains of *H. pylori* with MIC values of 0.125 µg/mL. To provide luminal concentrations above the MIC for an extended time, we decided to follow two different formulation strategies: effervescent granules and HPMC-based hydrogel matrix tablets. Among the granule formulations, only one batch was stable and demonstrated excellent performance with respect to drug content, effervescent action, and drug release. It was therefore selected for further *in vitro* studies. All matrix tablets showed the desired tablet quality requirements and drug release was scalable *in vitro* by the HPMC concentration.

In order to quantify PGS in various formulations and media, an HPLC method was developed and validated. Due to the stability concerns, the degradation behavior of PGS was studied at different pH. PGS was found to be unstable at acidic pH values, but its stability was higher at more neutral pH values. Sufficient stability was exhibited at pH values above pH 4.5. Due to the instability of PGS in acidic media, alkalizers were added to the matrix tablets to prevent the degradation of the drug within the tablet. Among the alkalizers tested, $NaHCO_3$ showed the most

promising results as it significantly enhanced the stability within the matrix and also the concentration of PGS in the dissolution media. The stabilizing effect was caused mainly by the modulation of the microenvironmental pH rather than a pH change in the dissolution media. As a result, these matrix tablets were selected for further *in vitro* characterization.

In order to guide formulation development, a flow-through model (FTM), which was able to simulate various physiological conditions of the gastric environment, was developed and applied. In contrast to compendial dissolution methods, the FTM allowed studying the effect of gastric secretion, mixing and emptying on the gastric concentration of the drug *in vitro*. It could be shown that the granules generated a high initial concentration, which decreased over time. On the contrary, the matrix tablets did not provide such a profile due to the absence of pressure events in the model. Further investigations of the matrix tablets in a dissolution stress test device revealed faster drug release if pressure events of physiological relevance are simulated.

In the last part of this thesis, the two formulation concepts were compared *in vivo* by using the salivary tracer technique. For this purpose, caffeine was used as a model drug. The *in vivo* investigations suggested that granules administered in a fed state demonstrated longer gastric retention than in a fasted state. In a fed state, effervescent granules provided longer gastric retention of caffeine in comparison to the matrix tablets. Interestingly, the administration of the granules together with 240 mL of tap water provided an even better gastric retention of caffeine than the smaller volume (20 mL). Additional MRI investigations after 4 h of tablets' intake revealed that the matrix tablets were already disintegrated *in vivo*.

In conclusion, effervescent granules dosed after food are expected to better maintain intragastric drug concentration over an extended period compared to matrix tablets. Moreover, the carbon dioxide generated after disintegration supports the mixing of the drug with the chyme and thus, provides a uniform distribution of the drug. By this, bacterial sanctuary sites within the stomach can be avoided. The major challenge could be the stability of PGS in acidic media. This problem could be addressed via concomitant administration of PPIs. H2 blockers could also be recommended to address nocturnal acid-breakthrough during the mid-night. In combination with an acid-reducing agent, PGS granule formulations alone or part of the treatment regimens could enable the local eradication of *H. pylori* directly within the stomach.

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Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Hilfsmittel benutzt und insbesondere die wörtlichen oder dem Sinne nach anderen Veröffentlichungen entnommenen Stellen kenntlich gemacht habe.

I hereby declare that this work has not yet been submitted by me to the Faculty of Mathematics and Natural Sciences of the University of Greifswald nor to any other scientific institution for the purpose of the doctorate.

Furthermore, I declare that I have written this work independently and have not used any aids other than those specified therein, and in particular have identified the literal or sensory passages taken from other publications.

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Taddese Mekonnen Ambay

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