Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Review Article

Gastroretentive Mucoadhesive Microsphere for the Management of Gastric Infection

Sri Prakash Mishra*, Amit Nayak, Dinesh Kumar Mishra, Abhay Kumar

Sarvepalli Radhakrishnan University, Bhopal (M.P.)

ABSTRACT

Gastric infections are mostly triggered by Helicobacter pylori (H. pylori), a fungus that colonizes the stomach mucosa of more than 50% of the i nhabitants of the world. Chronic H. Pylori diseasewas associated with stomach diseases such as peptic ulcer, chronic gastritis and stomach aden oc arcinoma. Current therapy for eradication relies on antibiotic-based therapies that are ineffective in about 20% of patients. Traditional method constraints optimize the creation of new techniques for fast, consistent and cost-effective H diagnosis. Infection with pylori. Wide-ranging study has been carried out over the previous few centuries to create a type of gastro-retentive dosage (GRDF). This sort of dosage form can advance the delivery and efficiency of stomach-active medicines because the GRDF enables the medication to remain in the stomach for a sufficient time period. Various methods were used to develop effective GRDFs such as high-density systems, low-density systems, swelling and expansion systems, hydrodynamically balanced systems, superporous hydrogels,. However, there are both merits and demerits in these kinds of schemes. Intra-individual and inter-individual dissimilarities are obstacles to the growth of effective GRDFs in gastric physiology. Examples of these individual differences include gastric pH and gastric motility that have a notable effect on the moment of stomach retention and delivery of drugs. Some of these obstacles can be overcome by developing a novel mucoadhesive microsphere. The mucoadhesive microsphere is characterized by close contact of the MDF with the mucosal layer, thereby increasing the localized absorption of the drug. H_2Receptor antagonists (H_2RAs) have become first-line therapy for acid related peptic disease and GRDF especially designed for H_2RAs and drugs against H. pylori, including specific targeting systems and leading to a marked development in the quality of life for a large number of patients. In this relationship, new formulations with improved absorption, improved bioavailability and improved acid-suppressing regimens are welcome

Keywords: H. pylori, gastro-retentive dosage, mucoadhesive microsphere

Article Info: Received 16 June 2019; Review Completed 23 July 2019; Accepted 29 July 2019; Available online 15 August 2019

Cite this article as:

Mishra SP, Nayak A, Mishra DK, Kumar A, Gastroretentive Mucoadhesive Microsphere for the Management of Gastric Infection, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):680-686 **http://dx.doi.org/10.22270/jddt.v9i4-s.3357**

***Address for Correspondence:**

Sriprakash Mishra, Sarvepalli Radhakrishnan University, Bhopal (M.P.)

Introduction

H. Pylori, a gram-negative microaerophilic spiral bacterium, were created in 1982 by Warren and Marshall as a significant cause organism for peptic ulcer[1]. Approximately half of the world's inhabitants are infected with this pathogen, but only a tiny percentage of infected inhabitants show clinical signs, mostly dependent on bacterial virulence dissimilarity and hostile factor. H. Pylori infection occurs in 90-100% of patients with duodenal ulcer and 60-90% of patients with stomach ulcer. The H has been shown by numerous researches. Pylori infection is associated with an enhanced danger of peptic ulcer disease of at least 3 to 4 folds and that of 10-15% H. Individuals infected with pylori will have the disease of peptic ulcer in their lifetime[2]. H. pylori is the major contributing organism of the unceasing gastritis [3], peptic ulcer[4], B-cell MALT lymphomas[5], gastric carcinoma[6], and childhood malnutrition-associated carcinoma[7]. All of these are associated with an increase in epithelial cell apoptosis[8]. WHO has programmed H. pylori-associated gastric carcinoma as one of the three major causes of cancer-related deaths universal, around 0.5 million deaths each year. Chemotherapy of gastric cancer has deprived clinical efficacy; however, the abolition of H. pylori infection could possibly stop gastric carcinoma and other associated diseases. It is now well recognized that the maximum incidence of H. pylori infection is additional in children of 11-16 years mainly for lower socioeconomic condition due to deprived level of hygiene[9]. The occurrence of H. pylori infection in India has been reported to be extremely high, ranging from 70% to 90% in patients with duodenal and peptic ulcer and 50% to 80% in patients with non-ulcer dyspepsia as well as fit asymptomatic adults[10]. A triple therapy containing 2 antibiotics and 1 proton inhibitor over a period of 2 weeks is optional universal for abolition of H. pylori. But deprived stability of antibiotics in acidic

environment and deprived permeation of antibiotics across the mucus layer cause incomplete abolition and systemic side effects leading to patient disobedience[11,12]. Numerous study studies on gastro-retentive drug delivery systems such as floating formulations, mucoadhesive drug delivery system, pH-sensitive gel system were conducted in order to increase gastric residence and local drug concentration at H. pylori-infected site. These studies indicated the advantage of targeting drug to the gastric mucosal layer by dropping the dose of antibiotic therapy as well as improved patient's compliance. In this review, we recapitulate the existing information on colonization of H. pylori and gastroretentive mucoadhesive microsphere studies conducted in past few years, so as to utilize the information for future research on H. pylori abolition therapy.

Colonization of *H. pylori*

H. pylori are a motile pathogen which lives profound in the gastric mucus layer close to the epithelial cells. In common, after the entrance of any bacteria into the stomach, gastric acidity and peristaltic movement slow down the bond and migration of the bacteria in the gastric mucus layer. The incessantly secreted mucus from glands of the epithelial cell pushes bacteria toward the luminal surface, where the extra acidic environment delays the colonization and motility property of the pathogens [13-15]. However, even in these aggressive conditions, H. pylori sticks to the mucus layer and penetrates deep in the mucus membrane close to the epithelial cells due to good motility of flagellae and various adhesions present on its surface as shown in Figure 1. Once the bacterium establishes the adhesion with the mucus layer, the enzyme urease secreted by H. pylori metabolizes gastric urea to produce Co_2 and ammonia, which produces a surrounding coat of buffered acid. previous it was assumed that H. pylori usually colonizes in the mucus just close nearby to epithelial cell and do not penetrate the epithelial cells[16] but one of the recent study showed the attack of H. pylori in the intercellular space of gastric epithelial cell[17]. Thus, migration of H. pylori in the gastric mucus layer is determined by various dangerous and aggressive factors. Urease and flagella are two most significant dangerous factors for successful migration of H. pylori[15,18,19]. Among aggressive factors, Lewis blood group antigens are most significant factors for mucosal adhesions of bacteria. Based on composition, Lewis antigen are of two types: type 1, mainly dispersed in the epithelium surface contain Lea, Leb and sialyl-Lea, while type 2 located deeper in the mucous/ parietal cell contain LeX, LeY and sialyl-LeX. It is also well recognized that Leb and LeX are major aggressive factors that are responsible for H. pylori adhesion to the gastric epithelial cell[20]. In addition to Lewis antigen, integrins are other factors for adhesion of H. pylori. Apart from lewis antigens, blood group antigen-binding adhesion (Bab A) protein[21]and Sialic acid-binding adhesion (Sab A) protein[22] are adhesion factors present on the outer membrane of H. pylori. Bab A and Sab A recognize hostile factors Leb and LeX respectively for adhesion on gastric epithelial cell [22,23]. Sheu et al. (2003) [24] reported the expression of Leb antigen as a cause behind nearly 73% of H. pylori infection. Once H. pylori adheres to the epithelial cell, it produces a direct injurious effect, which is augmented by production and release of vacuolating cytotoxin (VacA) [25,26]

.

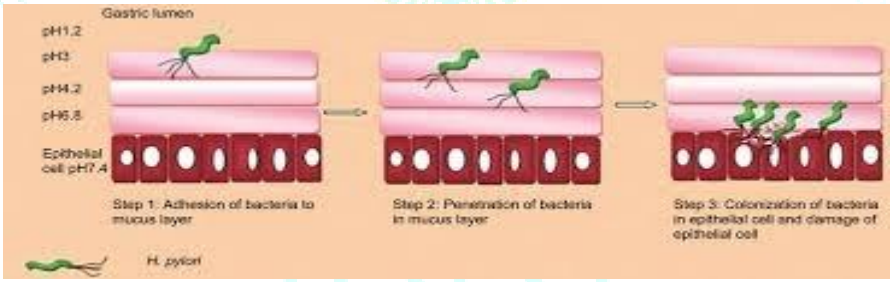

Figure 1: Steps involved in colonization of H. pylori

Present therapy and its limitations

The treatment chart presently adopted as a first-line option includes a combination of a proton pump inhibitor, amoxicillin and clarithromycin or metronidazole/tinidazole, according to International Guidelines[27,28]. This therapy continues during 7 to 14 days, twice a day. Abolition rates of H. pylori treated with a 14-day 3 therapy reached only 70% in non-ulcer dyspepsia patients and 80% in patients with peptic ulcer[27]. In Europe, Asia and North America rates of 20 to 45% have been reported[29]. This abolition rate is remote from the attractive rate to infectious diseases and from that proposed by the WHO[28,30]. The major limitation of the present therapy results from the lack of therapeutic compliance, due to the occurrence of side effects and the uneasiness resulting from the multiple doses[31,32]. These factors may lead to the expansion of antibiotic resistance[32]. Moreover, antimicrobial agents such as amoxicillin and clarithromycin are dishonored by gastric acid[30]. Therefore it is necessary to use higher doses, which is reflected in the increase of GIT side effects, namely diarrhea, nausea, vomiting, bloating and abdominal pain and as a result the discontinuation of the therapy[32]. Another important cause is the antibiotic resistance that H. pylori has been developing, for instance the resistance to metronidazole has reached around 40% in developed countries and exceeds 90% in developing countries[33]. The resistance to clarithromycin has also been increasing, reaching more than 20% in southern Europe[29]. The bacteria are sensitive to other antimicrobial drugs; nevertheless they cannot be used in acidic medium[30]. Not- withstanding, the antibiotic residence time in the stomach is inadequate to achieve significant concentrations capable of crossing the gastric mucosa and reaching the surface between the mucus gel layer and the epithelial cells, where the H. pylori resides. Although drug solutions reach the gastric luminal region, their absorption into deeper layers of the gastric mucosa is vulnerable by the mucous layer barrier[34]. In order to increase the efficacy of H. pylori abolition, different suggestions have been made, namely a bismuth-containing quadruple therapy, sequential and attendant treatment and the use of novel antibiotics, such as rifabutin[35,36]. However, these options may have to take into account that the difficulty of the treatment plan, including the switch middle in the sequential treatment and the large number of pills in attendant and BCQ therapy, may decrease therapeutic compliance. To overcome these limitations, novel effective therapies have been proposed: probiotics[37,38], phytomedicine, gastroretentive systems, namely floating drug delivery systems[39,40] and in a preventive approach, the attempt to develop an effective vaccine[29]. One of the leading

promising therapies that have newly emerged is based on the use of micro- or nanoparticles for direct contact with the H. pylori, through drug delivery techniques or mucoadhesive properties.

Novel drug delivery approaches for *H. pylori*

Literature review reveals that local use of antibiotics to gastric mucosa resulted in better abolition compared to systemically available antibiotic[41]. Hence, for successful abolition of H. pylori the DDS should sufficiently deliver the therapeutic agent in the close nearness of the gastric mucus membrane. In current years, various novel approaches are used for increasing the gastric residence time of the delivery system and local action of the drug in stomach. Different strategies utilized are:

(i) Density-based approaches including a high density system and a low density system

(ii) The floating drug delivery system,

(iii) The mucoadhesive/bioadhesive system

(iv) The swelling system for improving the gastric retention time of the system.

(v) Incorporation of passage delaying food agents

(vi) Ion exchange resins

(vii) Osmotic regulated systems

(viii) Hydrodynamically balanced systems

(ix) Gas-generating systems

(x) Raft-forming systems ect.

Physiological consideration

Over the past few decades, pharmaceutical study and formulation development scientists have documented the potential of site-specific ODDS. The stomach site has been recognized as a depot for CRDF. However, formulation scientists have to consider physiological variations such as gastric residence time, gastric emptying time and drug release from the dosage form. Once solid material (food and/or drug) is chewed and swallowed, the esophagus quickly transports it to the stomach. The stomach is mostly divided into two parts: the proximal stomach, consisting of the fundus and body and the distal stomach, consisting of the antrum (or pylorus). The proximal stomach serves as a food reservoir, whereas in the distal stomach the food is processed, forming chyme and proteins are partly broken down. The distal stomach (pylorus) acts as pump to help in GE. The rate of GE is prejudiced by both the volume of food and the composition of the gastric contents. However, the pattern of gastrointestinal motility varies significantly in both fasting and fed states, further influencing the GE time. The fasted state is characterized by an interdigestive series of electrical events which cycle both through the stomach and small intestine every 2-3 h[42]. This cycling event is termed the migrating myoelectric complex (MMC). The MMC is classified into 4 phases[43] as enumerated below and as depicted in Fig. 2. In the case of the fed state, the GE time is slowed as the onset of the MMC is interrupted. For example, the feeding state results in a lag time (i.e. 30 min-4 h) prior to the onset of GE.

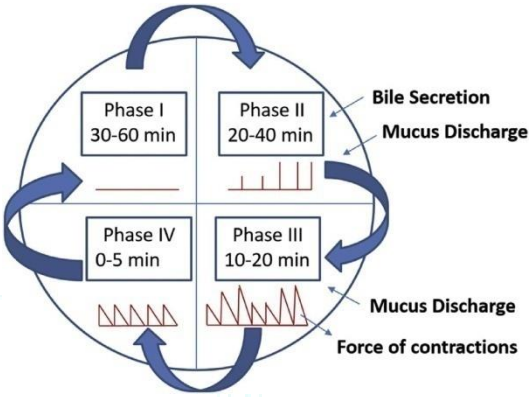

Fig. 2. Motility pattern in GIT

Composition of gastric mucus

The stomach wall is composed of numerous distinct layers of tissue: outer mucosa, inner submucosa, muscularis externa and serosa. The mucosa or lining of the stomach is comprised of columnar epithelial tissue, lamina propria (composed of areolar connective tissue) and a thin layer of smooth muscle. The mucosal cells (goblet cells) exude mucus (a translucent and glutinous secretion) which coats the stomach lining and stops its destruction from the gastric juice[43]. The mean thickness of the mucosal layer varies from about 50 to 450 mm in humans. Mucus is a complex mixture and its composition varies depending upon the source and the pathological state of the human[44,45] (Table 1).

Table 1 Composition of mucus[44,45].

Composition	Percentage (%)
Water	90-95%
Mucin	5-10%
Electrolyte	1%
Others ((Enzyme, Nucleic acid, Lipid, Plasma protein, Secretory IgA, Bacteria and their decomposition products)	4%

Mucoadhesive microspheres

Recent advances in polymer science and drug carrier technologies have promulgated the development of novel drug carriers such as mucoadhesive microspheres that have boosted the use of bioadhesion in the drug delivery[46]. Mucoadhesive microspheres include microparticles and microcapsules of 1 to 1000 µm in diameter consisting either completely of mucoadhesive polymer or having an outer coating with adhesive property[47]. Microspheres have the possible to be used for controlled as well as spatial drug delivery. Incorporating mucoadhesivenes to microspheres leads to competent absorption and enhanced bioavailability of drug. Precise targeting of drug to the absorption site is achieved by using homing devices (ligand) like plant lactin,

bacterial adhesion etc. on the surface of the microspheres. Mucoadhesive microspheres can be modified to stick to mucosal linings of GIT, thus offering the possibilities of localized as well as systemic absorption of drug in controlled manner[48,49].

Mechanism of mucoadhesion

Usually, mucoadhesion occurs from the interactions between the drug and carrier molecules with different mucus membranes in two steps: the contact stage and the consolidation stage (Fig. 3) [50]. However, the mechanism of mucoadhesion is highly complex and not yet fully understood. Different chemical interactions such as ionic bonds, covalent bonds, hydrogen bonds, Van der Waals forces and hydrophobic interactions are concerned in the mucoadhesion process that facilitates interactions of drug molecules across the interface. Several theories have been investigated to allow the understanding of mucoadhesion[51].

Electronic theory

Adsorption theory

Wetting theory

Diffusion theory

Fracture theory

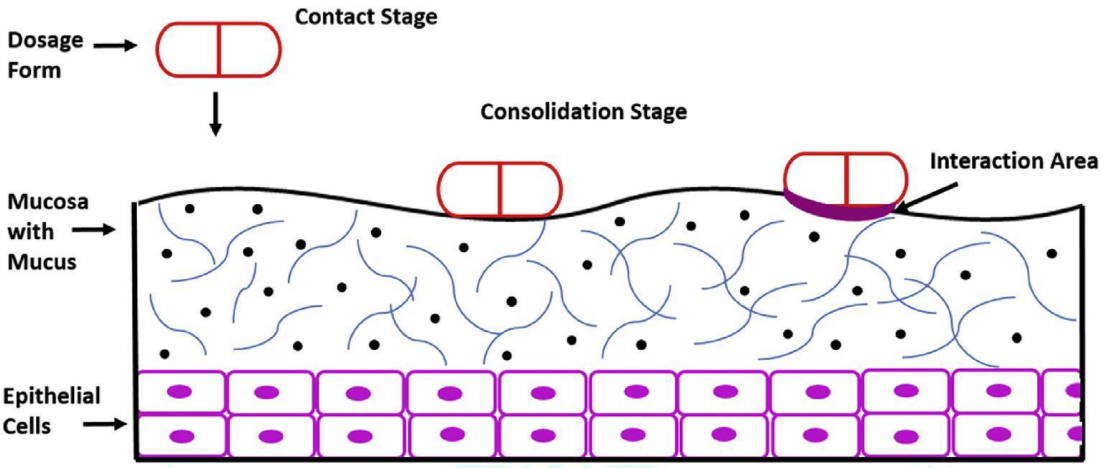

Fig. 3 The two stages in mucoadhesion

Types of Mucoadhesive Polymers

First generation mucoadhesive polymers

It may be divided into 3 main sub-categories, namely: Anionic polymers, Cationic polymers and non-ionic polymers. Amongst these anionic and cationic polymers have been exhibits the greatest mucoadhesive strength[52].

Anionic polymers

Anionic polymers are the most widely employed mucoadhesive polymers within pharmaceutical formulation due to their high mucoadhesive functionality and low toxicity. These include alginates, carrageenan, poly acrylic acid and its weakly cross-linked derivatives and sodium carboxymethylcellulose. PAA and NaCMC possess excellent mucoadhesive characteristics due to the formation of strong hydrogen bonding interactions with mucin[53]. Polycarbophil and carbomer (Carbopol, PAA derivatives have been studied extensively as mucoadhesive platforms for drug delivery to the GI tract[54,55]. Carbomers are cross-linked with allyl sucrose or allylpentaerythritol whereas polycarbophil polymers are cross-linked with divinyl glycol. Both compounds have the same acrylic backbone but vary in their cross-link density that is often tailored to suit pharmaceutical or cosmetic performance.

Cationic polymers

Chitosan is a cationic polysaccharide the most abundant polysaccharide in the planet, next to cellulose[56]. The most explored mucoadhesive polymers, chitosan is gaining increasing importance due to its good biocompatibility, biodegradability and due to their favourable toxicological[57].The linearity of chitosan molecules also ensures sufficient chain flexibility for interpenetration[58]. Chitosan may provide improved drug delivery via mucoadhesive mechanism; it has also been shown to improve drug absorption via the paracellular route through neutralization of fixed anionic sites within the tight junctions between mucosal cells[59,60].

Novel second-generation mucoadhesives polymers.

Second generation includes lectins and thiolated polymers.

Lectin is normally defined as proteins or glycoprotein complexes of non-immune origin that are able to bind sugars selectively in a non-covalent manner[61]. Lectins are capable of attaching themselves to carbohydrates on the mucus or epithelial cell surface and have been extensively studied, especially for drug-targeting applications[62,63]. These second-generation bioadhesives not only provide for cellular binding, but also for subsequent endo- and transcytosis.

Thiolated polymers, also designated thiomers, are hydrophilic macromolecules exhibiting free thiol groups on the polymeric backbone. Due to these functional groups, various features of polyacrylates and cellulose derivatives were strongly improved[64]. The presence of thiol groups in the polymer allows the formation of stable covalents bonds with cysteine-rich subdomains of mucus glycoproteins leading to increased residence time and improved bioavailability[65]. Other advantageous mucoadhesive properties of thiolated polymers include improved tensile strength, rapid swelling and water uptake behavior. .e.g- various thiolated polymers include chitosan-thioglycolic acid,chitosan-thioethylamidine, alginate-cysteine.

Methods of Preparation of Mucoadhesive Microspheres

- ✓ Emulsion cross-linking method.
- ✓ Single emulsion techniques.
- ✓ Ionotropic gelation.

- ✓ Phase inversion method.
- ✓ Spray drying and spray congealing method.
- ✓ Solvent removal method.
- ✓ Hot melt method.
- ✓ Polymerization

Conclusion

In gastric ulcers caused by H. pylori, the treatment requires high concentration of antibacterial agents like clarithromycin or metronidazole or amoxicillin in stomach and absorption through gastric mucosa. However, presently available conventional drug deliveries of these drugs fail to achieve the same. A lot of extraordinary novel drug delivery approaches making use of buoyancy and bioadhesion to increase the gastro-retention time have been developed for the treatment of H. pylori infection. Among many novel delivery systems investigated so far for gastric delivery of drugs for H. pylori, the mucoadhesive microspheres showed the great potential for the selectively delivering the drug at infection site. By modifying the surface groups present on mucoadhesive polymers, increased mobility of nanoparticles in the gastric mucus can be obtained for better abolition of H. pylori. These systems are connected with major problems like stability on prolonged storage, consistency of drug entrapment and drug release and industrial scale up.

Table 2: An over view of cited Mucoadhesive Microspheres delivery approaches for H. Pylori eradication

Polymers used	Drug	Mechanism	Salient features	Ref
Ethyl cellulose and carbomer 937	Amoxicillin	Microspheres	Protection of drug in stomach	66
chitosan	Amoxicillin	Microspheres	Increased gastric retention, gastric stability of drug and better H. pylori clearance effect than amoxicillin powder	67
Carbopol-934P	Amoxicillin	Microspheres	80% mucoadhesion after 1 h, Better H. pylori clearance effect than amoxicillin powder	68
CAB and cholestyamine	AHA	Microspheres	Increase gastric retention time up to 12 h	69
Polycarbonate	AHA	Microspheres	Increase gastric retention time up to 12 h	70
Modified gelatin	Amoxicillin	Microspheres	Increase mucoadhesion	71
Gelatin/Acrypol 934P	LEVOFLOXACIN	Microspheres	Increase mucoadhesion	72
carbopol 974P, Eudragit RS 100	Clarithromycin	Microspheres	Increase gastric retention time up to 12 h	73

References

1. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;342:1273-75.
2. Malfertheiner P, Selgrad M. Helicobacter pylori infection and current clinical areas of contention. Curr Opin Gastroenterol 2010;26:618-23.
3. Cover TL, Blaser MJ. Helicobacter pylori in health and disease. Gastroenterology 2009;136:1863-73.
4. Blaser MJ, Atherton JC. Helicobacter pylori persistence: Biology and disease. J Clin Invest 2004;113:321-33.
5. Graham DY. Campylobacter pylori and peptic ulcer disease. Gastroenterology 1989;96:615-25.
6. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127-31.
7. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, De Boni M, et al. Regression of primary low grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 1993;342:575-7.
8. Targa AC, Cesar AC, Cury PM, Silva, AE. Apoptosis in different gastric lesions and gastric cancer: Relationship with Helicobacter pylori, overexpression of p53 and aneuploidy. Genet Mol Res 2007;6:554-65.
9. Mishra S, Singh V, Rao GRK, Dixit VK, Gulati AK, Nath G. Prevalence of Helicobacter pylori in asymptomatic subjects—A nested PCR based study. Infect Genet Evol 2008;8:815-9.
10. Singh V, Mishra S, Maurya P, Rao G, Jain AK, Dixit VK, et al. Drug resistance pattern and clonality in H. pylori strains. J Infect Develop Count 2009;3:130-6.
11. Endo H, Yoshida H, Ohmi N, Ohta K, Higuchi S. Localization of [14C] amoxicillin in rat gastric tissue when administered with lansoprazole and clarithromycin. J Antimicrob Chemother 2001;48:923-6.
12. Endo H, Yoshida H, Ohmi N, Ohta K, Higuchi S, Suga T. Localization of [14C] clarithromycin in rat gastric tissue when administered with lansaprazole and amoxicillin. J Antimicrob Chemother 2002;50:285-8.
13. Schreiber S, Scheid P. Gastric mucus of the guinea pig: Proton carrier and diffusion barrier. Am J Physiol 1997;272:G63-70.
14. Worku ML, Sidebotham RL, Walker MM, Keshavarz T, Karim QN. The relationship between Helicobacter pylori motility, morphology and phaseof growth: Implications for gastric colonization and pathology. Microbiology 1999;145:2803-11.
15. Celli JP, Turner BS, Afdhal NH, Keates S, Ghiran I, Kelly CP, et al. Helicobacter pylori moves through mucus by reducing mucin viscoelasticity. Proc Natl Acad Sci U S A 2009;106:1421-6.
16. Ho SB, Shekels LL, Toribara NW, Kim YS, Lyftogt C, Cherwitz DL, et al. Mucin gene expression in normal, preneoplastic, andneoplastic human gastric epithelium. Cancer 1995;55:2681-90.
17. Ozbek A, Ozbek E, Dursun H, Kalkan Y, Demirci T. Can Helicobacter pylori invade human gastric mucosa? An in vivo study using electron microscopy, immunohistochemical methods, and real-time polymerase chain reaction. J Clin Gastroenterol 2010;44:416-22.
18. Eaton KA, Morgan DR, Krakowka S. Motility as a factor in the colonization of gnotobiotic piglets by Helicobacter pylori. J Med Microbiol 1992;37:123-7.
19. Eaton KA, Krakowka S. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by Helicobacter pylori. InfectImmun 1994;62:3604-7.
20. Moran AP. Relevance of fucosylation and Lewis antigen expression in the bacterial gastroduodenal pathogen H. pylori. Carbonhydr Res 2008;343:1952-65
21. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. Helicobacter pylori adhesion binding fucosylatedhisto-blood group antigens revealed by retagging. Science 1998;279:373-7.
22. Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, et al. Helicobacter pylori Sab Aadhesin in persistent infection and chronic inflammation. Science 2002;297:573-8.
23. Yamaoka Y. Roles of Helicobacter pylori BabA in gastroduodenal pathogenesis. World J Gastroenterol 2008;14:4265-72.
24. Sheu BS, Sheu SM, Yang HB, Huang AH, Wu JJ. Host gastric Lewis expressions determine the bacterial density of babA2-genopositve H. pyloriinfection. Gut 2003;52:927-32.
25. Papini E, Zorrati M, Cover TL. In search of Helicobacter pylori Vac A mechanism of action. Toxin 2001;39:1757-67.

26. Iijima K, Sekin H, Kokie T, Imatani A, Ohara S, Shimosegawa T. Long term effect of H. pylori eradication on the reversibility of acid secretion in profound hydrochlorhydria. Aliment Pharmacol Ther 2004;19:1181-8.
27. A. Zullo, C. Hassan, L. Ridola, V. de Francesco, D. Vaira, Standard triple and sequential therapies for Helicobacter pylori eradication: an update, Eur. J. Intern. Med. 24 (2012) 16–19.
28. P. Malfertheiner, F. Megraud, C.A. O'Morain, J. Atherton, A.T. Axon, F. Bazzoli, G.F. Gensini, J.P. Gisbert, D.Y. Graham, T. Rokkas, E.M. El-Omar, E.J. Kuipers, Management of Helicobacter pylori infection—the Maastricht IV/Florence Consensus Re port, Gut 61 (2012) 646–664.
29. M. Selgrad, P. Malfertheiner, New strategies for Helicobacter pylori eradication, Curr. Opin. Pharmacol. 8 (2008) 593–597.
30. P.-L. Bardonnet, V. Faivre, P. Boullanger, J.-C. Piffaretti, F. Falson, Pre-formulation of liposomes against Helicobacter pylori: characterization and interaction with the bacteria, Eur. J. Pharm. Biopharm. 69 (2008) 908–922.
31. J.K. Patel, M.M. Patel, Stomach specific anti-Helicobacter pylori therapy: preparation and evaluation of amoxicillin-loaded chitosan mucoadhesive microspheres, Curr. Drug Deliv. 4 (2007) 41–50.
32. A. Armuzzi, F. Cremonini, F. Bartolozzi, F. Canducci, M. Candelli, V. Ojetti, G. Cammarota, M. Anti, A. De Lorenzo, P. Pola, G. Gasbarrini, A. Gasbarrini, The effect of oral administration of Lactobacillus GG on antibiotic-associated gastrointestinal side-effects during Helicobacter pylori eradication therapy, Aliment. Pharmacol. Ther. 15 (2001) 163–169.
33. M. Obonyo, L. Zhang, S. Thamphiwatana, D. Pornpattananangkul, V. Fu, L. Zhang, Antibacterial activities of liposomal linolenic acids against antibiotic-resistant Helicobacter pylori, Mol. Pharm. 9 (2012) 2677–2685.
34. P. Jain, S. Jain, K.N. Prasad, S.K. Jain, S.P. Vyas, Polyelectrolyte coated multilayered liposomes (nanocapsules) for the treatment of Helicobacter pylori infection, Mol. Pharm. 6 (2009) 563–603.
35. S.D. Georgopoulos, V. Papastergiou, S. Karatapanis, Current options for the treat- 1298 ment of Helicobacter pylori, Expert. Opin. Pharmacother. 14 (2013) 211–223
36. J.-M. Liou, C.-C. Chen, M.-J. Chen, C.-C. Chen, C.-Y. Chang, Y.-J. Fang, J.Y. Lee, S.-J. Hsu, J.-C. Luo, W.-H. Chang, Y.-C. Hsu, C.-H. Tseng, P.-H. Tseng, H.-P. Wang, U.-C. Yang, 1346 C.-T. Shun, J.-T. Lin, Y.-C. Lee, M.-S. Wu, Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial, Lancet 381 (2013) 205–213.
37. A. Patel, N. Shah, J.B. Prajapati, Clinical appliance of probiotics in the treatment of Helicobacter pylori infection — a brief review, J. Microbiol. Immunol. Infect. (2013) 1–9.
38. J. Vítor, F.F. Vale, Alternative therapies for Helicobacter pylori: probiotics and phytomedicine, FEMS Immunol. Med. Microbiol. 63 (2011) 153–164.
39. P.L. Bardonnet, V. Faivre, W.J. Pugh, J.C. Piffaretti, F. Falson, Gastroretentive dosage forms: overview and special case of Helicobacter pylori, J. Control. Release 111 (2006) 1–18.
40. C.H. Prasanthi, N.L. Prasanthi, S.S. Manikiran, N.R. Rao, Focus on current trends in the treatment of Helicobacter pylori infection: an update, Int. J. Pharm. Sci. Rev. Res. 9 (2011) 42–51.
41. Cooreman MP, Krausgrill P, Hengels KJ. Local gastric and serum amoxicillin concentration after different oral application forms. Antimicrob Agents Chemother 1993;37:1506-9.
42. J.T. Fell, Targeting of drugs and delivery systems to specific sites in the gastrointestinal tract, J. Anat. 189 (Pt 3) (1996) 517e519.
43. C. Wilson, N. Washington, The Stomach: Its Role in Oral Drug Delivery, Physiological Pharmaceutical: Biological Barriers to Drug Absorption, Chichester, U. K. Ellis Horwood. 47, 70, 1989.
44. T. Ichikawa, K. Ishihara, Protective effects of gastric mucus, in: Dr Paola Tonino (Ed.), Gastritis and Gastric Cancer - New Insights in Gastroprotection, Diagnosis and Treatments, INTECH Open Access Publisher, Croatia, 2011, pp. 3e24.
45. E.A. Kharenko, N.I. Larionova, N.B. Demina, Mucoadhesive drug delivery systems (Review), Pharm. Chem. J. 43 (4) (2009) 200e208.
46. Sinha V R, Bansal K, Kaushik R, Kumria R, Trehan A, Polycaprolactone microspheres and nanospheres, International Journal of Pharmaceutics, 2004, 278(1), 1–23.
47. Mathiowitz E ,Langer R,Polyanhydride microspheres as drug carriers I: Hot-melt microencapsulation, Journal of controlled Release, 1987, 5(1), 13-22.
48. Gabor F, Wirth M, Jurkovich B, Haberl I, Theyer G, Walcher G, Hamilton G, Lectin mediated bioadhesion: Proteolytic stability and binding characteristics of wheat germ agglutinin and Solanum tuberosum lectin on Caco-2, HT-29 and human colonocytes, Journal of Controlled Release, 1997,49,27-37.
49. Haas J, Lehr CM, Developments in the area of bioadhesive drug delivery systems, Expert Opinion on Biological Therapy, 2002,2,287-298.
50. J.D. Smart, The basics and underlying mechanisms of mucoadhesion, Adv. Drug Deliv. Rev. 57 (11) (2005) 1556e1568.
51. J.K. Vasir, K. Tambwekar, S. Garg, Bioadhesive microspheres as a controlled drug delivery system, Int. J. Pharm. 255 (1) (2003) 13e32.
52. Ludwig A, The use of mucoadhesive polymers in ocular drug delivery, Advanced Drug Delivery Reviews, 2005,57,1595-1639.
53. Fefelova N, Nurkeeva Z, Mun G, Khutoryanskiy V, Mucoadhesive interactions of amphiphilic cationic copolymers based on [2 (methacryloyloxy)ethyl]trimethylammonium chloride, International Journal of Pharmacy, 2007,33,25-32.
54. Singla AK, Chawla M, Singh A, Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: A review, Drug Development and Industrial Pharmacy, 2000,26,913-924.
55. Khutoryanskiy VV, Hydrogen-bonded interpolymer complexes as materials for pharmaceutical applications, International Journal of Pharmaceutics, 2007,334,15-26.
56. He P, Davis S, Illum L, In vitro evaluation of the mucoadhesive properties of chitosan microspheres, International Journal of Pharmaceutics, 1998,166,75-88.
57. Teijeiro Osorio D, Alonso M, Remuñán López C, Development of chitosan sponges for buccal administration of insulin, Carbohydrate Polymers, 2007,68,617-625.
58. El-Kamel A, Sokar M, Naggar V, Al-Gamal S, Chitosan and sodium alginate based bioadhesive vaginal tablets, American Association of Pharmaceutical sciences, 2002,4, 40-44.
59. Soane RJ, Frier M, Perkins AC, Jones NS, Davis SS, Illum L, Evaluation of the clearance characteristics of bioadhesive systems in humans, International Journal of Pharmaceutics, 1999,178,55-65.
60. Bravo-Osuna I, Vauthier C, Farabollini A, Palmieri GF, Ponchel G, Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles, Biomaterials, 2007,28,2233-2243.
61. Smart JD, Nicholls TJ, Green KL, Rogers DJ, Cook JD, Lectins in Drug Delivery: a study of the acute local irritancy of the lectins from Solanum tuberosum and helix pomatia, Europian Journal of Pharmaceutical Sciences,1999, 9,93–98.
62. Naisbett B, Woodley J, The potential use of tomato lectin for oral drug delivery, International Journal of Pharmaceutics,1994,107,223–230.
63. Nicholls TJ, Green KL, Rogers DJ, Cook JD, Wolowacz S, Smart JD, Lectins in ocular drug delivery, An investigation of lectin binding sites on the corneal and conjunctival surfaces, International Journal of Pharmaceutics,1996,138,175– 83.
64. Hornof M, Weyenberg W, Ludwig A, Bernkop-Schnurch A, A mucoadhesive ophthalmic insert based on thiolated poly(acrylic) acid: Development and in vivo evaluation in human volunteers, Journal of Controlled Release, 2003, 89,419–428.
65. Albrecht K, Zirm EJ, Palmberger TF, Schlocker W, BernkopSchnurch A, Preparation of thiomer microparticles and in vitro evaluation of parameters influencing their mucoadhesive properties, Drug Development and Industrial Pharmacy, 2006,32,1149–1157.
66. Liu Z, Lu W, Qian L, Zhang X, Zeng P, Pan J. In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. J ContRel 2007;102:135-44
67. PatelJK, PatelMM. Stomach specific anti-Helicobacter pylori therapy: Preparation and evaluation of amoxicillin-loaded

chitosan mucoadhesive microspheres. Curr Drug Del 2007;4:41-50.
68. Patel JK, Chavda JR. Formulation and evaluation of stomach-specific amoxicillin-loaded carbopol-934P mucoadhesive microspheres for anti-Helicobacter pylori therapy. J Microencapsul 2009;26:365-76.
69. Umamaheshwari RB, Jain S, Tripathi PK, Agrawal GP, Jain NK. Floatingbioadhesive microspheres containing acetohydroxamic acid for the clearance of Helicobacter pylori. Drug Deliv 2002;9:221-33.
70. Umamaheshwari RB, Jain S, Bhadra D, Jain NK. Floating microspheres bearing acetohydroxamic acid for the treatment of Helicobacter pylori. J Pharm Phamacol 2003;55:1607-13.
71. Wang J, Yoshihiko T, Yoshiharu D, Kazuhiro M, Yasuhiko T, Yashito I. Positively charged gelatin microspheres as gastric mucoadhesive drug delivery system for eradication of H. pylori. Drug Deliv 2000;7:237-43.
72. Keerthi Sumana Murathoti, Trapti Saxena Development of mucoadhesive microspheres of levofloxacin for the treatment of h. Pylori infection Journal of Drug Delivery & Therapeutics. 2016; 6(1):34-45
73. Venkateswaramurthy N. Sambathkumar R, Perumal P Controlled release mucoadhesive microspheres of clarithromycin for the treatment of Helicobacter Pylori infection DER PHARMACIA LETTRE