REVIEW ARTICLE

Mucoadhesive and muco-penetrating delivery systems for eradication of *Helicobacter pylori*

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Helicobacter pylori (*H. pylori*), the major culprit for peptic ulcer, has a unique way of survival in harsh acidic environment of the stomach by colonizing deep in the gastric mucosal layer. Failure of conventional therapies against *H. pylori* for complete eradication has major limitations like low residence time of delivery system in stomach, poor penetration of drug in gastric mucosa, acidic degradation of antibiotics, and development of antibiotics resistance. The poor penetration of antibiotics through thick viscoelastic mucosal gel results in incomplete eradication of *H. pylori*. Various investigators have formulated novel gastro-retentive drug delivery systems such as floating systems, mucoadhesive systems, pH-sensitive gel systems, and muco-penetrating delivery systems for increasing the concentration of antibiotic in close proximity to the site of *H. pylori* infection. This review summarizes the novel drug delivery approaches investigated during the last few years and suggests that a high eradication rate can be achieved by therapy comprising of muco-penetrating delivery systems of antibiotics against *H. pylori*.

Key words: *Colonization, eradication therapy, floating drug delivery, Helicobacter pylori, liposomes, muco-penetrating nanoparticles, mucoadhesive systems, pH-sensitive gel systems*

INTRODUCTION

Helicobacter pylori (*H. pylori*), a gram-negative microaerophilic spiral bacterium, has been found to be a major causative organism for peptic ulcer by Warren and Marshall in 1982.[1] Around half of the world's population is infected by this pathogen but only a small percentage of infected population shows clinical symptoms, which mainly depends upon the difference in bacterial virulence and hostile factor. *H. pylori* infection is present in 90–100% of duodenal ulcer patients and in 60–90% of gastric ulcer patients. Several studies have shown that *H. pylori* infection is associated with at least three- to four-fold increased risk of peptic ulcer diseases and that 10–15% of *H. pylori*-infected individuals will have peptic ulcer disease in their lifetime.[2] *H. pylori* is the major causative organism of the chronic 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 listed *H. pylori*-associated gastric carcinoma as one of the three major causes of cancer-related deaths worldwide, around 0.5 million deaths every year. Chemotherapy of gastric cancer has poor clinical efficacy; however, the eradication of *H. pylori* infection could possibly prevent gastric carcinoma and other associated diseases. It is now well established that the maximum incidence of *H. pylori* infection is more in children of 11–16 years particularly for lower socioeconomic condition due to poor level of sanitation.[9] The prevalence of *H. pylori* infection in India has been reported to be very high, ranging from 70% to 90% in patients with duodenal and peptic ulcer and 50% to 80% in patients with non-ulcer dyspepsia (NUD) as well as healthy asymptomatic adults.[10] A triple therapy containing two antibiotics and one proton inhibitor over a period of 2 weeks is recommended worldwide for eradication of *H. pylori*. But poor stability of antibiotics in acidic environment and poor permeation of antibiotics across the mucus layer cause incomplete eradication and systemic side effects leading to patient noncompliance.[11,12] To sort out these problems, several research investigations on the gastro-retentive drug

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delivery systems like floating formulations, mucoadhesive drug delivery system, pH-sensitive gel system had been carried out for increasing the gastric residence and local concentration of drug at *H. pylori*-infected site. These studies indicated the benefit of targeting drug to the gastric mucosal layer by reducing the dose of antibiotic therapy as well as increased patient's compliance. In this review, we summarize the current information on colonization of *H. pylori* and novel delivery system studies conducted in past few years, so as to utilize the information for future research on *H. pylori* eradication therapy [Figure 1].

Colonization of *H. pylori*

H. pylori is a motile pathogen which lives deep in the gastric mucus layer close to the epithelial cells. In general, after the entry of any bacteria into the stomach, gastric acidity and peristaltic movement inhibit the adhesion and colonization of the bacteria in the gastric mucus layer. The continuously secreted mucus from glands of the epithelial cell pushes bacteria toward the luminal surface, where the more acidic environment retards the colonization and motility property of the pathogens.[13-15] However, even in these hostile conditions, *H. pylori* adheres to the mucus layer and penetrates deep in the mucus membrane close to the epithelial cells due to good motility of flagellae and various adhesins present on its surface as shown in Figure 2.

Once the bacterium establishes the adhesion with the mucus layer, the enzyme urease secreted by *H. pylori* metabolizes gastric urea to produce carbon dioxide and ammonia, which produces a surrounding coat of buffered acid. Earlier it was assumed that *H. pylori* usually colonizes in the mucus just close proximate to epithelial cell and do not penetrate the epithelial cells[16] but one of the recent study showed the invasion of *H. pylori* in the intercellular space of gastric epithelial cell.[17]

Thus, colonization of *H. pylori* in the gastric mucus layer is determined by various virulent and hostile factors as listed in Table 1. Urease and flagella are two most important virulent factors for successful colonization of *H. pylori*.[15,18,19] Among hostile factors, Lewis blood group antigens are most important factors for mucosal adhesions of bacteria. Based on composition, Lewis antigen are of two types: type 1, mainly distributed in the epithelium surface contain Le^a, Le^b, and sialyl-Le^a, while type 2 located deeper in the mucous/parietal cell contain Le^X, Le^Y, and sialyl-Le^X. It is also well established that Le^b and Le^X are major hostile 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 Le^b and Le^X respectively for adhesion on gastric epithelial cell.[22,23] Sheu *et al*. (2003)[24] reported the expression of Le^b 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 amplified by production and release of vacuolating cytotoxin (VacA).[25,26]

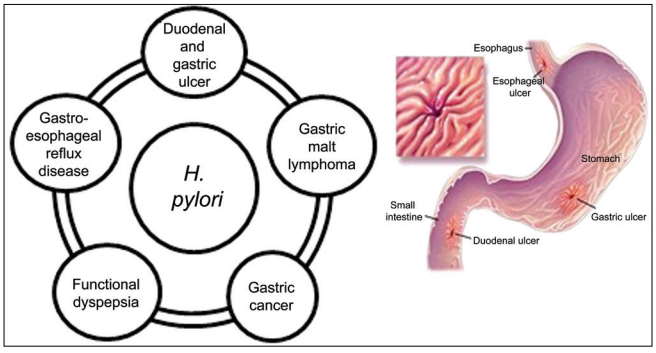

Figure 1: Pathophysiological conditions associated with *H. pylori*

Current Treatment Regimen for Eradication of *H. pylori* Treatment

Although, *H. pylori* is sensitive to many antibiotics during *in vitro* studies, yet no single antibiotic showed complete eradication *in vivo*. The various antibacterial agents used against *H. pylori* are reported in Table 2. Presently, these infections are treated with first-line triple therapy consisting of two antibiotics (amoxicillin along with clarithromycin/metronidazole) and a proton pump inhibitor (omeprazole/rabeprazole/lansoprazole). The proton pump inhibitor increases the pH within stomach to facilitate the local action of antibiotics by increasing their stability, absorption, and tissue penetration.[34,35] A quadruple treatment regimen was also tried for eradication of *H. pylori, w*hich include colloidal bismuth subcitrate (CBS), tetracycline, metronidazole, and omeprazole.[36] This therapy had advantage over the triple therapy because bismuth precipitates in and around *H. pylori* pathogens leading to lysis of the bacterial cell wall within 2 h after ingestion of drug.[37] Also heavy metals such as zinc, nickel, and bismuth compounds interfere with the activity of urease enzyme. By combining complicated therapies in a single dosage form, a capsule (Helicide) of bismuth subcitrate, metronidazole, and tetracycline, an effort has been made to improve patient compliance.[38]

Reason for Failure of *H. pylori* Treatment

In spite of various antibiotic combinations studied against *H. pylori,* none has shown complete eradication of the bacterium. The characteristic of *H. pylori* is its colonization

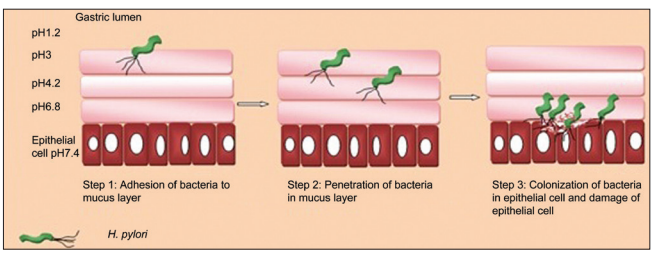

Figure 2: Steps involved in colonization of *H. pylori*

Table 1: Various virulent factors, their predictable role and association with *H. pylori*

Adhesions	Predicted role	Association with *H. Pylori*	References
Urease and its subunits urea, ureB	Survival in acidic environment, nutrient acquisition	Cytoplasmic, in part extracellular	Ghaira, 1997[27]
Flagella and flagellins FlaA, FlaB	Motility	Extracellular, but enveloped by flagellar sheath	Ghaira, 1997[27]
HspA, HspB	Heat shock proteins, Chaperonins	Intracellular, in part extracellular	Ghaira, 1997[27]
Catalase	Detoxification of oxygen radicals (H_2O_2)	Intracellular, in part extracellular	Ghaira, 1997[27]
CagA	Unknown function, marker for cag pathogenicity island	Cytoplasmic	Rad *et al.*, 2002[28]
VacA	Cytotoxic *in vitro*	Secreted extracellular	Rad *et al.*, 2002[28]
BabA	Binds to fucosylated blood group antigens on cells	It has been implicated in peptic ulcer disease and gastric cancer	Rad *et al.*, 2002[28]
SabA	Binds to sialyl-Le^a and sialyl-Le^x antigens and involved in activation of neutrophils	None	Tannes *et al.*, 2005[29]
SabB	Binding specificity is unknown	Absence of SabB expression via phase variation is associated with duodenal ulcers	Tannes *et al.*, 2005[29]
AlpA and AlpB	Inactivation of the AlpA and AlpB genes result in decreased adherence to gastric epithelial cells	Outer membrane protein	Jonge *et al.*, 2004[30]
IceA	It encodes a CATG-recognizing restriction endonuclease	It has been associated to PUD, but its association is not universal	Figueiredo *et al.*, 2000[31]
DupA	The DupA gene encodes a VirB4 ATPase homolog	Associated with duodenal ulcers but also with reduced risk for gastric atrophy and cancer	Lu *et al.*, 2005[32]
OipA	OipA assist in IL-8 induction, but this association is not universal	Expression of OipA is linked to *cag* status and development of duodenal ulcers and gastric cancer	Kudo *et al.*, 2004[33]

CagA, the protein encoded by cytotoxin-associated gene A; FlaA and B, Flagellins A and B; HspA and B, heat shock proteins A and B; VacA, vacuolating cytotoxin A; BabA, blood group antigen-binding adhesin; SabA and B, Sialic acid-binding adhesin; AlpA and B, adhesions lipo proteins; IceA, induced by contact with epithelium; OipA, Outer membrane protein; DupA, duodenal ulcer promoting protein

and survival in deep gastric mucosa and also in the intercellular space between epithelial cells.[17] Because of poor penetration power of antibiotics across the gastric mucus membrane, incomplete eradication results.[11,12]

The stability of commonly used antibiotics of triple therapy is another reason for treatment failure, which is not more than 3–4 h in gastric environment.[38] The most important issue in management of *H. pylori* is increased instances of antibiotic's resistance. *H. pylori* is known for its panmictic population structure[39,40] i.e. genetic recombination is so frequent that it randomizes the DNA sequences and generates linkage equilibrium.[40] There is general agreement that increasing antimicrobial resistance is related to the selection pressure exerted by the use of antibiotics. A significant variation in the resistance to antibiotics in *H. pylori*, especially to metronidazole, amoxicillin, and clarithromycin, has been reported across the globe. Development of resistance against antibiotic is mainly responsible for the declining rate of *H. pylori* eradication seen in many countries, more prominently in developing countries like China, India, Mexico, etc. A study in 2004 showed a 70% decline in eradication rates with clarithromycin-containing regimen in clarithromycin-resistant cases.[41]

Novel Drug Delivery Approaches for *H. pylori*

Literature review reveals that local application of antibiotics to gastric mucosa resulted in better eradication compared to systemically available antibiotic.[42] Hence, for effective eradication of *H. pylori* the drug delivery system should adequately deliver the therapeutic agent in the close proximity of the gastric mucus membrane. In recent years, various novel approaches are used for increasing the gastric residence time of the delivery system and local action of the drug in stomach as shown in Figure 3. 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 and, (iv) the swelling system for improving the gastric retention time of the system.

Table 2: Various classes of drugs used against *H. pylori* and their mechanisms of action

Class	Drugs	Mechanism	Use
β lactams	Amoxicillin, Penicillin, Ampicillin	Inhibit cell wall synthesis	Used in multi-drug eradication therapy
Macrolides	Clarithromycin, Erythromycin, Azithromycin	Inhibition of bacterial protein biosynthesis	Used in multi-drug eradication therapy
Nitroimidazoles	Metronidazole, Tinidazole	Inhibition of metabolic pathway	Used in multi-drug eradication therapy
H_2-receptor antagonists	Cimetidine, Ranitidine, Famotidine	Acid inhibition	*H. pylori*-negative peptic ulcer; replaced by PPI because of inferiority in acid suppression.
PPI	Rabeprazole, Esomeprazole, Omeprazole, Lansoprazole	Most potent acid inhibition	Standard treatment for all *H. pylori*-negative peptic ulcers; given intravenously in bleeding ulcers
Prostaglandin analogues	Misoprostol	Increase mucosal resistance	Weak acid inhibition, *H. pylori*-negative gastric ulcer; prevention of NSAID ulcers.
Bismuth salts	Bismuth subcitrate, Bismuth subsalicylate	Increases mucosal prostaglandin synthesis	In quadruple therapy for *H. pylori* eradication

Several mucosal protectives used in some countries (i.e. Sucralfate, Rebamipide, and others) do not have sufficient trial documentation to be included in the efficacy comparison with the listed standard therapies. PPI: Proton-pump inhibitor; NSAID: Non-steroidal anti-inflammatory drug

Density-based approaches involve the density difference of formulation in comparison to normal stomach content density (~1.0004 g/cm^3). These systems improve sustained release of drug along with prolonging the gastric residence time of the dosage form. In high-density based systems, tablets with 1.5–2.4 g/cm^3 densities are prepared, but effectiveness of these systems in humans have not been observed.[43] In the case of low density-based systems, bulk density is less than gastric fluid's density and remains buoyant in stomach without affecting the gastric emptying rate for a prolonged period and releases the drug slowly. By entrapment of air (e.g. hollow chambers)[44] or by incorporation of low-density materials (e.g. fatty materials, oils, or foam powder) low-density systems are prepared.[45,46] Floating drug delivery systems are based on the use of the low-density polymer or gas-generating agents. In the case of gas-generating agent, gas generated is entrapped in the polymer matrix, thus provide the floating property to the drug delivery system. Bio/mucoadhesive systems comprise of natural or synthetic polymer(s) capable of adhering to a biological membrane (bioadhesive polymer) or the mucus lining of the GIT (mucoadhesive polymer) by forming non-covalent bonds with the mucin–epithelial surfaces.[47] The adherence of the delivery system to the gastric wall increases residence time at a particular site, thereby improving bioavailability.[48] Swelling/expanding systems swell to a size that prevents their passage through the pylorus.[49] As a result, dosage form is retained in stomach for a long period of time. Sustained and controlled drug release may be achieved by selecting a polymer with high swelling properties due to physiochemical crosslink's in the hydrophilic polymeric network. These systems may also erode in the presence of gastric juices.[50]

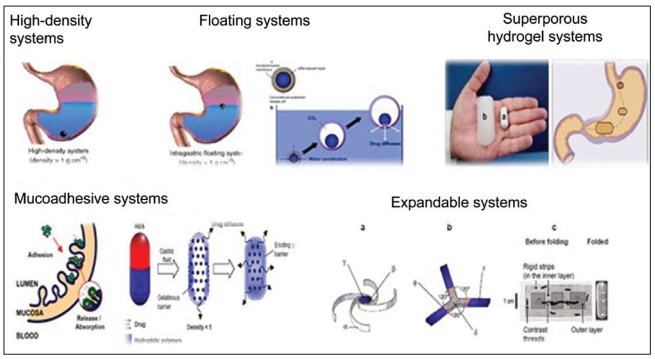

Figure 3: Various gastro-retentive drug delivery systems

Various researches based on these novel gastro-retentive systems against *H. pylori* are summarized below:

Floating systems

Floating or hydrodynamically balanced systems (HBS) are simple, most approachable systems to provide high gastric residence time, and sustained release of drug.[51] Floating tablets or buoyant systems using swellable polymers such as chitosan (CS), hydroxyl propyl methyl cellulose (HPMC), polyoxyethylene, carbopols, polycarbophils, guar gum,

xanthan gum, etc. have been prepared for various drugs with or without CO_2-generating agent.[51-54]

A gastro-retentive dosage form of clarithromycin was formulated on the principle of buoyancy using different gel forming polymers.[51] Xanthan gum showed least possible lag time (35 s) but the integrity of tablet was poor. While in the case of Carbopol 934P, the lag time was much higher (16.5 minutes) and hydroxy propyl cellulose showed undesired floating characteristics. By optimizing the sodium bicarbonate content, floating tablets with desired floating lag time of about 3 min were obtained using hydroxy propyl methyl cellulose (HPMC). The report indicated that the floating and drug release profile of the tablet depends on the ratio of gas forming agent and polymer. The *in vivo* studies in humans for evaluation of the gastric residence time of tablet were found to be 220±30 min.

Another study on floating tablets of levofloxacin utilized HPMC and gelucire in the presence of sodium bicarbonate which increased the floating lag time range from 258 to 464 s.[52] The presence of gelucire, a release retarding hydrophobic polymer, resulted in the sustained effect. Another study prepared floating matrix tablets of clarithromycin using HPMC K4M, HPMC K100LV and sodium carboxy methyl cellulose (CMC sodium) as a release-controlling polymer and sodium bicarbonate as the gas-forming agent for buoyancy, showed prolonged gastric residence time in stomach and controlled release behavior with increased bioavailability.

More than 6 h *in vivo* buoyancy was observed in the case of famotidine-floating tablets using chitosan as a polymer and sodium bicarbonate and citric acid as the effervescent-generating system.[53]

These single unit buoyant formulations are associated with drawback of "all or none" system and require sufficiently high stomach fluid to be buoyant. Hence, depending on size, floating tablets may cross over to small intestine during house-keeper waves.[55,56] This serious limitation can be overcome by making the buoyant system which also adheres to the mucous lining of the stomach wall.[57] Among various mucoadhesive polymers, CS offers a great advantage being polycationic in nature and also has some antibacterial activities. However, adhesion failure may occur when overhydration converts the chitosan gel network to slippery mucilage in gastric environment.[58] Therefore, the addition of other types of biodegradable polymers in the delivery system may provide control over the swelling of CS and thereby preventing adhesion failure.

Mucoadhesive systems

Mucoadhesive drug delivery systems are most suitable for local drug delivery in the gastric mucus layer. These systems provide an intimate contact with the mucus membrane for drug diffusion without acidic degradation. There are many theories given for the mechanisms of mucoadhesion like polyvalent adhesive interaction,[59] electrostatic attraction, H-bond formation, vander-Waal force, and other.[60] The gastric mucus mainly contains a negative charge on their surface due to the presence of carboxyl, sulfate group, and sialic acid in the mucus glycoprotein and hence mucoadhesion occurs by electrostatic attraction force. For the effective mucoadhesion, the drug delivery system should be positively charged. Figure 4 shows the different strategies for eradication of *H. pylori* by using microparticles/microspheres and nanoparticles.

Microspheres/beads

Microspheres or microcapsules are multi-particulate systems, preferred over the conventional dosage forms like tablet and capsule because of their increased surface area, thus increasing the absorption of the drug, reducing the dosing frequency, and improving the patient compliance. Such systems with a mucoadhesive property release the entrapped drug in proximity to the mucosal layer and beneficial for local treatment and enhanced bioavailability.

In 2000, Wang *et al*.[61] prepared positively charged modified gelatin microspheres of amoxicillin for the eradication of *H. pylori*. The aminated gelatin microspheres showed much higher mucoadhesion with gastric mucosa than gelatin microspheres for improving the mucoadhesion property. This is attributed to the strong electrostatic interaction between higher amino group content in modified gelatin and the mucus membrane. Increasing the concentration of the cross-linking agent like glutaraldehyde decreases the free amino group content of gelatin, which decreases the mucoadhesion property of microspheres. This study showed that the amino group content or the cationic charge on a polymer plays a crucial role in the mucoadhesion property of the particulate system.

Stability of antibiotic in acidic environment is one of the major limitations for the local delivery of drug in stomach.

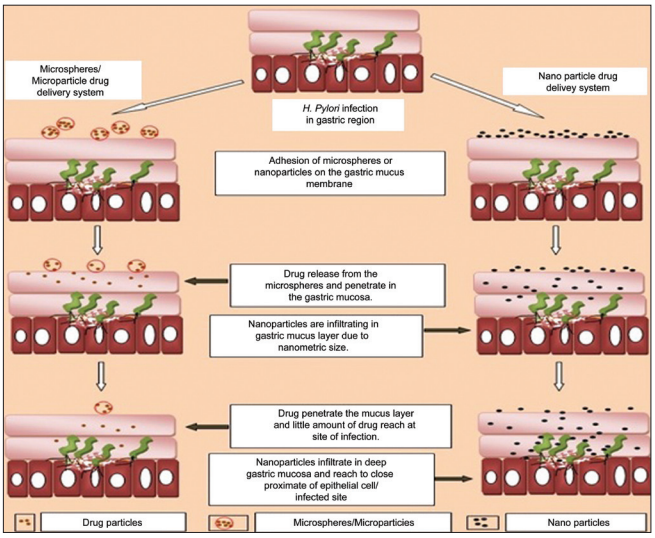

Figure 4: Different particulate strategies for *H. pylori* eradication

Several studies have been conducted for assessment of stability of antibiotics like amoxicillin, clarithromycin, metronidazole, most commonly used in *H. pylori* treatment. It is well documented that these antibiotics are unstable at acidic pH below 2.[62-64] In 2005, Liu *et al.*[65] prepared a amoxicillin-loaded mucoadhesive microsphere of ethyl cellulose and carbomer 937 by the solvent evaporation method and observed low acidic degradation of amoxicillin in the prepared microspheres. Also, mucoadhesive microspheres of amoxicillin could stay in the gastrointestinal tract for a longer period of time and resulted in higher *H. pylori* clearance.

Patel and Patel (2007) formulated chitosan mucoadhesive microspheres of amoxicillin by the emulsification phase separation technique using glutaraldehyde as a cross-linking agent.[66] Microspheres were discrete, spherical, free flowing, showing high drug entrapment efficiency and strong adherence to the gastric mucous layer. The prepared amoxicillin mucoadhesive microspheres showed increased gastric retention, gastric stability of amoxicillin, and better clearance effect than amoxicillin powder.

In 2009, Patel and Chavda[67] developed mucoadhesive amoxicillin microspheres using carbopol-934P as a mucoadhesive polymer and ethyl cellulose as a carrier polymer by the emulsion–solvent evaporation technique. The optimized formulation exhibited 80% mucoadhesion after 1 h and *in vivo H. pylori* clearance tests on amoxicillin mucoadhesive microspheres under fed conditions at single dose or multiple oral dose(s) showed better clearance effect than amoxicillin powder.

Narkar *et al.*, 2010, developed amoxicillin-loaded mucoadhesive gellan beads by the cation-induced ionotropic gelation method using acidic and alkaline cross-linking media.[68] The beads prepared in an alkaline cross-linking medium showed higher yield and entrapment efficiency than the acidic cross-linking medium. Chitosan-coated gellan beads exhibited *in-vitro* drug release up to 7 h in a controlled manner. Complete growth inhibition of *H. pylori* was observed due to good mucoadhesion of beads.

The mucoadhesive formulations face a drawback of washing out from stomach along with the mucus layer. As the mucus layer turn out from stomach, it carries the adhesive particles along with to intestine. This is a major limitation of the mucoadhesive system in the treatment of *H. pylori* eradication.

To sort out this problem, some researcher tried to formulate floating bioadhesive microspheres for prolonging the gastric retention time. In 2002, Umamaheswari *et al.* utilized both the above mechanisms for improving the gastric residence time of the drug delivery system by preparing cellulose acetate butyrate (CAB)-coated cholestyamine microcapsule of acetohydroxamic acid (AHA) along with a gas-generating agent, sodium bicarbonate.[69] The buoyancy of prepared microcapsules was about 85% till 12 h, sufficient time for effective delivery of drug to stomach. Higher coat to core ratio and sodium bicarbonate content resulted in high buoyancy. Because of CAB coating on the cholestyramine, electrostatic charge got hindered which decreased the mucoadhesion of microcapsules compared to the uncoated resin–drug complex. In 2003, the same group of researcher prepared AHA-loaded polycarbonate (PC) hollow microspheres by the emulsion solvent evaporation method in the size range of 240–288 mm for improving the gastric retention property of dosage form.[70]

Ishak *et al.* 2006 prepared chitosan-treated alginate beads of metronidazole by the ionotropic gelation method.[71] Histopathological examination of mice stomach after the *in vivo H. pylori* clearance test using 5, 10, 15, and 20 mg/ kg as single daily dose for 3 successive days showed 100% clearance rate with 15 mg/kg dose of metronidazole-loaded chitosan-treated alginate beads in comparison to only 33.33% with 20 mg/kg dose of metronidazole suspension.

In 2007, Rajnikanth and Mishra prepared floating bioadhesive beads of AHA using gellan gum as a polymer.[72] AHA can permeate intact bacterial cells and play an important role in the chemotactic motility of *H. pylori*.[73] Gellan gum has the property of temperature dependent and cation-induced gelation property. The prepared floating beads of gellan by cross linking with calcium ions showed the barrier property in acidic medium and also resulted in the sustained release matrix system.

Mucoadhesive *in situ* Gels

The liquid gel system has an advantage of patient compliance due to ease of administration. Also, the mucoadhesive polymeric gel system forms a protective layer over the gastric mucosal surface, thus preventing mucosa from further acidic damage.

In 2007, Rajnikanth *et al.* prepared floating *in situ* gel system of gellan gum and calcium carbonate for local delivery of amoxicillin in stomach.[74] The formulation showed sol to gel rheological property due to gelation of gellan in acidic environment and cross linking with calcium ion. In an acidic condition, carbon dioxide generated from calcium carbonate got entrapped in the gel matrix to show buoyancy. Higher concentration of calcium carbonate resulted in rapid and rigid gel in minimum time, which helped the formulation to stay in stomach for more than 24 h against peristaltic movement. The *in vivo H. pylori* clearance study showed higher efficacy of amoxicillin *in situ* gel in dose of 1.0 mg/kg compared to 10 mg/kg amoxicillin suspension.

Mucoadhesive Nanoparticles

Floating or mucoadhesive microparticulate systems release

their content in gastric medium or on the surface of gastric mucosa. Thus, the mucus penetration property of drug is essential for effective clearance of *H. pylori*. So it is believed that particle with nanometric size with mucoadhesive property can adhere to gastric mucosa and infiltrate deep into the mucus membrane toward the gastric epithelial cell and even protect the drug from degradation by acidic environment.

Nanoparticles are made from biocompatible and biodegradable materials such as polymers, either natural (gelatin, albumin), synthetic (polylactides, polyalkyl cyanoacrylates), or solid lipids. In body, drug loaded in nanoparticles is released from matrix by diffusion, swelling, erosion, or degradation. Advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, drug targeting and feasibility of variable routes of administration including oral and inhalation.

In 2008, Ramteke and Jain prepared and evaluated oral mucoadhesive-sustained release nanoparticles of clarithromycin and omeprazole against *H. pylori* in order to improve patient compliance and therapeutic effect by reducing dose-related side effects.[75] This study observed the long residence period of drug-containing nanoparticles in the stomach, which is not possible with conventional systems. They concluded that drug-containing gliadin nanoparticles with dual therapy may provide greater antibacterial activity than the plain drug formulations.

In 2010, Chang *et al.* prepared amoxicillin-loaded chitosan-poly–g-glutamic acid nanoparticles by the ionic gelation method and incorporated in the pH-sensitive calcium alginate–gelatin gel for preventing the acidic degradation of nanoparticles.[76] The effect of pH on the stability of chitosan-poly–g-glutamic acid nanoparticles indicated the instability and protonation of the carboxylic group of g-glutamic acid at pH 1.2 and pH 7.0, resulting in collapse of nanoparticles. Degradation of nanoparticles at pH 1.2 results in burst release of drug and hence for controlled drug release and stability of nanoparticles, incorporation of nanoparticles in pH-sensitive gel was proposed. According to researcher's proposal, hydrogels first adhere to the gastric mucosa in acidic environment and then swell or disintegrate, followed by release of incorporated nanoparticles at suitable pH condition of the gastric mucosal surface. This resulted in deep infiltration of drug-loaded nanoparticles into the mucus membrane. In the presence of type A gelatin, a positively charged polymer, sodium alginate, interacts with Ca^{2+} ions to form polyionic hydrogel. The swelling characteristics of the gel studied at different pH conditions showed shrinking of hydrogel at acidic pH ($\leq$4) due to hydrogen bond formation between the -COOH and -OH group, which helps in protection of entrapped nanoparticles from acidic degradation. At higher pH, the carboxylic acid group of alginate gets deprotonated, resulting in formation of carboxylate ions, which cause an expulsive force within the gel due to electrostatic repulsion to release of the incorporated nanoparticles.

Moogooee *et al.* (2011) prepared novel amoxicillin-loaded nanoparticles using cross-linked *N*-isopropyl acrylamide-acrylic acid-hydroxyethyl methacrylate, which due to its mucoadhesive property delivered the drug in close proximate of gastric mucosa.[77]

Liposomes

Liposomes are concentric bilayered vesicles mainly composed of natural and synthetic phospholipids enclosing an aqueous core. Lipid molecules are usually phospholipid–amphipathic moieties with a hydrophilic head group and two hydrophobiclipidic tails. On addition of excess water, such lipidic moieties spontaneously originate to give the most thermodynamically stable conformation, in which polar head groups face outwards into aqueous medium, and the lipidic chains turns inward to avoid the water phase, giving rise to double layer or bilayer lamellar structures. Both water and lipid soluble drugs can be entrapped into the liposomes. Hydrophilic drugs can be entrapped in aqueous environment and lipophilic drugs remain within the bilayer region.

Umamaheshwari and Jain, 2004, formulated AHA-loaded phosphatidyl ethanolamine (PE) liposomes anchored polyvinyl alcohol (PVA) xerogel beads (lipobeads) as a receptor-mediated drug delivery system for use in blocking adhesion of *H. pylori*.[78] PVA beads containing AHA were prepared by emulsification followed by the low-temperature crystallization method. Surface acylation with a fatty acid chain was accomplished by treating PVA bare beads with palmitoyl chloride. The inhibitory efficacy of lipobeads was significantly higher compared to that of PVA bare beads, proving their potential as the targeted drug delivery system in the treatment of *H. pylori*.

Bardonnet *et al.*, 2009, prepared fucosylated-targeted liposomes loaded with antimicrobial agents (ampicillin and metronidazole) against *H. pylori*.[79] Incorporation of fucosyled glycolipids in the vesicle membrane leads to liposome–bacteria interactions, in both spiral and coccoid forms of bacteria. The formulated liposomes seemed to be promising against *H. pylori* infection.

Jain *et al.*, 2009, designed a gastro-retentive drug delivery system incorporated with amoxicillin and metronidazole, specifically suited for the eradication of *H. pylori*.[80] The system possessed the advantages of both vesicular and particulate carriers, and it was prepared by alternative coating of polyanion (poly(acrylic acid), PAA) and polycation (poly(allylamine hydrochloride), PAH) using liposomes as the core. They compared the conventional liposomes and the polyelectrolyte based multi-layered system (nanocapsules)

and found that multi-layered system gave prolonged drug release in simulated gastric fluid, suitable for drug delivery against *H. pylori* infection. Newly developed composite nanocapsules of combination therapy proved to have commendable potential in *H. pylori* eradication as compared to conventional drug delivery systems.

Targeted Nanoparticles

The most prominent advantage of the nano-colloidal drug delivery system over the conventional delivery system is the option of selective delivery to the site of action and hence termed as targeted drug delivery. Active targeting can be achieved by modification of the polymeric colloidal particles' surface by attaching some ligand or targeting entity, which have affinity toward bacteria.

Some researchers found lectin-binding affinity of carbohydrates present on microorganisms such as *Helicobacter*[81,82] and *Streptococcus*.[83] In 2000, Khin*et al*. found mannose- and fucose-specific carbohydrate residue on bacterial surface during the lectin–agglutination assay.[84] Probably, these residues may help in adherence of bacteria to the gastric mucosal layer or epithelial cells. A carbohydrate moiety present on bacterial surface may contribute in targeting the drug delivery system for *H. pylori*. Hence, it was proposed that the lectin-conjugated nanoparticulate system may bind to the carbohydrate residue present on the bacterial surface and hence shall play an effective tool for eradication of *H. pylori*.

Umamaheswari and Jain (2003) prepared AHA-loaded glidian nanoparticles conjugated with lectins [UlexEuropaeus Agglutinin I (UEA I) and Conconavalin A (Con A)] with average size of 412 nm, zeta potential 26 mV, and 72% drug entrapment efficiency.[85] Lectin conjugation inhibited initial burst release of AHA due to covering of surface adsorbed drug. Also, lectin conjugation increased interaction of nanoparticles with mucus by four folds and interaction between *H. pylori* and lectin-conjugated formulation as confirmed by the agglutination assay. *In vitro H. pylori* growth inhibition results showed almost two-fold increased inhibition for lectin-conjugated glidian nanoparticles than glidian nanoparticles.

In 2008, Ramteke *et al*. also studied lectin-conjugated nanoparticles containing drug combinations of amoxicillin, clarithromycin, and omeprazole, a standard therapy for eradication of *H. pylori*.[86] The triple drug regimen showed a synergetic and additive effect on the eradication study. The same research group in 2009 reported triple drug therapy-based fucose-conjugated chitosan–glutamate nanoparticles for eradication of *H. pylori*.[38] The triple therapy included proton pump inhibitor, omeprazole, and antibiotics, amoxicillin and clarithromycin, that minimized the chances of drug resistance and provided better gastric stability.[87,88] The ionic interaction of chitosan and glutamic acid decreased the rapid release of the drug from the nanoparticles due to low solubility of glutamate salt in acidic medium. Conjugation of fucose on chitosan–glutamate nanoparticles may assist targeting of nanoparticles on *H. pylori* and also help in sustained release of drug from nanoparticles (~80% in 8 h). Histopathological studies after the *H. pylori* eradication study showed higher eradication than plain drug or simple nanoparticles. Thus, systems with increased potential of gastric retentivity due to the mucoadhesive property of polymer and with targeting affinity for *H. pylori* in the presence of lectins and fucose on the nanoparticles surface could be one of the possible delivery systems for eradication of *H. pylori* in near future.

Muco-penetrating Systems

Mucoadhesion though increases the gastric residence time of particles, thick viscoelastic mucosal gel does not allow antimicrobial drugs to penetrate through it uniformly. Swelling of polymer may hinder docking it in gastric mucus and strong mucoadhesion decreases mobility and thus inter-penetrability into mucus.[89] In addition, gastric motility and proteolytic activity make mucus turnover intense, thereby making gastric residence of formulation shorter. Hence, efficient adherence to mucus could make the system incapable of penetrating across the mucus layer and entering the underlying epithelia.[90]

To overcome limitations of mucoadhesive systems, particulate systems are required to penetrate the mucus membrane and deliver the drug in close proximity to the site of *H. pylori* infection. Many researchers reported particulate systems capable of penetrating the mucus membrane. Some of these reports include polyethylene glycol (PEG)-coated polystyrene-based non-adhesive nanoparticles effectively penetrating sputum of cystic fibrosis patients,[91] PEG-PSA(poly sebacic acid)-based biodegradable nanoparticles rapidly penetrating the human mucus barrier,[92] insulin-loaded polyethylene glycol-grafted chitosan (PEG-g-chitosan) nanoparticle for the nasal absorption,[93] DNA-coated biodegradable (poly lactide co-glycolic acid) PLGA nanoparticle for the gene delivery in gastric mucus.[89] These studies emphasized on modifying the surface chemistry of the particulate system such as CS, to minimize the mucoadhesion property by shielding the cationic charge. Along with shielding charge, particle size may also play a very crucial role in penetration of particles. Particle size less than mesh size of mucin fiber are reported to exhibit good mucin penetration property.[76]

In 2009, Lin *et al*.[90] prepared pH responsive chitosan–heparin nanoparticles for stomach-specific delivery of antibiotics in *H. pylori* eradication. They used chitosan for its mucoadhesive and antimicrobial properties and heparin for its anticoagulant property in accelerating ulcer healing and mucosal regeneration. For gastric mucosal adhesion, infiltration of nanoparticles deep into the mucus layer and effective delivery of drug near epithelial cells, the particulate

system must have smaller size less than 200 nm and a low zeta potential value.[94-96] Particle size of nanoparticles at pH 1.2–2.5 was larger compare to that at pH 4.5–6.5 which may be due to the protonation of amino groups [NH_3] of chitosan and the carboxylic ions [$-COO^-$] on heparin at low pH resulting in a polyelectrolyte complex with relatively weaker electrostatic interaction. The study revealed that nanoparticles are stable in acidic conditions and able to protect the antibiotic agent. Prepared nanoparticles infiltrate deep in to mice stomach where breakdown of nanoparticles resulted in drug release due to lysosomal degradation and cytoplasm pH of epithelial cells. This pH-sensitive nanoparticulate system satisfied requisite conditions for drug delivery against *H. pylori*.

Another study by Arora *et al*, 2011,[97] showed a novel muco-penetrating CS-ALG PEC nanoparticulate system composed of chitosan and sodium alginate. According to researchers, by electrostatic interactions between anionic groups from sodium alginate and cationic groups from chitosan, a microporous matrix structure of the polyelectrolyte complex can be formed which results in decreased mucoadhesion and increased muco-penetration as well as localization of nanoparticles in the deep mucosal region can be obtained. Results proved the concept of increased mobility of nanoparticles in the gastric mucus by decreasing the surface charge on polymers like chitosan and its utilization for transmucosal delivery of antibacterial drugs in eradication of *H. pylori*.

A summarized report on the novel delivery systems investigated against eradication of *H. pylori* is presented in Table 3.

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 remarkable 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 nanoparticulate system 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 eradication of *H. pylori.* These systems are associated with major problems like stability on prolonged storage, consistency of drug entrapment, and drug release and industrial scale up.

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Table 3: An over view of cited drug delivery approaches for *H. Pylori* eradication

Polymers used	Drug	Mechanism	Salient features	Reference
A-Matrix tablets				
HPMC K4M, Carbopol, Xanthan Gum	Clarithromycin	Floating system in presence of $NaHCO_3$	*In vitro* FLT-33 s, Buoyancy>12 h, *In-vivo* Gastric retention 220 min	Nama *et al.*, 2008[51]
Gelucire43/01, HPMC K4M, sod. carbonate	Levofloxacin	Floating tablet	*In –vitro* floating time – 10 hours, FLT decrease with increase in HPMC conc.	Thakker *et al.*, 2008[52]
HPMC, Sod. CMC	Clarithromycin	Controlled release floating tablets	*In –vitro* floating time – 12.5 h, percent drug release at 12 h was 99.99%	Barhate *et al.*, 2009[53]
Chitosan, MCC	Famotidine	Floating tablets	Buoyancy time more than 6 h	Gnanaprakash *et al*., 2010[54]
B-Mucoadhesive Microsphers/beads				
Modified gelatin	Amoxicillin	Microspheres	Increase mucoadhesion	Wang *et al.*, 2000[61]
Ethyl cellulose and carbomer 937	Amoxicillin	Microspheres	Protection of drug in stomach	Liu *et al.*, 2005[65]
chitosan	Amoxicillin	Microspheres	Increased gastric retention, gastric stability of drug and better *H. pylori* clearance effect than amoxicillin powder.	Patel and Patel, 2007[66]
Carbopol-934P	Amoxicillin	Microspheres	80% mucoadhesion after 1 h, Better *H. pylori* clearance effect than amoxicillin powder	Patel and Chavda 2009[67]

Contd...

Table 3: (*Contd*..)

Polymers used	Drug	Mechanism	Salient features	Reference
CAB and cholestyamine	AHA	Microspheres	Increase gastric retention time up to 12 h	Umamaheswari *et al*., 2002[69]
Polycarbonate	AHA	Microspheres	Increase gastric retention time up to 12 hours	Umamaheswari *et al*., 2003[70]
Chitosan and sodium alginate	Metronidazole	Beads	High *in vivo* H. pylori activity with 50% reduction in dose	Ishak *et al*., 2006[71]
Gellan gum	Clarithromycin	Beads	Better *H. pylori* clearance effect than clarithromycin suspension	Rajnikanth and Mishra, 2008[72]
C-Mucoadhesive Gels				
Gellan gum in the presence of calcium carbonate	Amoxicillin	*In-situ* gel in acidic pH	High *in vivo* H. pylori clearance with 1.0 mg/kg amoxicillin *in situ* gel compare to 10 mg/kg amoxicillin suspension	Rajnikanth *et al*., 2007[74]
D-Mucoadhesive Nanoparticles				
Gliadin	Clarithromycin and omeprazole	Nanoparticles	The gliadin nanoparticles showed high drug entrapment, yield and greater eradication *in vitro* effect.	Ramteke and Jain, 2008[75]
Chitosan, Poly-γ-glutamic acid Sod. alginate, Gelatin	Amoxicillin	Nanoparticles in pH-sensitive gel	Increased drug stability in stomach and sustained drug release	Chang *et al*., 2010[76]
Cross-linked *N*-isopropyl-acrylamide-acrylic acid- hydroxyethyl methacrylate	Amoxicillin	Nanoparticles	Increases drug penetration in gastric mucosa	Moogooee *et al*., 2011[77]
E-Liposomes				
Phosphatidyl ethanolamine, polyvinyl alcohol	AHA	Multilayered liposomal delivery	Mucoadhesion, active targeting at the bacterial surface hence higher eradication rate	Umamaheshwari and Jain, 2004[78]
Fucose-linked glycolipids	Ampicillin, metronidazole	Multilayered liposomal delivery	The presence of cholesterol in liposomes helped in specific interaction with *H. pylori* surface and better eradication in presence of antibiotics.	Bardonnet *et al*., 2008[79]
Poly acrylic acid, poly allylamine liposomes	Amoxicillin, metronidazole	Multilayered liposomal delivery	The system showed prolonged drug release in SGF, successful *in vitro* activity and surface binding to *H. pylori*	Jain *et al*., 2009[80]
F-Targeted Nanoparticles				
UEA I and Con A-conjugated gliadin nanoparticles	AHA	Nanoparticles	Mucoadhesion, active targtting at the bacterial surface hence higher eradication rate	Umamaheswari and Jain, 2003[85]
Lectin-conjugated gliadin nanoparticles	Amoxicillin, Clarithromyci nomeprazole	Nanoparticles	Triple therapy show better eradication profile due to synergetic and additive effect with active targeting	Ramteke *et al*., 2008[86]
Fucose-conjugated chitosan -glutamate	Amoxicillin, Clarithromycin, nomeprazole	Nanoparticles	Controlled the release behavior of drug from nanoparticles with selective targeting	Ramteke *et al*., 2009[38]
G-Mucopenetrating Nanoparticles				
Chitosan	-	Nanoparticles	Muco-penetration into deep gastric mucosal layer	Lin *et al*., 2009[90]
Chitosan– Alginate	Amoxicillin	Nanoparticles	Muco-penetration into deep gastric mucosal layer	Arora *et al*., 2010[97]

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. pylori*infection. 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. Ghiara P. Therapeutic intragastric vaccination against *Helicobacter pylori* in mice eradicates an otherwise chronic infection and confers protection against reinfection. Infect Immun 1997;65:4996-5002.
28. Rad R, Gerhard R, Lang M. The *Helicobacter pylori* blood group antigen-binding adhesin facilitates bacterial colonization and augments a nonspecific immune response. J Immunol 2002;168:3033-41.
29. Tannaes T, Bukholm IK, Bukholm G. High relative content of lysophospholipids of *Helicobacter pylori* mediates increased risk for ulcer disease. Immunol Med Microbiol 2005;44:17-23.
30. Jonge D, Durrani RZ, Rijpkema SG. Role of the *Helicobacter pylori* outer-membrane proteins AlpA and AlpB in colonization of the guinea pig stomach. J Med Microbiol 2004;53:375-9.
31. Figueiredo C, Quint WG, Sanna R. Genetic organization and heterogeneity of the iceA locus of *Helicobacter pylori*. Gene 2000;246:59-68.
32. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology 2005;128:833-48.
33. Kudo T, Nurgalieva ZZ, Conner ME, Crawford S, Odenbreit S, Haas R. Correlation between *Helicobacter pylori*OipA protein expression and oipAgene switch status. J Clin Microbiol 2004;42:2279-81.
34. Pedrazzoli J Jr, Calafatti SA, Ortiz RA, Dias FE, Deguer M, Mendes FD, *et al*. Transfer of clarithromycin to gastric juice is enhanced by omeprazole in *Helicobacter pylori*-infected individuals. Scand J Gastroenterol 2001;36:1248-53.
35. Uygun A, Kadayifci A, Yesilova Z, Ates Y, Safali M, Ilgan S, *et al*. Poor efficacy of ranitidine bismuth citrate – based triple therapies for eradication. Indian J Gastroenterol 2007;26:174-7.
36. Bhatia SJ, Kulkarni SG. Cost effectiveness of *Helicobactor pylori* Eradication in India: To live and let live… expensively?. Indian J Gastroenterol 1997;16:S25.
37. Kato M, Yamaoka Y, Kim JJ, Reddy R, Asaka M, Kashina K, *et al*. Regional differences in metronidazole resistance and increasing clarithromycin resistance among *H. pylori* isolates from Japan. Antimicrob Agent Chemother 2000;44:2214-6.
38. Ramteke S, Ganesh N, Bhattacharya A, Jain NK. Amoxicillin, clarithromycin, and omeprazole based targeted nanoparticles for the treatment of *H. pylori*. J Drug Targeting 2009;17:225-34.
39. Achtman M, Azuma T, Berg DE, Ito Y, Morelli G, Pan ZJ, *et al*. Recombination and clonal groupings within *Helicobacter pylori* from different geographical regions. Mol Microbiol 1999;32:459-70.
40. Suerbaum S, Smith JM, Bapumia K, Morelli G, Smith NH, Dyrek EKI,*et al*. Free recombination within *Helicobacter pylori*. Proc Nat Acad Scien 1998;95:12619-24.
41. Mégraud F. *H. pylori* antibiotic resistance: Prevalence, importance, and advances in testing. Gut 2004;53:1374.
42. Cooreman MP, Krausgrill P, Hengels KJ. Local gastric and serum amoxicillin concentration after different oral application forms. Antimicrob Agents Chemother 1993;37:1506-9.
43. Moes AJ. Gastric retention systems for oral drug delivery. Business Briefing: Pharmatech; 2003. p. 157-9.
44. Krogel I, Bodmeier R. Development of a multifunctional matrix drug delivery system surrounded by an impermeable cylinder. J Control Release 1999;61:43-50.
45. Sriamornsak P, Thirawong N, Puttipipatkhachorn S. Emulsion gel beads of calcium pectinate capable of floating on the gastric fluid: Effect of some additives, hardening agent or coating on release behavior of metronidazole. Eur J Pharm Sci 2005;24:363-73.
46. Streubel A, Siepmann J, Bodmeier R. Floating microparticles based on low density foam powder. Int J Pharm 2002;241:279-92.

47. Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Del Rev 2009;61:158-71.
48. Wilding IR, Davis SS, O'Hagan DT. Targeting of drugs and vaccines to the gut. Pharmacol Ther 1994;62:97-124.
49. Caldwell LJ, Gardner CR, Cargill RC. Drug delivery device which can be retained in the stomach for a controlled period of time. US Patent 4735804, April 5,1988.
50. Kagan L, Hoffman A. Systems for region selective drug delivery in the gastrointestinal tract: Biopharmaceutical considerations. Expert Opin Drug Del 2008;5:681-92.
51. Nama M, Gonugunta CSR, Veerareddy PR. Formulation and evaluation of gastroretentive dosage forms of Clarithromycin. AAPS PharmSciTech 2008;9:231-37.
52. Thakkar VT, Shah PA, Soni TG, Parmar MY, Gohel MC, Gandhi TR. Fabrication and evaluation of levofloxacin hemihydrate floating tablet. Research in Pharmaceutical Sciences 2008;3:65-72.
53. Barhate SD, Patel MM, Patil AB, Pawar SR, Rathi SR.Formulation and optimization of controlled released floating tablets of Clarithromycin. J Pharm Res 2009;2:445-8.
54. Gnanaprakash K, Chandhrashekhar KB, Chetty CM. Floating tablets of Famotidine with natural polymer: An approach for gastric treatment. J Pharm Sci Res 2010;2:657-62.
55. Timmermans J, Andre JM. Factors controlling the buoyancy and gastric retention capabilities of floating matrix capsules: New data for reconsidering the controversy. J Pharm Sci 1994;83:18-24.
56. Vyas SP, Khar RK. Gastroretentive systems in: Controlled drug delivery: Concepts and advances. 1st ed. New Delhi: Vallabh Prakashan; 2002. p.196-217.
57. Chitnis VS, Malshe VS, Lalla JK. Bioadhesive polymers-synthesis, evaluation and application in controlled release tablets. Drug Dev Ind Pharm 1991;17:879-92.
58. Asane GS, Nirmal SA, Rasal KB, Naik AA, Mahadik MS, Rao YM. Polymers for mucoadhesive drug delivery system: A current status. Drug Dev Ind Pharm 2008;34:1246-66.
59. Cone RA. Mucus. In: Mucosal Immunology. In: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR, editors. 2nd ed. San Diego: Academic Press; 1999. p. 43-64
60. Lehr CM, Bodde HE, Bouwstra JA, Junginger HE. A surface energy analysis of mucoadhesion. II. Prediction of mucoadhesive performance by spreading coefficients. Eur J Pharm Sci 1993;1:19-30.
61. 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.
62. Mascher HJ, Kikuta C. Determination of amoxicillin in human serum and plasma by high-performance liquid chromatography and on-line post column derivatisation. J Chromatogr A 1998;812:221-6.
63. Erah PO, Goddard AF, Barrett DA, Shaw PN, Spiller RC. The stability of amoxicillin, clarithromycin and metronidazole in gastric juice: Relevance to the treatment of *Helicobacter pylori* infection. J Antimicrob Chemother 1997;39:5-12.
64. Chadha R, Kashid N, Jain DV. Kinetic studies of the degradation of an amino penicillin antibiotic (amoxicillin trihydrate) in aqueous solution using heat conduction microcalorimetry. J Pharm Pharmacol 2003;55:1495-503.
65. 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.
66. PatelJK, PatelMM. Stomach specific anti-*Helicobacter pylori* therapy: Preparation and evaluation of amoxicillin-loaded chitosan mucoadhesive microspheres. Curr Drug Del 2007;4:41-50.
67. 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.
68. Narkar M, Sher P, Pawar A. Stomach-specific controlled release gellan beads of acid-soluble drug prepared by ionotropic gelation method. AAPS PharmSciTech 2010;11:267-77.
69. Umamaheshwari RB, Jain S, Tripathi PK, Agrawal GP, Jain NK. Floating-bioadhesive 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. Ishak AHI, Gehanne ASA, Mortada ND. Preparation, *in vitro* and *in vivo* evaluation of stomach-specific metronidazole-loaded alginate beads as local anti-*Helicobacter pylori* therapy. J Cont Rel 2007;119:207-14.
72. Rajnikanth PS, Mishra B. Preparation and *in vitro* characterization of Gellan based floating beads of acetohydroxamic acid for eradication of *H. pylori*. Acta Pharm 2007;57:413-27.
73. El. Nujumi AM, Dorrian CA, Chittajallu RS, Neithercut WD, McColl KE. Effect of inhibition of *Helicobacter pylori* urease activity of acetohydroxamic acid on serum gastrin in duodenal ulcer subjects. Gut 1991;32:866-70.
74. Rajnikanth PS, Balasubramaniam J, Mishra B. Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of *Helicobacter pylori*. Int J Pharm 2007;335:114-22.
75. Ramteke S, Jain NK. Clarithromycin and omeprazole-containing gliadin nanoparticles for the treatment of *Helicobacter pylori*. J Drug Targeting 2008;16:65-72.
76. Chang CH, Lin YH, Yeh CL, Chen YC, Chiou SF, Hsu YM, *et al.* Nanoparticles incorporated in pH-sensitive hydrogels asamoxicillin delivery for eradication of *Helicobacter pylori*. Biomacromolecules 2010;11:133-42.
77. Moogooee M, Ramezanzadeh H, Jasoori S, Omidi Y, Davaran S. Synthesis and *in vitro* studies of cross- linked hydrogel nanoparticlescontaining amoxicillin. J Pharm Sci 2011;100:1057-66.
78. Umamaheshwari RB, Jain NK. Receptor-mediated targeting of lipobeads bearing acetohydroxamic acid for eradication of *Helicobacter pylori*. J ContRel 2004;99:27-40.
79. Bardonnet PL, Faivre V, Boullanger P, Ollivon M, Falson F. Glycosylated liposomes against *Helicobacter pylori*: Behavior in acidic conditions. Biochem Biophys Res Commun 2009;383:48-53.
80. Jain P, Jain S, Prasad KN, Jain SK, Vyas SP. Polyelectrolyte coated multilayered liposomes (nanocapsules) for the treatment of *Helicobacter pylori* infection. Mol Pharm 2009;6:593-603.
81. Wong KH, Skelton SK, Feeley JC. Strain characterization and grouping of *Campylobacter jejuna* and *Campylobacter coli* by interaction with lectins. J Clin Microbiol 1986;23:407-10.
82. Ascencio F, Guruge JL, Ljungh A, Megraud F, Wei S, Wadstrm T. Lectin typing of *Helicobacter pylori*. In: Malfertheiner P, Ditschuneit H, editors. *Helicobacter pylori*, gastritis and peptic ulcer. Berlin: Springer Verlag; 1990. p.105-9.
83. Kellens JIC, Jacobs JA, Peumans WJ, Strobbersingh EE. The agglutination of a haemolytic streptococcus by lectins. J Med Microbiol 1993;39:440-8.
84. Khin MM, Hua JS, Ng HC, Wadstrom T, Bow H. Agglutination of *Helicobacter pylori* coccoids by lectins. World J Gastroenterol 2000;6:202-9.
85. Umamaheshwari RB, Jain NK. Receptor mediated targeting of lectin conjugated gliadin nanoparticles in the treatment of *Helicobacter pylori*. J Drug Target 2003;11:415-24.
86. Ramteke S, Ganesh N, Bhattacharya S, Jain NK. Triple therapy-based targeted nanoparticles for the treatment of *Helicobacter pylori*. J Drug Target 2008;16:694-705.
87. Unge P, Gad A, Gnarpe H, Olsson J. Does omeprazole improve antimicrobial therapy directed towards gastric *Campylobacter pylori* in patients with antral gastritis–a pilot study. Scand J Gastroenterol 1998;24:49-54.
88. Sjostrom JE, Fryklund J, Kuhler T, Larsson H. *In vitro* antibacterial activity of omeprazole and its selectivity for Helicobacter spp. are dependent on incubation conditions. J Med Microbiol 1996;441:425-33.
89. Dawson M,Krauland E, Wirtz D, Hanes J. Transport of polymeric nanoparticle gene carriers in gastric mucus. Biotechnol Prog 2004;20:851-7.
90. Lin YH, Chang CH, Wu YS, Hsu YM, Chiou SF, Chen YJ. Development of pH-responsive chitosan/heparin nanoparticles for stomach- specific anti-*Helicobacter pylori* therapy. Biomaterials 2009;30:3332-42.

91. Suk JS, Lai SK, Wang YY, Ensign LM, Zeitlin PL, Boyle MP, *et al.* The penetration of fresh undiluted sputum expectorated by cystic fibrosis patients by non-adhesive polymer nanoparticles. Biomaterials 2009;30:2591-7.
92. Tang BC, Dawson M, Lai SK, Wang YY, Suk JS, Yang M, *et al.* Biodegradable polymer nanoparticles that rapidly penetrate the human mucus barrier. Proc Natl Acad Sci U S A 2009;106:19268-73.
93. Zhang X, Zhang H, Wu Z, Wang Z, Niu H, Li C. Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles. Eur J Pharm Biopharm 2007;68:526-34.
94. van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. Chitosan and its derivatives in mucosal drug and vaccine delivery. Eur J Pharm Sci 2001;14:201-7.
95. Fernandez-Urrusuno R, Romani D, Calvo P, Vila-Jato JR, Alonso MJ. Development of a freeze-dried formulation of insulin-loaded chitosan nanoparticles intended for nasal administration. STP Pharma Sci 1999;9:429-36.
96. van der Lubben IM, Verhoef JC, van Aelst A, Borchard G, Junginger HE. Chitosan microparticles for oral vaccination: Preparation, Characterization and preliminary *in vivo* uptake studies in murine Peyer's patches. Biomaterials 2001;22:687-94.
97. Arora S, Gupta S, Narang RK, Budhiraja RD. Amoxicillin loaded chitosan–alginate polyelectrolyte complex nanoparticles as muco-penetrating delivery system for *H. pylori*. Sci Pharm 2011;79:673-94.

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