REVIEW ARTICLE

DRUG DELIVERY STRATEGIES FOR *HELICOBACTER PYLORI* INFECTION MANAGEMENT: AN OVERVIEW

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ABSTRACT

Helicobacter pylori, profoundly termed as *H. pylori*, *is* a gram negative microorganism and a main causative pathogen for gastritis, peptic ulcers, duodenal ulcers and mucosa associated lymphatic tissue (MALT) lymphoma that leads to gastric cancer in infected patients, if uncontrolled in the stipulated time. Until 1994, half of the total world population was suffering from *H. pylori* infection. Based on a WHO survey it is predicted that by 2020, *H. pylori* infection will be the top ten of the leading causes of death worldwide. This current scenario indicates that it is high time for pathologists, pharmacologists and pharmaceutical formulation development scientists to come together to address the challenge of managing *H. pylori* infection. This article briefly highlights symptoms, diagnostic tests and various treatment regimens reported for the management of the *H. pylori* infection. The present article mainly focuses upon novel drug delivery systems developed in the last decade, with special emphasis on the need of gastro retentive drug delivery systems (GRDDS), for effective management of *H. pylori* infection. Targeted drug delivery to the stomach mucosal layer is believed to provide a site-specific effect for eradication of *H. pylori*. The authors have analysed various reported approaches to deliver drugs for the management of *H. pylori* infection. It is evident that efficacious results can be obtained with a multi-particulate drug delivery system as compared to a conventional single unit dosage form.

Keywords: Diagnostic test, *Helicobacter pylori* infection, Gastro retentive drug delivery, Gastric cancer, *H. pylori* treatment regimen.

Abbreviations: **IMMC**, Interdigestive myoelectric motor complex;

INTRODUCTION:

Helicobacter pylori is a Gram negative microphilic, flagellated and spiral shaped organism with a unipolar-sheathed flagellum. Its spiral shape and high motility helps it to penetrate deep into the mucus layer, resist gastric emptying and remain in the host gastric mucosa[16]. This infection is silent when talking about symptoms but has a potential to present some serious diseases at later stages, including gastritis, peptic ulcer, duodenal ulcer, gastric cancer and Mucosa Associated Lymphoidal Tissue (MALT) Lymphomas. From an extensive literature search, it has been observed that until 1994, around fifty percent of the total global population was suffering from this particular bacterial infection. The survey statistics state that about 80-90% of the population in developing countries and 10-50% of population of developed countries will be suffering from *H. pylori* infection by 2020[105]. The present review article summarises the basic etiology, symptoms and diagnostic tests and gives a wide array of treatment regimens starting from mono-therapy to quadruple therapy containing combinations of antibiotics and antacids along with reported novel formulations[16,39,40].

The complete etiology of *H. pylori* infection is not known but the infection occurs mainly during early childhood through oral ingestion of bacterium and it has a capacity to last until death with intra familiar transmission. Food and water borne routes of transmission are also significant. *H. pylori* infection may transfer from mother to fetus[16,39,40]. The common feature of *H. pylori* infection is formation of lesions which leads to disruption of the gastric mucosal layer thereby affecting gastric motility. The *H. pylori* bacterium adheres to the epithelial cell lining and produces several lesions which then further progress by the production and release of a vacuolating cytotoxin,

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Table I: Different treatment regimens for *H. pylori* infection[13,38,42]

Drug regimen	Drug combination	Duration	Comment
Two drug regimen	PPI (omeprazole 40mg-QID or Lansoprazole 30mg -TID) with Clarithromycin 500 mg -BID OR Amoxicillin 1gm- BID. PPI to be continued for 2 more weeks	2 week	Eradication rate 60-85%. This is choice as second option due to low eradication rate
	Ranitidine bismuth citrate (RBC) 400 mg –BID with Clarithromycin 500 mg- TID OR BID. RBC to be continued for 2 more weeks	2 weeks	Eradication rate 60-85%. This is choice as second option due to low eradication rate
Three drug regimen	Proton pump inhibitor - BID with Clarithromycin 500mg -BID and Amoxicillin 1gm - BID Market product: Prevpac™	10-14 days	FDA Approved regimen. Eradication rate 75-85%. Consider for non-penicillin allergic patients who have not previously treated with macrolide.
	Bismuth subsalicylate 525mg - QID with Tetracycline 500mg – QID and Metronidazole 500mg orally TID to QID.	14 days	Cheapest option but efficacy reduced without PPI.
	Proton pump inhibitor BID with Clarithromycin 500mg BID and Metronidazole 500mg BID	10-14 days	Eradication rate 75-85%. Consider for penicillin allergic patients who have not previously treated with microlide or are unable to tolerate bismuth quadruple therapy
	Ranitidine bismuth citrate 400mg BID with Clarithromycin 500mg BID and Amoxicillin 1gm BID	7-10 days	
	PPI- BID with Amoxicillin 500mg -BID OR TID and Metronidazole 500mg- BID or TID	10-14days	
	Ranitidine bismuth citrate 400mg BID with Clarithromycin 500mg-BID and Metronidazole 500mg-BID.	7 days	
Four drug regimen	Proton pump inhibitor BID with Bismuth subsalicylate 525mg QID and Tetracycline 500mg QID and Metronidazole 250mg TID or QID	10-14 days	Eradication rate 75-90%. Consider in penicillin allergic patients

Standard dosages for PPIs are as follows: - Omeprazole 20mg, Lansoprazole 30mg, pantoprazole 40mg, Rabeprazole 20mg, esomeprazole 40mg BID.

VacA. This bacterium produces several enzymes, the presence of which can be characterised by high urease activity during the diagnosis process. The resultant urea is converted into bicarbonate and ammonia. This cascade of reactions and the by-products of urea protect the *H. pylori* bacterium in the acid environment of the stomach. High levels of ammonium ions produced can be toxic to the gastric upper mucosal epithelial cells, which lead to the formation of lesions. *H. pylori* urease triggers inflammatory cytokine production and also activates mononuclear phagocytes, although, after colonisation, the host immune defences are triggered and higher secretory IgA (sIgA) level is detected in the gastric mucosa along with a raised level of specific IgG. Colonisation of *H. pylori* bacterium results in chronic gastric inflammation. An uncontrolled and prolonged *H. pylori* infection has also shown a significant link between *H. pylori* infection and the risk of gastric cancer. The general symptoms of this infection include ulcers, pain or discomfort (usually in the upper abdomen), bloating, feeling full after eating a little portion of food, lack of appetite, nausea or vomiting[9,16,21,26,33,39,40,44].

Diagnostic tests for detection of *H. pylori* are categorised into two main classes as a) Endoscopic Testing:- that includes Histology, that involves evaluation of pathologic changes associated with H. pylori infection such as inflammation and intestinal metaplasia, Rapid Urease Testing, which detects change in color of pH sensitive indicator due to metabolism of urea to ammonia in presence of urease, Culture which involves characterisation of antimicrobial sensitivity and Polymerase Chain Reaction which involves rapid production of multiple copies of a target DNA sequence to identify *H. pylori* and b) Non Endoscopic testing: - that includes Antibody testing, that identifies IgG antibodies specific to *H. pylori* in serum, whole blood or urine using ELISA, complement fixation and latex agglutination, Urea Breath Test which involves Urea, labelled with either, the nonradioactive isotope ^{13}C or the radioactive isotope ^{14}C, results in the production of labelled CO_2 which can be quantities in breath and Fecal Antigen Testing in which *H. pylori* antigen is identified in the stool by ELISA with the use of polyclonal or monoclonal anti *H. pylori* antibody[22,41].

Helicobacter pylori Treatment Regimen

Since 1990, a number of regimens have been evaluated for *H. pylori* infection management. Although there are a number of case studies available for the *H. pylori* infection, yet no optimal therapeutic regimen is available[109]. The current treatment therapies are presented in Table I. They are broadly classified into (i) Mono drug regimen, (ii) Two drug regimen, (iii) Three drug regimen and (iv) Quadruple or four drug regimen. Although there are various mono drug regimens proposed for the initial treatment of *H. pylori* infection, they have been found to be ineffective due to several reasons such as bacterial resistance and difficulty in achieving bactericidal concentration in the gastric mucosa. The drawback of single drug therapy led to a regimen including more drugs to ensure high eradication rates[16,39,40]. US-FDA approves *H. pylori* management using a two drug regimen includes a proton pump inhibitor (PPI) along with one or two antibiotics[106]. This combination has also not shown effective eradication of *H. pylori* bacterium. Hence, double drug therapy is considered as a second-tier option.

Triple drug therapy is mainly adopted when a patient shows <15 percent clarithromycin resistance. Although the *H. pylori* organism is sensitive to a number of antibiotics *in vitro,* it does not reproduce similar results *in vivo* due to deeper residence of the bacterium in the gastric mucosal layer which remains adhered to the gastric epithelium[9]. Hence, the drug molecules show a limited access to this deeper layer of the mucosa. Another reason of drug resistance could be due to the acquired resistance of the bacterium to the commonly used antimicrobial agents[42]. The most commonly recommended triple drugs regimen as a first line therapy includes a proton pump inhibitor (PPI) along with two antibiotics[107]. The higher incidences of increasing resistance to the current antibiotics are driving research in the direction of alternative therapies[108]. This may be the reason that the current recommended regimen of triple therapy (metronidazole, tetracycline or amoxicillin, bismuth) exhibits an eradication rate of 60%-80% only. The other reason which may contribute to the lower rate of efficiency could be patient compliance, side-effects and/or bacterial resistance[16,39,40]. Quadruple drug regimen is adopted when clarithromycin resistance is high (≥15 percent), as an alternative to triple therapy. It includes colloidal bismuth sub-citrate, tetracyclines, metronidazole and Omeprazole. Graham *et al* (2004) and W.A. de Boer *et al* (2004) have stated that the compliance with such a dosage regime could be a debatable issue. Compliance can be improved if all four drugs are combined in one or two dosage forms. It has also been reported that the resistance to metronidazole can be reduced by increasing the duration of the treatment. Tables II and III give a review of all the treatment regimens and the effect of different compounds. Alternative to the standard quadruple therapy is levofloxacin containing quadruple therapy, which also was found to give better eradication and is recommended after the failure of first line treatment with clarithromycin and metronidazole or amoxicillin. (rescue therapy)[19,24,25,27]

Table II: Curing rate of drugs used for *H. pylori*[13,38,40,55]

Drugs	Cure rate as single agent (%)
Colloidal bismuth subcitrate (De-Nol)	30-40
Bismuth subsalicylate (Pepto-Bismol)	5-10
Amoxicillin or Ampicillin	15
Tetracycline	5
Doxycycline	5
Furazolidone	20-40
Nitrofurantoin	10-15
Metronidazole	5
Tinidazole	5
Erythromycin base	15
Clarithromycin	40-60
Ciprofloxacin (Ofloxacin, Norfloxacin, Levofloxacine etc.)	10

Table III: Important aspects for choosing drug and its combinations[13,15,19,24,27,32,38,42,60]

Amoxicillin	It has better minimum inhibitory concentration; Less resistant to microbes. It is effective when administered with a PPI or H_2RA. Contraindications: There are chances of penicillin allergy. If prior pre-treatment is given with PPI, it becomes less effective Side effects: diarrhoea and candidiasis
Bismuth subsalicylate (BSS) (Peptol Bismol)	It is topically active. It shows cytoprotective and antimicrobial activity. BSS should be used as suspension when co-administered with tetracycline It increases warfarin effect and decreases tetracycline/doxycycline absorption. It has side effects like blackening of tongue and stool as well as tinnitus.
Clarithromycin (Biaxin)	It is highly effective as an anti-*H. pylori in vivo* but it is very expensive too. It shows drug interactions with cyclosporin, theophylline, cisapride, terfenadine, astemizole, and warfarin It has side effects like taste disturbance
Metronidazole	This drug shows regional variation for rate of resistance, i.e, 11-38%. It reduced the chances of resistance when co-administered with BSS or clarithromycin. This drug has very significant interaction with alcohol, i.e. disulfiram-like reaction. The side effects of this drug are furry coated tongue, metallic taste, diarrhoea, dyspepsia and nausea. Neuropathic disorders are observed rarely for short term administration of this drug.
Tetracycline	Tetracyclin shows good minimum inhibitory concentration with very less resistance. But this drug has to be given with frequent dosing such as four times a day. Tetracyclines make complexes with metals, hence, dairy products and antacids should be avoided. Also, it is contraindicated in pregnant women and children during their teething age. This drug also reduces the effectiveness of oral contraceptives.
Levofloxacine	Effective when given with PPI. Mainly used in cases of resistance to clarithromycin and metronidazole. Considered as second or third line of treatment (rescue treatment).
Proton Pump Inhibitors (PPI's)	PPIs enhance antimicrobial activity of certain antibiotics like, clarithromycin and amoxicillin. Frequently used PPIs are omeprazole, lansoprazole, and pantoprazole.

For the successful elimination of *H. pylori* infection, follow-up is required to check whether the colonisation of the bacterium has been eradicated completely. Any of the diagnostic tests, either a urea breathe test or a stool test along with endoscopy is advisable to ensure that the infection has been cured. During follow-up, blood tests are not recommended as even after complete elimination of the infection, the *H. pylori* antibodies most of the time remain in the blood circulation for almost four months or longer. *H. pylori treatment* failure has been observed in more than 20 percent of the population. Under this situation, a second treatment or repeat treatment was recommended with a full 14 days' regimen with two antibiotics along with a proton pump inhibitor of which at least one of the antibiotics would be different from the one taken during earlier treatment.

Current Formulation Strategies for *H. pylori* Infection Management

Considering the wide spread prevalence and severity of *H pylori* infection, a lot of research work has been carried out in the last few years. The colonisation of

Table IV: Approaches based on nano particulate drug delivery for management of *H. pylori infection.*

Sr No	Active Ingredient	Formulation approach	Remarks	Reference
1	Amoxicillin	Crude fucoidan was depolymerized to obtain low molecular weight fucoidan for preparing LMWF/CS-*N*-Arg Nano Particles (NP). The NPs were further cross-linked with genipin to obtain the pH-responsive nanogels (FCSA nanogels).	Nanogels were able to bind to *H. pylori* for specific delivery of amoxicillin to the sites of *H. pylori* infection, and significantly inhibited *H. pylori* growth and efficiently protected AGS gastric epithelial cells against *H. pylori* induced cytotoxicity.	[35]
2	*Helicobacter pylori* vaccine-encapsulated acid-resistant HP55/ PLGA	Nanoparticles were synthesized by using a membrane emulsification technology and double-emulsion solvent evaporation technique.	Oral subunit vaccine had clear advantages due to theirsafety, noninvasive nature and cost-effectiveness. They emerged as a promising oral delivery system for clinical applications and contributed to the development of an efficient oral protein vaccine.	[74]
3	Amoxicillin	Ureido-conjugated chitosan derivatives (UCCs) /TPP nanoparticles were prepared using the ionic gelation method with magnetic stirring at room temperature.	Nanoparticles exhibited pH-sensitive release profiles. Various studies verified that amoxicillin-UCCs/TPP nanoparticles can deliver the drug efficiently to get more specific and effective eradication of *H. pylori.*	[31]
4	Amoxicillin	Dual templates (MNQA and AmoNa) imprinted nanoparticle were prepared via inverse microemulsion polymerization.	*In vitro* drug release showed a burst effect during the first hour, which is helpful in achieving effective amoxicillin concentration in gastric tissue for *H. pylori* clearance.	[62]
5	Acetohydroxamic acid (AHA) and Clarithromycin (CLR)	PLGA nanoparticles were prepared by solvent evaporation method. The conjugation of lectin (Con A) to PLGA nanoparticles was performed through carbodiimide technique.	Con-A-conjugated PLGA nanoparticles offer a potential approach to be employed for the incorporation of other antibiotics along with urease inhibitor for the effective treatment of *H. pylori* infection.	[30]
6	Amoxicillin (AMX) and a novel anti *H. pylori* adhesion material pectin sulphate (PECS)	Lipid polymer nanoparticles (LPN) were prepared by the emulsification-solvent evaporation method. The outer layer of nanoparticles is designed to contain the mixed lipids of rhamnolipid and phospholipid. The inner core contains first-line antibiotics amoxicillin and pectin sulphate.	Mixed lipids as the outer layer of nanoparticles and PECS as the inner core produces a system capable of significantly disrupting *H. pylori* biofilm by eliminating the extracellular polymeric substances as well as inhibiting the adherence and colonization of bacteria.	[10]

this bacterium is the major cause of the development of peptic and duodenal ulcers ultimately (if untreated) leading to gastric cancer. The most effective 7-day triple therapy with > 90% eradication rate is unknown due to poor patient compliance and bacterial resistance towards antibiotics. It is an enormous and challenging task for clinical pharmacologists to address these issues as well as to identify novel targets. As pharmaceutical formulation scientists, the authors feel that novel approaches for delivering the drugs to the deeper layers of the stomach lining can play a significant role in patient compliance and a higher rate of treatment success. Following are the examples of novel approaches that have been reported in literature where the bacterium develops resistance to antibiotics or the bioavailability of the drug is quite low as the bacterium resides in the deep linings of stomach thus making the drug inaccessible. Hence, this is where, novel formulation approaches come into play. Besides effective regimens and novel drug delivery systems, vaccination is also proposed to prevent or treat the infection. The proposed vaccine has demonstrated its effectiveness in animal models, but it has not yet presented encouraging results in human beings[18,63].

Several approaches have been studied for an effective treatment for the *H. pylori* infection. The most studied approach is the Gastroretentive drug delivery system. This drug delivery system ensures the delivery of a higher drug concentration of drugs on the stomach linings and also gives a prolonged residence time for the drugs to show local as well as systemic absorption from the stomach endothelium. Several approaches such as floating (buoyant) drug delivery systems, expandable (swellable) drug delivery systems and bioadhesive (mucoadhesive) systems as single or multi-unit dosage forms have been studied. All these approaches, except the mucoadhesive system have certain drawbacks such as lack of specificity and retention in the stomach that depends on mucus turnover and gastrointestinal motility patterns during fasting and non-fasting states. Mucoadhesive/ bioadhesive systems present themselves as one of the most promising approaches to address the issue of gastric retention. The close proximity of the dosage form and interpenetrating polymeric layers with the mucus membrane help in prolonging total retention time along with better absorption of the drug through the epithelial layers[8,14,50,65].

Mucoadhesive drug delivery system

Mucoadhesion or bioadhesion is a phenomenon in which the drug delivery system adheres to the mucus layer lining the internal walls of the gastrointestinal tract. This generally tak place due to the secretion and deposition of mucin onto these gastrointestinal wall which in-turn interacts with the bioadhesive polymers incorporated in the dosage form leading to mucoadhesion. Mucoadhesive polymers have hydrophilic moieties such as amide, sulphate and hydroxyl, which attach onto the cell wall or mucus membrane by hydrogen bonding leading to swelling exposing maximum adhesive sites. As *H pylori* resides deep down in the villi of the gastrointestinal lining making it inaccessible to most of the conventional dosage forms, thus hampering the effective treatment strategy, this is where mucoadhesion plays a vital role in the treatment efficiency and ultimately eradication of the bacteria[52].

Number of natural (chitosan, alginates), semi-synthetic (cellulosic derivatives like HPMC, HPC etc.)and synthetic polymers (acrylates) have played a very vital role in improving mucoadhesive drug delivery systems. This is due to either a functional group which connects with mucus or swelling-gelling behaviour of the polymers. The mucoadhesive force and duration may vary with the change in the chemical structure of the polymer. The next part of the article deals with the role of various polymers studied by various research groups. Chitosan, a polysaccharide and its derivatives have been well accounted for their Mucoadhesive property. Chitosan is composed of *N*-acetyl-D-glucosamine and D-glucosamine and its multiple units are linked by a 1,4-β-glycosidic bonds which account for the formation of hydrogen and covalent bonding, leading to mucoadhesion. The hydroxyl and amino groups present in its molecular structure play an essential role in providing a unique solubility attribute. Hou et al. [66] formulated alginate-chitosan mucoadhesive microparticles containing puerarin by emulsification-internal gelation method using chitosan and calcium ions and alginate as complimentary ionic components. Parameters such as surface morphology, particle size, drug loading, encapsulation efficiency and swelling ratio, *in vitro* drug release, *in vivo* evaluation of mucoadhesiveness and fluorescence imaging of the gastrointestinal tract were determined. The *in vitro* release test, mucoadhesive tests and fluorescence imaging suggested that the mucoadhesion and cumulative release of puerarin mucoadhesive microparticles were influenced by the pH of the test medium along with prolonged adherence time thus improving bioavailability[83]. Considering the synergistic effect of mucoadhesive and floating system, Thombre and Gide [56] have formulated floating-bioadhesive gastroretentive *Caesalpinia pulcherrima*-based beads of amoxicillin trihydrate for eradication of *H. pylori* provoked infected sites more effectively. Chitosan-coated amoxicillin trihydrate-loaded *Caesalpinia pulcherrima* galactomannan

(CPG) - alginate beads showed promising results by eradicating *H. pylori* infection with a lower dose, reduced adverse effect and enhanced bioavailability. Similarly, Clarithromycin-loaded calcium alginate beads were prepared using olive oil (reported for *H. pylori* treatment activity) along with chitosan (mucoadhesive polymer) to control the drug release to ensure more effective targeting to bacteria and reduced drug resistance. *Adebisi et al*[2] formulated calcium alginate beads containing metronidazole by first adding olive oil and then coating with chitosan to improve drug release and floating profile. Upon modification, buoyancy improved and drug release was sustained leading to improved eradication of the bacteria[1,2,56].

Many cellulosic derivative polymers like hydroxy methyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC) and Carbopol have been widely attributed for mucoadhesive drug delivery, thus also providing gastro-retentive property to the particular dosage form. These polymers have been used either alone or in combination with each other to provide the optimum release profile as required in the studies planned. Various dosage forms like table, nanoparticles and microspheres are formulated using these polymers, of which some have been discussed as follows: *Zhao et al*[65] have evaluated the potential of intragastric floating tablets of ascaridole for achieving increased gastric residence time and localised action in the proximal part of the gastrointestinal tract. Similarly, an attempt to formulate effervescent floating tablets of levofloxacin using sodium bicarbonate, citric acid as a gas forming agent and polymers, like HPMC and Carbopol 974P for improving therapeutic efficacy due to increase in gastric residence time and reduction in dosing frequency has been made by *Shah et al*[51,64]. Another attempt to combat the complications associated with conventional triple-therapy (TT) by formulating curcumin loaded mucoadhesive microspheres was made by *Pandit et al* (2014). Microspheres containing Carbopol 934P and ethyl cellulose were prepared using an emulsion-solvent evaporation technique. Floating curcumin microsponges to increase absorption of curcumin in the gastric region for the treatment of gastric cancer were formulated. The *in vitro* evaluation proved that good adhesion of microsponges helps the permeation of curcumin through the gastric mucin layer and provides site specific release of curcumin in the gastric region more efficiently[3,5]. Singh formulated a gastroretentive matrix tablet of clarithromycin whereas Shah et al designed gastroretentive multi-layer coated tablets of gatifloxacin using HPMC K4M, HPMC K15M, HPMC K100M and Eudragit RL 30D with $NaHCO_3$ as a gas forming agent for the *H.pylori* infection. The hydrophilic matrix entrapped the generated gas and imparted buoyancy to the tablet. The authors concluded that these dosage forms can be used for a combination of drugs which can be targeted to the stomach to eradicate *H. pylori* infection. Similarly, Badhan formulated sustained release gastroretentive mini-matrices using xanthan gum, HPMC, Carbopol 974P and gas generating agents like sodium bicarbonate and citric acid by a non-aqueous granulation technique using a solution of PVP K30 in isopropyl alcohol. *In vitro* evaluation results of formulation showed sustained drug release and gastric retention capability, which could be very useful for an effective treatment of *H. pylori* infection[7,51,57].

Swelling/gelling properties of the polymers have been well accounted for increase in the retention time of the drug delivery system. Similar approaches were applied by various research groups, one of which has been mentioned here. *Farshforoush et al*[20] have explored the capability of hydrogels for localized delivery of metronidazole in the stomach for treatment of *H. pylori*. Hydrogel was formulated using dimethylaminoethyl methacrylate (DMAEMA) as a biocompatible monomer along with methylene bisacrylamide (MBA) and triethylene glycol diacrylate (TEGDA) as cross-linkers. The developed formulation exhibited high drug loading capacity, which may pave the way for new horizons in the treatment of *H. pylori*[20]. Rajinikanth and Mishra [47] have developed a floating *in situ* gelling system (FIGC) of clarithromycin and amoxicillin. In this formulation, gellan was used as gelling polymer and calcium carbonate as a gas forming agent for treating gastric ulcers caused due to *H.pylori*. On comparing the formulated systems with suspension of clarithromycin and amoxicillin, efficacy was improved a few folds. They concluded that prolonged gastrointestinal residence time and enhanced stability of the drug contributed towards the complete eradication of *H. pylori*[47].

Multiparticulate drug delivery system

Multiparticulate units like microspheres, microencapsulated particles and pellets are multiple unit dosage forms, which consist of a fixed unit dose of the drug, which may or may not vary in their drug release profile. These are miniature micron sized particulates that give them the advantage to leach down deep into the congested mucus lined villi in the gastrointestinal lining, hence providing targeted delivery. Multiparticulate systems can be used for pulsatile, controlled and delayed release of the drugs depending on the target site and they are generally administered orally. Some of the multiparticulate approaches have been explained below.

Microparticles are the miniature particulates comprised of phospholipid fragments with an internal drug core enabling it as a targeted drug delivery system. It generally comprises of microspheres, microcapsules, microballoons and *Nohemann et al*[43] have developed effervescent floating microparticles for ensuring reliable, reproducible and uniform drug absorption. Floating microparticles were formulated using swellable polysaccharide (chitosan), hydrophilic (HPMC) or hydrophobic (EC) polymer. Although drug release from the floating micro particle was a critical parameter, floating ability was equally considered to exhibit its effect for targeting the infection[43]. Floating microspheres containing acetohydroxamicacid (AHA) using an emulsion (O/W) solvent evaporation technique for the treatment of *H. pylori* were developed by *Umamaheshwari et al.* The authors concluded that more gastric retention time of microspheres give better efficacy against *H. pylori* as compared to a conventional formulation[59]. Umamaheshwari and Jain[58] developed xerogel beads containing acetohydroxamic acid (AHA) using the emulsification method. These beads were prepared using phosphatidyl ethanolamine (PE) liposomes which were attached with polyvinyl alcohol (PVA). These beads performed as a receptor-mediated drug delivery system which further could block an adhesion of *H. pylori*[58]. With an aim to achieve admirable buoyancy *in vitro* and prolonged gastric retention time, *Choudhary et al* [29] have formulated microballoons of rabeprazole and amoxicillin whereas *Awasthi and Kulkarni* [6] have prepared amoxicillin loaded hollow microballoons for a longer-lasting and more consistent release of drugs. Both formulations depicted a lower ulcer index and a higher ulcer preventive potential. In addition to it was observed that the combined effect of four antibiotics (rabeprazole and amoxicillin) helped to aggravate the potential of rabeprazole during the course of chronic therapy[6,29]. Zou *et al.* formulated a low density system of metronidazole-loaded porous Eudragit® RS microparticles with high drug loading capacity (>25%) via electrospray method and suggested that prepared porous microparticles have the potential to provide better treatment for the H. pylori infection[66]. Ishak et al prepared and optimized metronidazole (MZ) containing chitosan-treated alginate beads by the ionotropic gelation method using methyl cellulose, Carbopol 934P and κ-carrageenan. After *in vitro* and *in vivo* evaluation, the histopathological results revealed that the group receiving floating beads of MZ showed a better effect than MZ in suspension form. The authors reported that floating beads of MZ at the dose of 15 mg/kg showed 100% eradication as compared to an MZ suspension at a dose of 20 mg/kg [33.33% eradication][28]. Bhople *et al.* formulated and optimized mucoadhesive microcapsules of clarithromycin and omeprazole for the treatment of the *H. pylori* infection. These microspheres were evaluated for micromeritics studies, drug entrapment study, percentage recovery of microspheres, swelling and adhesion properties, *in vitro* drug release and surface topography study of the uncoated and coated microsphere. The study concluded that a microcapsule containing microspheres of clarithromycin and omeprazole increases gastric residence time and hence bioavailability which is found to be more beneficial to treat *H. pylori* infection. Various formulations such as floating gellan gum beads of amoxicillin and clarithromycin, mucoadhesive microspheres of clarithromycin using sodium alginate and HPMC have been prepared by employing the ionic gelation method. The formulation exhibited pH-sensitive controlled drug release in the stomach along with showing good anti-microbial activity against *H. pylori* strain[46,57]. Patel et al prepared chitosan based mucoadhesive microspheres of amoxicillin for gastroretentive anti-*H. pylori* therapy by an emulsification phase separation technique using glutaraldehyde as a cross-linking agent. An *in vitro* mucoadhesion evaluation test revealed strong mucoadhesion of amoxicillin microspheres to the gastric mucous layer. The formulation showed prolonged gastric retention and increased amoxicillin stability which helps in complete eradication of *H. pylori*[45].

Nano particle or emulsion drug delivery

The problems with the conventional drug delivery system involve adherence of the dosage form and irrational drug delivery hence insufficient drug bioavailability. Mucoadhesive property of polymers help in adherence but the viscoelastic gel matrix does not allow uniform drug delivery through it at the target site. Gastric motility and proteolytic activity shorten the mucoresidence time by increasing the mucus turnover rate[110]. This has led to the development of nanoparticulate drug delivery systems. Nanoparticles vary in the range of 10nm to 1000nm in size. Generally, 200-300nm sized particles are incorporated into the drug delivery systems for an optimum therapeutic effect. The major advantage of nanoparticles is that they provide a wide array of applications for different target sites. They can be easily formulated as drug carriers due to their higher drug loading capacity, better stability as compared to macro-sized particulates, and incorporation of both lipophilic and hydrophilic components in their matrix[23]. Major works for treatment therapy are described below.

During an *H. pylori* infection, a major imbalance occurs in the pH of the stomach and various novel approaches have been worked out considering that change in pH of

the stomach. In order to acquire more efficient and specific system for the eradication of *H. pylori*, Muhammad Arif *et al.*[4], have observed a cysteine-conjugated chitosan derivative with amide linkage (Cys-CS) by $^{1}H_{-NMR}$ and FTIR spectroscopy. The loading efficiency, pH-sensitive behaviour to go to gastric mucus layer and growth inhibition of the ideal cysteine-conjugated chitosan/ PMLA nanoparticles encapsulated amoxicillin have been assessed[4]. Similarly, *Wu et al* [60] prepared pH-responsive nanoparticles by an instantaneous addition of a solution of heparin to a solution of chitosan with continuous stirring at room temperature. The study showed that the prepared nanoparticles can adhere to and interact locally with *H. pylori* infection sites in spaces between the cells[61].

In order to obtain an effective delivery system for eradication of *H. pylori*, *Min Luo et al.* [36], have reported a formulation containing PLGA and urea-modified chitosan derivative UCCs-2 to target the urea transport channel protein UreI of *H. pylori*, to construct pH-sensitive bilayer PLGA/UCCs-2 nanoparticles for specific anti-*H. pylori* treatment. When using amoxicillin (AMX) as a model drug, the nanoparticles were prepared by double emulsion-solvent evaporation method and the formulations of AMX-PLGA/UCCs-2 were optimized by orthogonal design, mainly with pH-sensitive drug release properties. The pH sensitivity, cytotoxicity, *in-vitro* and *in vivo* anti- *H. pylori* effects, and targeted uptake mechanism of AMX-PLGA/UCCs-2 nanoparticles were investigated in detail to demonstrate the favourable delivery efficiency[36]. Tan *et al* (2016) have synthesized acid-resistant HP55/PLGA nanoparticles with an aim to prevent the *H. pylori* infection. These vaccine-encapsulated acid-resistant HP55/PLGA nanoparticles showed promising results in mice model by promoting their immune protection against *H.pylori* infection. Results obtained from mice vaccinated with CCF-encapsulated HP55/PLGA NPs showed elevated levels of antigen-specific antibodies, switched IgG2a/ IgG1 ratio and pro inflammatory cytokines[74].

Nanostructured lipid carriers have solid and liquid lipids as its core thus providing increased solubility, enhanced drug loading capacity, improved bioavailability and permeability. In recent years, multiple research groups have formulated NLCs for various classes of drug and a few have been quoted in the article. *Seabra et al.* [76] formulated nanostructured lipid carriers (NLCs) loaded with docosahexaenoic acid (DHA). DHA is an essential polyunsaturated fatty acid which is generally found in fish oil and the authors have hypothesized that DHA would emerge as a promising component in treatment of *H. pylori* infection. NLCs were produced by hot homogenisation technique followed by ultrasonication using lipids like Precirol-AT05 and Miglyol-812 along with surfactants like Tween 60. The final formulation was evaluated for antibacterial activity on human isolated *H. pylori* strain J99 and mouse adapted strain SS1. It was observed that the NLCs had an interaction with the bacterial strains without being affected by the presence or absence of DHA molecule, although DHA molecule had an additive effect in bactericidal properties as compared to NLC alone. The bactericidal effect is achieved by disruption of the plasma membrane from the outer membrane of the bacterium, showing leakage of cytoplasmic components. Thus, DHA-NLCs can be explored as a substitute in the modern treatment therapy[76]. *Sunil K. Jain et al.* [30] have developed lecithin-conjugated nanoparticles containing acetohydroxamic acid (AHA) and clarithromycin (CLR) for effective eradication of *H. pylori* infection. The PLGA nanoparticles were prepared by solvent evaporation method and the conjugation of PLGA with lecithin (Con-A) was performed through the carbodiimide technique. Preliminary results suggested that the formulation can be a potential approach for incorporation of other antibiotics along with urease inhibitor for the effective treatment of *H. pylori* infection[30].

Lin et. al [34] had developed positively charged W/O nanoemulsion particles containing amoxicillin, chitosan and heparin against *H. Pylori* infection. The *in vivo* evaluation revealed that amoxicillin-loaded nanoemulsion particles had greater *H. pylori* eradication ability than the amoxicillin solution when given alone. Novel nanoparticles of berberine with the carrier heparin were formulated by Chang *et al* to avoid the side effects of antibiotics during the treatment of *H. pylori* infection. The authors concluded that berberine nanoparticles significantly reduced proliferation of *H. pylori* and decreased cytotoxic effect in the cells infected with *H. Pylori*[12,34].

In a study carried out by *Seven et al.* [49], efficacy of triple therapy containing levofloxacin, amoxicillin and PPI was evaluated. Authors suggested that this therapy could be an option for first line and second line treatment of *H. pylori* infection. The study was carried out on 2 groups of patients using triple therapy with two different dose regimens [Group 1 (N=60) treated with levofloxacin (500 mg o.i.d), amoxicillin (1 g bid) and PPI (bid) for the duration of 10 days and Group 2, (n=50) treated with a levofloxacin (500 mg bid), amoxicillin (1 g bid) and PPI (bid) for the duration of 10 days]. The result showed an eradication rate of the Group 1 was 60% and 72.2% in Group 2[49]. *Malfertheiner et al* [37] carried out a randomised, non-inferiority, open-label, phase 3 trial and compared efficacy of a capsule of quadruple therapy [bismuth subcitrate potassium, metronidazole, and tetracycline

with omeprazole] against clarithromycin containing triple therapy for the treatment of *H. pylori* infection. The authors suggested that quadruple therapy gives better eradication with comparable safety and tolerability. It further may help in reducing the chances of development of resistance to clarithromycin[37].

A few other nanoparticulate approaches have been further mentioned in Table IV.

Novel formulation aspects in recent years

In recent years, novel formulation approaches have been well defined for the treatment of *H pylori* infection which involves certain modifications in the previously existing treatment therapies or development of a new formulation all-together that is supposed to give an equivalent or better management of the bacterial infection. A few studies by research groups have been mentioned here with their contribution to the particular field. Fusogenic cationic liposomes (DSPE-PEGylated liposomes) comprising of nucleic amino acids (NAMs/ oligonucleotides) were formulated by *Santos et al*[48]. Since *H pylori* resides within the gastric mucus layer as well as in close proximity with the epithelial cells underlying the mucus layer, fusogenic stealth liposomes were evaluated for their abilities as nanocarriers for NAMs to target *H pylori* infections. They have shown that 2,3- DOTAP (Dioleoyloxy-propyl)-trimethyl ammonium chloride) and DOPE (1,2-dioleoyl-sn134-glycero-3 phosphoethanolamine) liposomes post-PEGylated with DSPE-PEG (DSPE Lpx) can successfully deliver NAMs into *H. pylori*, simultaneously offering protection to the NAMs from binding and inactivation in gastric mucus isolated from pigs. This particular research portrayed that PEGylated liposomes represent a valuable opportunity in the post-antibiotic era to deliver NAMs as a novel class of therapeutic antimicrobials and diagnostic agents[48].

Silva-Freitas et al[53] formulated magnetic microparticles coated with Eudragit S100 and amoxicilin. The trationale for this formulation approach includes dual responsive (both pH and magnetic field sensitive) polymeric magnetic particles loaded with drug was explored as a smart drug carrier for enhanced penetration into the mucus layer and *in situ* drug release. Mesoporous polymer-coated amoxicillin microparticles, exhibited deep mucus targeted antimicrobial delivery in the treatment of peptic ulcers[53].

Iannuccelli et al[11] have explored the benefits provided by montmorillonite (organically modified nanoclay) for management of *H. pylori* infection. Montmorillonite nanoclay was optimised by intercalating tetracycline into montmorillonite nanoplatelets with two different pH reaction conditions. The prepared nanoclays exhibited sufficient mucoadhesiveness and drug desorption processes thus representing an effective approach in *H. pylori* infection perspective[11].

PATENTS

A total of 214 patents have been filed in the literature till date for the treatment/ eradication of *H. pylori*. Some of the recent patents are mainly focusing on novel formulations aspects for Gastroretentive system containing more than one active ingredients.

CN106421768 (2017), claims an effective and extensive immunoprotection based vaccine system containing inosine 5'-monophosphate dehydrogenase, Type II citrate synthase and urease subunit beta. The developed vaccine is a multi-subunit vaccine based on CD4+T cellular immunity. Gastric retention controlled release formulation (gastric floating system) comprising of capitatum and berberine was claimed in WO2017020861A1 (2017). The formulation as per the claim is supposed to give a synergistic effect along with an improved rate of elimination of infection. WO2017173544A1 (2017) claims to have formulated a lyophilized powder containing TRPML Agonist (ML-SA1, SF-22, SF-51, MK6-83). This powder has to be reconstituted with sterile water or saline prior to administration. The combination of TRPML agonist with an antibiotic showed a synergistic treatment and/or preventative effect as per the claim. CN107536843A (2017) claims an immediate release capsule comprising of azithromycin and atractylone. The innovator claims to have a synergistic effect of the active ingredients in the product. Wax Pellets containing sulfur, *Lygodium japonicum* and dragon's bone in a certain order were prepared for the treatment therapy claimed in CN107362181A (2017). The developed pellets exhibited a rapid effect with no recurrence of *H. pylori*. CN106075436A (2016) claims elimination of drug resistance by formulating oral formulation (powders, tablets, pills, capsules or oral solution) containing egg yolk immunoglobulin, 2-5 parts of lactoferrin and 2-5 parts of chlorophyll in barley green. The combination eliminated the potential of relapse of the infection along with greatly decreasing the adverse effect rate. Capsules filled with granules of raw herbs was claimed under CN104958672A (2015). The herbs were common andrographis herb, Rhaphidophora hongkongensis, Semen lepidii, Hovenia dulcis thumb, hairystalk loosestrife herb, Baikal betony rhizome, *Polygonum dissitiflorum, Palmate girardinia herb, Rhizoma bletillae, Peucedanum morisonii bess, Chinaroot greenbrier, Stephania sinica diels,* wild grape vine*, Semen*

coicis, phoenix-tail fern*, Sculellaria barbata, Stichopus japonicus, Alpinia katsumadai, Agastache rugosus,* oriental wormwood, talcum*, Rhizoma acori graminei, Scutellaria baicalensis, Fructus forsythiae,* blackberry lily*, Lysimachia christinae hance, Rhizoma corydalis, Radix glehniae* and black jasmine. These herbs effectively help in reduction of *H. pylori*. It does not affect appetite, meanwhile helps restoring the body, with rapid onset, high efficiency, efficacy, short treatment course, toxic side effects and low price advantage. WO2016108774 (2014) claims improved drug release from encapsulated essential oils in chitosan/nanoclay microspheres which are localized in gastrointestinal system. the essential oils (cinnamon, thyme, lemongrass, lemon and clove) were spray dried into microspheres which were further incorporated in organic modified montmorillonite clay[92]. Carbon Nano-pellets were formulated by mixing Carbon Nano-spheres with deionized water under the patent CN105998065A (2016). Carbon was used as the active ingredient as it has the advantages of low cost, easiness for manufacturing, good stability, high safety and good anti-*helicobacter pylori* infection effect. CN106729719A (2016) claims eradication of *H. pylori* was improved due to increment in effective rate and reduction in drug resistance rate adverse reactions. Oral formulation such as granules, pills, capsules or tablets containing combination of proton pump inhibitor with an antibiotic and a volatile extract were claimed for the eradication of the bacteria.

CONCLUSION

The total eradication of *Helicobacter pylori* from the embedded layers of gastric mucosa is a challenge in the *H. pylori* infection management. The multiple drug regimens, like triple and quadruple therapies including antibiotics and proton pump inhibitors in combination, have proven as effective treatment regimens. Also, it has been studied that the absorption of an antibiotic via mucus layer results in an effective cure for *H. pylori* treatment rather than absorption by blood. Also, types of dosage forms play a vital role in the management of the *H. pylori* infection. The current conventional dosage forms lack pharmacological effect (lesser bioavailability and targeting to *H. pylori*), due to a short residence time in the stomach thus giving systemic effect instead of targeted action. *Helicobacter pylori* organisms can be eradicated if antibiotics are formulated as sustained release in the stomach giving a release profile for more than 10-12 hours. Several formulation strategies like, mucoadhesion, swelling, raft forming and gas generating stomach specific drug delivery systems have been suggested to improve the bioavailability of these drug molecules by increasing dosage form residence time in the gastric region. It has been concluded in previous works that the drug released will cover a larger surface area of the stomach and small intestine for absorption and thereby enhance the absorption of the drug when given in multiparticulate drug delivery systems.

Hence, with the current scenario of the widespread prevalence of the *H. pylori* infection, it is time to take a step forward for effective management of *H. pylori* treatment. The authors believe that this can be possible when a group of pharmacologists, pathologists and pharmaceutical formulation scientists work together using novel techniques of formulation development.

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