1. INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is reported to be an important etiologic factor in the development of the gastritis, gastric ulcer and gastric carcinoma in human stomach (Marshall and Warren, 1984). *H. pylori* reside mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach (Peterson, 1991). Although the microorganism is highly susceptible to many antimicrobial agents *in vitro*, clinical trials with a single antimicrobial agent have resulted in a low eradication rate of *H. pylori* (Labenz, 2001).

The antimicrobials used in the therapeutic regimes of these diseases are amoxicillin, clarithromycin and metronidazole (Hentschel *et al*., 1993). Triple therapies (one or two antibiotics combined with a proton pump inhibitor) are proved effective in clinical application. However, some other reports and clinical trials indicate that the therapies cannot bring out complete eradication of *H. pylori* (Lin *et al*., 2002, & Kawabami *et al*., 2001). The classical way to cure *H. pylori* infection is to use a 7 days triple therapy based on two antibiotics (amoxicillin, and clarithromycin) and one proton pump inhibitor (omeprazole, lansoprazole, and pantoprazole). However, because of the high level of antibiotic resistance to *H. pylori* and the poor patient compliance in a lesser measure (Mc Loughlin *et al*., 2004).

There are two major reasons for the failure of *H. pylori* eradication with conventional dosage forms of antibiotics. One of the reasons for incomplete eradication may be the degradation of antimicrobial agents such as amoxicillin and clarithromycin by gastric acid (Lin *et al*., 2002). In an effort to overcome this problem, concomitant administration of antimicrobial agents and drugs which inhibit gastric acid secretion such as H$_2$ receptor antagonists and proton pump inhibitors (PPI) have been tried, but complete eradication has not been achieved (Schwartz *et al*., 1998). Therefore, the administration of high doses of antimicrobial agents on a daily basis is necessary for *H. pylori* eradication, but they are usually accompanied by adverse effects and poor patient compliance (Kawabami *et al*., 2001).
Another reason for incomplete eradication is probably that the residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where \textit{H. pylori} exists (Cuna \textit{et al.}, 2001; Hirayama \textit{et al.}, 1996, & Katayama \textit{et al.}, 1999).

Clarithromycin is a macrolide, orally absorbed, broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric \textit{H. pylori} infection combined with a second antibiotic and an acid-suppressing agent like proton pump inhibitors. Clarithromycin has highest rate of eradication of \textit{H.pylori} in monotherapy \textit{in vivo}, though it is unstable and rapidly undergo degradation in low pH of gastric acid (Myung \textit{et al.}, 2005). Access of antimicrobial drugs to the site is restricted from both the lumen of the stomach and the gastric blood supply. As conventional drug delivery systems do not remain in the stomach for prolonged periods, they are unable to deliver the antibiotics to the site of infection in effective concentrations and in fully active forms. Therefore, it is necessary to design drug delivery systems that not only alleviate the shortcomings of conventional delivery vehicles but also deliver the antimicrobials to the infected cell lines. The absorption of antibiotics into the mucus through the mucus layer (from the gastric lumen) is believed to be more effective for \textit{H. pylori} eradication than absorption through the basolateral membrane, i.e. from blood (Katayama \textit{et al.}, 1999).

Proton pump inhibitors play an important role in increasing the bioavailability of antibiotics in the gastric mucus by altering gastric volumes and increasing the stability of antibiotics. Increasing the doses of potent proton pump inhibitors has small effects on eradication rates but there may be differences between proton pump inhibitors (Vakil, 2005).

Proton pump inhibitors are used in \textit{H. pylori} treatment regimens because they have a profound effect on the delivery of antibiotics to the organism. Gastric acid degrades antibiotics in the stomach. The antibiotics used in \textit{H. pylori} regimens differ in their susceptibility to acid, clarithromycin is particularly sensitive to degradation with acid and has a half-life of less than 1 h at pH 2 (Goddard, 1998, & Erah \textit{et al.}, 1997). The stability of the pantoprazole in aqueous solution is pH-dependent. The rate of degradation
increases with decreasing pH. At ambient temperature, the degradation half life is approximately 2.8 h at pH 5 and approximately 220 h at pH 7.8. Most of the PPI’s are administered as enteric coated formulations as these drugs are degraded by the acidic environment of stomach. The PPI reduces the stomach pH by acid suppression via proton pump inhibition.

The use of proton pump inhibitors in antimicrobial regimens is therefore important in preventing degradation of clarithromycin by acid. Antibiotics move across cells by lipid diffusion, i.e., solution of the drug in the lipids of the membrane followed by passive transfer across the lipid by a concentration gradient (Goddard, 1998).

Differently from omeprazole, pantoprazole kinetics appears not to be influenced at all by clarithromycin. This is likely to be due to the stereo-selective metabolism of the drug by CYP2C19 (Bliesath et al., 1996) and essentially no handling by CYP3A. Reports indicate that treatment with pantoprazole and clarithromycin (with or without additional tinidazole or bismuth subnitrate) will result in eradication of H. pylori and of gastroduodenal (GD) ulcer similar to omeprazole/clarithromycin (Kromer et al., 1990).

There were many drug delivery systems reported for H. pylori eradication, including sustained release ampicillin preparation by sodium alginate by Ca$^{2+}$ ion induced ionotropic gelation method (Katayama et al., 1999).

Effective eradication of H. pylori, antibiotics need to penetrate through the gastric mucus layer and maintains a concentration sufficient for antibacterial activity for long enough time in the infected site. Other than the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate H. pylori from the stomach including mucoadhesive drug delivery and floating drug delivery system, which can deliver the antibiotics at stomach site where H. pylori exist. Floating and gastroretentive drug delivery system have been gaining more attention due to its ability to deliver the antibacterial locally at stomach site and enhances local antibiotics concentration and prolongs the residence time of formulation (Rajinikanth et al., 2007).

Prolonging the GRT of therapeutic agents is thought to be beneficial especially under several circumstances such as for drugs acting topically on the gastric region, for
drugs with a narrow therapeutic window or for drugs with the major absorption site in the upper GI tract.

Various approaches have been proposed to retain the dosage form in the stomach. Gastroretentive floating drug delivery systems were developed based on the principle of effervescence by carbon dioxide generation by many researchers (Dettmar and Lloyd-Jones, 1994, & Yang et al., 1999), and hollow microspheres called microballoons also capable of floating in the gastric fluid also were reported (Umamaheshwari et al., 2002).

Mucoadhesive drug delivery systems were developed by many scientists produced longer gastric residence time and better efficacy for drugs like amoxicillin (Nagahara et al., 1998, & Liu et al., 2005) and ampicillin (Katayama et al., 1999). There were reports of cross linked chitosan microspheres of tetracycline with prolonged gastric retention (Hejazi and Amiji, 2003, & Naisbett and Woodley, 1995).

Most of the gastroretentive formulations reported were based on the carbon dioxide gas generation and already there is a controversy over the usage of antacids containing bicarbonates. These types of the formulations are not suitable for H. pylori therapy since H. pylori produces an enzyme called urease and which is degraded in to bicarbonate and ammonia. This bicarbonate increases the pH of the surroundings to 4 to 5.7, which is a favorable environment for the organism to lodge into the mucosa of the antral region of the stomach, and hence we thought of making a formulation based on the floating principle but without gas generation. The required floating can be achieved by freeze drying of the chitosan formulations.

Chitosan is a polycationic, nontoxic, mucoadhesive polymer, which has been proven to be safe (Mansouri et al., 2006, & Jin et al., 2004). It allows a prolonged interaction between the delivered drug and the membrane epithelia, facilitating more efficient drug diffusion into the mucus/epithelium layer (Thanou et al., 2001, & Hejazi and Amiji, 2004).

Chitosan is a biocompatible and biodegradable polysaccharide soluble only in aqueous media of low pH and showing extremely low toxicity. It forms gel beads with
multivalent counter ions via ionotropic gelation (Kawashima et al., 1985; Shiraishi et al., 1993, & Shu and Zhu, 2000).

In order to increase the local drug concentration by inhibiting the degradation of clarithromycin in acidic environment of the stomach an Eudragit S100 enteric coated formulation contains the proton pump inhibitor pantoprazole sodium sesquihydrate also prepared using different polymers such as sodium alginate, low methoxy pectin and combination of these two polymers.

In order to improve the therapeutic efficiency of the drug in the form of a floating drug delivery system of chitosan and administered along with an enteric coated multiparticulate dosage form of PSS is also investigated in this work.

Pantoprazole is highly unstable in the gastric medium and an enteric formulation is required to protect it from degradation. Eudragit S100 enteric coated polymeric delivery systems based on the ionotropic gelation of sodium alginate, low methoxy pectin with anhydrous calcium chloride can be explored in developing the formulations. These polymers are biodegradable, non toxic and having no pharmacological activity.

Since the clarithromycin formulation is expected to remain buoyant on the stomach contents for the duration of the dosing period, a full characterization of the clarithromycin loaded chitosan beads was necessary. The effect of PSS loaded formulation on the gastric levels of clarithromycin was also needed to be explored after characterization of various pantoprazole loaded enteric coated polymeric beads.

To overcome above discussed problems associated with clarithromycin we proposed to develop floating delivery system of clarithromycin loaded chitosan gel beads prepared by trisodium citrate and subsequent freeze drying of the product to achieve the required floating characteristics of the dosage form and to effectively check the gastroretentive properties in vitro and in situ.

With this background it was thought to develop a gastroretentive floating delivery system of clarithromycin using chitosan and an enteric coated polymeric delivery system of pantoprazole for better efficacy compared to the existing dosage forms for the H. pylori therapy.