8. SUMMARY AND CONCLUSIONS

There are many 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 clarithromycin by gastric acid. In an effort to overcome this problem, concomitant administration of antimicrobial agents and drugs which inhibit gastric acid secretion such as H₂ receptor antagonists and proton pump inhibitors (PPI) are in use.

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, in the present studies an attempt has been made to design a better 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 *H. pylori* eradication than absorption through the basolateral membrane, i.e. from blood.

Clarithromycin is a macrolide, orally absorbed, broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent like proton pump inhibitors. Clarithromycin has highest rate of eradication of *H. pylori* in monotherapy *in vivo*, though it is unstable and rapidly undergo degradation in low pH of gastric acid.

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.

Chitosan is a polycationic, nontoxic, mucoadhesive polymer, which has been proven to be safe. It allows a prolonged interaction between the delivered drug and the membrane epithelia, facilitating more efficient drug diffusion into the mucus/epithelium layer.
Pantoprazole is highly unstable in the gastric medium and an enteric formulation is required to protect it from degradation. 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 pharmacologically inactive. Eudragit S 100 coating of these PSS loaded alginate and pectin formulations can serve the purpose of protection of PSS from the gastric environment.

Keeping the above points in mind, we in the present studies developed a floating gastro retentive delivery system clarithromycin and an enteric coated formulation of pantoprazole using different natural polymers.

The clarithromycin formulations were prepared based on the interaction of a strong polycation, chitosan, with the trisodium citrate resulted in a poly cation multivalent anion complex by ionotropic gelation. The freeze drying of the beads increases the porosity, so that the beads float in the aqueous gastric medium.

The pantoprazole formulations were prepared using cat-ion induced ionotropic gelation method using alginate, pectin, alginate and pectin combinations and subsequent coating with enteric polymer Eudragit S 100.

The preformulation studies, such as DSC, FTIR, and XRD carried out to study the drug polymer interactions proved that there is no such interaction between the drug, polymer and the other excipients used in the formulations.

The product optimization tool, a $3^2$ factorial design was employed efficiently to study the effects of independent variables in the formulations.

Nine formulations were prepared for clarithromycin at varying drug to polymer ratios and % trisodium citrate used in the formulation to obtain a 100% release for the formulation in 8 h. The formulation CLCH6 was found to be the best among the nine formulations as it fulfilled the response requirement within the criteria selected for various factors in the factorial design. This formulation was further subjected for in vitro studies, in situ, and pharmacokinetic analysis.
Nine formulations each (a total of 27 formulations) were prepared for pantoprazole at varying drug: polymer ratios and % Eudragit coating used in the formulation to obtain a 100% release for the formulation in 8 h. The formulation CLCH6 was found to be the best among the nine formulations as it fulfilled the response requirement within the criteria selected for various factors in the factorial design. Quadratic and interactions were studies and the equations were reduced by omitting the non significant terms.

The evaluation of the clarithromycin formulation for the *in vitro* buoyancy of clarithromycin chitosan beads after 8 h, it was found that CLCH6 with drug to polymer ratio of 1:1 and 2% w/v of trisodium citrate has a maximum percentage buoyancy of 55.82% which indicates that the formulation will float in the stomach for long time for the better delivery of drug in the upper GIT.

The apparent particle density of clarithromycin chitosan beads was determined and all the formulations found to have a density less than 1; hence these formulations have buoyancy in the aqueous GI fluids. This is attributed by the fact that the freeze drying of the clarithromycin beads yield highly porous stricture, which renders the particles to float.

Apparent particle density of the sodium alginate beads from ALG1 to ALG9 were found to be above 1 g cm$^{-3}$ which is attributed by the fact that the beads will sink in the aqueous GI fluids. This was almost true for all the formulations of LM pectin and combination of alginate and LM pectin formulations.

SEM analysis of clarithromycin particles showed that the interior of the microspheres had large open channels or interconnected pores and drug crystals embedded in the solid matrix. These porous cavities may help the particles to float in the medium for longer period and this may be the reason that the particles processed densities less than 1.

The SEM analysis of the Eudargit coated PSS beads revealed that the particles were spherical in shape and the coating was clearly visible in the higher magnifications.
Volume weighted mean particle size of clarithromycin loaded chitosan beads were ranged between 932.24 to 1382.67 µm. The higher polymer concentration will have an opposite effect on the particle size as the concentration increases there is a decrease in the particle size was observed for most of the formulations.

Volume weighted mean particle size of Eudragit coated PSS alginate beads determined by laser diffraction method using Malvern master seizer and the particle size ranged between 922.10 to 1342.67 µm and that of Eudragit coated PSS pectin beads were between 754.80 to 1281.36 µm. The combination of alginate and pectin produced microbeads of size ranging between 853.24µm to 1142.37 µm.

The in vitro dissolution profile of clarithromycin from the nine formulations shows a near about 100% release for the formulation CLCH6. The formulation CLCH 6 has shown a first order concentration dependent kinetics, and the diffusion exponent close to Fickian kinetics was selected for in situ and in vivo. The formulation CLCH 6 has shown a first order concentration dependent kinetics, and the diffusion exponent close to Fickian kinetics was selected for in situ and in vivo.

The dissolution studies of PSS alginate formulations study showed that near about 100% release from the formulations were obtained at the end of 24 h. But in all formulations the maximum accumulative amount of drug released was at 24 h and this was not a good formulation for oral administration.

In the pectin formulations there was a burst release of PSS from all the batches in 4-6 h. The best fit model of first order kinetics was obtained for PECT9 with R² value 0.9803 and in the Peppas model it was observed a case II transport mechanism.

Though the drug release from pectin formulations showed an initial burst release, most of the formulations the drug release was at a very fast rate i.e. within 4-6 hours, and the drug release from alginate formulation was very slow i.e. 100% of drug release was found in any of the formulations in 24 h we explored the combination of the two polymers for the preparation of Eudragit coated PSS loaded alginate pectin formulations.
The maximum % CR was obtained for formulations ALG-PECT1 with low drug: polymer ratio and low % coating and ALG-PECT6, with drug: polymer ratio 1:3 and 5% coating. The best fit kinetic models revealed that a first order model for the formulation ALG-PECT6 and the Peppas model with a best fit of n=0.7290 was found to be the best formulation among all the formulations of alginate, pectin, and alginate and pectin combinations.

The formulation ALG-PECT6 subjected for long term stability testing revealed that the product remain stable for a period of one year with degradation constant of 0.138% per month.

The in situ gastro retention study conducted in rats showed a significant amount of CLCH9 remained in the rat stomach after 1, 2, 4 hours respectively Compared to PSS formulation significantly (P<0.05) higher amount clarithromycin beads were retained in the stomach after ever time interval of 1, 2, And 4 hours respectively. The reason for this is attributed by the effect of floating behavior of the beads may be the interaction of the mucin with the cationic polymer chitosan.

The gastric mucosal concentration of clarithromycin in presence of the pantoprazole beads, the delivery of CL from CLCH6 beads alone and in combination with ALG-PECT6 can ensure high concentration of CL in the gastric mucosa in comparison with clarithromycin suspension.

Similar results were obtained for study of clarithromycin concentration in the blood of the rats. The plasma CL concentrations obtained for the CLCH6 beads, it was found that there were significantly (p<0.05) higher levels plasma concentration concentrations compared with that of CL suspensions.

The pharmacokinetics of PSS from the ALG-PECT6 formulation and clarithromycin from the CLCH6 formulation carried out and various pharmacokinetic parameters such as $C_{\text{max}}$, $T_{\text{max}}$ and AUC were established.
Finally the floating clarithromycin formulation has proved *in situ* that 45.40% of the floating beads retained in the stomach at the end of 4 h. and this study has to be carried out in healthy volunteers to prove its worthiness as dosage form in healthy human beings followed by clinical usage for *H. pylori* therapy.

**Conclusions**

1. The clarithromycin loaded chitosan formulations were prepared by ionotropic gelation followed by freeze drying of the beads to increase the porosity, so that the beads float in the aqueous gastric medium.

2. The pantoprazole formulations were prepared using cat-ion induced ionotropic gelation method using alginate, pectin, alginate and pectin combinations and subsequent coating with enteric polymer Eudragit S 100.

3. The preformulation studies, such as DSC, FTIR, and XRD carried out to study the drug polymer interactions proved that there is no such interaction between the drug, polymer and the other excipients used in the formulations.

4. The product optimization tool, a $3^2$ factorial design was employed efficiently to study the effects of independent variables in the formulations.

5. The formulation CLCH6 was found to be the best among the nine formulations as it fulfilled the response requirement within the criteria selected for various factors in the factorial design.

6. In *in vitro* buoyancy of clarithromycin chitosan beads it was found that CLCH6 with drug to polymer ratio of 1:1 and 2% w/v of trisodium citrate has a maximum percentage buoyancy so that the formulation will float in the stomach for long time for the better delivery of drug in the upper GIT.

7. Clarithromycin formulations found to have a density less than 1; hence these formulations have buoyancy in the aqueous GI fluids.
8. Apparent particle density of the sodium alginate formulations beads were found to be above 1 g cm$^{-3}$ which is attributed by the fact that the beads will sink in the aqueous GI fluids.

9. SEM analysis of clarithromycin particles showed that the interior of the microspheres had large open channels which may help the particles to float in the medium for longer period

10. The SEM analysis of the Eudargit coated PSS beads revealed that the particles were spherical in shape and the coating was clearly visible in the higher magnifications.

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16. The best fit kinetic models revealed that a first order model for the formulation ALG-PECT6 and the Peppas model with a best fit of $n=0.7290$ was found to be the best formulation.

17. The formulation ALG-PECT6 subjected for long term stability testing revealed that the product remain stable for a period of one year with degradation constant of 0.138% per month.

18. The *in situ* gastro retention study conducted in rats showed a significant amount of CLCH9 remained in the rat stomach after 4 hours; The reason for this is attributed by the effect of floating behavior of the beads may be the interaction of the mucin with the cationic polymer chitosan.

19. The gastric mucosal concentration of clarithromycin in presence of the pantoprazole beads, the delivery of CL from CLCH6 beads alone and in combination with ALG-PECT6 can ensure high concentration of CL in the gastric mucosa in comparison with clarithromycin suspension.

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