SUMMARY

Oral route is considered as the most effective route of drug delivery because of ease of administration which leads to better patient compliance. Effectiveness of the drug delivered through oral route is affected by physiological factors such as gastric motility and physicochemical properties of drug moiety such as its solubility and permeability at specific region of gastrointestinal tract. There are many therapeutic moieties that are primarily absorbed in stomach and upper parts of small intestine (e.g. Levodpa, furosemide, riboflavin) or intended for local action in stomach (e.g. antibiotics against H.Pylori). Such moieties should remain in stomach for longer duration for better therapeutic effect. These moieties can not be delivered through conventional oral formulations because of their short gastric residence time and ultimately their inability in holding the drug in stomach for longer time.

Helicobacter pylori lives deep within the gastric mucus layer. Therapeutic moieties should be delivered locally at this site for its complete eradication. Antibiotics administered through conventional drug delivery systems can not eradicate H.pylori completely due to their inability in maintaining effective bactericidal concentration for longer time in stomach. Amoxicillin, levofloxacin and clarithromycin are widely explored antibiotics in anti H.Pylori therapy and are currently delivered through conventional drug delivery systems. But failure rates are high because conventional formulations can not maintain higher antimicrobial concentration for longer duration in stomach. A logical way to improve the effectiveness of therapy is to develop a gastroretentive drug delivery system which can reside in the stomach for longer duration and release drug as long as possible in the ecological niche of the bacterium.

Gellan gum based intra-gastric floating in-situ gelling system, gliadin nanoparticles, pH-sensitive chitosan / polyvinyl pyrrolidone based controlled drug release system and mucoadhesive microspheres have been reported for amoxicillin. Chitosan-based mucoadhesive microspheres and chitosan and carboxymethylcellulose sodium interpolymer complexes have been reported for clarithromycin.

In the present investigation, novel sustained release gastroretentive formulations of amoxicillin, levofloxacin and clarithromycin have been developed in the form of minimatrices and softgel.

Analytical methods

UV spectrophotometric method was developed for estimation of amoxicillin and levofloxacin while colorimetric method was developed for estimation of clarithromycin. These methods were utilized for estimating drug release during in vitro dissolution study. HPLC method was developed for estimation of all the three moieties and it was used for estimating drug content (assay).

UV spectrophotometric method has shown maximum absorbance for amoxicillin at 229 nm in 0.1N HCl. Linearity was plotted in the range of 5 to 35 mcg/ml. HPLC method involved phosphate buffer pH 5 and acetonitrile (96:4) as mobile phase. Separation was carried out on 250 mm x 4.6 mm C18 column at 230 nm. Linearity was plotted in the range 5 to 35 mcg /ml.

In UV spectrophotometric method, levofloxacin shows maximum absorbance at 293.6 nm in 0.1N HCl. Linearity was plotted in the 2 to12 mcg per ml. HPLC method was developed by using water: acetonitrile: triethylamine (50:50:0.1) as mobile phase and 250 mm x 4.6 mm C18 column. Detection wavelength was 293 nm. Linearity was plotted in the range 2 to14 mcg per ml.

Colorimetric method was developed for estimation of clarithromycin. A coloured complex was developed by treating clarithromycin with Folin ciocalteu reagent. This complex shown maximum absorbance at 760 nm. Linearity was plotted in the range of 10 to 60 mcg/ml. HPLC method was developed by using phosphate buffer and acetonitrile in 30:70 proportion as mobile phase. C18 column having 250 mm x 4.6 mm dimensions was used. Detection wavelength was 205 nm.

Minimatrices of Amoxicillin

Multiparticulate formulation in the form of minimatrices having floating and mucoadhesive feature were developed. Formulations were designed by central composite design. Xanthan gum, rate controlling polymers (HPMC K100M CR and polyox WSR coagulant), carbopol 974P and gas generating couple were

selected as formulation variables. Total 26 experiments were designed which included 16 factorial, 8 axial and 2 center points. Effect of formulation variables was studied on buoyancy lag time, drug release at 1 hour, time required for 95% drug release, swelling index and bioadhesive strength. Drug excipient compatibility was proved by DSC study. Preliminary formulation trials were carried out for screening suitable excipients and preparation technique. Direct compression was not feasible. Hence non- aqueous granulation technique was used for preparation of the minimatrices. Prepared minimatrices were evaluated for thickness, hardness, friability, buoyancy lag time, drug release pattern, swelling properties, bioadhesion strength and drug content. Drug release data was fitted to zero order, first order, Higuchi and Ritger and Peppas equation to ascertain the drug release mechanism. Regression analysis was carried out to find out significance of the formulation variables on particular response variable.

Sodium bicarbonate and citric acid was used in 3:1 proportion as gas generating couple. It significantly decreased buoyancy lag time. HPMC K100M CR and polyox WSR coagulant were used in 1:1 proportion as rate controlling polymers. These polymers along with xanthan gum and carbopol 974P were found to play important role in sustaining drug release at initial as well as later phase. Amongst the designed formulations, best formulation was selected by desirability approach. Formulation AGT 09 was found as best formulation. It was having minimum buoyancy lag time, high bioadhesion strength and was capable to sustain drug release upto 12 h. Drug release profile of this formulation best fitted to Higuchi's equation. Significance of the effect of xanthan gum on swelling index was interpreted from regression analysis. Increase in Carbopol 974P quantity significantly increased bioadhesion strength. Gastric residence time of the formulation was determined in healthy human volunteers by gamma scintigraphy technique which shown that the minimatrices stayed in stomach till 8 h. stability studies of the optimum formulation was carried out as per ICH guidelines and the formulation was found stable at accelerated condition till 6 months as there was no significant change in drug content and drug release pattern.

Softgel of Amoxicillin

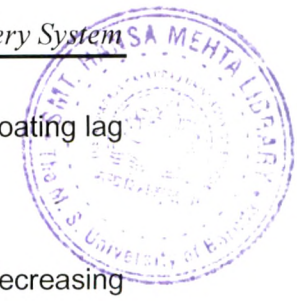
A novel softgel formulation with floating feature was developed for stomach specific drug delivery of amoxicillin. Preliminary studies were carried out to select

appropriate excipients for imparting floating feature and sustaining drug release and suitable vehicle for preparing encapsulation blend. Polyethylene glycol 400 was finalized as a vehicle for preparing encapsulation blend. HPMC K100M CR was selected as rate controlling polymer and sodium bicarbonate and citric acid as gas generating couple. Full factorial design (3^2) was implemented for selecting appropriate level of these variables and studying effect of combination of the variables on various formulation parameters such as buoyancy lag time, drug release pattern and swelling index. Total 9 experiments were designed. Response variables were fitted to quadratic model and regression analysis was carried out to get a quantitative relationship between formulation and response variables.

Increasing amount of gas generating couple significantly decreased floating lag time. Polymer hydration phenomenon kept the formulation floating for longer duration. Increasing levels of HPMC increased swelling index and decreased drug release. Increasing levels of gas generating couple significantly increased drug release and hampered the sustained release feature. Formulations with high level of gas generating couple and low level of HPMC were having low swelling index as the matrix was unable to hold absorbed fluid for longer duration. Hence faster drug release was also observed. Formulation ASF 12 was selected as an optimum formulation by desirability approach. It followed first order release kinetics. This formulation contained high level of rate controlling polymer and low level of gas generating couple. It was having minimum floating lag time and sustained drug release for maximum duration. In vivo studies were carried out in healthy human volunteers by gamma scintigraphy technique. Optimum formulation was found to have gastric residence time of 9 h. Stability study of the optimum formulation was carried out as per ICH guidelines and it was found stable till 6 months as there was no significant change in drug content and dissolution profile.

Minimatrices of Levofloxacin

In the present investigation levofloxacin minimatrices were developed for sustained delivery in stomach. Formulation optimization exercise was done by 3^2 full factorial design. Total 9 formulations were designed. HPMC K100M CR and polyox WSR coagulant in 1:1 ratio and sodium alginate, each at three different



level, were selected as formulation variables. Its effect was studied on floating lag time, drug release pattern and swelling index.

HPMC K100M CR and polyox were found to play important role in decreasing drug release at initial hour. Scanning electron microscopy studies were carried out to observe surface properties of the minimatrices in swollen state. Optimum formulation was selected by desirability approach. Formulation LMT 03 was found optimum and its release profile best fitted to zero order release kinetics. HPMC and polyox (1:1 ratio) and sodium alginate both significantly affected swelling index but effect of the HPMC and polyox was more prominent than alginate. In vivo study shown presence of the minimatrix formulation upto 9 h in stomach. Stability study of the optimum formulation was carried out as per ICH guidelines and it was found stable till 6 months as there was no significant change in floating lag time, drug content and dissolution profile.

Softgel of levofloxacin

In the present investigation, gastroretentive softgel with floating feature has been developed. Preliminary trials have shown promising results for HPMC K100M CR and sodium alginate. Hence systematic optimisation was carried out by full factorial design. HPMC K100M CR (X_1) and sodium alginate (X_2) each at three different levels were selected as formulation variables. Effect of these formulation variables was observed on drug release at 1 hour, time required for 50% drug release, time required for 95% drug release and swelling index. These response variables were fitted to quadratic model and regression analysis was carried out to get a quantitative relationship between formulation and response variables.

Sodium alginate shows synergistic action regarding sustained release phenomenon along with HPMC K100M CR. As HPMC K100M CR level increased from lower to higher, levofloxacin release was significantly decreased. At specific level of HPMC K100 CR, increase in sodium alginate level decreased levofloxacin release. Xanthan gum was one of the formulation component. This component mainly helped in retarding drug release by creating viscous network in the polymeric drug diffusion channels and helped in maintaining matrix integrity. Optimum formulation LSF 13 was having release exponent 0.614 which indicate anomalous type of drug release.

Results of regression analysis indicated that formulation variables X_1 and X_2 significantly decreased drug release at 1 h and increased the time required for 95% drug release and swelling index. Time required for 50% drug release was significantly increased due to X_2. An in vivo study in healthy human volunteers was carried out by gamma scintigraphy technique. The images captured by gamma camera shown presence of the softgel upto 10 h in stomach. There was no significant change in floating lag time, drug content and dissolution profile when the optimum formulation was subjected for stability study at accelerated condition for 6 months.

Minimatrices of clarithromycin

Multiparticulate formulation of clarithromycin was developed in the form of minimatrices. Formulations were optimized by 3^2 full factorial design which involve study of two factors at three different levels. Total 9 formulations were designed. Polyox WSR coagulant and chitosan were selected as formulation variables. Effect of different levels of the formulation variables was studied on floating lag time, drug release pattern, swelling index and bioadhesive strength.

Polyox and chitosan together played crucial role in decreasing drug release at initial hour. Increase in chitosan level decreased drug release which may be due to solubility of chitosan in acidic atmosphere. SEM images have shown prominent pores on minimatrix surface which were openings of the drug release channels. Optimum formulation CMT 07 best fitted to Ritger and Peppas equations as maximum R^2 value of 0.995 was observed for this equation. Value of release exponent "n" was 0.561 indicating anomalous type of drug release. As chitosan is soluble in acidic atmosphere, formulations containing high level of this variable were found to have less swelling index which may be due to improper matrix integrity over the period of time. Formulations containing high amount of chitosan was found to have higher bioadhesive strength. Optimum formulation was found to have gastric residence time of 8 h which was determined in vivo by gamma scintigraphy technique in healthy human volunteers. Stability study of the optimum formulation was carried out as per ICH guidelines and it was found stable till 6 months as there was no significant change in floating lag time, drug content and dissolution profile.

Softgel of clarithromycin

Clarithromycin softgel formulations were designed by 3^2 full factorial design technique. HPMC K100M CR and Polyox in 1:1 ratio (X_1) and sodium alginate (X_2) each at three different levels were selected as formulation variables. Total 9 experiments were designed. Effect of these formulation variables was studied on drug release at 1 hour, time required for 50% drug release, time required for 95% drug release and swelling index.

Combination of both the polymers was found effective in sustaining clarithromycin release. As the developed formulation is intended to release clarithromycin in acidic atmosphere of stomach, sodium alginate was considered as one of the potential excipient for sustaining drug release. Results of drug release study proved this assumption as increase in level of this variable decreased clarithromycin release at 1 h and increased time for 50% and 95% drug release. Optimum formulation CSF 09 followed Ritger and Peppas equation with "n" value of 0.524 indicating anomalous type of drug release. Optimum formulation was subjected to in vivo studies in healthy human volunteers which shown that developed softgel formulation could remain in stomach upto 8 h.

CONCLUSION

Gastroretentive formulations in the form of minimatrices and softgel were successfully developed for sustained and stomach specific delivery of amoxicillin, levofloxacin and clarithromycin. Formulations were designed and optimized by systematic approach of experimental design technique. Optimum formulations were selected by various in vitro tests and were subjected for in vivo studies. In vivo studies have shown that the developed minimatrices as well as softgel formulation were capable to remain in stomach for 8 to 10 h. Sustained release feature ensure delivery of these therapeutic moieties for longer duration which in turn would maintain effective bactericidal concentration for longer time in stomach. As local drug delivery for longer duration is the prerequisite criteria for complete eradication of H. pylori, the developed formulations have strong potential for achieving this therapeutic target.

The development work may be more useful as it can be produced on large scale by using existing technological facilities. Scale up of the minimatrices can be done by using existing tablet manufacturing facility and softgel can be produced

on large scale by using existing soft gelatin capsule manufacturing facility. Thus the formulations developed in the present investigation have therapeutic as well as manufacturing advantages.

The proposal for present investigation was scrutinized by Indian Council of Medical Research (ICMR) and fellowship was awarded for carrying out the said research work. It shows strong therapeutic potential of the present investigation