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Development and Evaluation of a Novel Floating Insitu Gelling System of Levofloxacin Hemihydrate

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ABSTRACT

The aim of this study was to develop a novel gastro retentive oral floating in situ gelling system for controlled release of levofloxacin hemihydrate (LIG) for the eradication of *Helicobacter pylori* (*H. pylori)* and other micro organisms causing local complications in stomach and proximal small intestine. Polymer based floating in situ gelling systems of levofloxacin hemihydrate were prepared by dissolving varying concentrations of gellan gum and sodium alginate in deionised water containing sodium citrate, to which varying concentrations of drug and calcium carbonate are added and dissolved by stirring. Calcium carbonate added to the formulation provides calcium ions and carbon dioxide. Calcium ions, due to ion interactions with the polymer, help in gelation. Carbon dioxide entraps in the gel and facilitates buoyancy of the gel. The formulation variables, concentration of gellan gum and sodium alginate significantly affected the in vitro drug release of drug from the prepared LIG. Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) were used to check the presence of any interaction between the drug and the excipients. The drug release from the *in-situ* gels follows the Hixson-crowell model, which indicates a drug release through the polymeric matrix. A stomach specific *in-situ* gel of Levofloxacin hemihydrate could be prepared using floating mechanism to increase the residence time of the drug in stomach and thereby increase the absorption.

Keywords: Floating insitu gelling system, Levofloxacin hemihydrate, polymeric matrix.

INTRODUCTION

Micro organisms responsible for serious stomach specific infections include Proteus, Klebsiella, Salmonella, Shigella, Harmful strains of E. coli, *Helicobacter pylori (H. pylori)* (Marshall BJ and Warren JR, 1984). Among all *H. pylori is* one of the most widespread pathogenic bacterial infections, causing serious gastric diseases like peptic ulcers, gastric lymphoma and acute chronic gastritis (Crescenzi V *et al*., 1990). *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 WL, 1991). Most antibacterial agents have low minimum inhibitory concentrations (MIC) against *H. pylori* in culture. And also single antibiotic therapy is not efficient for the eradication of *H. pylori* infection in vivo. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid and short residence time of the antibiotic in the stomach (Shah S *et al*., 1999). Patient compliance, side effects and bacterial resistance are the other problems. Other than the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate *H. pylori* from the stomach. One way to develop the efficacy in eradicating the *H. pylori* and other infections is to deliver the antibiotic locally in the stomach (Yokel RA *et al*., 1995). Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on infectious organisms (Umamaheshwari RB *et al*., 2004).

Alginic acid is a linear block polysaccharide copolymer made of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues connected by 1,4 glycosidic linkages. The proportion and the arrangement of the blocks along the polymer chain much depend on the salgal source. The aqueous alginate solutions could form firm gels in presence of di- and tri-valent metal ions by a cooperative process involving consecutive guluronic residues in the G blocks of the alginate chain. This property has been widely used for preparation of vehicles for sustained delivery of the bioactive molecules (Grant

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GT *et al*., 1973; Morris ER *et al*., 1978). The optimum quantities of sodium citrate that maintained the fluidity of the formulation before administration and resulted in gelation after being added to simulated gastric fluid (pH = 1.2), was reported earlier. The calcium carbonate present in the gelling formulation released carbon dioxide in gastric environment thereby making the formulation porous and buoyant and prolonging the residence time. This floating in stomach provides the potential to sustain the drug release over a long period of time.

Gellan gum is a multi-functional gelling agent can be used alone or in combination with other products to produce a wide variety of textures. Gellan gum acts as a thickening or gelling agent and can produce textures in the final product that vary from hard, non-elastic, brittle gels to fluid gels. The formulation adopted was a gellan solution containing sodium citrate (as a source of $Ca2^{+}$), which complexes the free $Ca2^{+}$ ions and releases them only in the acidic environment of the stomach. In this way, the formulation remains in liquid form until it reaches the stomach, where gelation of gellan gum is instantaneous (Jigar and N. Shah, 2007).

Levofloxacin Hemihydrate is a fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria and atypical respiratory pathogens. Several randomized comparative trials have confirmed the efficacy of levofloxacin in the treatment of infections of the respiratory tract, genitourinary tract, skin and skin structures (Gisbert JP and Pajares JM, 2005). Recently, some studies have evaluated the efficacy of new fluoroquinolones, such as levofloxacin, that could prove to be a valid alternative to standard antibiotics not only as first-line therapies but, more interesting, as rescue-regimens (Croom KF and Goa KL, 2003).

MATERIALS AND METHODS

Materials

Levofloxacin hemihydrate was received as gift sample by Kwality pharmaceuticals pvt. Ltd (Punjab), gellan gum and sodium alginate MMC Healthcare, (Himachal Pradesh). Calcium carbonate and sodium citrate was purchased from S.D. Fine Chemicals, Mumbai. All other chemicals were used of analytical grade.

Method

Preparation of insitu gelling solution (Rishad R *et al*., 2010)

Specified quantity of Levofloxacin hemihydrate, calcium carbonate, sodium citrate, different polymers such as sodium alginate and gellan gum were weighed according to formula given in Table 1. The mixture of gellan gum and Sodium alginate solutions of different concentrations were prepared in deionized water containing 0.25% of sodium citrate. Low concentrations of cations in solution were sufficient to hold the molecular chains together and inhibit hydration. Sodium alginate solution was heated to 70^{o}C with stirring. After cooling to below 40^{o}C, different concentrations of calcium carbonate and the drug were added and dispersed well with continuous stirring. The resulting sodium alginate and gellan gum in-situ gelling solution containing Levofloxacin hemihydrate was finally stored in amber coloured bottles until further use.

Differential scanning calorimetry (DSC)

The DSC thermograms were recorded on a DSC (model Dsc-60, Shimadzu). Samples of were heated in hermetically sealed aluminium pans over temperature range of 10^{0}C – 300 ^{0} C at a constant rate of 10^{0}C/min under nitrogen purge.

Fourier Transform Infra Red Spectroscopy (FTIR)

FTIR spectra were obtained on FTIR-8400S (Shimadzu). Samples were prepared in KBr disks. Data were collected over a spectral region from 4000 to 400 cm-1.

Gel strength (Jayvadan K *et al*., 2010)

Gel strength is calculated using the gel strength apparatus. It contains two tubes; upper tube is attached with pan through thread in which weights are added. Two surfaces are tightly covered with egg membrane. 1 gm of gel was kept between the two surfaces. The weights are added into pan. The weight at which the two surfaces detach is noted and the gel strength is calculated by using formula:

Gel strength = Mg/a

M: Weight at which the two surfaces detaches

g: gravitational force

a: Area of surfaces

***In-vitro* buoyancy** (Rishad R *et al*., 2010)

The *in-vitro* buoyancy study was performed using the USP dissolution apparatus II, model USP TDL-08L with 500 mL of simulated gastric fluid (pH = 1.2) and the temperature of the medium maintained at 37^{o}C. A 10 mL sample of the prepared solution (*in-situ* gelling formulation) was drawn up with the help of a disposable syringe and placed into a Petri dish. Then, the Petri dish was placed in the dissolution vessel containing the medium without much turbulence. The time for the gel to come to surface (floating lag time) and the time the gel remained floated on the medium surface (floating time) were recorded (PS. Rajinikanth *et al*., 2007).

Microbiological studies

Four petri plates with nutrient agar medium were prepared. Using micropipette, 0.2ml of the seeded broth containing test organisms E.coli, Klebsiella, Staphylococcus, Streptococcus, were inoculated on four plates of solidified agar and spreaded uniformly with a glass spreader. Then four wells were cut out in agar layer of each plate with an aluminum bore of 5mm diameter to contain equal volume of test and standard drug solution. In each plate fill the test and standard alternatively in zig zag manner. All the work was carried out under strict aseptic conditions. The plates were then incubated at 37^{0}c ± 1 for 24 hours. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well were measured and were tabulated in table.3.

***Invitro* drug release study**

The study of the Levofloxacin hemihydrate release from the in-situ gelling preparation was carried out with some modification using USP dissolution test apparatus II with paddle stirrer; model USP TDL-08L at a rate of 50 rpm. The slow speed prevented breaking of the gelled

formulation and ensured a low level of agitation. The dissolution medium used was 500 mL of a 0.1 N solution of HCl (pH = 1.2), and the temperature was kept at 37°C. A 10 mL sample was withdrawn using a disposable syringe; the needle was then wiped clean and the excess sample removed from the needle end. The sample was then gently transferred into a petri dish which was then immersed into the dissolution medium without much turbulence. At 1 hr intervals, an accurately measured sample of the dissolution medium was removed for analysis and replaced with the same amount of the pre-warmed (37°C) fresh medium. The absorbance of the sample was measured at 294 nm using a UV spectrophotometer for analysis of Levofloxacin hemihydrate. Each experiment was performed for a period of 8 hr in triplicate.

Analysis of *Invitro* release

The *in- vitro* drug release data were analyzed by fitting them into different kinetic models in order to investigate the release mechanism of Levofloxacin hemihydrate from the gel systems (Higuchi I, 1962; Higuchi T, 1961; Korsmeyer R *et al*., 1983).

RESULTS AND DISCUSSION

IR spectroscopic studies were conducted to determine possible drug-polymer interactions. IR spectra of pure drug Levofloxacin hemihydrate, sodium alginate, gellan gum and physical mixture of Levofloxacin hemihydrate with sodium alginate and gellan gum were obtained which shows all the characteristic peaks of Levofloxacin hemihydrate and polymers were present in the physical mixture. The major peaks C=O peak at 1724.81cm-1, aromatic C-H peak at 2935.62cm-1 and OH group of carbonyl moiety at 3265.81cm-1 which were present in pure drug Levofloxacin hemihydrate are also present in the physical mixture which indicates that there is no interaction between drug and the polymers, which confirms the stability of drug.

The pure drug showed a sharp endothermic peak at 227 °C corresponding to its melting point. The gellan gum and sodium alginate showed endothermic peak at 252.5 °C and 255 °C respectively The appearance of two or more endothermic peaks in the heating DSC curves of Levofloxacin hemihydrate-gellan gum may be an indication of the presence of junction zones with different bonding energies or different rotational freedoms (Nishinari K, 1997). Higher temperature endothermic peaks, as observed for Levofloxacin hemihydrate-gellan gum in comparison to gellan gum, could be attributed to the melting of the zones with higher bonding energies or with lower rotational freedoms. The DSC showed that the Levofloxacin hemihydrate-sodium alginate and Levofloxacin hemihydrate-gellan gum form more thermally stable junctions.

Sol to gel transformation of polymers occurs in the presence of either monovalent or divalent cations in contact with the gastric fluids. The floating ability of the formulations was due to the presence of calcium carbonate. The calcium carbonate effervesced, releasing carbon dioxide and calcium ions. The released carbon dioxide is entrapped in the gel network producing buoyant formulation and then calcium ion reacted with gellan produced a cross linked three-dimensional gel network that might restrict the further diffusion of carbon dioxide and drug molecules and has resulted in extended period of floating and drug release, respectively. The prepared formulations floated more than 24hr (Chandrasekaran R and Thailambad VG, 1990). The microbial studies of prepared oral floating insitu gels are effective compared to standard drug.

For LIG1 and LIG2 the intial burst effect was 51.3% and 84.3% and drug release was upto 97.2 and 97.8% respectively at the end of 8h. LIG3 formulation with high concentration of gellan gum polymer, the intial brust effect was 39.1% and drug release was upto 87% at end of 8h. LIG4 with equal concentration of sodium alginate and gellan gum, intial brust effect was 38.7% and drug release was 98.1% at end of 8h. LIG5 and LIG6 the release was upto 99% and 95.8%. A decrease in the rate and extent of drug release was observed with the increase in polymer concentration in in situ gels and is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to traverse. Formulation with high concentration of gellan gum has shown decreased rate and extent of drug release with sustained effect. The intial high release and moderate release second, this bi-phasic pattern of release is a characteristic of the matrix diffusion kinetics (Lemoine D *et al*., 1998).

Table.1 Formulation of Levofloxacin hemihydrates

S.NO	Formulation	Levofloxacin hemihydrate (%)	Sodium alginate (%)	Gellan gum (%)	Calcium carbonate (%)	Sodium citrate (%)
1	LIG-1	1	1	-	0.5	0.25
2	LIG-2	1	2	-	0.5	0.25
3	LIG-3	1	-	1	0.5	0.25
4	LIG-4	1	0.5	0.5	0.5	0.25
5	LIG-5	1	0.7	0.3	0.5	0.25
6	LIG-6	1	0.3	0.7	0.5	0.25

Table.2 Gel strength and Buoyancy studies

Formulation	Gel strength (N / m2)	Lag time (min)	Floating time (hr)
LIG-1	2029.58	<1	>24
LIG-2	2398.5	<1	>24
LIG-3	2767.5	<1	>24
LIG-4	2216.1	<1	>24
LIG-5	2031.4	<1	>24
LIG-6	2216.1	<1	>24

Table.3 Microbial studies of LIG formulations

S.NO	Formulations	ZONE OF INHIBITION (cm)			
		E.coli	Klebsiella	Staphylococcus	Streptococcus
1	Standard drug	1.75	1.80	1.55	1.60
2	LIG-1	1.70	1.65	1.30	1.25
3	LIG-2	1.65	1.65	1.45	1.35
4	LIG-3	1.65	1.70	1.40	1.45
5	LIG-4	1.60	1.75	1.40	1.40
6	LIG-5	1.75	1.70	1.50	1.40
7	LIG-6	1.70	1.70	1.40	1.45

Table.4 Release kinetics Levofloxacin hemihydrate (LIG1-LIG6)

Model	Parameter	LIG1	LIG2	LIG3	LIG4	LIG5	LIG6
Zero order	R^2	0.908	0.967	0.892	0.874	0.932	0.921
First order	R^2	0.976	0.942	0.767	0.990	0.913	0.975
Higuchi	R^2	0.968	0.988	0.802	0.949	0.945	0.969
Korsmeyer Peppas	R^2	0.985	0.975	0.757	0.959	0.945	0.978
Hixson-crowell	R^2	0.989	0.979	0.817	0.978	0.978	0.980

Figure.1 DSC thermograms, A: Levofloxacin hemihydrate, B: Sodium alginate, C: Gellan gum, D: Levofloxacin hemihydrate + sodium alginate, E: Levofloxacin hemihydrate + gellan gum

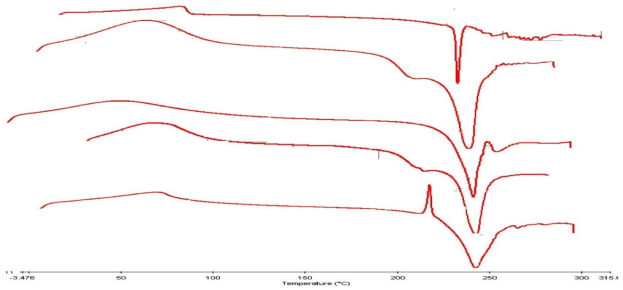


Figure.2 FTIR of A: Levofloxacin hemihydrate, B: Gellan gum, C: Sodium alginate, D: Levofloxacin hemihydrate + gellan gum, E: Levofloxacin hemihydrate + sodium alginate

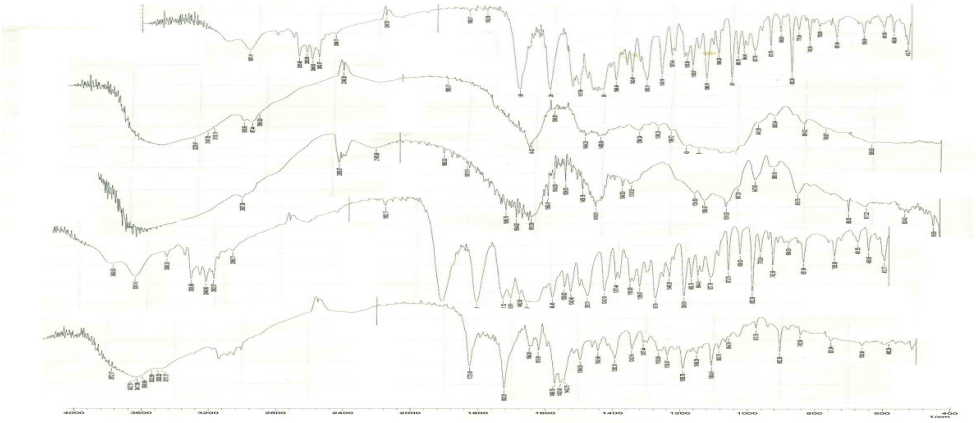


Figure.3 *Invitro* drug release of different LIG formulations

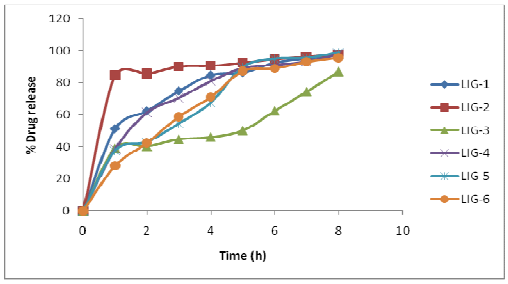


Figure.4 Zero order plot of LIG formulations

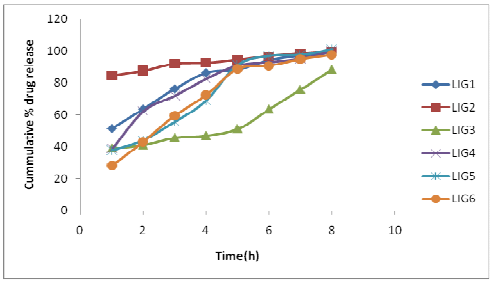


Figure.5 First order plot of LIG formulations

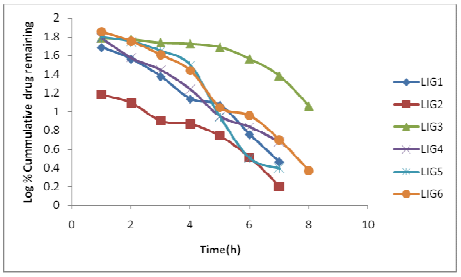


Figure.6 Higuchi plot of LIG formulations

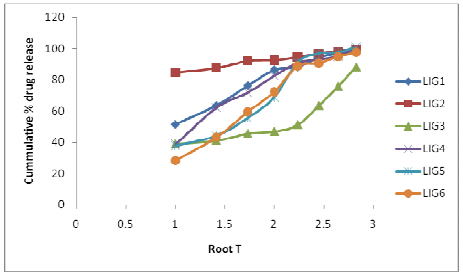


Figure.7 Krosmeyer plot of LIG formulations

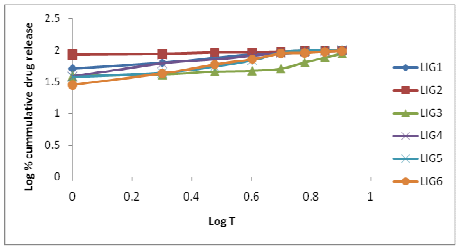
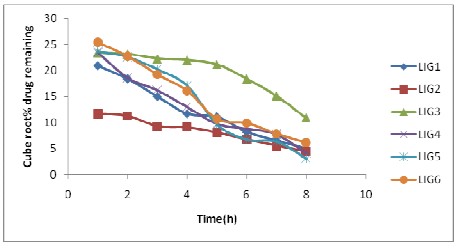


Figure.8 Hixon-Crowell plot of LIG formulations



The order of drug release was found to be Hixson-crowell cube root for LIG1, LIG5 and LIG6, in which regression value was close to 1. It was assumed that release rate is limited by drug particles dissolution rate and not by diffusion that might occur through the polymeric matrix. Where LIG3 follows zero order release. LIG2 follows Higuchi, the drug release was matrix diffusion. LIG4 follows first order.

CONCLUSION

This study showed the feasibility of *invitro* gel forming from aqueous solutions of sodium alginate and gellan gum containing Ca++ ions in a complexed form. The in situ formed gel preserved its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally. The developed formulations met all prerequisites to become an in situ gelling floating system, gelled, and floated instantaneously in the pH conditions of the stomach. It was observed that the resulting gel remained buoyant for 24 h and slowly released Levofloxacin hemihydrate during the 8 h period. It is concluded that Levofloxacin hemihydrate could be targeted to stomach and be released slowly over a period of time.

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