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Research Article

FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF LEVOFLOXACIN

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ABSTRACT

Purpose: The objective of present study is to develop site-specific drug delivery system for controlled release of levofloxacin for eradication of H. pylori for the treatment of peptic ulcer. Methodology: Floating microspheres of Levofloxacin were prepared by solvent evaporation method. The drug was encapsulated with Eudragit and Ethyl cellulose in various combinations of polymers ratios. Findings: The prepared microspheres are subjected to evaluation for particle size, % buoyancy, in-vitro release study and stability studies. The percentage buoyancy was found to be in the range of 67.12% to 94.21%. The good buoyancy behaviour of the microspheres revealed that the microspheres are hollow in nature and retained for more than 12 hr in the upper part of the GIT in order to enhance gastric residence time. Research Implications: The prepared formulations can be tested clinically to assure the in vivo performance. Value of paper: The current study compared the combination of polymers and revealed their effect on drug release and various other parameters for the preparation of floating microspheres.

Keywords: Floating, microspheres, gastro retentive and sustained release.

INTRODUCTION

Historically, oral drug administration has been the predominant route for drug delivery[1]. Oral route has high patient acceptability, primarily due to easy of administration[2]. It is necessary to optimize residence time of system within the gastrointestinal tract as well as release of drugs from system for developing successful oral drug delivery system. Drugs that show good absorption from the gastrointestinal tract but have short elimination half-life are disappearing quickly from the blood circulation. So frequent dosing is requiring for such kind of drugs. To overcome these problems, the oral controlled release formulations can be developed to release the drug in controlled manner into the gastrointestinal tract to maintain the constant drug concentration in the serum for longer period of time[3]. In the pathogenesis of peptic ulcer diseases *H. pylori* infection play a crucial role. More than 70%-80% of patients suffering from the gastric ulcers are *H.pylori* positive & 95% of patient suffering from duodenal ulcers. *H. pylori* is a gram negative motile, microaerophilic, curved bacillus that is found in the mucus layer overlying the gastric epithelium. The aim of this study is to develop a gastroretentive particulate drug delivery system for controlled-release of Levofloxacin drug for eradication of Helicobacter pylori in the treatment of peptic ulcer. To improve the gastric stability & short residence time of Levofloxacin due to entrapment within the microsphere gives controlled release in the gastric environment make complete eradication of *H. pylori* from GIT more effectively than conventional dosage form.

MATERIAL AND METHODS

Levofloxacin, Eudragit S100, eudragit L100, HPMC, ethylcellulose was purchased from yarrow chem. Product. All the chemical and reagents were used of analytical grade.

Preparation of Levofloxacin microspheres

The floating microspheres of Levofloxacin were prepared by using solvent evaporation method. HPMC K4M, Eudragit S-100, HPMC K100, Eudragit L-100 & Ethyl cellulose polymers were selected for the preparation of six batches of floating microspheres of Levofloxacin. Combination of Eudragit L100 & Eudragit S100, Ethylcellulose & Eudragit S100 was tried in development of formulation batches at 300 rpm stirring speed. Drug and polymer combination were dissolved in 1:1 mixture of Ethanol & DCM. The clear solution of drug and polymer was poured into a 100 ml of distilled water maintained at a room temperature. The solution was continuously stirred at specified speed of 300 rpm for 2 hours. The volatile solvent was evaporated completely. The microspheres were collected by filtration and dried for 1 hr at 60^0 C and subsequently stored in desiccators over fused calcium chloride**.** The compositions of batches are tabulated in Table 1.

Table 1: Composition of floating microspheres

Formulation	F1	F2	F3	F4	F5	F6
Levofloxacin Drug (mg)	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Eudragit (S100 + L100) (mg)	300 mg	400 mg	500 mg	---	---	---
Eudragit S100 + Ethyl cellulose (mg)	---	---	---	300 mg	400 mg	500 mg
Distilled water	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Tween 80 (%)	0.1	0.1	0.1	0.1	0.1	0.1

Preformulation Studies

UV Spectrophotometric Analysis

Determination of Λ max (UV Spectroscopic Method): Stock solution of 100µg/ml of levofloxacin was prepared in 0.1 N HCl. To obtained concentration 100µg/ml 0.1 N HCl was appropriated diluted with this solution. The spectrum of this solution was run in 200 to 400 nm range on Shimadzu Double beam UV-Visible Spectrophotometer. The λ max of Levofloxacin was found to be 293 nm in 0.1 N HCl[4].

Calibration curve of Levofloxacin in 0.1 N HCl: The aliquots of stock solution 100µg/ml of levofloxacin were subsequently diluted to obtain a series of dilutions containing 1, 2, 3, 4, 5 µg/ml of drug. The absorbance of these solutions was measured by using UV-Visible spectroscopy at 293 nm using 0.1 N HCl as a blank.

FTIR Spectrophotometric Analysis

The compatibility between drug and polymer was analysed by using FTIR Spectrophotometer. The FTIR spectra of drug with polymers were compared with standard FTIR spectrum of pure drug. Samples were prepared for drug levofloxacin, polymer Eudragit S100, Eudragit L 100, Ethyl cellulose and mixture of drug and polymer[5].

Evaluation of floating microspheres

Particle size analysis

Size and shape of floating microspheres particles were evaluated by optical microscope. The formulated microspheres particles were examined on an optical microscope and the size of microspheres was measured by pre-calibrated ocular micrometer (Leica software).

Scanning Electron Microscopy

The surface topography, morphology, cross-section, particle size etc. was determined by Scanning Electron Microscopy and Pictures of microspheres were taken by random scanning of the stub[6].

% Buoyancy

Microspheres were added to the basket of USP XXIV type II dissolution apparatus. 900 ml of 0.1 N HCl was taken as media for buoyancy study with 0.02% tween 80. The media was stirred with a paddle which was rotating at 100 rpm. The test was carried out for 6 hrs. The floating microspheres at the surface were collected out by pipette and the settled portion of microspheres particles were decanted separately. The collected microspheres were dried in an oven at 60^0 C for 1 hour. The buoyancy % was calculated by the formula as follow[7-8].

$$\% \text{ Buoyancy} = W_f / (W_f + W_s) \times 100$$

Where, W_f = weight of microspheres found floated at the end,
W_s = weight of microspheres settled at the end

Drug Entrapment Efficiency (DEE)

The all formulations were subjected to estimate drug content. The floating microspheres equivalent to 50 mg of Levofloxacin from all batches was accurately weight and crushed. The powdered microspheres were dissolved in 5 ml of ethanol and diluted up to 0.1 N HCl. The sample was filtered by Whatmann filter paper. Then the sample was analyzed by UV spectrophotometer. The absorbance of samples was taken at wavelength maxima of 293 nm against 0.1 N HCl as a blank[9-10]. The % entrapment was calculated as follows:

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoritical drug concentration}} \times 100$$

In-vitro drug release study

The *in-vitro* drug release studies were conducted by taking 0.1 N HCl as dissolution media. The dissolution was performed for 12 hours. The drug release of formulated microspheres was determined using USP XXIV type II dissolution apparatus[11]. A weight number of floating microspheres was transferred in dissolution vessel containing 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C. The rotation of apparatus shaft was fixed at a speed of 100 rpm. 5 ml of sample was pipette out at periodic time interval and the volume of dissolution fluid was maintained by adding 5ml of fresh dissolution fluid after each withdrawn. Then sample was passed through 5µm membrane filter and analyzed spectrophotometrically at 293 nm.

Study of drug release kinetic

The *in-vitro* release data was plotted to different kinetic models such as zero, first-order and Higuchi kinetics. Further data was fitted in Korsemeyer-Peppas equation for getting the mechanism of drug release[12].

Stability Studies

Stability studies were performed according to ICH guidelines. All the six formulation of Levofloxacin microspheres were tested for stability studies in stability chamber. Formulation was exposed up to 30 and 60 days of stability studies at 40°C ± 2°C and 75% ± 5% RH[13-14].

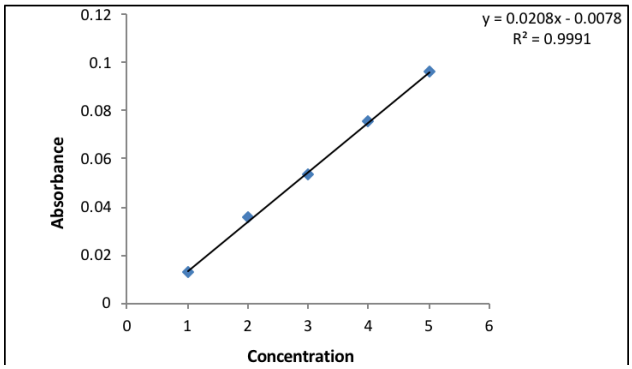
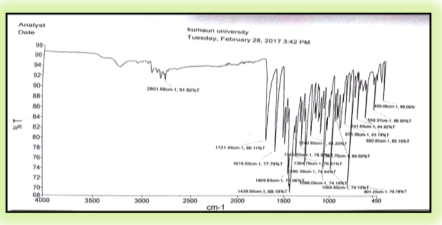


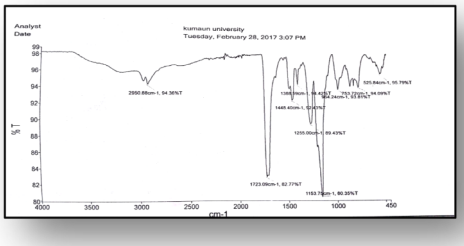
Fig 1: Calibration curve of Levofloxacin in 0.1 N HCl

Table 2: Characteristic peaks of Levofloxacin

S.no.	Reference peak (cm^{-1})	Obtained peaks (cm^{-1})	Functional group	Stretching/Bending
1.	2500-3300	2801.88	-COOH	Stretching
2.	1710-1720	1721.49	C=O	Stretching
3.	1030-1230	1240.83	C-N	Stretching
4.	1000-1350	1004.89	C-F	Stretching
5.	1085-1150	1088.09	C-O-C	Stretching



a)



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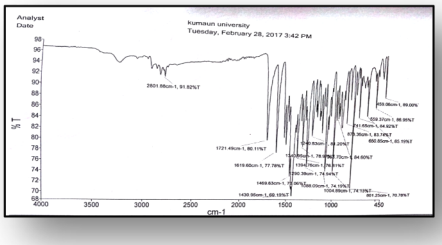

c)

Fig 2: a) FTIR of pure drug Levofloxacin, b) Drug + Eudragit S100 +Eudragit L100 , c) Drug + Eudragit S100+ Ethylcellulose

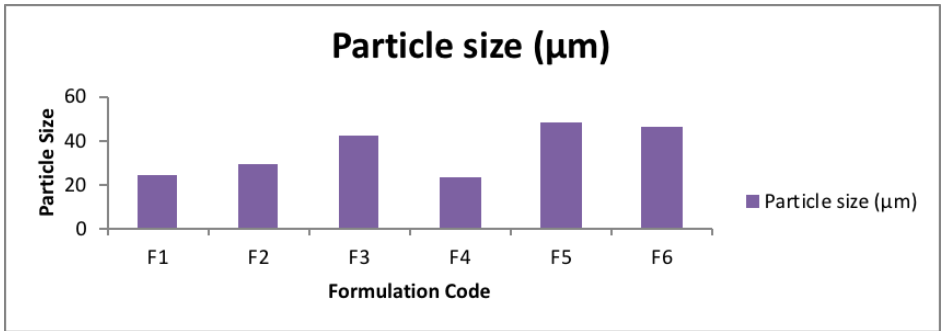

Fig 3: Particle size of microspheres

Fig 4: SEM image of formulation F5

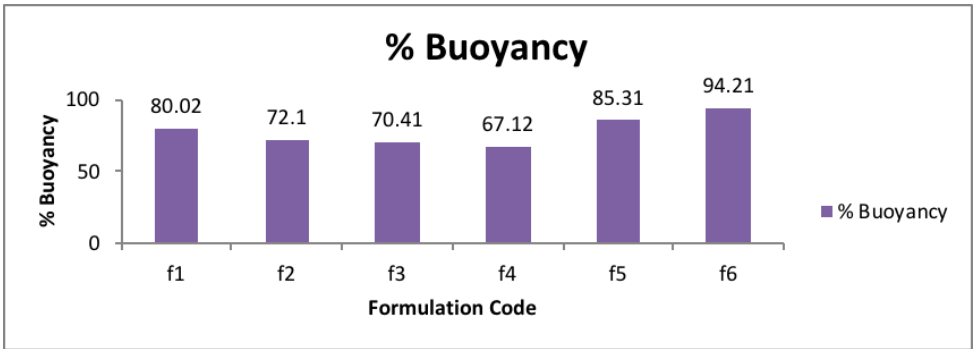

Fig 5: Percentage buoyancy of floating microspheres

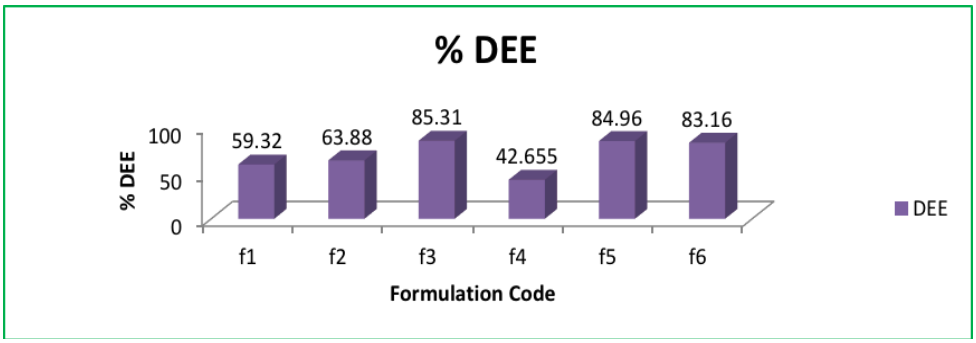

Fig 6: Percentage DEE of floating microspheres

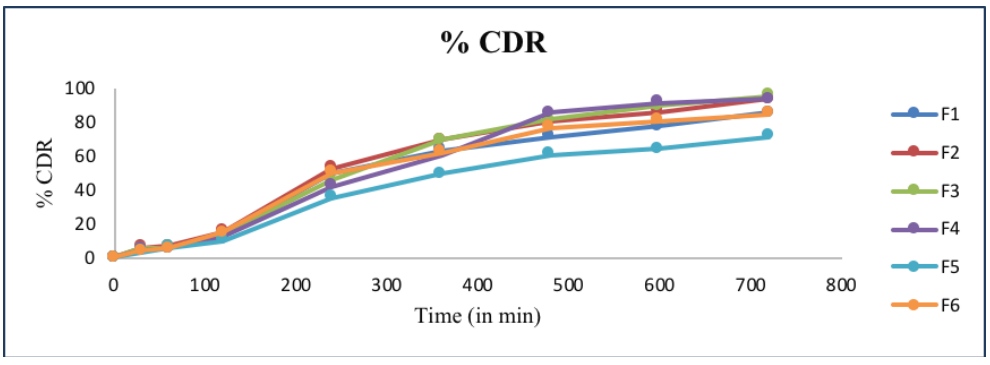

Fig 7: Percentage CDR of floating microspheres of levofloxacin

Table 3: Model fitting release profile of formulations F1 to F6

Formulations	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Korsmeyer – Peppas	
				(r^2)	n value
F1	0.875	0.957	0.899	0.906	1.122
F2	0.880	0.980	0.905	0.915	1.084
F3	0.898	0.984	0.904	0.917	1.090
F4	0.911	0.970	0.900	0.926	1.186
F5	0.905	0.950	0.912	0.942	1.127
F6	0.871	0.948	0.901	0.923	1.146

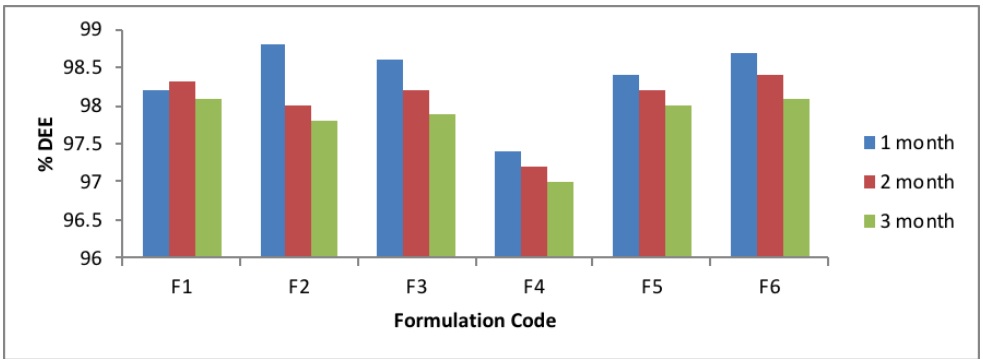

Fig 8: Accelerated Stability data for % DEE at 40°C ± 2°C and 75 % ± 5% RH for 1, 2, 3 months

RESULT AND DISCUSSION

UV Spectrophotometric Analysis

Identification of drug ((λmax VALUE): Levofloxacin shows maximum absorbance at 293 nm in 0.1 N (pH 1.2) HCl and the Calibration curve represented in Fig 1.

FTIR Spectrophotometric Analysis

FT-IR Spectra of Levofloxacin and its combination with Eudragit S100 and Eudragit L100 and Ethyl cellulose indicate that Levofloxacin is not involved in any chemical interaction. The FT-IR spectra of Levofloxacin and its combination with polymers are represented in Fig 2. and the FT-IR of pure drug levofloxacin are summarized in Table 2.

Particle Size Analysis

The average particle size range for formulation F1 to F6 was found to be 24.13 µm - 46.53 µm. In formulation F1 to F4 the particle size increases with increase in polymer concentration respectively except formulation F5 and F6. This is because the consistency of polymer was increases which decrease the stirring efficiency, which results in increased particle size. The particle size is represented in Fig 3.

Scanning Electron Microscopy

SEM analysis showed that the prepared floating microspheres were having size in micrometers and the particles were nearly spherical. SEM image of formulation F5 represented in Fig 4**.**

Percentage Buoyancy

The % buoyancy of formulations F1 to F6 was determined and it was found in between 67% to 94%. The formulations F5 and F6 showed good floating properties. In the test of floating time, the microspheres remain floating for more than 12 hr. The buoyancy profile of the microspheres revealed that the microspheres might be hollow in nature, which retained for a longer period of time in the upper part of the GIT in order to enhance gastric residence time of the drug. As the concentration of polymers increases, buoyancy also increases. The percentage buoyancy is represented in Fig 5.

Drug Entrapment Efficiency (DEE)

The drug entrapment efficiency of all formulation was found to be in the range from59.32% to 84.96%. While increasing polymers Ethyl cellulose and Eudragit S100 concentration, increased entrapment efficiency due to increased polymers content, more particles of drug would be coated leading to higher entrapment efficiency. The percentage DEE is represented in Fig 6.

In-vitro drug release study

In-vitro drug release studies were performed in 0.1 N HCl for 12 hours. It was observed that as the concentration of polymer increases, the drug release was reduced. It is due to the polymer matrix acts as a barrier for the drug release. Finally, it was observed that the formulations prepared with polymer Ethyl cellulose and Eudragit S100 showed better controlled release than formulations F1 to F3. The percentage cumulative drug release for different formulations is represented in Fig 7.

Release Kinetic Study

The data obtained for in-vitro release were fitted into the equations for the Zero-order, First-order, Higuchi plot and Peppas kinetics models. The in-vitro drug release showed that the all formulation has highest regression coefficient values for first-order kinetics, indicating swelling diffusion to be the prominent mechanism of drug release. The model fitting release profile of formulations F1-F6 is represented in Table 4.

Stability Studies

Physical appearance for formulation F1 to F6 shows no colour change while in formulation F2, F4 shows weight loss in 30 and 60 days. Accelerated stability studies of different formulations for 1,2,3 months for F1 to F6 at 40°C ± 2°C & 75% ± 5% shows in Fig 8.

CONCLUSION

Gastro retentive microspheres of Levofloxacin were prepared by solvent evaporation method. The concentration of polymer influences the physical characteristic as well as floating behaviour of the microspheres. The formulation F5 considered good flow properties, including floating ability (85.31%), DEE (84.96%), particle size (48.53 µm) and continuous release more than 12 hours due to ethyl cellulose and eudragit S100 polymer combination which enhance the GRT as compare to conventional dosage form. Prolonged gastric resistance time of the formulation and its controlled release in the gastric environment makes complete eradication of *H.pylori* from GIT.

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