Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Design and Characterization of Eudragit Coated Floating Microsphere of Clarithromycin Prepared by Emulsion Solvent Diffusion

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ARTICLE DETAILS

Article history:
Received on 5 March 2016
Modified on 25 March 2016
Accepted on 28 March 2016

Keywords:
Heliobacter pylori,
Clarithromycin,
In -vitro buoyancy,
SEM.
In- vitro release

ABSTRACT

In the present study, an attempt has been made to prepare floating microspheres of clarithromycin designed as gastroretentive dosage form for the treatment of Heliobacter pylori. To develop oral drug delivery systems, it is necessary to optimize both the residence time of system within the gastrointestinal tract and release of drug from the system .The floating microsphere is prepared by the emulsion solvent evaporation method. Formulations were characterized for their particle size, practical yield, entrapment efficiency, in vitro buoyancy, scanning electron microscopy (SEM) and in vitro drug release. Scanning electron microscopy shows that spherical microspheres with porous surface were formed. The *In-Vitro* release was significantly decreased with increase in polymer concentration.

INTRODUCTION

In the field of pharmaceutical technology; great efforts are being directed towards the refabrication of existing drug molecules in a fashion, capable of solving problem related to poor water solubility, poor bioavailability, dosing problem, stability, toxicity, etc. This trend of working has lead to development of new drug delivery system. Even today, conventional drug delivery systems are primary pharmaceutical products commonly seen in prescriptions and 'over the counter' market place. They provide prompt release of the drug but in order to achieve as well as maintain drug concentration within therapeutically achieved range, it is often necessary to administer it several times a day. Conventional drug therapy results in significant fluctuations of drug concentration in systemic circulation causing either lethal effect or no therapeutic action[1]. Basic goal of drug therapy is to provide therapeutic amount of drug to proper site in body to promptly achieve and then maintain desired drug concentration. This idealized objective points to two aspects most important to the drug delivery, namely spatial placement and temporal delivery of drug.

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Spatial placement relates to targeting a drug to specific organ or tissue while temporal delivery refers to controlling rate of drug delivery to that specific organ or tissue [2]. Oral controlled release dosage forms have been developed over past three decades. These drug delivery system have a great potential of solving problems associated with conventional multiple dosing system like strict adherence to timely dosing, flip flop plasma concentration, associated side effects due to systemic accumulation of drug. Thus, there are numerous advantages such as improved efficacy, reduced toxicity, improved patient compliance and convenience, reduction in health care cost, etc. [4]. The past two decades have been characterized by an increased understanding of causes of low bioavailability and great deal of innovation in oral delivery technologies, marked by an unprecedented growth of drug delivery industry [6].

MATERIALS AND METHOD

Clarithromycin obtained gift sample from Cipla Pvt. Ltd. Mumbai, Ethylcellulose, HPMC, Ethanol, Dichloromethane, Hydrochloric acid, Polyvinyl alcohol, Tween-80, Tween-20, Acetone, SD Fine Che Ltd, Mumbai. Eudragit S 100 & L 100, Deggusa India Pvt. Ltd Mumbai.

Table 1: Formulation table of floating microspheres of Clarithromycin (EU1 to EU6)

Ingredients	Formulation code					
	EU_1	EU_2	EU_3	EU_4	EU_5	EU_6
Clarithromycin (mg)	500		500	500	500	500
Eudragit S 100 (gm)	1.00	1.50	2-00	-	-	-
Eudragit L 100 (gm)	-		-	1.00	1.50	2.00
Ethanol (ml)	20		20	20	20	20
Dichloromethane (ml)	20		20	20	20	20

Preparation of Floating Microspheres Loaded With Clarithromycin [10-13]

The floating microspheres loaded with clarithromycin were prepared by emulsion solvent evaporation and emulsion solvent diffusion method using different polymers as follows:

Emulsion Solvent Diffusion [14]

Weighed amount of clarithromycin was mixed with Eudragit S 100 / Eudragit L 100 drug: polymer ratio (1:1, 1:2, 1:3) in a solution of ethanol: dichloromethane (1:1) at room temperature. The resulting drug polymer solution was poured slowly using glass tube into 200 ml of water containing 0.75 % w/v polyvinyl alcohol, maintained at constant temperature of 40^0 c and preparation was stirred at 300 rpm for 1 hr. The finely developed floating microspheres were then filtered, washed with water and sieved between 50 and 30 mesh size and dried overnight at 40^0 c. The formulation table of floating microspheres of Clarithromycin showed in Table 1.

Scanning Electron Microscopy [10-12]

The external and internal morphology of the microspheres were studied by scanning electron microscopy (SEM). The sample for SEM was prepared by lightly sprinkling the powder on a double adhesive tape stuck to an alminum stub. The stubs were then coated with gold to a thickness of about $300A^0$ under an argon atmosphere using a gold sputter module in a high vaccum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Joel JSM-1600, Tokyo, Japan). SEM photographs are shown in Fig. 1.

Micromeritic properties:[13, 14]

The microspheres were characterized by their micromeritic properties such as particle size, bulk density, tapped density, compressibility index. Hauser's ratio and angle of repose.

***Particle size* [15]**

The particle size was measured by microscopic technique. In this method suspension of floating microspheres was prepared using castor oil. A drop of suspension was mounted on a slide and observed under optical microscope about 600 particles were measured with the help of the eye piece micrometer. All the microspheres in a field was counted.

***Bulk density* [16,17]**

In this method floating microspheres are transferred to a measuring cylinder and is tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

Bulk density = Mass of microspheres / Bulk Volume

***Tapped density* [18, 19]**

In this method floating microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density floating microspheres.

Tapped density = Mass of microspheres/ Volume of Microspheres after tapping

Percent Compressibility index was determined by using the formula:

% Compressibility index = $1- V/V_0 \times 100$

Here V and Vo are the volumes of the sample after and before the standard tapping, respectively.

***Hauser's Ratio* [20]**

Hauser's ratio of microspheres was determined by comparing tapped density to bulk density using the equation

$$\text{Hauser's ratio} = \frac{\text{Bulk density}}{\text{Tapped density}}$$

Angle of Repose [19]

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder was measured and angle of repose was calculated using the following equation:

$$\theta = \tan^{-1} h/r$$

Where,
θ - Angle of repose
h - Height of granules above the flat surface
r - Radius of the circle formed by the granule heap.

Yield of Floating Microspheres [18]

The prepared floating microspheres were collected and weighed. The measured weight was divided by total amount of all non-volatile components which were used for the preparation of microspheres.

% Yield = Actual weight of product / Total Weight of excipents and drugs X 100

In-Vitro Buoyancy [20]

Floating microspheres were dispersed in 900ml of 0.1 N hydrochloric acid solution containing 0.02% tween 80 to simulate gastric fluid at 37°. The mixture was stirred with a paddle at 100 rpm and after 12 hr, the layer of buoyant microspheres (W_f) was pipetted and separated by filtration simultaneously sinking microsphere ($W_{s)}$ was also separated. Both microspheres type were dried at 40°C overnight. Each weight was measured and buoyancy was determined by the weight ratio of the floating microspheres to the sum of floating and sinking microspheres.

$$\text{Buoyancy \%} = Wf/ Wf+ Ws \text{ X } 100$$

Where,
W_f and W_s are the weights of the floating and settled microspheres, respectively.

All the determinations were made in triplicate.

Incorporation efficiency [19]

Floating microspheres were dissolved in a minimum amount of methanol and drug was extracted into 0.1 N hydrochloric acid by evaporating methanol. The solution was filtered through whatman filter paper, diluted suitably and analyzed for drug content spectrophotometrically at 762 nm using 0.1N hydrochloric acid as blank.

In-Vitro Drug release [20]

The *In-Vitro* release of drug from floating microspheres was carried out using paddle type Electro lab tablet dissolution tester USP XXIII. Drug loaded microspheres equivalent to 500 mg of drug was introduced into 900 ml of the dissolution medium (0.1N HCl) maintained 37±0.5ºC with paddle rotating at 100 rpm. Aliquots were withdrawn at regular interval and analyzed spectrophotometrically using Shimadzu-1700 UV-visible spectrophotometer. The dissolution studies were carried out in triplicate in 0.1N HCl for 12 hours. The volume of the dissolution medium was adjusted to 900 ml at every sampling time by replacing 5ml with same dissolution medium. The released data obtained was fitted into various mathematical models as under to know which mathematical model is best fitting the obtained release profile**.** The amount of drug released was analysed at 762 nm using shimadzu UV visible spectrophotometer.

Release Kinetics [21,22]

The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas and finding the R2 values of the release profile corresponding to each model results are shown in Table 4.

Statistical analysis

Experimental results were expressed as mean ± SD. Student's t-test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release from different formulations. Differences were considered to be statistically significant at $P< 0.05$.

Short Term Stability Studies

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish retest period for the drug substance or a shelf-life for the drug product and recommended storage condition. Stability studies for selected formulations (EU1, EU2, EU5, EU6) were carried out by storing 1 gm of microspheres in an amber colored screw capped bottle at 40 ± 0.5ºC and 71.0 ± 5% RH for a period of 3 month. The microspheres were visually examined for any physical change and drug content was estimated at the end of every month. Results are shown in Table 5.

Table 2: Micromeritic properties of floating microspheres of Clarithromycin

Formulation	Mean Particle Size	Compressibility index (%)	Tapped density (gm/cm^3)	True density (g/cm^3)	Angle of repose
EU_1	127.30± 15.27	23.52 ± 0.05	0.6847 ± 0.07	0.699± 0.01	15.81 ± 1.43
EU_2	162.35± 11.01	22.29 ± 0.02	0.6730 ± 0.03	0.725± 0.03	19.05 ± 2.42
EU_3	190.35± 27.50	19.24 ± 0.03	0.7129 ± 0.05	0.731± 0.02	20.07 ± 1.68
EU_4	105.61± 21.12	25.11 ± 0.04	0.6350 ± 0.05	0.743± 0.01	17.71 ± 1.59
EU_5	125.65± 19.60	21.45 ± 0.03	0.6940 ± 0.05	0.755± 0.03	18.07 ± 1.68
EU_6	153.72± 27.53	18.68 ± 0.02	0.7339 ±0 .01	0.760± 0.02	14.25 ± 2.40

*All values are represented as mean ± standard deviation (n=3)

Table 3: Practical yield, *in-vitro* buoyancy and entrapment efficiency, cumulative percent release of floating microspheres of Clarithromycin

Formulation code	Practical yield (mean±SD) (%)	*In-Vitro* buoyancy (mean±SD) (%)	Drug entrapment efficiency (mean±SD) (%)	Cumulative Percent Drug Released
EU_1	62.55± 0.72	82.55 ± 2.08	65.35± 1.58	78.760
EU_2	78.70± 2.00	86.16 ± 1.00	85.21± 2.02	75.765
EU_3	80.45± 1.50	90.62 ± 1.52	89.40± 2.28	71.573
EU_4	61.78± 2.05	78.25 ± 4.04	62.68± 0.98	79.359
EU_5	74.49± 1.51	86.76 ± 1.52	80.85± 2.34	76.664
EU_6	79.42± 1.97	92.00 ± 3.05	83.15± 2.00	74.567

*All values are represented as mean + standard deviation (n=3)

Table 4: Kinetic values obtained from in-vitro release profile for floating microspheres of clarithromycin

Formulation Code	EU1	EU2	EU3	EU4	EU5	EU6
Zero order kinetic data	0.985	0.982	0.983	0.985	0.981	0.983
First order kinetic data	0.910	0.915	0.929	0.907	0.913	0.915
Higuchi Matrix kinetic data	0.856	0.845	0.844	0.856	0.814	0.850
Peppas kinetic data	0.994	0.984	0.992	0.994	0.977	0.992

Table 5: Stability data of clarithromycin loaded floating microspheres

Sampling Time (months)	Drug Entrapment Efficiency (%)			
	EU1	EU2	EU5	EU6
0	80.30±2.72	69.15±2.59	65.35±1.58	62.68±0.98
1	79.80±0.65	68.85±1.40	65.25±1.10	62.52±0.70
2	79.30±0.88	68.40±0.75	65.10±0.75	62.32±0.35
3	79.02±0.96	68.10±0.35	64.86±0.92	62.15±0.05

*All values are represented as mean + standard deviation (n=3)

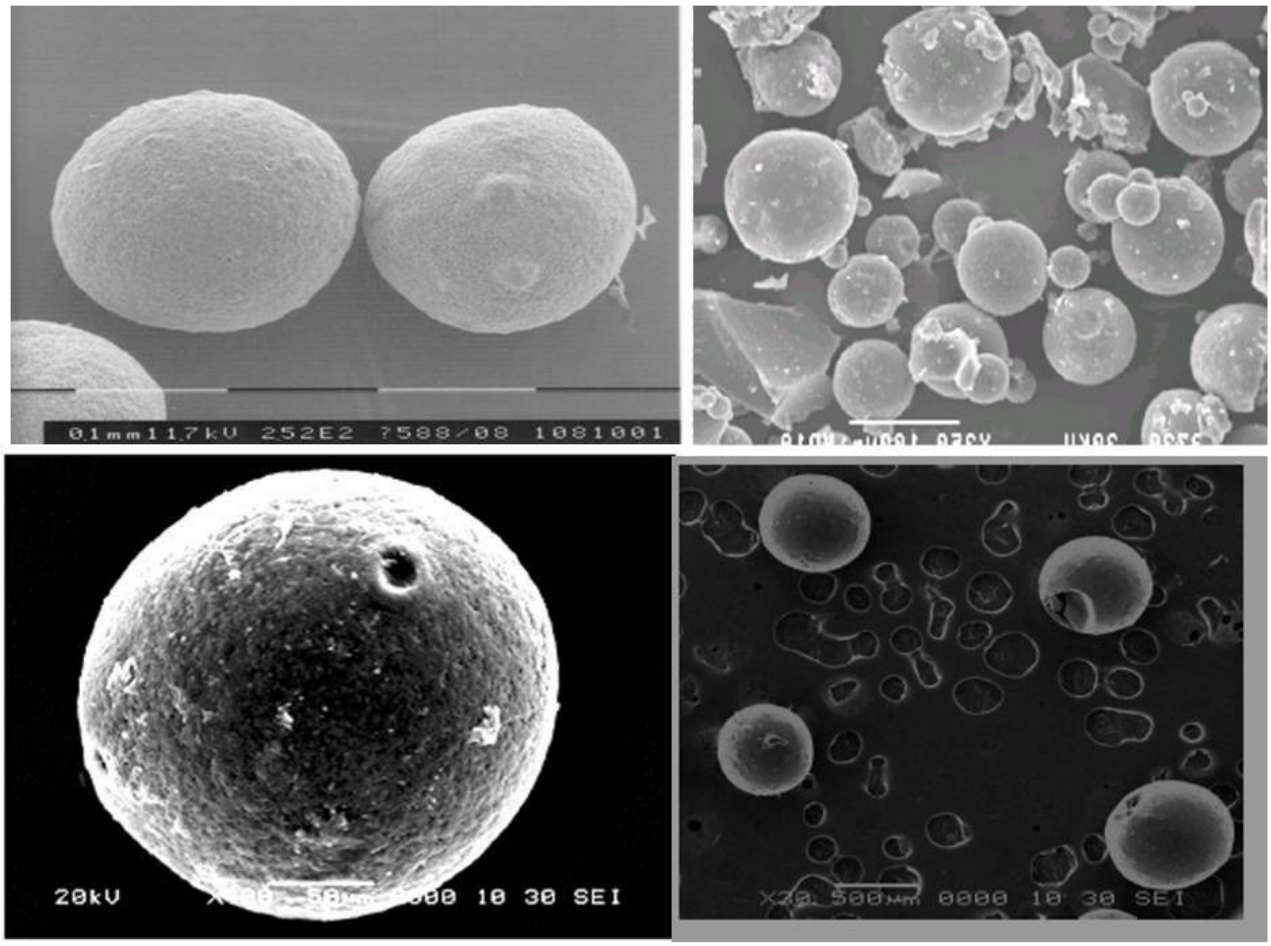

Figure 1: Scanning electron microphotographs of clarithromycin loaded floating microsphere of Eudragit L 100 & Eudragit S 100

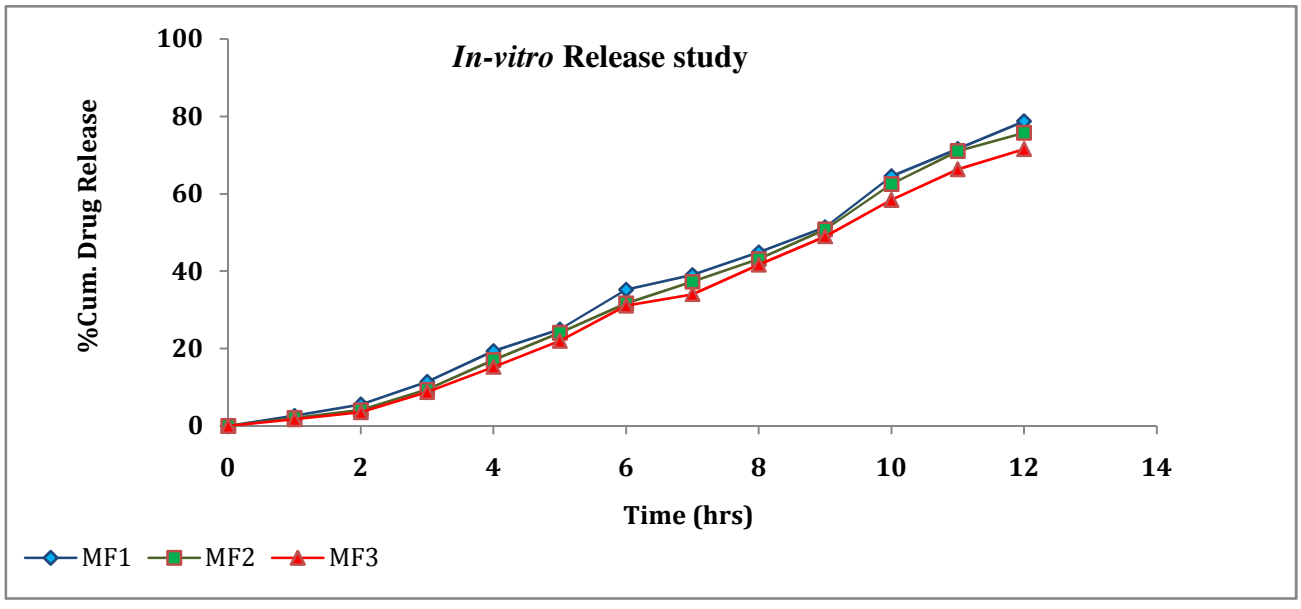

Figure 2: Cumulative percentage drug release of clarithromycin from formulation EU1 to EU3

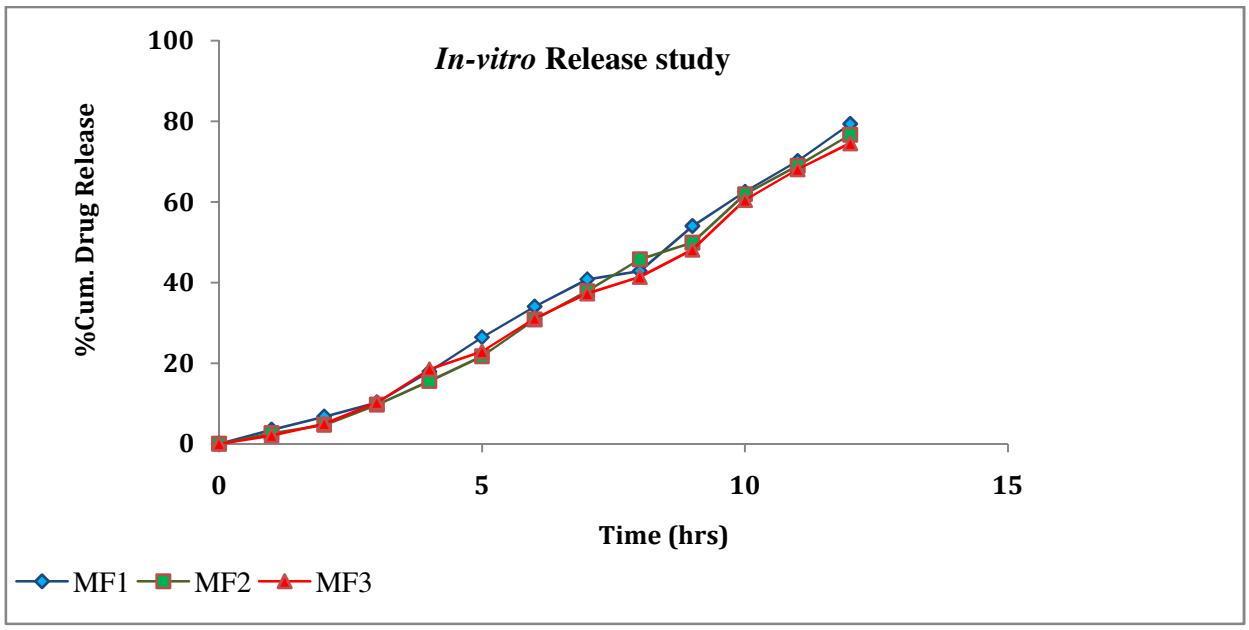

Figure 3: Cumulative percentage drug release of clarithromycin from formulation EU4 to EU6

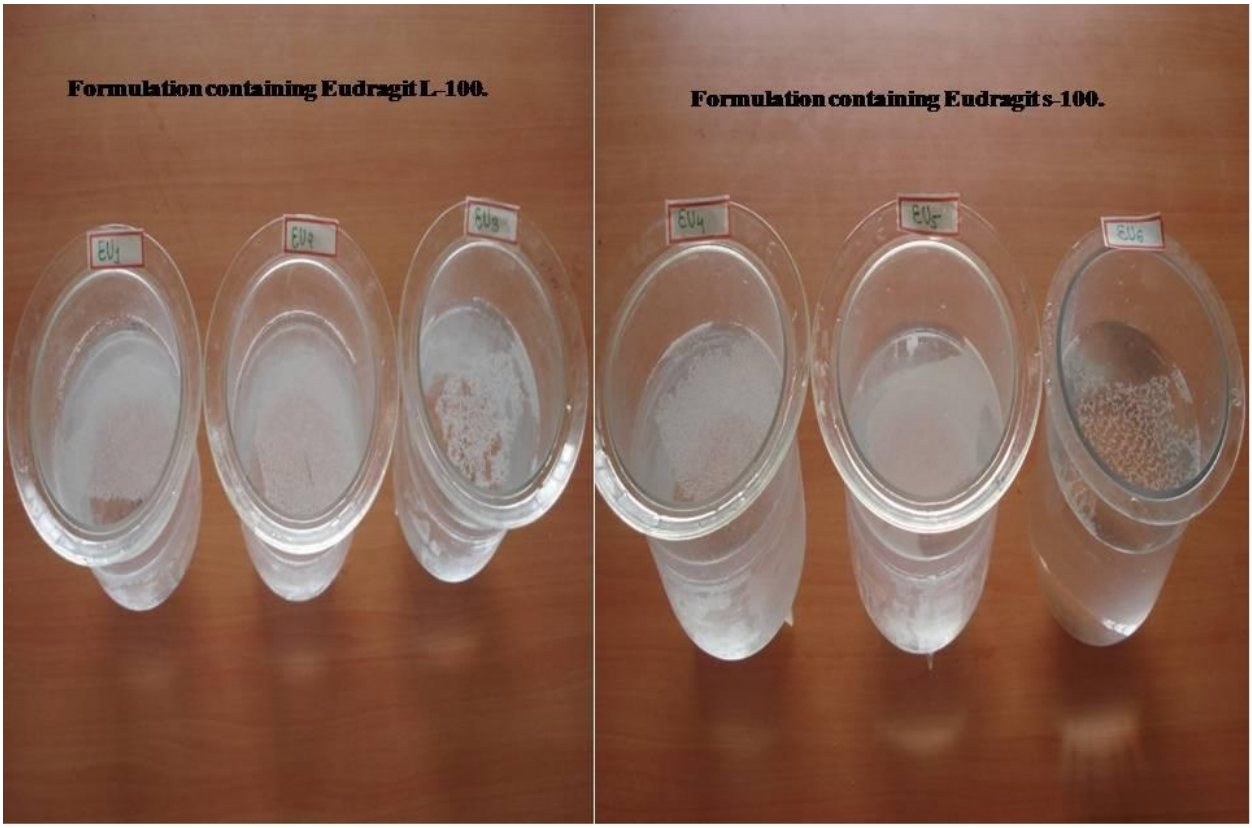

Figure 4: *In-vitro* Buoyancy floating microsphere of clarithromycin (a) Formulation EU1 to EU3 (b) Formulation EU4 to EU 6

RESULTS AND DISCUSSION

Floating drug delivery system have a bulk density less than gastric fluids and thus it remains buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from stomach. This results in an increased gastric residence time and a better control of the fluctuation in plasma drug concentration. Single unit formulations (floating tablet) are associated with problems such as sticking together or being obstructed in the gastrointestinal tract. The aim of present study was to develop floating microspheres of clarithromycin for treatment of H. pylori by emulsion solvent evaporation and emulsion solvent diffusion method by using HPMC, Ethylcellulose and eudragit S-100, eudragit L-100 as polymers. In the present study, drug entrapment efficiency from the formulation was estimated after extracting in 0.1 N HCL. Floating microspheres of clarithromycin using Eudragit S -100 and Eudragit L -100 as polymer prepared by emulsion solvent diffusion method as shown in table 1.Morphology of microspheres was examined by scanning electron microscopy. The view of the microspheres showed a hallow spherical structure with smooth surface morphology shown in Fig. 1 The Micromeritic properties of floating microsphere of clarithromycin are given Table 2. *In-Vitro* drug release studies of clarithromycin from floating microspheres were performed in 0.1 N HCL for 12 hours using USP Type I dissolution test apparatus. *In-Vitro* release of clarithromycin was good at end of 12 hr from all the formulation shown in Fig.2 and Fig 3. The Practicle yields, In-Vitro buoyancy shown in Fig. 4 and entrapment efficiency of floating microspheres of Clarithromycin are given in Table 4. The drug was get released from the microspheres by entry of dissolution medium through porous surface and that there was no polymer dissolution or chain relaxation due to non-swelling nature of polymers, No burst effect was observed from any of prepared microspheres. The *In-Vitro* release of clarithromycin significantly decreased with increase in polymer concentration in each type of preparation.

CONCLUSION

In vitro data obtained for floating microspheres of clarithromycin showed excellent floatability, good buoyancy, when increased density of polymer matrix at higher concentration results in an increased diffusional path length. This may decrease overall drug release from the polymer matrix. Furthermore, smaller microspheres were formed at lower polymer concentration and have large surface area exposed to dissolution medium giving rise to faster drug release. From the result it was observed that drug**:** polymer ratio influence the particle size, *In-Vitro* buoyancy as well as drug release pattern of floating microsphere. Hence, the multiple-unit floating systems of clarithromycin are expected to provide clinician with a new choice of safe and more bioavailable formulation in the management of bacterial infections. The study reveals satisfactory results with a further scope of pharmacokinetic and pharmacodynamic parameters.

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