

Research Article

Development and Characterization of Novel Sustained Release Floating Microspheres for Gastric Retention

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ABSTRACT

In this article author developed floating microspheres of Eudragit S100 containing Clarithromycin drug in it. For developing microspheres different process variables were optimized. Optimum surfactant concentration was found to be 0.75 % w/v, optimum stirring speed was found to be 400 rpm, optimum temperature was found to be 37°C. Particle size was determined by ocular micrometer and average particle size was found to be less than 2 µm. floating microspheres resulted in sustained and prolonged release of drug in the GIT fluids. It was found that more than 80 % of entrapped drug was released in 24 hours.

INTRODUCTION

To develop oral drug delivery systems, it is necessary to optimize both the residence time of the system within the gastrointestinal tract and the release rate of the drug from the system. Various attempts have been made to prolong the residence time of the dosage forms within the stomach (Moe, 1993; Deshpande et al., 1996). The prolongation of the gastric residence time (GRT) of delivery devices could be achieved by adhesion to the mucous membranes (Akiyama, et al., 1995), by preventing their passage through the pylorus (Fix et. al, 1993) or by maintaining them in buoyant fashion in gastric juice (Desai and Bolton 1993; Oth et al., 1992; Whitehead et al., 1998). With regard to the floating devices, Innuccelli et al., 1998 reported that an air-contained multiple-unit compartment system showed excellent buoyancy in vitro and prolonged GRT relative to the controls in vivo in the fed state.

Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration.

When microspheres come in contact with gastric fluid the gel formers, polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is

maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However a minimal gastric content needed to allow proper achievement of buoyancy. Hollow microspheres of Acrylic resins, Eudragit, PMAA, Polyethylene oxide, and Cellulose acetate; Polystyrene floatable shells; Polycarbonate floating balloons and Gelucire floating microspheres are the recent developments (Shiv kr.et al., 2004).

In the present investigation we have developed and characterized the Floating microspheres of Clarithromycin to increase bioavailability of the drug by increasing its gastric retention.

MATERIALS AND METHODS

Material

Clarithromycin was obtained as a gift sample from Ind-swift India limited, Chandigarh, India. Eudragit S100 obtained as a gift sample from Evonik Industries Ltd., Mumbai, India, 0.45µm syringe filter was obtained as a gift sample from Millipore Millex HN, USA, Polyvenyl Alcohol (PVA), Dichloromethane (DCM), Acetone was purchased from sigma aldrich, India. All other chemicals were used of extra pure grade.

Method

Eudragit S100- PVA complexed floating microspheres were prepared by solvent diffusion method as reported by Kawashima et al., (2001) with slight modification briefly, Eudragit S100 solution (100 mg) was

dissolved in 8.0 ml of DCM, and 8.0 ml of acetone, drug solution (clarithromycin solution in acetone & DCM) was also added to it. An oil-in-water (o/w) emulsion was formed by injecting the polymer solution in 100 ml of polyvinyl alcohol (PVA). Then this solution is stirred at 350-400 rpm using a mechanical stirrer (Remi india) equipped with a blade propeller for 3-4 hrs. After complete removal of organic phase microspheres were collected by filtration, washed three times with distilled water and dried at room temperature for 24 hrs.

PVA solution was chosen as the external phase because ethanol/dichloromethane (DCM) mixture as an internal phase is not miscible with PVA solution and the Eudragit S100-PVA complex is not soluble in it. As the dispersed droplets of Eudragit S100 solution collided with those of PVA solution, they formed an interpolymer complex. The droplets of Eudragit S100/PVA complex gradually solidified and hardened as ethanol and DCM diffused out of the internal phase.

Particle size and size distribution

Microspheres were studied microscopically for their size and size distribution using calibrated ocular micrometer. Least count of the ocular micrometer was calculated as 16.2 μm . Around 100 particles from each formulation were seen and the observed data for each formulation are recorded.

Surface Morphology (SEM)

The samples for SEM were prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å using a sputter coater. All samples were examined under a scanning electron microscope (LEO 435 VP, Eindhoven, Netherland) at an acceleration voltage of 30 kV, and photomicrographs were taken.

Particle Morphology (TEM)

Transmission electron microscope was used as a visualizing aid for particle morphology. The sample (10 μL) was placed on the grids and allowed to stand at room temperature for 90 sec. Excess of fluid was removed by touching the edge with filter paper. Samples were examined after negative staining with phosphotungstic acid under a transmission electron microscope (Philips Morgagni 268, Eindhoven, Netherland) at an acceleration voltage of 20 kV and images were taken.

Drug content

100 mg of microspheres was dispersed in 100 mL of PBS (pH 7.4) and shaken vigorously for 10 min. and supernatant was kept aside. Similarly, the sediment was again treated in the same manner and second supernatant was mixed with first supernatant. The microspheres obtained after two washings were dissolved in 20 mL of PBS (pH 7.4) for 2 hrs and was centrifuged at 3000 rpm for 5 min. The solution was then filtered through 0.45 μm syringe filter (Millipore Millex HN, USA) and the filtrate was assayed for clarithromycin spectrophotometrically. The percent drug entrapped was calculated and reported.

In vitro drug release study in simulated gastrointestinal fluids of different pH

All formulations of microspheres were evaluated for the in vitro drug release study. The dissolution test of clarithromycin microspheres was carried out by the paddle type dissolution apparatus specified in USP XXIII.

50 mg of clarithromycin loaded microspheres was weighed accurately and gently spread over the surface of 500 mL of dissolution medium. The content was rotated at 100 rpm and thermostatically controlled at $37\pm 0.5^\circ\text{C}$. Perfect sink condition was prevailed during the drug dissolution. The release was tested in dissolution medium of pH 1.2 and pH 7.4 phosphate buffer solutions. An aliquot of the release medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added to the release medium. The collected samples were filtered through 0.45 μm -syringe filter (Millipore millex HN) and analyzed spectrophotometrically. Drug release profiles were observed and recorded.

RESULTS AND DISCUSSION

It was also observed that Eudragit S100 and PVA aggregated and precipitated out in ethanol-DCM mixture in a relatively short period of time, resulting in the formation of a PVA/Eudragit S100 interpolymer complex, suggesting that the intensity of hydrogen bonding between Eudragit S100 and PVA is quite strong. It was believed that this strong complexation could be utilized to prepare floating microspheres.

The effect of formulation variables e.g. drug concentration, solvent ratio of internal phase (ethanol/DCM), surfactant concentration and process variables e.g. stirring speed and temperature were studied in order to optimize the formulation. The results suggested that

(Table No 1, Fig No. 1) these variables influence the shape, size and size distribution, total drug loading efficiency and in vitro drug release. Hence these parameters were optimized to prepare microspheres of small size with narrow size distribution, good drug loading efficiency.

During optimization of drug concentration the optimal concentration of drug was found to be 80 % in respect to the polymer weight. When the concentration of drug increases the matrix of polymer might has been fully saturated that's why the additional drug was leached out from the matrix.

Effect of polymer concentration was found to influence the particle size and entrapment efficiency. The polymer concentration was found to be optimal in the 100 mg % of drug weight. The highest entrapment was found in 100% of drug weight. The lower polymer concentration resulted in low entrapment efficiency and exceeding the polymer concentration above 100 mg further reduced the entrapment efficiency from 78.33% to 73.29%. The reason behind this might be that increase in polymer concentration increases matrix density and thus all drug moieties was encapsulated and thus the entrapment efficiency was low due to increase of matrix density.

The surfactant concentration was optimized on the basis of particle size and entrapment efficiency of microspheres. In case of 0.50 % w/v surfactant concentration, the particle size was slightly more than that of 0.75 % w/v. The reason behind this is that 0.50% w/v surfactant concentration was not sufficient to reduce the surface charge so that the particles were aggregated and resulted in increase in particle size, where as the entrapment efficiency was

found high might be because increase in particle size resulting increase in entrapment efficiency of drug into the increased matrix. Whereas above the 0.75% concentration of surfactant it results in reduction in drug loading due to increase in surface charge by the surfactants it makes the particles smaller and thus matrix was unavailable to get entrapped.

Stirring speed was optimized to get optimum particle size and percent drug entrapment. The results confirmed that stirring speed of 400 rpm results in good particle size and better entrapment efficiency. The drug entrapment was found low when stirring speed was 300 which may be due to, reduction in shearing stress. This resulted in increase in leaching of drug from matrix cavity to external phase and thus entrapment efficiency was low.

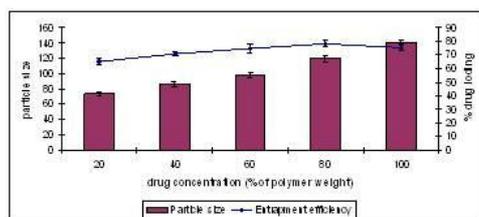
The surface and particle morphology (SEM) (TEM) confirmed the shape and size of microspheres, the particle size was found to be less than 200 μ m as shown in Fig. 3 & 4. Similarly surface morphology was found be plain and spherical.

The in vitro drug release study performed in all pH, confirmed that floating microspheres resulted in sustained and prolonged release of drug in the GIT fluids. It was found that more than 80 % of entrapped drug was released in 24 hours (Fig. No. 4).

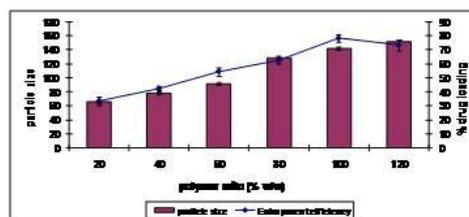
The microscopic examination of microspheres revealed that the mean diameter of Eudragit S100-PVA complexed microspheres varied from 121.24 μ m to 143.24 μ m on varying the concentration of all variables. Total drug loading efficiency varied from 67.76 to 79.22%. Thus it may be concluded that the prepared microspheres were of spherical shape with good entrapment efficiency.

Table 1: Optimized formulation on the basis of Formulation & process variables

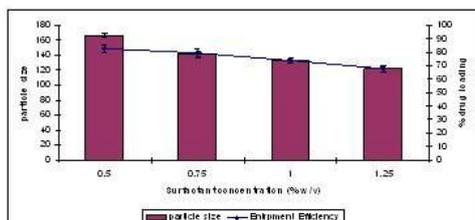
S. No.	Optimizing parameter	Optimized parameter code
1.	Drug concentration	80 % w/v
2.	Polymer concentration	100 % w/v
3.	Surfactant concentration	0.75 % w/v
4.	Stirring speed	400rpm
5	Temperature	37 °C



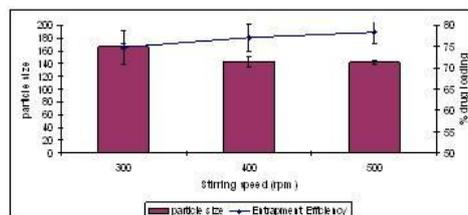
Effect of Clarithromycin concentration (% of polymer weight) on particle size and clarithromycin loading of microspheres



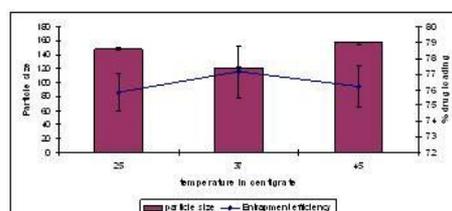
Effect of polymer ratio on particle size and clarithromycin loading of microspheres



Effect of surfactant concentration on particle size and clarithromycin loading of microspheres



Effect of stirring speed on particle size and clarithromycin loading of microspheres



Effect of temperature on particle size and clarithromycin loading of microspheres

Fig. 1: Different process variables for optimizing the formulation

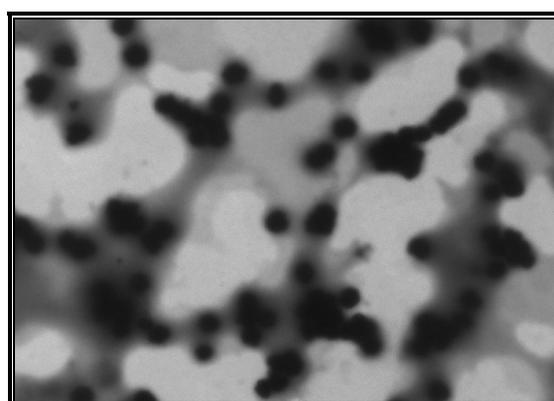


Fig. 2: TEM photomicrograph of floating microspheres

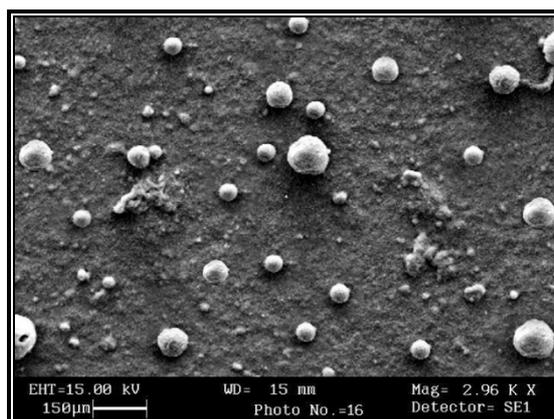
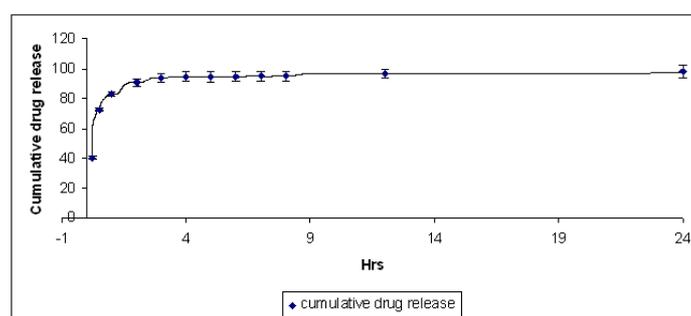


Fig. 3: SEM photomicrograph of floating microspheres



n=3; \pm SD

Fig. 4: Cumulative % clarithromycin release from PVA-Eudragit S100 microspheres at pH 7.4

CONCLUSION

It is suggested that the work should further be elaborated in the field of targeted drug delivery systems like, binding of molecules (ligands) to the microspheres surface, which have to exhibit the ability to recognize cell surface structure such as, lectin, adhesion invasins, antibodies or sugars, which may offer site specific drug delivery. Further in vivo bacterial clearance and their study with infected animal model also recommended for the future work.

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REFERENCES

1. Moe's AJ. Gastroretentive dosage forms. *Crit Rev Ther Drug Carrier Syst.* 1993;10:143-195.
2. Deshpande AA, Rhodes CT, Shah NH and Malick AW. Controlled release drug delivery systems for prolonged gastric residence: an overview. *Drug Dev Ind Pharm.* 1996;22:531-539.
3. Akiyama Y, Nagahara N, Kashihara T, Hirai S and Toguchi H. In vitro and in vivo evaluation of mucoadhesive microspheres prepared for the gastrointestinal tract using polyglycerol esters of fatty acids and a poly (acrylic acid) derivative. *Pharm Res.* 1995;12:397-405.
4. Fix JA, Cargill R and Engle K. Controlled gastric emptying. Part 3. Gastric residence time of a nondisintegrating geometric shape in human volunteers. *Pharm. Res.* 1993;10:1087-1089.
5. Desai S and Bolton S. A floating controlled-release drug delivery system: in vitro-in vivo evaluation. *Pharm Res.* 1993;10:1321-1325.
6. Oth M, Franz M, Timmermans J and Moe's A. The bilayer floating capsule: a stomach-dried drug delivery system for misoprostol. *Pharm Res.* 1992;9:298-302.

7. Whitehead L, Fell JT, Collett JH, Sharma HL and Smith AM. An in vivo study demonstrating prolonged gastric retention. *J Contr Release*. 1998;55:3-12.
8. Iannuccelli V, Coppi G, Bernabei MT and Cameroni R. Air compartment multiple-unit system for prolonged gastric residence. Part I. Formulation study, *Int J Pharm*. 1998;174:47-54.
9. Iannuccelli V, Coppi G, Sansone R and Ferolla G. Air compartment multiple-unit system for prolonged gastric residence. Part II. In vivo evaluation. *Int J Pharm*. 1998;174:55-62.
10. Iannuccelli V, Coppi G, Leo E, Fontana F and Bernabei MT. PVP solid dispersions for the controlled release of furosemide from a floating multiple-unit system. *Drug Dev Ind Pharm*. 1998;26:595-603.
11. Shiv Kr HG, Vishakanta GD and Pramod Kr. *TM .I.J.P.E*. 2008;38.