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Original Article

Floating Microspheres Encapsulating Amoxicillin for Effective Treatment of Peptic Ulcer

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ABSTRACT
Although oral route of drug administration is preferred route of administration, drug absorption from the gastrointestinal (GI) tract may be very less and highly variable in certain circumstances. Certain drugs are required longer residence in upper GI tract. The gastro-tentative drug delivery is the approach for the such drugs which require longer residence in stomach and for the drugs with shorter half-life. Floating microspheres of amoxycillin trihydrate were prepared solvent evaporation method using polymethyl methacrylate. The effect of drug concentration, stirring speed and process temperature were optimized in the study. The *in vitro* drug release studies performed in the simulated gastric fluid shows 89.6±2.6% cumulative drug release at the end of 12 hours with the optimized batch, following Higuchi diffusion drug release kinetics. The prepared floating microspheres of the amoxycillin trihydrate were found to have adequate stability profile at ambient conditions.
Keywords: Amoxycillin trihydrate, polymethyl methacrylate, microspheres, floating microsphere, gastro retentive drug delivery, Peptic ulcer

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INTRODUCTION

Gastroretentive Floating Drug Delivery Systems (FDDS) is popular approach are among the several approaches that have been developed in order to increase the gastric residence time (GRT) of dosage forms. Single and multiparticulate unit systems have been developed by researchers. The single-unit floating systems are more popular but higher variability of gastroretentive systems and variable transit time are major problems. The multiparticulate formulation are better because they have potential to reduce the biological variation in subjects during absorption and lower the chances of dose dumping. Such a dosage form can be distributed widely throughout the gastrointestinal tract (GIT), affording the possibility of a longer gastric residence time and more reliable drug release from FDDS.

Both natural and synthetic polymers have been used to prepare floating microspheres. Kawashima et al. reported microspheres of ibuprofen by the emulsion-solvent diffusion method using acrylic polymers. The microspheres exhibited good in vitro floatability and drug release decreased drastically with increasing polymer concentration. Methylcellulose and chitosan micropellets of lansoprazole with lower density than gastric contents and exhibited better encapsulation efficiencies.

Amoxycillin trihydrate is a moderate-spectrum, bactericidal, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is better

absorbed from the upper part of GIT, following oral administration, with a half-life of 1.5 hr and bioavailability is about 30%. Amoxycillin trihydrate is prescribed for the treatment of *H. pylori* induced peptic ulcers alone or in combination with other drugs for the eradiation from the human body. Conventional oral dosage form of Amoxycillin trihydrate cannot bring out complete eradication of *H. pylori* probably due to the short residence time in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucosal layer or epithelial cell surfaces where *H. pylori* exist. Other reason may be due to the degradation of amoxicillin trihydrate in gastric acid.

Hence the major objectives of the present study are to develop an intragastric floating and sustained release floating microspheres for gastric retention using polymethyl methacrylate (PMMA) as floating carrier and to study the effect of important formulation and processing variables on the floating and drug release behaviour of these systems.

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2. MATERIALS AND METHODS

Amoxicillin Trihydrate was generously supplied as a gift samples by Dr. Reddy's Laboratories, Hyderabad**.** Polymethylmethacrylate**,** Dichloromethane and Dimethylformamide were procured from C.D. Fine Chemicals, Mumbai, India. All other chemicals and reagents were used of analytical grade.

Preformulation study

The obtained drug was evaluated for the organoleptic characteristics, solubility, melting point and partition coefficient.

Partition coefficient study

The partition coefficient of Amoxycillin Trihydrate was determined in n-octanol/water (pH 7.0), and n-octanol/PBS (pH 7.4) system employing the method reported by (Dearden JC 1988). 10 mg of Amoxycillin Trihydrate was accurately weighed and taken in a stopper tube containing 10 ml each of n-octanol and aqueous phase. The tubes were placed on a wrist shaker for 6 hours until equilibrium was reached. Phases were separated using the separating funnel and the aqueous phase was analysed for amount of drug after appropriate dilution.

Solubility study

When the excess of a solid is brought into contact with a liquid, the molecules of the former are removed from its surface until equilibrium is established between the molecules leaving the solid and those returning to it. The resulting solution is said to be saturated at the temperature of the experiment and the extent to which the solute dissolves is referred to as its solubility (Veseli A, 2019). The solubility determination of Amoxycillin Trihydrate was carried out in various common solvents.

Fourier Transform Infrared Spectroscopy

The drug sample was scanned on IR spectrophotometer between 400-4000 cm^{-1} using KBr disc. The obtained IR spectrum was interpretated with the structure of amoxycillin trihydrate.

Preparation of Floating Microspheres By Solvent Evaporation Method

Floating microspheres were prepared by Solvent evaporation (oil-in–water emulsion) technique. In this 500 mg polymethyl methacrylate (PMMA) were dissolved in a mixture of dimethyl formamide and dichloromethane (1:1) at room temperature.

And 500mg Amoxicillin were added in the above mixture. This was poured into 500ml water containing 0.1% span 80, maintained at a temperature 30-40^{o}C and subsequent stirred at ranging agitation speed for 20 minute to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried in vacuum.

Table 1 Master Formula

S. No	Name of Ingredient/ Drug	Amount
1	Amoxicillin Trihydrate	500 mg
2	Polymethyl methacrylate (PMMA)	500 mg
3	Dimethylformamide	25 ml
4	Dichloromethane	25 ml
5	Span 80	0.5 ml

Optimization of Formulation and Process Variables

Various formulation and process variables i.e. drug concentration, stirring speed and effect of temperature which could affect the preparation and properties of floating microspheres were identified and studied. The optimization was done on the basis of particle size and drug loading efficiency

Optimization of drug (Amoxicillin Trihydrate) concentration

For optimization of drug concentration, the floating microspheres were prepared with varying percentage of drug from 25 to 100% of the formula value, while keeping other variables constant. Optimization was done on the basis of drug loading and particle size.

Optimization of stirring speed

Stirring speed from mechanical stirrer was varied from 200 to 500 rpm for floating microspheres preparation with selected formulation ATF3 while keeping the other variables constant.

Optimization of temperature

For optimization of temperature, formulation ATF3R3 was selected while keeping the other variables constant. Floating microspheres formulations were prepared at different temperatures *viz*. 25^{0}C, 37^{0}C and 45^{0}C.

Characterization of Prepared Floating Microspheres

The prepared floating microspheres were characterized for shape and surface morphology, size, percent drug loading and *in vitro* drug release in different GIT pH.

Shape and surface morphology

In order to examine the surface morphology, the formulations were viewed under scanning electron microscopy. The samples for SEM were prepared by lightly sprinkling the floating microspheres powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300Å using a sputter water. The samples were then randomly scanned for studying surface morphology but show the images of coating to prove internal surface.

Particle size determination

The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer.

Drug Entrapment

The percent drug entrapped was calculated using following formula

$$\% \text{ DE} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

***In vitro* Buoyancy Test of Optimized Amoxicillin Trihydrate Floating Microspheres**

The floating test of the prepared optimized Amoxicillin Trihydrate floating microspheres formulation was carried out using dissolution test apparatus USP XXII method II. 500 mg of floating microspheres were immersed in 900 ml simulated gastric fluid SGF (pH 1.2) maintained at 37 ± 2°C, which was agitated by a paddle rotated at 100 rpm. The paddle blades were positioned at the surface of dissolution medium. The floating microspheres floating on the surface of SGF (pH 1.2) were recovered with a sieve No. 120 (34µm) at every 1 hr time interval for 12 hours. The floating microspheres so collected were dried and weighed. The floating percentage of the floating microspheres was defined as the weight ratio

of the floating microspheres against the total weight of floating microspheres in the floating test. The buoyancy of the floating microspheres was calculated by the following equation:

$$\text{Buoyancy}\,(\%) = \frac{Q_f}{Q_f + Q_s} \times 100$$

Where Q_f and Q_s are the weights of the floating and settled floating microspheres respectively.

***In vitro* drug release in different gastrointestinal fluids**

Optimized formulation (ATF3R3T2) was evaluated for the *in vitro* drug release study in different GIT fluids. The dissolution test of Amoxicillin Trihydrate floating microspheres was carried out by the paddle type dissolution apparatus specified in USP XXIII under perfect sink condition.

1000 mg of floating microspheres was weighed accurately and gently spread over the surface of 1000 mL of dissolution medium. The media was rotated at 100 rpm and thermostatically controlled at 37±2°C. Perfect sink condition was prevailed during the drug dissolution. The release was tested in dissolution medium of pH 1.2, pH 6.8 and pH 7.4 solutions. An aliquot of the release medium was withdrawn at every 1 hr time interval and an equivalent amount of fresh medium was added to the release medium.

Effect of Storage on Structural Integrity of Optimized Floating Microspheres

The optimized formulation (ATF3R3T2) was stored in amber colored glass bottles at 4±1°C, 25±1°C and 40±1°C for a period of 6 months and observed for any change in particle size (optical microscopy) and surface morphology by phase contrast microscope (Leica MPS, Germany).

Effect of Storage on Residual Drug Content

Stability of floating microspheres formulations on storage is of great concern as it is the major factor in their development as marketed preparation. The prepared formulation was tested for stability at 4±1°C, 25±1°C and 40±1°C temperatures. Formulation was stored in amber colored glass vials, and then it was evaluated after for 6 months for change in residual drug content. For the determination of residual drug content floating microspheres formulation were dissolved in 3ml dichloromethane filter through polycarbonate membrane (Millipore, USA) of 200 nm pore size than after suitable dilution with PBS (pH 7.4) the drug content estimated spectrophotometrically using UV-visible spectrophotometer (Shimdazu 1800, Japan).

3. RESULTS AND DISCUSSION

Among the solvents used, highest solubility of drug was found in methanol, ethanol (95%), DMSO and pH1.2, soluble in water, DMF, pH 6.8 and 4.5, slightly soluble behaviour in chloroform, toluene and dichloromethane. The partition coefficient of Amoxycillin Trihydratein n-octanol/distilled water (pH 7.0) was found to be 0.78 and n-octanol/PBS (pH 7.4) 0.49 respectively. Partition coefficient value of drug also revealed its polar and hydrophilic nature.

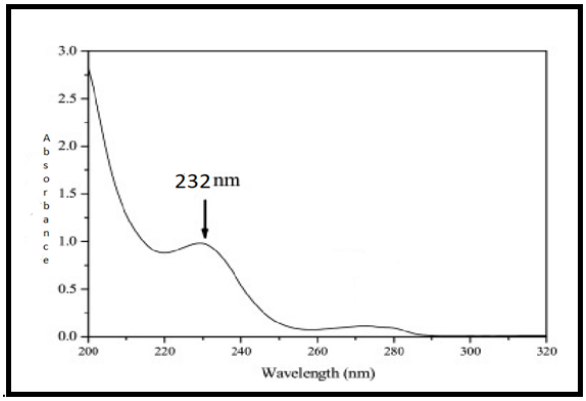

Figure 1 UV absorption maxima of Amoxycillin Trihydratein PBS (pH 7.4) at λ max 232 nm

The amoxycillin trihydrate was identified by U.V spectroscopy showing absorption maxima at 232nm.

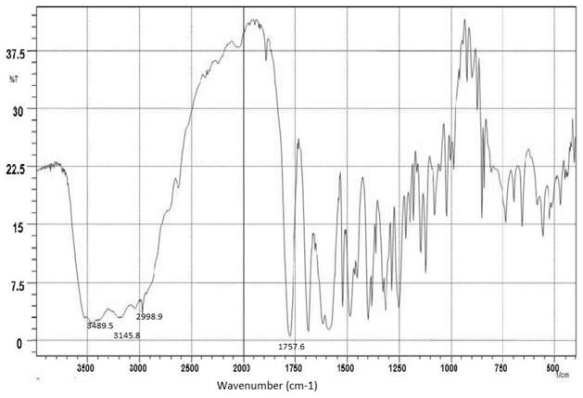

Fig 2 FTIR Spectra of amoxycillin trihydrate

Amoxycillin trihydrate was identified by FTIR study peaks at 3489.5 cm^{-1} can be assigned to the OH-streching, 3145.8 cm^{-1} can be assigned to NH stretching, 2998.9 cm^{-1} can be assigned to the CH stretching and 1757.6 cm^{-1} can be assigned to the C=O stretching,

The entrapment of drug was also optimized on the basis of average particle size and maximum drug entrapment efficiency. For this floating microsphere formulation were prepared with varying concentration amoxycillin trihydrate *viz*. 25, 50,75%. It was observed that on increasing the concentration of drug, the entrapment efficiency increased upto 75 % shown in table 2. while on further increasing drug concentration the entrapment efficiency gradually decreased. So that formulation ATFD3 was selected for further optimization process.

Table 3 Effect of drug concentration on particle size and drug entrapment in floating microspheres

Formulation code	Drug concentration (%)	Particle size (nm)	Drug Entrapment (%)
ATF1	25	756.25± 28	76.8±1.2
ATF2	50	654.66± 33	65.5±1.9
ATF3	75	556.33± 48	81.2±1.4
ATF4	100	826.89± 37	61.2±2.8

Mean SD ± (n=3)

The process variable, stirring speed was optimized in terms of average particle size and maximum drug entrapment efficiency. In this experiment results were concluded that increase in stirring speed decrease an average particle size of floating microspheres. At 400 rpm formulation ATF3R3 produced size distribution 526.4±1.4nm with floating and spherical shape with 87.9±1.2% drug entrapment efficiency. The particle size and percent drug loading were determined which are recorded in Table 4 and fig.3.

Table 4 Effect of stirring speed on particle size and drug entrapment of floating microspheres

Formulation code	Speed (rpm)	Particle size (nm)	Drug Entrapment (%)
ATF3R1	200	754.3±2.9	61.8±2.4
ATF3R2	300	838.9±1.8	75.3±2.8
ATF3R3	400	526.4±1.4	87.9±1.2
ATF3R4	500	709.3±1.7	88.5±2.3

Mean SD ± (n=3)

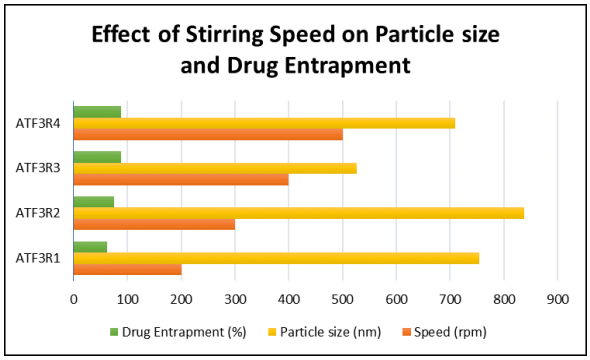

Fig. 3: Effect of stirring speed on particle size and drug entrapment of floating microspheres

Average particle size of floating microspheres reduces with increased in temperature. Narrow size distribution 531.4±1.6nm and 87.5±1.4% entrapment efficiency was found to formulation ATF3R3T2 at 35^0C temperature. The effect of temperature on formulation is reported in Table 5 and shown in Fig.4

Table 5 Effect of temperature on particle size and drug entrapment in floating microspheres

Formulation code	Optimization of temperature (^{0}C)	Particle size (nm)	Drug Entrapment (%)
ATF3R3T1	30	654.3±2.9	69.8±1.5
ATF3R3T2	35	531.4±1.6	87.5±1.4
ATF3R3T3	40	722.4±1.7	77.2±1.8
ATF3R3T4	45	789..5±1.3	75.5±2.3

Mean SD ± (n=3)

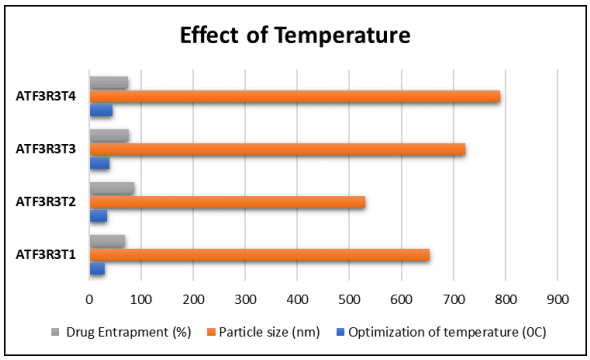

Fig. 4: Effect of temperature on particle size and drug entrapment in floating microspheres

On the basis of formulations and process variables, the optimized floating microspheres formulation was prepared by the parameters recorded in Table 6.

The scanning electron microscopic (SEM) photomicrograph of the optimized floating microspheres formulations (ATF3R3T2). These results of SEM support that floating microspheres where spherical in shape.

Table 6 Optimized floating microspheres formulation on the basis of formulation & process variables

S. No.	Optimized parameter	Value	Particle Size (nm)	Entrapment Efficiency (%)
1.	Drug concentration	75 %	556.33±48	81.2±1.4
2.	Stirring speed	400 rpm	526.4±1.4	87.9±1.2
3.	Temperature	35^{0}C	531.4±1.6	87.5±1.4

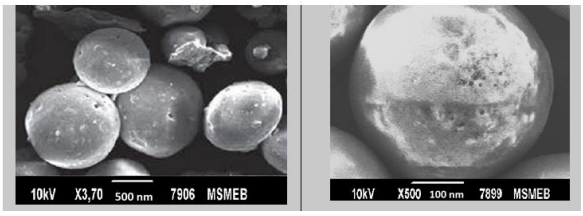

Fig. 5: SEM photograph of Amoxicillin Trihydrate floating microsphere

100 mg of floating microspheres was dissolved in 3 ml of dichloromethane and shaken vigorously for 2 min. The solution was then filtered through 0.45µm syringe filter (Millipore Millex HN, USA). After suitable dilution with PBS (pH 8.4) solution was assayed for Amoxicillin Trihydrate spectrophotometrically.

In vitro floating test of optimized floating microspheres formulation was studied in SGF (pH 1.2) showed that microsphers are significantly buoyant for 12 hrs. The collected samples were filtered through 0.45µm-syringe filter (Millipore millex HN) and after suitable dilution sample were analyzed spectrophotometrically. % cumulative drug release are recorded. *In vitro* amoxycillin trihydrate release from optimized floating of floating microspheres formulation was significantly decreased after 5 microspheres (ATF3R3T2) were carried out in SGF (pH 1.2), SIF (pH 6.8) and PBS (pH 7.4) by dissolution test of floating microspheres was carried out by the paddle method specified in the U.S.P. XXI. No initial burst release was observed in any

medium suggested that the amoxycillin trihydrate molecules are entrapped in floating microspheres. Nearly linear relationship between the % cumulative release of amoxycillin trihydrate and the square root of time was obtained for the 12 hrs. suggested that the floating microspheres formulation follows a diffusion-controlled Higuchi drug release mechanism with r2 value 0.9908. The % Cumulative amount of drug release was found 89.6±2.6% in SGF (pH 1.2), 90.6±4.9 % in SIF (pH 6.8) and 95.2±4.5% PBS (pH 7.4) upto 12 hrs as shown in figure. The results clearly suggest that floating microspheres formulation could also be utilized for sustained and drug delivery purpose.

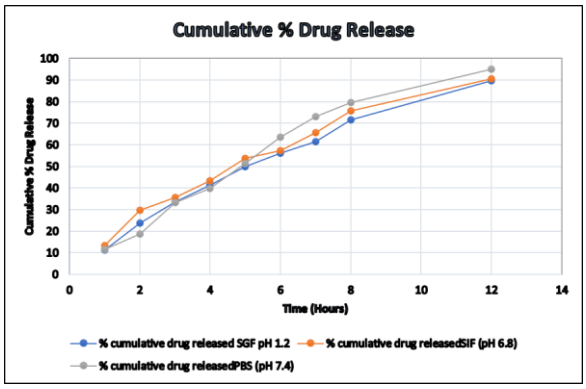

Fig. 6 Cumulative percent drug release in of optimized formulation

Effect of storage temperature on particle size and surface morphology of optimized floating microspheres formulation (ATF3R3T2) was studied. The particle size was almost constant at 4±1°C and 28±1°C. The change in shape and size was observed at 40±1°C which may be attributed to higher temperature.

Table 7. Stability studies

S. No.	Formulations code (s)	Storage Temperature	Particle size (nm)				Vesicles shape at end of stability test
			Initial	1 month	3 months	6 months	
1	(ATF3R3T2)	4±1°C	526.4± 2.5	525.6± 1.5	526.3± 3.1	527.4± 3.3	Spherical
		28±1°C	529.4± 2.6	526.5± 3.4	527.9± 3.7	527.5± 2.9	Spherical
		40±1°C	531.4± 3.3	546.4± 3.9	589.5± 4.5	662.2± 2.6	Change in shape

Mean SD ± (n=3)

Percent residual drug content in optimized floating microspheres formulation stored at different temperatures is shown in following data shows that the formulation should be stored at 4±1°C as the drug content was reduced at 25±1°C and 40±1°C.

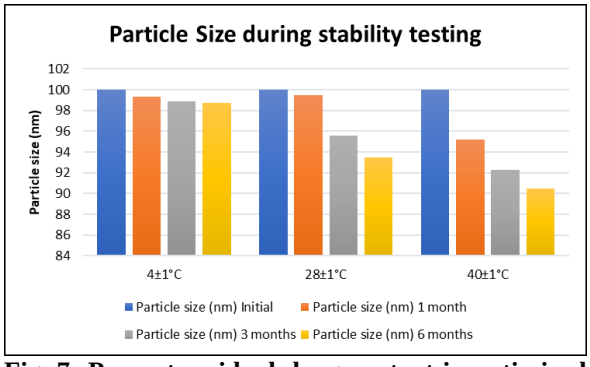

Fig. 7: Percent residual drug content in optimized floating microspheres formulation stored at different temperatures

CONCLUSION

The result obtained from all the experiments perform as a part of project work suggested that it is possible to prepare an intragastric floating and sustained release floating microspheres preparation using Polymethylmethacrylate, solvent evaporation method floating microspheres drug delivery system provides the possibility of enhancing the bioavailability and control the release of nizatidine hydrochloride exhibiting absorption window by prolonging the gastric emptying time of the dosage form ensuring availability of drug at the absorption site for the desired period of time. As the floating microspheres showed a good buoyancy and drug release properties so that it has a great potential for its use both in powder form for dry suspension and granular form for

tableting.

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