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Research Article

"Formulation and Characterization of Azithromycin Microspheres for Gastroretentive drug Delivery System"

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Abstract

Gastro-retentive drug delivery system (GRDDS) has gained immense popularity in the field of oral drug delivery recently. It is a widely employed approach to retain the dosage form in the stomach for an extended period of time and release the drug slowly that can address many challenges associated with conventional oral delivery, including poor bioavailability. Mucoadhesive Microspheres of Azithromycin were prepared by using isolated TSP by single emulsion method for eradication of H.Pylori. The formulation was optimized using a three-factor, three-level Box–Behnken design. The prepared microspheres were evaluated by mean % Mucoadhesion, % Drug Entrapment Efficiency, Cumulative % Drug Release and Microsphere Size. The formulation was optimized by the mathematical relationship in the form of quadratic model by Design-Expert-10 software. Mucoadhesive microsphers containing capsules were compared with marketed modified release tablet and found comparable drug release up to 24 hrs with added advantage of gastroretntion.

Keywords : Gastro-retentive, Microspheres, Azithromycin. *Helicobacter pyroli*

Introduction

Dosage forms that can be retained for longer time in stomach are called Gastroretentive drug delivery systems (GRDDS). GRDDS can improve controlled delivery of drugs that have an absorption window by continuously releasing the drug for a prolonged period of time before it reaches its absorption site, thus ensuring its optimal bioavailability. The development of oral drug-delivery systems for a specific drug involves the optimization of the dosage form as per characteristics of GI physiology. Although significant advances have been made to develop the drug-delivery systems, most of the dosage forms are still designed on the empirical basis. For oral solid-delivery systems, drug absorption is unsatisfactory and highly variable between the individual's despite of excellent *in-vitro* release patterns.[1] The major problem is the physiological variability such as GI transit and gastric retention time (GRT). Gastric retention time plays a dominating role in the overall transit of the dosage form and physiological variability makes it difficult to label the drug-delivery systems with a definite *in-vivo* performance. . In the oral controlled-release (CR) systems the slow release can be achieved because the drug released after passing the absorption site (upper position of small intestine) but the GRT of the delivery system is less than 12 hr. Therefore, it is not possible to deliver the drug for more than 12 h through the oral route.[2] This has encouraged various researchers to retain the drug delivery systems in the stomach for prolonged and predictable time. This type of prolonged gastric retention not only controls the time but also provides the space in the stomach for maintaining the delivery system positioned at a steady site.

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Drugs having narrow absorption window are mostly associated with improved absorption at jejunum and ileum due to their enhanced absorption properties e.g. large surface area, or because of enhanced solubility in stomach as opposed to the more distal parts of the GIT. Gas-generating system[56]: Gas-generating system consisting of an expandable asymmetric triple layer tablet. One layer was the swellable gas-generating layer poly (ethylene oxide), HPMC and sodium bicarbonate/calcium carbonate (1:2 w/w). The second one was the expandable/sustainable drug-containing layer poly(ethylene oxide), tetracycline hydrochloride and Metronidazole. The third one was a rapidly dissolving drug layer bismuth salt. Cellulose Acetatebutyrate (CAB) coated Cholestramine microcapsules as a intragastric floating drug delivery system endowed with floating ability due to the carbon dioxide generation when exposed to the gastric fluid. The microcapsules also have a mucoadhesive property. Ion-exchange resin particles can be loaded with bicarbonate followed by Acetohydroxamic Acid (AHA) and coated with CAB by emulsion solvent evaporation method. The drug concentration was monitored to maintain the floating property and minimum effective concentration. The amount of CAB-coated Cholestramine microcapsules that remained in the stomach was slightly lower than that of uncoated resin particles. Cholestramine microcapsules were distributed throughout the stomach and exhibited prolonged gastric residence via mucoadhesion. These results suggest that CAB-coated microcapsules could be a floating as well as a mucoadhesive drug delivery system. Thus, it has promise in the treatment of *H. pylori*. A preparation that spreads out and adheres to the gastric mucosal surface, and continuously releases antibiotics should be highly effective against *H. pylori*. All the experiments performed in the present work suggest that the developed system may be successful in the treatment of *H. pylori*. The in vitro and in vivo growth inhibition studies with isolated *H. pylori* strains to evaluate the efficiency of formulations may further substantiate the present conclusions. Proposed gastric retentive systems for the enhancement of local drug delivery include floating systems, expandable or swellable systems and bioadhesive systems. Generally, problems with these formulations are lack of specificity, limited to mucus turnover or failure to persist in the stomach. Gastric mucoadhesive systems are hailed as a promising technology to address this issue, penetrating the mucus layer and prolonging activity at the mucus-epithelial interface.

Materials and Methods[6-13]

Azithromycin was purchased from Pharmasynth Formulations Ltd. New Delhi as gift sample. Tamarind seeds was purchased from Phool Chand & Sons, Bhopal, India. Methanol, Citric Acid, Span-80, Magnesium Stearate & Talc was purchased from Central Drug House Pvt. Ltd.,New Delhi. Ferric chloride, Potassium Cyanide purchased from HiMedia Laboratories, Mumbai. Light Liquid paraffin purchased from Delta Chemsol,Mumbai. All other reagents and chemicals used were of analytical grade.

Pre-formulation Studies

Organoleptic evaluation

The Organoleptic studies of drug like general appearance like color, odor and appearance etc were observed.

Melting point

The Melting point was determined by the capillary method using melting point apparatus. . The melting point was compared with reference and it was found within the given range of (IP.2014) monograph.

Solubility analysis:

Quantitative solubility of drug in different solvents was determined according to USP NF , 2007. Drug (1 mg) was accurately weighed and transferred into 10 ml test tube; then it was dissolved in the respective solvents (1 ml each) such as Methanol, Ethanol, Acetone, Chloroform, Benzene, DMSO, Phosphate buffer pH-2, 0.1 N HCl (pH-1.2), Simulated Gastric fluid.(SGF)

pKa determination

The pKa or ionization constant is defined as the negative logarithm of the equilibrium coefficient of the neutral and charged forms of a compound. This allows the proportion of neutral and charged species at any pH to be calculated, as well as the basic or acidic properties of the compound to be defined. This is important in respect to stability of drug under acidic and basic conditions.

Determination of partition coefficient

The partition coefficient of drug was determined by shake flask method between n-octanol and water.

Bulk characterization studies

a.Bulk density

The bulk density of a powder is the ratio of the mass of an untapped sample powder sample and its volume including the contribution of the interparticulate and intraparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density was determined by observing the volume occupied by 50gm of drug using densitometer.

b.Tapped density

The tapped density was determined by mechanically tapping the dry drug powder in the graduated measuring cylinder. The mechanical tapping was attained by raising the cylinder and allowing it to drop, under its own mass from height of 14±2 mm of height for 500 times. Finally tapped volume (V_{500}) was determined in gm/ml and tapped density was calculated

c.Powder flow properties:

The flow properties of powders are critical parameter during the pre- formulation evaluation of the drug substance, therefore, its flow characteristic should be studied, especially when the anticipated dose of the drug is large. Powders may be free flowing or cohesive (non free flowing).Flow properties are affected by changes in particle size, density, shape, electrostatic charges, and adsorbed moisture. It is characterized by Carr's index and Hausner ratio

Quantitative Estimation by UV

Thiocynate The analytical investigation of drug and drug products is very important. UV/Vis spectroscopy method was found suitable method for estimation of drug. Estimation of drug was performed using the visible range in spectrometer using $FeCl_3$ (Ferric Chloride) and KSCN (Potassium).

Standard solution of Drug was prepared by dissolving accurately weighed 10 mg of Azithromycin with 5 ml of water solvent, in 10 ml volumetric flask. The volume was made up to 10 ml with water to obtain a stock solution of 1000 µg/ml. 1 ml of this stock solution was taken and then diluted up to 10 ml using respective solvent to obtain a solution that has a concentration 100 µg/ml which is standard stock solution .

IR Study

IR spectrum of drug recorded over the range of 4000 to 400 cm^{-1} by KBr pellet method using a IR spectrophotometer. The KBr disc was prepared using 1 mg of drug and 100 mg of spectroscopic grade KBr which was previously dried using IR lamp.

Preparation of Azithromycin Microspheres

Four formulation factors were found to have significant effects on the mucoadhesion and controlled release. Mucoadhesive Microspheres prepared by Single Emulsion Technique. The mathematical relationship in the form of polynomial equation for the measured responses obtained with Design- Expert-10 software. Mucoadhesive Microspheres of Azithromycin were prepared by Single Emulsion Technique. Mucoadhesive TSP was dissolved in 10 ml warm water (50ºC) and then the Drug Azithromycin was dispersed in this solution. Azithromycin-TSP aqueous dispersion was added drop by drop in to a 500ml beaker containing 200 ml Light paraffin oil and Span-80. The Mucoadhesive Microspheres were gradually hardened and the hardened microspheres were collected by vacuum filtration. The microspheres were washed several times with petroleum ether and dried in a vacuum desecrator at an ambient temperature for 24 hr.

Table 1: Composition of Formulation

S. No.	Mucoadhesive TSP (%)	Light paraffin oil and Span-80 (%)	Stirring Time (min)	Temperature
1	1.5	10	30 min	30º C
2	2.0	20	30 min	30º C
3	2.5	30	30 min	30º C
4	3.0	40	30 min	30º C
5	3.5	50	30 min	30º C

Evaluation parameters of Drug Microspheres Blend Formulation

Zeta Potential

The zeta potential was analyzed by Malvern zetasizer Nano Z instrument.

Particle Size

The Particle Size was analyzed by Malvern zetasizer Nano Z instrument.

Entrapment efficiency

To calculate entrapment efficiency, weighed the quantity of drug microspheres with 5 ml of ethanol in a volumetric flask was shaken for 1 min using vortex mixer .

Formulation of Blend of Mucoadhesive Microspheres for capsule

S.No	Ingredient	Quantity of ingredient Per capsule
1	Mucoadhesive Microspheres of Azithromycin	625mg (Equivalent to 250 mg of Azithromycin)
2	Magnesium Stearate	15mg
3	Talc	10 mg

Flow properties of Mucoadhesive Microspheres and its blend

The mucoadhesive microspheres and the formulation blend were evaluated for their flow properties such as Bulk density, Tapped Density, Hausner's Ratio and Carr's Index.

Evaluation of Capsules Containing Mucoadhesive Microspheres

The prepared capsules containing the mucoadhesive microspheres of Azithromycin were evaluated for various pharmacopoeial tests.

Disintegration Test

This test determines whether capsules disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions. Complete disintegration is defined as that state in which no residue of the capsule, except fragments of insoluble coating or capsule shell, remain on the screen of the test apparatus or adhering to the lower surface of the discs.

Weight Variation Test

The prepared capsules were subjected to weight variation test. Weight variation test was conducted as per pharmacopeial norms and found that weight variation for all 20 capsules was within the permissible limit.

Content Uniformity Test

This test is applicable to all capsules which are meant for oral administration. Weighed amount of the Mucoadhesive microsphere blend equivalent to 10 mg of Azithromycin was dissolved in 100 ml of 0.1 N HCl and kept for 24 hrs, The samples was filtered through a 0.45 micron filter. The filtrate was analyzed by UV-VIS spectrophotometer.

Dissolution Profile

Ultimate objective of dissolution testing is to ensure adequate and reproducible bioavailability. Dissolution testing is an *in-vitro* method that characterizes how a drug is extracted out of a solid dosage form. It can indicate the efficiency of *in-vivo* dissolution but does not provide any information on drug substance absorption.

Batch	Angle of Repose(θ)	Bulk Density (gm/cm3)	Tapped Density (gm/cm3)	Hausner's Ratio	Carr's Index (%)
Mucoadhesive Microsheres	26.5±0.21	0.952±0.011	0.986±0.016	1.035	3.44
Blendof Mucoadhesive Microspheres	21.3±0.14	0.958±0.013	1.052±0.019	1.098	93

Table 1: Flow properties of Mucoadhesive Microspheres and its blend

S.No.	Wavenumber (cm^{-1})	Characteristic absorption
1	1051.01	C-O stretching vibrations
2	1169.58	C-H stretching vibrations
3	1106.52	C-O-C stretching vibrations
4	1731.76	C=O stretching vibrations
5	2976.59	O-H stretching vibrations

Table 2 : Positions of some characteristic absorption peaks in the mixture of Azithromycin and TSP (1:1)

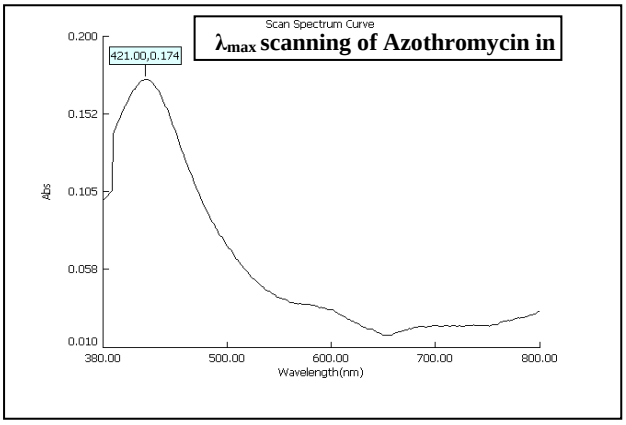


Figure 1.:Standard calibration curve data of Azithromycin containing concentration and respective absorbance.

Result and Discussion

S.No.	Solvents	Observed solubility in (gms/ml) ± SD
1	Distilled water	0.0036±0.0001
2	Methanol	0.368±0.021
3	Ethanol	0.517±0.016
4	Acetone	0.764±0.025
5	Chloroform	0.184±0.008
6	Benzene	0.0140±0.008
7	DMSO	0.632±0.014
8	Phosphate buffer pH-2	0.581±0.019
9	0.1 N HCl (pH-1.2)	0.782±0.013
10	Simulated Gastric fluid.(SGF)	0.756±0.015
11	Phosphate buffer pH-7.4	0.01780±0.006
12	0.1 N NaOH	0.02370±0.006
13	Petroleum ether	0.00021±0.00002

Table 3: Solubility of Azithromycin

Figure 2: Zero order release kinetics from Mucoadhesive microspheres of Azithromycin

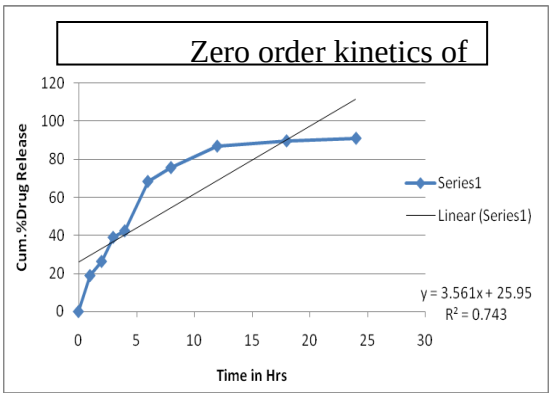


Figure 3: First order release kinetics from Mucoadhesive microspheres of Azithromycin

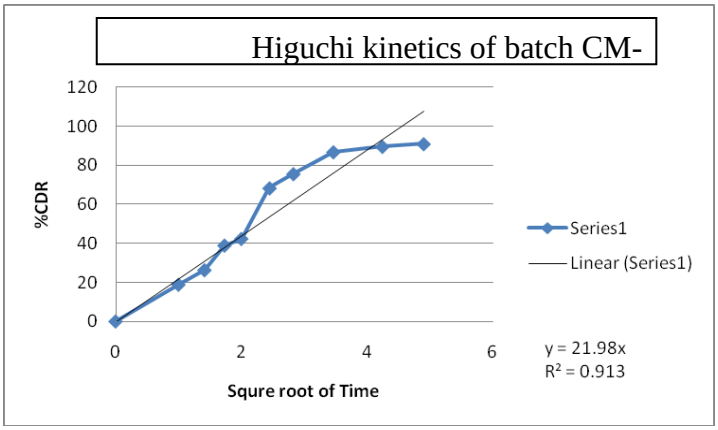
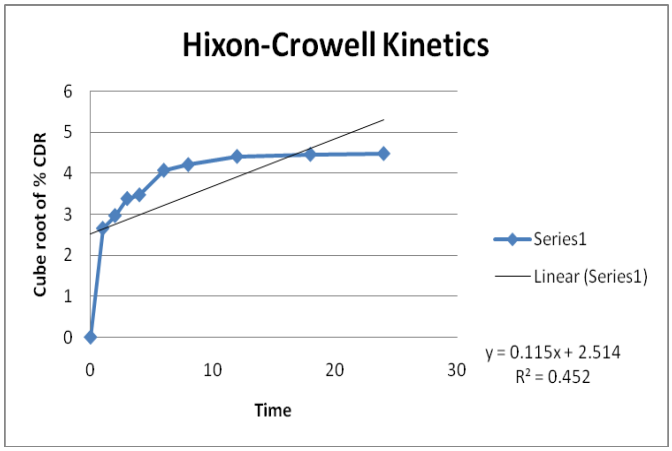


Figure 4: Higuchi release kinetics from Mucoadhesive microspheres of Azithromycin



Discussion

The total 29 batches were prepared as generated by software and evaluated. Percentage mucoadhesion was varied from 46.1 to 91.2, % Drug Entrapment Efficiency from 35.8 to 90.1%, Cumulative % Drug Release (for 8 hours) from 35.5 to 94.2 and average size of microsphere 119 to 194 µm. The optimized formulation was found using point prediction, and formulation CM-9 showed optimum results. The Gamma-scintigraphy study result showed that adhesion of Azithromycin microspheres were prolonged in TSP containing microspheres. Finally capsules were prepared containing mucoadhesive microsphere and evaluated for pharmacopeial standards. Mucoadhesive microsphers containing capsules were compared with marketed modified release tablet and found comparable drug release up to 24 hrs with added advantage of gastroretntion.

The Preformulation studies were carried out in terms of tests for identification (physical appearance, melting point and IR spectra) of drug, solubility profile, drug partition coefficient, drug excipient interaction and quantitative estimation of drug. Coherent preformulation studies of Azithromycin were performed. The Azithromycin was observed as white odorless

powder having melting point 220^{0}C. The quantitative solubility of Azithromycin was determined at 25±1^{o}C using conventional shake flask method in various polar and non-polar solvents. Azithromycin exhibited good solubility in polar organic solvents like methanol and ethanol, less solubility in water and was soluble in 0.1 N HCl and Simulated Gastric Fluid (SGF). The partition coefficient of Azithromycin was found to be 3.19 which indicated the lipophilic nature of the drug. Bulk density, tapped density, compressibility index and Hausner ratio were found to be 0.5263 gm/cm^3, 0.9090 gm/cm^3, 42.1 and 1.727 respectively. The Azithromycin was identified by FT-IR spectra. The sample showed its characteristics absorption peaks for various functional groups which were in accordance with the reference spectra. The IR spectra of Azithromycin with TSP showed no major change in characteristic peaks of drug confirming no interaction between drug and excipients used in the present investigation. Mucoadhesive Microspheres of Azithromycin were prepared by Single Emulsion Technique. Mucoadhesive TSP was dissolved in 10 ml warm water (50^{o}C) and then the Drug Azithromycin was dispersed in this solution. Azithromycin-TSP aqueous dispersion was added drop by drop in to a 500ml beaker containing 200 ml Light paraffin oil and Span-80. The Mucoadhesive Microspheres were gradually hardened and the hardened microspheres were collected by vacuum filtration. The microspheres were washed several times with petroleum ether and dried in a vacuum desecrator at an ambient temperature for 24 hr.The optimizations of Mucoadhesive Microspheres were accomplished by using a response surface design that is appropriate to the problem with Design-Expert-10 software. To include a response in the optimization criteria it must have a model fit through analysis or supplied via an equation only simulation. Factors are automatically included “in range”. Numerical optimization were used to the models to search the factor space for the best trade-offs to achieve multiple goals. The process of optimization has the following steps: Selection of the preferred goal for each factor and response from the menu. The possible goals are: maximize, minimize, target, within range, none (for responses only) and set to an exact value from factors only.Preference of a minimum and a maximum level form each parameter included. The default is for all goals to be equally important at a setting of 3 pluses (+++). If you want one goal to be most important, you could change it to 5 pluses (+++++).Selection of 3D surface plots of the desirability function at each optimum batch that was be used to explore the function in the factor space. Also, any individual response may be graphed to show the optimum point.The Design-Expert program seeks to maximize these functions. The goal seeking begins at a random starting point and proceeds up the steepest slope to a maximum. There may be two or more maximums because of curvature in the response surfaces and their combination into the desirability function. By starting from several points in the design space chances improve for finding the "best" local maximum. A Report is generated by The Design-Expert program. This most detailed report optional views of optimization outcomes. It presents its results in several sections: The Design-Expert program eliminates multiple starts that lead to the same optimum, so fewer results than the number of starting points may be reported. The list of solutions will be sorted with the highest desirability first. Only solutions that meet the criteria are reported. The optimized batch was selected on this report. Mucoadhesive microspheres shows smaller p-values have the larger the significance because it tells signifies the consideration of hypothesis. Scanning electron microscopic analysis of microspheres revealed that they were spherical in shape and ranging from 100-150 µm. The zeta potential of final optimized batch was determined at Malvern zetasizer Nano Z instrument. This report estimated the zeta potential of prepared mucoadhesive microspheres to be +0.54mV. The positive nature of zeta indicates that microspheres will have affinity to protein. Evaluation of optimized Mucoadhesive microspheres of Azithromycin were carried out for Drug Release in modified dissolution apparatus. The drug release from mucoadhesive microspheres followed the Non-Fickin diffusion.Results relating to the fate of formulations of Mucoadhesive Microspheres of Azithromycin microspheres and Azithromycin plain in rabbit stomach were evaluated to determine as (a) Gastroretentive system (b) comparison between two formulations. The results indicated that adhesion of Azithromycin microspheres was prolonged in case TSP containing mucoadhesive microspheres.Finally hard gelatin capsule containing mucoadhesive microspheres of Azithromycin were formulated and evaluated.The capsules passed the test as weight variation was found to be in the range of ±7.5% of the average weight.The disintegration time for hard gelatin capsule containing microspheres was found to be in the range of 6:23±0:6.02 minutes which was well within the range of Pharmacopeial standards.Capsules were evaluated for Content uniformity. The percentage drug content of all the capsules was found to be between 99.12±1.730, which was within the acceptable limits of Pharmacopeia. *In-vitro* drug release was studied and compared with the marketed modified release tablet of Azithromycin. The rate of release from capsule was comparable to the rate of release from the modified release tablet.

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