International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 3, 2024

Original Article

DEVELOPMENT AND OPTIMIZATION OF RABEPRAZOLE CHRONO-MODULATED DRUG DELIVERY SYSTEMS

PHANEENDRA KURAPATI[1*], SANTHIVARDHAN CHINNI[2]

[1]Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Ananthapuramu-515002, Andhra Pradesh, India.
[2]Department of Pharmaceutical Sciences, Raghavendra Institute of Pharmaceutical Education and Research, Ananthapuramu-515721, Andhra Pradesh, India
*Corresponding author: Phaneendra Kurapati; *Email: phanipharma3@gmail.com

Received: 20 Dec 2023, Revised and Accepted: 02 Feb 2024

ABSTRACT

Objective: Development and optimization of chrono-modulated pulsatile drug delivery systems (CPDDS) loaded with Rabeprazole for treating nocturnal acid breakthrough in ulcer patients was set as the major objective of this work.

Methods: CPDDS were developed to provide drug release as two pulses with predetermined gap. Separate microparticles for delayed instant release (DIR) and delayed extended-release (DER) were formulated. Through the optimization of several formulation and process parameters, ER microparticles were created as matrix microspheres. Central composite design was used to understand how the factors affected the responses. The optimized ER microspheres and plain drug were separately subjected to enteric coating to obtain DER and DIR portion microparticles, respectively.

Results: With the exception of stirring speed's impact on drug release, every other factor was found to have a significant influence ($p<0.05$) on every response. The mechanism underlying the Rabeprazole's delayed prolonged release was explained by the SEM images. The microspheres made with Eudragit RSPO at 0.72 g and polyethylene oxide at 0.5 g for 1 g of Rabeprazole at 400 rpm were shown to be the optimal formulation based on the graphical optimization results. After being coated with a terminal enteric coating, this formulation showed delayed release for a duration of 6 h.

Conclusion: After oral administration of equal doses of DIR microcapsules along with the optimized DER microspheres could release Rabeprazole effectively as two different pulses at the desired time intervals.

Keywords: Chrono-modulated pulsatile drug delivery systems, Rabeprazole, Nocturnal acid breakthrough, Delayed release, Optimization

DOI: https://dx.doi.org/10.22159/ijap.2024v16i3.50189 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Proton pump inhibitors (PPIs) like Rabeprazole (RBP) are the first-choice agents used to treat duodenal and stomach ulcers [1]. Delayed-release tablets and capsules of these PPIs are frequently seen on the market [2, 3]. However, the basic enteric coating simply controls the release of the drug until the tablet reaches the small intestine, which is about one to two hours after delivery. Usually, these formulations are taken twice a day. One typical characteristic sign of *Helicobacter pylori*-negative ulcers that affects about 70% of patients is nocturnal acid breakthrough (NAB) [4-6]. The NAB causes the stomach pH to drop to less than 4 for at least an hour straight throughout the late night and early morning. The patients experience extreme agony as a result while they sleep. Because of this, we need special ways to deliver drugs that can keep therapeutic plasma concentrations high during possible NAB times [7, 8].

Pulsatile drug delivery systems (PDDS) release the medicine in a pulse, with a set amount of time between doses [9, 10]. This is done to match the body's natural circadian rhythm with the person's illness. Many physiological disorders, such as NAB, have a chronological course, and managing them effectively necessitates adjusting drug plasma levels to coincide with the symptoms. A significant body of research supports the use of PDDS to achieve chronomodulated drug delivery. Compression-coated aceclofenac tablets were developed for chronomodulated drug delivery to treat arthritis; according to Rashid R *et al.* [11], using the combined matrix of Eudragit and HPMC, a predetermined lag time of five to six hours was attained. Additionally, Mahalakshmi P *et al.* [12] created coated tablets by compressing Eudragit RSPO into the tablet to achieve the necessary delay before pantoprazole releases. The combination of HPMC, EC, and xanthum gum matrix, according to Garg AK *et al.* [13] and Kharwade R *et al.* [14], provided the required lag-time and prompt release of the included medicines from the tablets formed by compression coating. According to this literature, a combination matrix of water-swellable and water-insoluble polymers can be used to successfully produce the appropriate lag-time before the drug release. However, the disadvantage of single-unit dosage forms is the potential for coating rupture, which could lead to dosage form failure [15]. Hence, multi-particulate methods successfully provide the required chronomodulated delivery while avoiding this possible disadvantage. Tekade AR *et al.* [16] used a new swellable polymer to make microspheres that release theophylline in pulses in the colon to treat asthma.

Even after a careful study of the existing research, there is still a lot to learn about how to make pulsatile microparticulate systems that could allow time-controlled delivery [17] of drugs like PPIs. The proton pump inhibitor (PPI) formulations that are currently available for immediate release or delayed release, when taken twice daily, cannot sufficiently control gastric acid secretion overnight. Therefore, the PPI used in this investigation was Rabeprazole, and the development of a PDDS was the goal. Two PPI doses, one for delayed immediate release (DIR) and the other for delayed extended release (DER), are included in the proposed PDDS. The hypothesis to achieve the desired drug release and, thus, the plasma drug concentrations is as follows: The initial dose of this PDDS capsule must be released from the DIR portion after the capsule's contents have been emptied into the small intestine, which should be taken right before dinner, at approximately 8 or 9 p. m. The second dose of the drug must then be released from the DER portion at around 3 or 4 a. m. The outer enteric coating of the DER microparticles prevents drug release in the stomach. Later, the polymer matrix should prevent the drug release for a period of around 4 h in the intestine, which sums up to a total lag of around 6 h, which is when the nocturnal acid breakthrough usually happens [4–6].

DIR microcapsules were planned to be developed by the emulsion solvent evaporation technique from the plain drug. Whereas, the formulation of the matrix microspheres for the DER component is essential to fulfilling the current research's goal. Therefore, to develop DER portion microparticles, the quality by design (QbD) approach [18, 19] was put into practice utilizing Stat-Ease Design Expert software. Particle size and drug released at different times were considered the

dependent variables (responses); the independent variables (factors) were the type of swellable polymer, amount of swellable polymer, amount of insoluble polymer, and stirring speed. The experimental design used to examine how the factors affected the responses was a central composite design (CCD). Following that, graphical optimization found the design space and the optimized formulation of the DER microparticles. Finally, one dose-equivalent DIR microcapsules and one dose-equivalent DER microspheres were filled into a capsule and subjected to drug release studies to inspect whether the desired lag was achieved or not.

MATERIALS AND METHODS

Materials

Rabeprazole was acquired from Mylan Laboratories Ltd., Hyderabad; Eudragit RSPO, polyethylene oxide (PEO N60K) and hydroxypropyl cellulose (HPC MF) were purchased from Sigma Aldrich Mumbai; dichloromethane, methanol and all other chemicals are of analytical grade and were acquired from SD Fine Chemicals, Mumbai.

Development of DER portion microspheres

QbD aspects

Using the Quality by Design (QbD) approach, the matrix microspheres for the development of DER-part microspheres of RBP were planned. The desired drug release profile from this DER was a lag of 4 h in the small intestine, followed by the completion of release in one to two hours. Following an extensive review of the literature and testing in experiments, the following formulation and process parameters were taken as the factors: A: the amount of Eudragit RSPO (0.33–0.67g), B: the amount of hydrophilic polymer (0.33–0.67g), C: stirring speed (400–550 rpm), and D: hydrophilic polymer type (PEO N60K/HPC MF). Particle size and percent drug release at 4, 5, and 6 h under intestinal circumstances as D4%, D5%, and D6%, respectively, were taken as the responses. The most appropriate CCD was chosen based on the number and types of the chosen factors. Table 1 displays the combination of the factors and their levels in accordance with the chosen design.

Table 1: Compositions of various formulations of the matrix microspheres as per the CCD

Formulation code	Factor A	Factor B	Factor C	Factor D
MSF1	0.6	0.6	348.87	PEO N60K
MSF2	0.4	0.4	400.00	PEO N60K
MSF3	0.8	0.4	400.00	PEO N60K
MSF4	0.4	0.8	400.00	PEO N60K
MSF5	0.8	0.8	400.00	PEO N60K
MSF6	0.6	0.26	475.00	PEO N60K
MSF7	0.26	0.6	475.00	PEO N60K
MSF8	0.6	0.6	475.00	PEO N60K
MSF9	0.94	0.6	475.00	PEO N60K
MSF10	0.6	0.94	475.00	PEO N60K
MSF11	0.4	0.4	550.00	PEO N60K
MSF12	0.8	0.4	550.00	PEO N60K
MSF13	0.4	0.8	550.00	PEO N60K
MSF14	0.8	0.8	550.00	PEO N60K
MSF15	0.6	0.6	601.13	PEO N60K
MSF16	0.6	0.6	348.87	HPC MF
MSF17	0.4	0.4	400.00	HPC MF
MSF18	0.8	0.4	400.00	HPC MF
MSF19	0.4	0.8	400.00	HPC MF
MSF20	0.8	0.8	400.00	HPC MF
MSF21	0.6	0.26	475.00	HPC MF
MSF22	0.26	0.6	475.00	HPC MF
MSF23	0.6	0.6	475.00	HPC MF
MSF24	0.94	0.6	475.00	HPC MF
MSF25	0.6	0.94	475.00	HPC MF
MSF26	0.4	0.4	550.00	HPC MF
MSF27	0.8	0.4	550.00	HPC MF
MSF28	0.4	0.8	550.00	HPC MF
MSF29	0.8	0.8	550.00	HPC MF
MSF30	0.6	0.6	601.13	HPC MF

Preparation of ER matrix microspheres

The process of emulsion solvent evaporation was used to form the microspheres [20]. Specified quantities of the hydrophilic and hydrophobic polymers listed in table 1 were dissolved in 15 ml of a solvent mixture comprising a 1:1 combination of dichloromethane and methanol. Using a cylcomixer (Remi CM 101), 1 g of RBP was dissolved in the aforementioned polymer solution. A 250-mL beaker containing 80 ml of liquid paraffin was placed separately on a hot plate (Metalab) that had been preheated to 45 °C. A paddle-style mechanical stirrer blade (Remi RQ-5 Plus) was dipped into this beaker. The stirrer was set to a speed specific to each formulation, as mentioned in table 1. Next, while continuously stirring, the drug-polymer dispersion was gradually added to the liquid paraffin in the form of drops. This resulted in tiny drops of volatile solvent that contained an emulsion of drug-polymer monolith in liquid paraffin. The stirring was maintained until the volatile solvent had totally evaporated and the produced droplets had solidified into matrix microspheres, which took four to five hours. After that, the dispersion was filtered to separate the microspheres, and any attached paraffin was removed by washing them with petroleum ether. The microspheres were then promptly dried after being cleaned with distilled water. The free-flowing, dry ER matrix microspheres were collected and suitably preserved for further use.

Characterization studies on the ER matrix microspheres

Percentage yield

The microspheres were carefully weighed. Using the following formula, the yield (%) was calculated

$$\text{Yiled (\%)} = \frac{\text{Weight of the obtained microspheres}}{\text{Total weight of the drug and the polymers taken}} \text{x100}$$

Entrapment efficiency (EE)

The microspheres weighing 100 mg of RBP equivalent weight were collected, ground, and mixed with water using a rotary shaker.

Samples were taken out and subjected to spectrophotometric analysis (using Thermo Scientific Evolution One) for absorbance at regular intervals until a consistent absorbance was achieved. The amount of RBP contained in the microspheres was estimated using the final absorbance. Then, the EE was calculated using the formula below.

$$\text{EE (\%)} = \frac{\text{Amount of RBP present in the microspheres}}{\text{Theoretical amount of RBP taken}} \text{x100}$$

Particle size

The microspheres' size was determined using the microscopy technique [21, 22]. On a glass slide, a small number of microspheres were dispersed and focused under an optical microscope. Feret's diameter was calculated for 200 particles using an eyepiece micrometer that had been previously calibrated. The acquired data was used to compute the arithmetic mean diameter.

Studies on drug release

These were carried out using Lab India Disso 8000, a USP type II equipment running at 100 rpm. The medium was 1000 ml of 0.6M Tris buffer, pH 8.0 [23]. The test was started after 20 mg of RBP equivalent microspheres were put into the vessel. At regular intervals, we took out 5 ml samples and placed them into test tubes with stoppers that contained 1 ml of 0.5N NaOH solution. The samples were then stored in a dark area until further examination. At its maximum wavelength of 285 nm, spectrophotometric analysis was carried out to quantify RBP.

Design validation and optimization

Design Expert software was used to analyze the design of experiments (DoE) [24]. To develop the regression model for each response with the factors, the sequential model sum of squares analysis was run for each response. An ANOVA was then performed to determine the suitability of the accepted design and model for optimization, as well as the significance of the model and its parameters. Through the use of desirability functions, graphic optimization was carried out. The objective of optimization was to get the required drug release profile, meaning that the drug release should be at least minimal for the first four hours and maximal for the last two hours.

Scanning electron microscopy (SEM)

Using a SEM (ZEOL JSM–5610), the morphological characterization of microspheres was investigated in accordance with the methodology described by Srikar G *et al.* [18]. The SEM studies were also carried out to the ER microspheres remained after drug release studies. The images obtained before and after the drug release studies were compared to find out any potential mechanism of drug release.

Enteric coating and characterisation of the optimized ER matrix microspheres

The optimized ER matrix microspheres were transformed into DER-part microspheres in this stage. The optimized ER microspheres underwent enteric coating to prevent RBP release in the stomach area. Eudragit S 100 was the enteric film-forming substance utilized in this stage. For the optimum outcome, three distinct extended enteric coating solution formulations (EECF), as indicated in table 2, were designed and validated. Each comprising varying amounts of the film former and viscosities. 100 g of the optimized microspheres were put into the coating pan, which had a 50 rpm rotation. Drying hot air was introduced into the coating pan at a temperature of 40 °C. At a predetermined, optimal rate of 5 ml/min, the coating solution was sprayed onto the microspheres. The coated microspheres were removed and visually inspected for any adhering after they had dried.

Table 2: Composition of extended enteric coating solution formulations

S. No.	Ingredients	Quantity		
		EECF1	EECF2	EECF3
1	Eudragit S 100	8g	10g	10g
2	PEG 400	1.2g	1.5g	1.5g
3	Talc	0.1g	0.1g	0.1g
4	Span 20	0.1g	0.1g	0.1g
5	Isopropyl alcohol q. s.	50 ml	50 ml	40 ml

The resulting enteric-coated matrix microspheres (DER microspheres) were evaluated using the previously described techniques for yield, drug content, and particle size. Drug release studies were carried out in acid stage and buffer stage. For the acid stage, the paddle apparatus revolved at 100 rpm; 700 ml of 0.1N HCl was taken as the medium and the test was carried out for 2 h. Later, the buffer stage was carried out using the conditions described for the ER matrix microspheres.

DIR portion microcapsules preparation and characterization

When the microcapsules reach the small intestine, this part of the microcapsules must release the drug right away, avoiding it in the gastric area. The enteric polymer Eudragit S100 was employed to coat the plain RBP via the emulsion solvent evaporation process, with the core material being disseminated in the polymer solution [25]. Three distinct immediate enteric-coated microspheres formulations (IECF) containing Eudragit S100 (as indicated in table 3) were dissolved in the ethanol, followed by dispersing the RBP in them. In a beaker, liquid paraffin containing 0.2% v/v of Span 20 was added. The beaker was then stirred at 550 rpm (Remi RQ-5 Plus) and kept at 45 °C. Under these circumstances, the dispersion of RBP in the polymer solution was poured drop-wise into this beaker. Stirring was continued until the solvent evaporated, which took around 3.5 h. Filtration separated the resulting microcapsules from the liquid paraffin, followed by washing away any attached paraffin with petroleum ether. Ultimately, the microcapsules underwent two rounds of water washing before being placed in a hot air oven to dry. The RBP enteric-coated microcapsules (DIR microcapsules) were dried and kept for later research.

Using the same methods as described for the matrix microspheres, these DIR microcapsules were also examined for yield, EE, and particle size. Similar to enteric-coated DER portion microspheres, drug release studies were conducted in the acid and buffer stages.

RESULTS

Matrix microspheres

Yield and EE

As seen in table 4, every microsphere formulation produced a significantly high yield, ranging from 87.3 to 94.2%. Table 4 also displays the EE of all of the microsphere formulations, which ranged between 67.4 and 89.3%.

DoE analysis of the response–particle size

The particle size analysis results are presented in table 4. Using the Design Expert software, sequential sum of squares analysis was used to evaluate the regression model type to demonstrate the factors' effect on this response. It was discovered that the parameters had a quadratic on the particle size, and fig. 1 illustrates this effect. ANOVA revealed that all four factors' influences were significant at $p<0.05$.

Design validation and optimization

The sequential sum of squares analysis revealed that the factors had a linear impact on the response particle size, D4% and D5%, whereas the quadratic effect of D6%. These appropriate models were used to examine

each response. Fig. 3 shows the predicted vs. actual plots for each of the responses. To match the desired drug release profile, graphical optimization was carried out by imposing limitations or desirability criteria on the responses. Therefore, D4 was set to be a minimum of below 10%; D5 was set to be between 45 and 55%; and D6 was set to be between 90 and 99%. The particle size was set to a minimum of below 250 µm. The graphical optimization was carried out within these restrictions, and fig. 4 displays the overlay plot that was produced. Table 5 displays one of the software's most optimal combinations, together with the predicted values of the responses. ER matrix microspheres were made using the recommended optimum combination, and their response values were assessed, which are presented in table 5.

Table 3: Formulation composition of immediate enteric coating microcapsules of rabeprazole

S. No.	Formulation	Quantities			
		RBP	Poloxamer 188	Eudragit S100	Ethanol
1	IECF1	0.5g	0.05g	0.125g	5 ml
2	IECF2	0.5g	0.05g	0.25g	5 ml
3	IECF3	0.5g	0.05g	0.375g	5 ml
4	IECF4	0.5g	0.05g	0.5g	5 ml

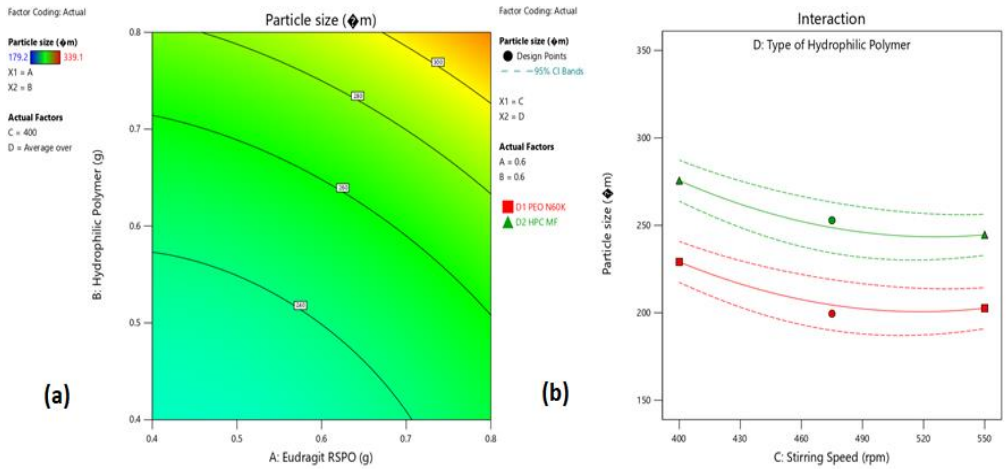

Fig. 1: Illustration of the effects of the factors on the particle size (a) Contour plot showing the effect of the factors A and B; and (b) Interaction plot showing the effect of the factors C and D

Table 4: Results of various characterization studies, including the responses

Formulation code	Yield (%)	EE (%)	Particle size (µm) (R1)	D4 (%) (R2)	D5 (%) (R3)	D6 (%) (R4)
MSF1	90.2±2.5	85.7±3.6	271.4±12.6	16.8±2.5	52.6±3.2	98.1±1.7
MSF2	93.8±1.9	76.3±4.1	192.3±10.8	22.1±0.9	63.4±3.4	100±3.7
MSF3	89.4±3.2	78.1±3.2	215.7±15.3	7.5±2.4	46.7±1.6	97.4±2.2
MSF4	88.1±4.6	87.4±2.5	253.8±17.2	18.6±1.7	56.9±2.7	99.6±1.8
MSF5	85.6±2.8	89.8±1.8	302.6±24.7	4.7±1.2	40.2±2.4	85.3±2.4
MSF6	92.7±3.7	71.3±5.2	185.4±16.9	20.4±2.6	61.5±1.9	100±1.9
MSF7	93.1±1.4	72.6±3.9	216.1±13.4	25.5±3.4	70.3±3.1	100±3.4
MSF8	89.6±3.5	75.4±2.2	199.5±18.7	19.3±1.2	53.8±3.7	100±2.8
MSF9	85.2±2.6	76.1±5.7	235.2±20.5	8.1±1.3	40.1±1.9	68.5±1.5
MSF10	86.4±2.7	80.5±3.4	274.9±23.6	11.7±0.8	46.3±2.6	70.2±1.9
MSF11	93.4±2.1	71.8±6.3	179.2±16.2	26.2±3.1	72.4±4.2	100±3.3
MSF12	90.5±3.6	74.7±2.9	198.4±17.1	9.1±2.2	49.6±1.8	98.4±2.7
MSF13	88.9±4.2	75.9±3.7	236.7±20.5	20.6±1.5	60.7±2.9	100±2.6
MSF14	86.3±1.9	81.2±4.1	269.5±24.3	8.4±0.9	42.8±3.1	91.3±1.3
MSF15	91.1±2.7	72.3±5.8	211.8±15.7	22.9±1.1	59.5±2.4	100±3.1
MSF16	88.5±3.1	89.2±1.3	313.5±27.8	12.6±1.7	46.1±1.5	94.1±2.6
MSF17	91.8±2.2	80.4±4.6	247.9±21.4	17.4±2.3	60.9±4.5	96.2±4.2
MSF18	89.1±4.3	83.7±2.8	271.4±24.9	5.2±0.6	45.2±2.8	93.7±2.8
MSF19	90.4±1.5	84.9±3.3	290.3±16.2	9.4±1.1	52.4±1.6	95.5±3.6
MSF20	84.7±5.4	91.3±1.4	339.1±30.1	3.9±0.4	41.7±3.3	66.4±5.7
MSF21	92.3±1.9	73.8±4.9	254.3±18.4	21.9±2.7	54.8±1.6	99.2±1.3
MSF22	91.6±2.9	76.7±1.5	241.6±22.7	22.7±1.4	69.5±4.2	100±2.5
MSF23	90.8±2.3	82.5±3.4	252.9±14.6	16.5±1.3	49.3±2.9	92.3±3.9
MSF24	85.9±4.6	84.1±2.6	287.4±25.2	6.3±0.8	42.1±2.1	72.5±1.7
MSF25	84.4±3.8	86.3±2.3	309.1±27.8	9.2±2.1	47.6±2.4	76.1±2.2
MSF26	92.5±2.4	75.8±4.7	237.6±17.5	23.1±1.9	68.5±3.8	100±2.6
MSF27	90.3±3.1	78.2±3.9	255.8±23.4	9.2±0.6	47.4±2.2	92.4±3.4
MSF28	88.7±4.5	81.4±1.8	260.4±24.9	15.4±1.5	53.2±1.7	97.4±4.5
MSF29	85.2±2.7	86.3±2.6	301.5±27.3	6.3±0.6	38.7±2.5	62.5±3.1
MSF30	89.6±3.2	80.6±3.4	243.2±21.2	18.2±2.4	54.6±4.3	95.2±2.9

The results are expressed as mean±SD, deviation for n = 3

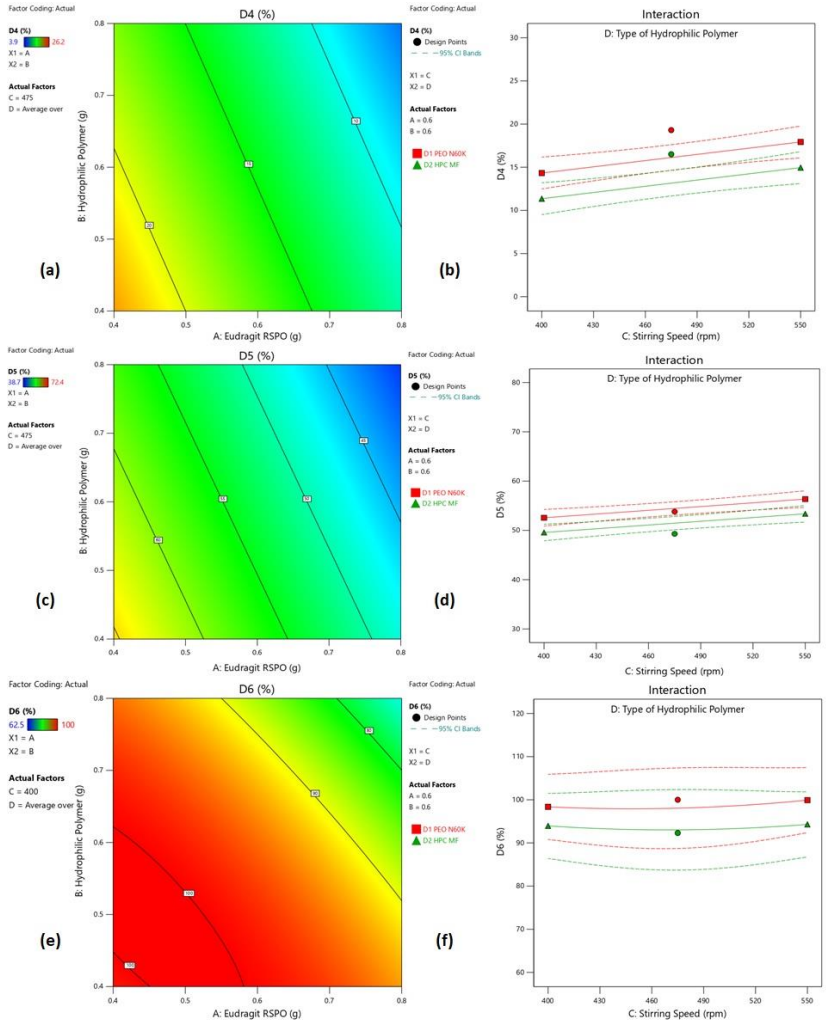

Fig. 2: Illustration of the effects of the factors on the Responses (a) Contour plot of effects of the factors A and B on D4%; (b) Interaction plot of the effects of the factors C and D on D4%; (c) Contour plot of the effects of the factors A and B on D5%; (d) Interaction plot of the effects of the factors C and D on D5%; (e) Contour plot of the effects of the factors A and B on D6%; (f) Interaction plot of the effects of the factors C and D on D6%

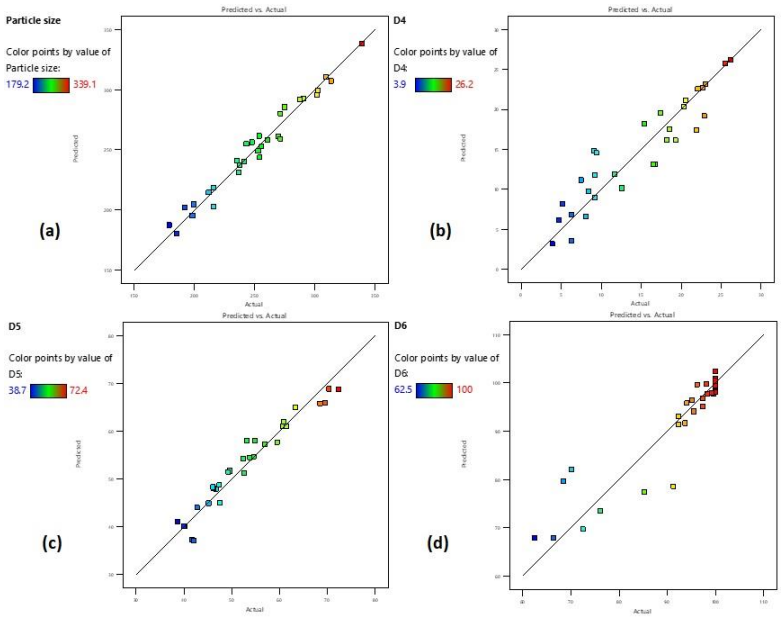

Fig. 3: Predicted vs actual plots for the responses (a) R1–Particles size; (b) R2–D4%; (c) R3–D5%; and (d) R4–D6%

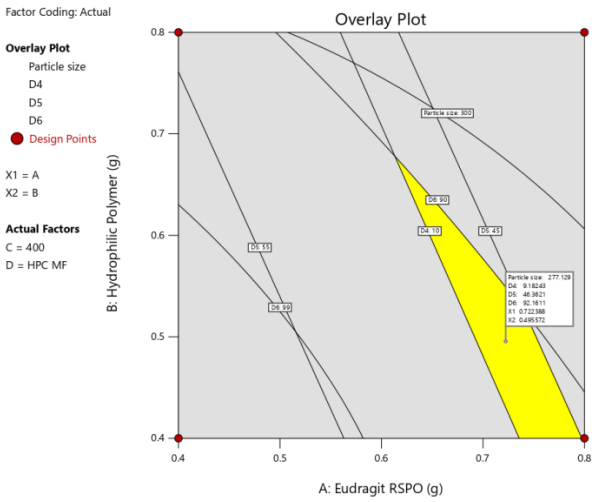

Fig. 4: Overlay plot illustrating the design space in yellow colour region

Table 5: Comparison of the predicted and observed values of the responses for the optimized ER microspheres formulation of RBP

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: Eudragit RSPO–0.72g	R1: Particle size (µm)	277.5	266.2	288.7	273.8
B: Hydrophilic Polymer–0.5g	R2: D4 (%)	9.1	7.1	11.2	8.6
C: Stirring speed–400	R3: D5 (%)	46.3	44.4	48.2	47.5
D: Type of Hydrophilic polymer–HPC MF	R4: D6 (%)	92.0	84.8	99.2	94.3

SEM examination

Before, during, and after the drug release investigation, the matrix microspheres' surface shape was examined; the results are shown in fig. 5. Fig. 5(a) depicts the surface of the microspheres prior to their exposure to drug release. Fig. 5(b) illustrates the microspheres' surface four hours into the drug release investigation. Once more, at the conclusion of the 6 h drug release research, fig. 5(c) displays the obtained SEM picture.

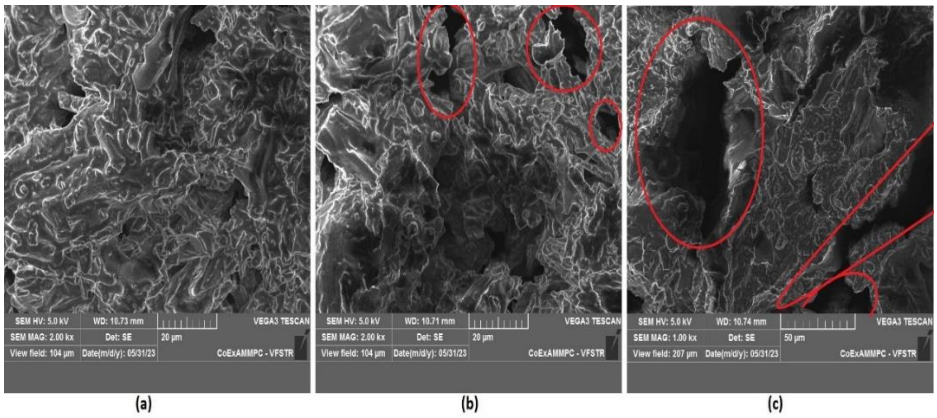

Fig. 5: SEM images of the optimized microspheres (a) before; (b) after 4 h; and (c) after 6 h of drug release study. The small encircled regions in the (b) show initiation of dissolution of the HPC from the matrix to allow the drug release and the (c) shows development into large pores at the end of the drug release

Table 6: Characteristics of the DER microspheres of RBP

S. No.	Characteristic property	Result/Observation		
		EECF1	EECF2	EECF3
1	Physical observation	Free-flowing microspheres	Free-flowing microspheres	Moderate extent of sticking
2	Weight (g)	106.7±2.4	109.8±1.1	108.3±1.7
3	Particle size (µm)	289.4±12.9	295.2±10.6	312.5±18.2
4	% Drug released in acid stage after 2 h (AD2%)	8.7±1.5	5.6±0.8	--
5	% Drug released in buffer stage after 4 h (D4%)	14.1±1.2	9.3±0.5	--
6	% Drug released in buffer stage after 5 h (D5%)	50.3±4.6	48.1±2.9	--
7	% Drug released in buffer stage after 6 h (D6%)	94.9±2.7	95.7±3.1	--

The results are expressed as mean±std. deviation for n = 3

Enteric-coated optimized ER microspheres: characterization

The ER microspheres in the preceding section were tuned to produce a 4 h delay in the release of RBP in the intestinal media. The microspheres require an enteric coating to prevent drug release in the gastric environment because they need to initially come into contact with the stomach environment after administration. Three distinct coating solution formulations (EECF1–EECF3) based on Eudragit S 100 were examined to determine the ideal composition. Table 6 displays the characterization study results following the enteric coating of the optimized ER microspheres, which hence become DER microspheres and fig. 6(a) displays the drug release profiles.

Studies on the DIR microcapsules

Since RBP dissolves in ethanol very little, it simply dispersed in the Eudragit S100's ethanolic solution. As the polymer solution settled on the RBP particles during emulsification, the emulsion solvent evaporation process in this instance, created microcapsules. Rigidifying the Eudragit S100 over the RBP particles followed by allowing the remaining ethanol to evaporate produced the microcapsules. These were called delayed immediate release (DIR) microcapsules because Poloxamer 188 was used as a surfactant to improve drug dissolution once the enteric polymer was dissolved upon reaching the small intestine following oral administration [26]. Four distinct formulations of the DIR microcapsules (IECF1–IECF4) were prepared and studied for various characterization studies, with the results displayed in table 7. Fig. 6(b) displays the dissolution profiles of these DIR microcapsules.

Drug release studies on the DER microspheres and the DIR microcapsules together

To create the final pulsatile drug delivery system, one dose (20 mg of RBP) equivalent DIR microcapsules and another 20 mg equivalent DER microspheres were combined and put inside a hard gelatin capsule. Using the previously described protocols, this was subjected to drug release investigation in both acid and buffer stages. Fig. 6(c) displays the dug release profile that was achieved.

Table 7: Characteristics of the DIR microcapsules of RBP

S. No.	Characteristic property	Result/Observation			
		IECF1	IECF2	IECF3	IECF4
1	Physical observation	Free-flowing microspheres	Free-flowing microspheres	Free-flowing microspheres	Free-flowing microspheres
2	Yield (%)	89.5±3.4	84.7±3.1	82.9±2.2	81.7±1.8
3	EE (%)	81.3±2.5	87.2±1.7	86.6±3.4	89.1±2.3
4	Particle size (µm)	109.3±10.2	117.6±8.3	125.2±13.1	136.8±11.7
5	%Drug released in acid stage at 2 h (AD2%)	17.7±3.3	11.3±2.4	7.5±0.9	6.2±1.1

The results are expressed as mean±std. deviation for n = 3

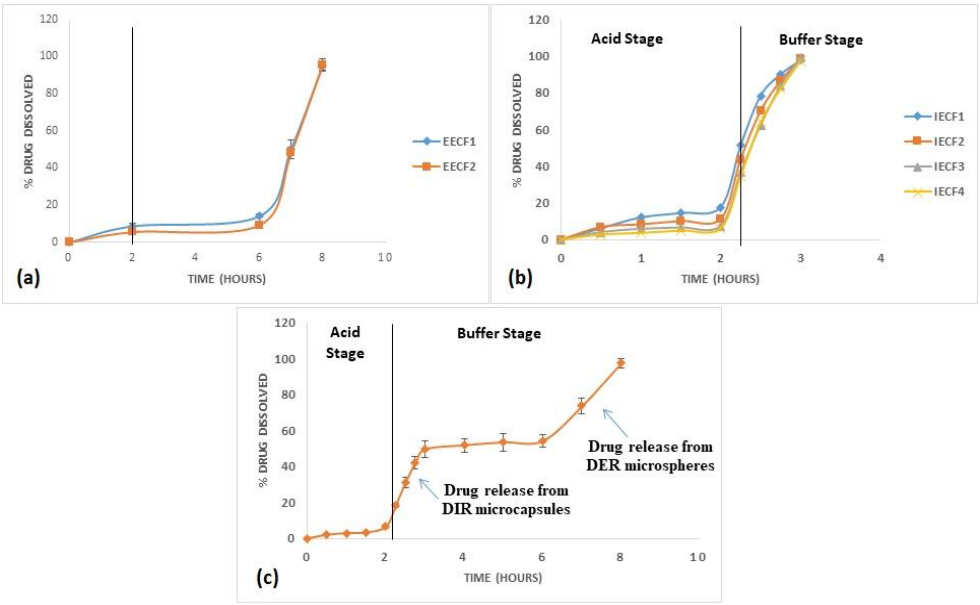

Fig. 6: Overall drug release profiles of RBP from (a) DER portion microspheres; (b) DIR portion microcapsules; and (c) Pulsatile drug delivery capsule containing both DIR and DER portions. (Results are expressed as mean±SD for n = 3)

DISCUSSION

The high yield numbers indicate that the chosen processing parameters are appropriate for the microspheres' composition. The high yield numbers indicate that the chosen processing parameters are appropriate for the microspheres' composition, demonstrating that the chosen approach and factors promote microsphere formation.

According to the results of the EE analysis, there was a positive correlation between the amounts of hydrophilic and hydrophobic polymers. This may be because the drug adheres to polymers more readily and entraps them. However, increasing the stirring speed resulted in a decrease in the EE. Before the microspheres hardened, high-speed mixing conditions might provide quick drug diffusion out of the polymer matrix. Therefore, the rigidized polymer matrix that formed the microspheres would contain less of the RBP after the volatile solvent was completely removed. The EE values obtained were in agreement with the findings published by Pavelkova MHJMKKVDSPM *et al.* [27]. The hydrophilic polymer type also had an impact on the EE. When compared to their similar formulations made with PEO N60K, the microspheres made with HPC MF showed

greater EE values. This could be because HPC MF has a higher viscosity (4000–65000 cps at 2% w/v in water) than PEO N60K (2000–4000 cps at 2% w/v in water). Increased viscosity of the polymer matrix prior to its stiffening would reduce the drug's diffusion out of the matrix, perhaps leading to a greater EE [18, 19].

Variables A and B both had a positive impact; increasing the levels of any polymer also increased the sizes. This might be the result of the polymer solution's higher viscosity, which might prevent the globules from breaking down during emulsification. The research findings published by Pavelkova MHJMKKVDSPM *et al.* [27] and Dashora K *et al.* [28] supported the hypothesis that this would lead to increased particle size upon solvent evaporation. It was found that factor C had a negative influence on the particle size, i.e., the size decreased upon increasing the stirring rate. During emulsification, the high-speed stirring generated more energy, leading to a size reduction of the dispersed phase globules. These globules eventually solidify into smaller microspheres when the solvent evaporates [28, 29]. Factor D increased the size when the polymer was changed from PEO to HPC, which could be attributed to the viscosities of both these polymers [30]. High-viscosity formulations incorporating HPC MF may prevent the globule size from breaking down at the same stirring speed, which could lead to larger particle sizes.

Drug release at three-time points, viz. at 4, 5, and 6 h, were taken as the responses. At each of these time points, it was discovered that components A and B had a negative impact on the amount of drug release; that is, when the levels of any polymer increased, the drug release decreased. This could be due to the drug's strong binding to the polymer matrix at higher polymer amounts [31]. Moreover, higher concentrations of hydrophobic polymer may impede the hydrophilic polymer's dissolution and impede drug release [32]. Lastly, higher concentrations of hydrophilic polymer may take longer to swell and dissolve, which may further impede drug release. Furthermore, compared to formulations including HPC, those containing PEO showed faster drug release. This may be due to the HPC's increased viscosity and molecular complexity, which would require more time to swell and dissolve [33]. As a result, HPC MF delayed drug release more than PEO 60K.

The ANOVA test results showed that each of the regression models of the four responses was significant. The predicted versus actual plots of all four responses indicated that the data points were uniformly distributed around the 45° line. This further confirmed that the models were significant and did not require any transformation. Therefore, these findings suggest that the chosen models may be further optimized since they were well-fit to depict the influences of the factors on the responses [19].

To attain the desired response values corresponding to the objective, graphical optimization was carried out by imposing limitations or desirability criteria on the responses. After a lag period of four hours after entering the small intestine, these ER matrix microspheres should release the contained dose. Additionally, the drug should start to release after four hours and then finish in two hours so that effective plasma concentrations can be sustained at the intended time. As a result, D4 was set to a minimum of 10% with an upper limit; D5 was set to be between 45 and 55%; and D6 was set to be between 90 and 99%. A minimum particle size of 250 µm was established as the upper limit. The graphical optimization was carried out within these restrictions, and fig. 4 displays the resultant overlay plot. A microsphere formulation with the intended response values would be produced by combining any point in the design space region (the region with the yellow color). Table 5 provides one such optimal combination along with the software's predicted response values. ER matrix microspheres were made using the recommended optimum combination, and their response values were assessed. Table 5 displays the obtained responses, which were determined to fall within the 95% confidence interval of the predicted values. The EE of 87.4% and the necessary drug release properties were effectively obtained through tuning of the matrix microspheres, as evidenced by this. Hence, this formulation was considered as the optimized ER matrix microspheres, which was then subjected to further enteric coating to obtain DER microspheres.

SEM examination was conducted on the surface and shape of the matrix microspheres before, during, and after the drug release investigation, with the results presented in fig. 5. As seen in fig. 5(a), the surface of the microspheres was continuous with the polymer matrix prior to being exposed to the drug release. After being removed from the dissolution vessel after 4 h of the drug release study and dried with filter paper to remove any remaining water, the microspheres were examined [34]. Fig. 5(b) shows that the microspheres at this point revealed a few tiny pores, suggesting the dissolution of the hydrophilic polymer. Once more, after the six hours of the drug release study, the obtained image, as seen in fig. 5(c), showed wide pores, which might have resulted from the hydrophilic polymer's potential for total dissolution, thus permitting the drug to be released completely from the matrix. These SEM images amply demonstrate the role of the combined Eudragit RSPO and HPC in delivering the intended delay in the commencement of the drug release. The Eudragit RSPO polymer delayed drug release by preventing rapid dissolution of HPC. To postpone the drug release by four hours, the amount of Eudragit RSPO was optimized. These SEM pictures also showed that, four hours after the drug release test began, tiny pores on the matrix caused by the HPC's breakdown were seen. Furthermore, the drug was released concurrently and completely in two hours after the optimal amount of HPC was dissolved. The optimum concentrations of Eudragit RSPO and HPC successfully produced the intended delay in the release of RBP from the matrix microspheres, according to the results of the drug release and SEM studies.

Following coating, free-flowing microspheres were produced using the EECF1 and EECF2 formulations. The high viscosity of the coating solution may have contributed to the sticking of the EECF3-coated microspheres, which is why they were left out of the drug release investigation. Fig. 6(a) illustrates how the drug release in the acid stage was limited to less than 10% in both of the two formulations and how the buffer stage was delayed by an additional 4 h. The drug release from these DER microspheres was eventually completed in two hours, resulting in a six-hour overall delay. While the EECF1-coated DER microspheres showed 8.7% of drug release in the acid stage, the EECF2-coated DER microspheres limited the drug release to only 5.6%. The higher enteric polymer content of EECF2 may offer stronger resistance to drug release [35]. As a result, the EECF2 coated microspheres were chosen as the ideal DER-part microspheres.

The yield, ranging from 81.7% to 89.5%, indicated that the process conditions were suitable for forming the microcapsules. It was discovered that the EE and particle size values (table 7) rose from IECF1 to IECF4, which may have been brought on by the added polymer. An increased polymer can encapsulate more amount of drug, increasing the effectiveness of entrapment. Additionally, larger polymer concentrations cause the dispersed phase's viscosity to rise, which prevents the globules' size from decreasing during emulsification under the same experimental conditions. As a result, the particle size rises at higher polymer concentrations [27, 28].

The findings of the dissolution testing from the acid stage which are displayed in table 7 and fig. 6(b), showed that IECF1 and IECF2 were unable to control the drug release below the 10% maximum limit. After two hours, all three of the remaining formulations were able to effectively limit the drug release to less than 10% in the acid stage. The drug dissolution in the buffer stage was seen to be nearly the same across all formulations, with the complete dosage dissolving in less than an hour. Despite the fact that IECF3 and IECF4 all met the delayed release formulations' dissolution requirements, IECF3 was selected as the optimal formulation because it offered a comparable level of effectiveness while carrying the least amount of enteric polymer, which might lower the formulation's final weight.

Hard gelatin capsules of Size 4 were filled with 36.2 mg of DIR microcapsules (equivalent to 20 mg of the drug) and 50.3 mg of DER microspheres (equivalent to 20 mg of the drug), respectively, based on the EE results of the DIR and DER microspheres as well as the enteric coat weight of the DER microspheres. The drug release from these capsules was investigated. According to the drug release profile displayed in fig. 6(c), the obtained dug release during the first two hours of the acid stage was significantly less than 5.9% of the total dose. This suggested that the technologies under development

could successfully stop the release of RBP in the stomach. Drug release was discovered to be 49.6% after an hour in the buffer stage, which may have been caused by the DIR microcapsules. This suggests that one of the two doses was entirely released as a single pulse within an hour after entering the small intestine. Additionally, the release of the second dose from the DER microspheres was initiated as the second pulse and finished in two hours following a prearranged lag of almost four hours in the buffer stage. Consequently, the RBP pulsatile drug delivery system could efficiently create the drug release as two pulses, the first occurring immediately upon reaching the small intestine and the second occurring four hours later.

CONCLUSION

The PDDS of RBP was created as a capsule dosage type that would deliver the drug in two separate pulses. By using the solvent evaporation method to microencapsulate the RBP with Eudragit S100, one dosage of the medication was created as DIR microcapsules. Because of their enteric layer, these microcapsules stop the drug from releasing in the stomach and release it as soon as they enter the small intestine. Once the second dose as the DER microspheres entered the small intestine, it was designed to release as another pulse four hours later. The development of RBP as hydrophilic-hydrophobic matrix microspheres and enteric coating-known as DER microspheres were the steps used to accomplish this. The drug release in the gastric acidic medium was inhibited by the outer enteric coat, and the optimal amounts of Eudragit RSPO and HPC produced the anticipated 4 h lag prior to the onset of matrix disintegration and subsequent drug release. Therefore, the first dose of this PDDS capsule will be released once the capsule's contents have been emptied into the small intestine from the DIR section, which should happen shortly before the night meal at around 9 PM. The second dose of the medication will then be released from the DER section at around 3 or 4 am, which is when the nocturnal acid breakthrough usually occurs, following a 6 h lag. Therefore, by releasing the drug at the appropriate time, this pulsatile drug delivery capsule dosage form with chronomodulated drug release features, comprising one dose of DIR microcapsules and one dose of DER microcapsules, can successfully avoid the nocturnal acid breakthrough. As a result, the quality-by-design strategy was successfully applied to accomplish the goal of the current study project.

ACKNOWLEDGEMENT

We are acknowledged to the management of Jawaharlal Nehru Technological University, Ananthapuramu and Raghavendra Institute of Pharmaceutical Education and Research, Ananthapuramu, Andhra Pradesh.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All others have contributed equally

CONFLICT OF INTERESTS

There are no conflicts of interest

REFERENCES

1. Brisebois S, Merati A, Giliberto JP. Proton pump inhibitors: review of reported risks and controversies. Laryngoscope Investig Otolaryngol. 2018;3(6):457-62. doi: 10.1002/lio2.187, PMID 30599030.
2. Shin JM, Kim N. Pharmacokinetics and pharmacodynamics of the proton pump inhibitors. J Neurogastroenterol Motil. 2013;19(1):25-35. doi: 10.5056/jnm.2013.19.1.25, PMID 23350044.
3. Hua S. Advances in oral drug delivery for regional targeting in the gastrointestinal tract-influence of physiological, pathophysiological and pharmaceutical factors. Front Pharmacol. 2020;11:524. doi: 10.3389/fphar.2020.00524, PMID 32425781.
4. Tutuian R, Katz PO, Castell DO. Nocturnal acid breakthrough: pH, drugs and bugs. Eur J Gastroenterol Hepatol. 2004;16(5):441-3. doi: 10.1097/00042737-200405000-00001, PMID 15097034.
5. Tutuian R, Castell DO. Nocturnal acid breakthrough-approach to management. Med Gen Med 2004;6(4):11. PMID 15775838.
6. Karyampudi A, Ghoshal UC, Singh R, Verma A, Misra A, Saraswat VA. Esophageal acidification during nocturnal acid-breakthrough with ilaprazole versus omeprazole in gastroesophageal reflux disease. J Neurogastroenterol Motil. 2017;23(2):208-17. doi: 10.5056/jnm16087, PMID 27585842.
7. Agarwal V, Bansal M. Statistical optimization and fabrication of a press coated pulsatile dosage form to treat nocturnal acid breakthrough. Curr Drug Deliv. 2013;10(4):444-52. doi: 10.2174/1567201811310040009, PMID 23253145.
8. Mogal RT, Galgatte UC, Chaudhari PD. Floating pulsatile drug delivery of ranitidine hydrochloride for nocturnal acid breakthrough: design, optimization, *in vitro* and *in vivo* evaluation. Int J Pharm Pharm Sci. 2013;5(3):722-7.
9. Ali J, Ahuja A, Baboota S, Qureshi J, Arora S. Pulsatile drug delivery systems: an approach for controlled drug delivery. Indian J Pharm Sci. 2006;68(3):295-300. doi: 10.4103/0250-474X.26655.
10. Ciancia S, Cafarelli A, Zahoranova A, Menciassi A, Ricotti L. Pulsatile drug delivery system triggered by acoustic radiation force. Front Bioeng Biotechnol. 2020;8:317. doi: 10.3389/fbioe.2020.00317, PMID 32411680.
11. Rashid R, Zaman M, Ahmad M, Khan MA, Butt MH, Salawi A. Press-coated aceclofenac tablets for pulsatile drug delivery: formulation and *in vitro* evaluations. Pharmaceuticals (Basel). 2022;15(3):326. doi: 10.3390/ph15030326, PMID 35337124.
12. Mahalakshmi P, Suriyaprakash TN, Prabu SL. Chronomodulated delivery of pantoprazole for nocturnal hyperacidity. Int J Pharm Sci Nanotechnol. 2015;8(4):3038-44.
13. Garg AK, Kumar A, Rani S, Singh M, Sharma A, Kumar R. Formulation and evaluation of chronotherapeutic pulsatile drug delivery system containing rabeprazole sodium. J Appl Pharm Sci. 2017;7(2):93-100.
14. Kharwade R, Nair H, Masurkar D, Pise A, More S, Pise S. Formulation and evaluation of chronomodulated pulsatile drug delivery system for nocturnal hyperacidity. Res J Pharm Technol. 2022;15(4):1449-54. doi: 10.52711/0974-360X.2022.00240.
15. Srikar G, Shanthi D, Ramesh J, Kalyani V, Nagamma I. Floating microspheres: a prevailing trend in the development of gastroretentive drug delivery system. Asian J Pharm. 2018;12(4):235-42.
16. Tekade AR, Gattani SG. Development and evaluation of pulsatile drug delivery system using novel polymer. Pharm Dev Technol. 2009;14(4):380-7. doi: 10.1080/10837450802712625, PMID 19235549.
17. Dhurke R. A review on chronomodulated drug delivery systems. Int J Recent Sci Res. 2020;11(8):39578-87.
18. Srikar G, Rani AP. Study on influence of polymer and surfactant on *in vitro* performance of biodegradable aqueous-core nanocapsules of tenofovir disoproxil fumarate by response surface methodology. Braz J Pharm Sci. 2019;55:e18736. doi: 10.1590/s2175-97902019000118736.
19. Srikar G, Rani AP. Tenofovir loaded poly (lactide-co-glycolide) nanocapsules: formulation optimization by desirability functions approach. Indian J Pharm Educ Res. 2020;54(2S):s230-40. doi: 10.5530/ijper.54.2s.79.
20. Datta A, Das A, Ghosh R. Eudragit® RL100 microspheres as a delayed-release system for ibuprofen: *in vitro* evaluation. Int J Pharm Pharm Sci. 2022;14(12):6-10. doi: 10.22159/ijpps.2022v14i12.45838.
21. Neelam S, Meenakshi B. Formulation and evaluation of polymeric microspheres using box–behnken design. Asian J Pharm Clin Res. 2022;15(10):47-55. doi: 10.22159/ajpcr.2022.v15i10.45250.
22. Allen T. Particle size analysis by image analysis. In: Allen T, editor. Powder sampling and particle size determination. 1st ed. Vol. 3. Elsevier; 2003. p. 142-207.

23. Tirpude RN, Puranik PK. Rabeprazole sodium delayed-release multiparticulates: effect of enteric coating layers on product performance. J Adv Pharm Technol Res. 2011 Jul;2(3):184-91. doi: 10.4103/2231-4040.85539, PMID 22171316.
24. Srikar G, Rani AP. Aqueous-core nanocapsules of lamivudine: optimization by design of experiments. Asian J Pharm. 2019;13(4):318-27.
25. Hasan S, Kumar N, Bakan JA. Microencapsulation. In: Khar RK, Vyas SP, Ahmad FJ, Jain GK, editors. The theory and practice of industrial pharmacy. 4th ed New Delhi: CBS Publishers and Distributors; 2013. p. 579-96.
26. Bodratti AM, Alexandridis P. Formulation of poloxamers for drug delivery. J Funct Biomater. 2018;9(1):11. doi: 10.3390/jfb9010011, PMID 29346330.
27. Pavelkova Mhjmkkvdspm, Vyslouzil Jmdvj. Influence of emulsification mode, stirring speed and volume on ibuprofen-loaded PLGA microparticles. Ceska Slov Farm. 2021;70(1):32-40. PMID 34237951.
28. Dhas SK, Deshmukh G. Formulation and evaluation of meloxicam microspheres for colon targeted drug delivery. Asian J Pharm Clin Res. 2021;14(8):45-51. doi: 10.22159/ajpcr.2021.v14i8.38482.
29. Nguyen TTU, Hamid ZAA, Nurazreena A. Effect of stirring speed on the formation and characteristic of alginate microspheres via gelation/ emulsification method. Key Eng Mater. 2022;908:171-6. doi: 10.4028/p-4c2w46.
30. Rowe RC, Sheskey PJ, Owen SC, editors. Handbook of pharmaceutical excipients. London: Pharmaceutical Press and American Pharmacists Association; 2006.
31. Gunasheela S, Chandrakala V, Srinivasan S. Development and evaluation of microsponge gel of an antifungal drug. Int J Curr Pharm Sci. 2023;15(1):30-41. doi: 10.22159/ijcpr.2023v15i1.2069.
32. Nikhil B, Allimalarkodi S. Formulation and evaluation of ciprofloxacin microspheres designed by using natural polymers by ionic gelation technique. Int J Pharm Pharm Sci. 2024;16(1):8-13. doi: 10.22159/ijpps.2024v16i1.49336.
33. Ghori MU, Grover LM, Asare Addo K, Smith AM, Conway BR. Evaluating the swelling, erosion, and compaction properties of cellulose ethers. Pharm Dev Technol. 2018;23(2):183-97. doi: 10.1080/10837450.2017.1389958, PMID 28985687.
34. Okunlola A, Odeniyi MA, Arhewoh MI. Microsphere formulations of ambroxol hydrochloride: influence of Okra (Abelmoschus esculentus) mucilage as a sustained release polymer. Prog Biomater. 2020;9(1-2):65-80. doi: 10.1007/s40204-020-00132-5, PMID 32504415.
35. Cui C, Sun J, Wang X, Yu Z, Shi Y. Factors contributing to drug release from enteric-coated omeprazole capsules: an *in vitro* and *in vivo* pharmacokinetic study and IVIVC evaluation in beagle dogs. Dose-Response. 2020;18(1):1559325820908980. doi: 10.1177/1559325820908980, PMID 32180688.