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Original Article

Pulsatile Drug Delivery Systems of Esomeprazole: Optimization through Quality by Design

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ABSTRACT

Background: The current research work was aimed to optimize and develop Pulsatile Drug Delivery Systems (PDDS) of esomeprazole so as to control the nocturnal acid breakthrough in ulcer patients. **Materials and Methods:** Microparticles separately for Delayed Immediate Release (DIR) and Delayed Extended Release (DER) were developed. DER microparticles were developed as matrix microspheres by optimizing different formulation and process parameters followed by enteric coating of the optimized formulation. Stirring speed, amount of Eudragit RSPO, type and amount of hydrophilic polymer were taken as the independent factors. Particles size and drug release at various characteristic time points were taken as the response variables. Central composite design was employed to elucidate the effect of the factors on the responses followed by optimization. **Results:** Except stirring speed on the drug release, all the other factors were observed to have significant effect ($p<0.05$) on all the responses. The SEM images described the mechanism responsible for delayed extended release of the esomeprazole. The results of graphical optimization indicated that the microspheres prepared with Eudragit RSPO at 0.67 g and polyethylene oxide at 0.33 g for 1 g of esomeprazole at 550 rpm as the optimized formulation. This formulation upon terminal enteric coating exhibited delayed release for an extended period of 6 hr, later the drug was released within 2 hr. **Conclusion:** Equal doses of simple enteric coated drug particles as DIR microcapsules along with the optimized DER microspheres could release esomeprazole effectively as two different pulses at the desired time intervals upon oral administration.

Keywords: Pulsatile drug delivery systems, Esomeprazole, Nocturnal acid breakthrough, Central composite design, Optimization.

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INTRODUCTION

Proton Pump Inhibitors (PPIs) are the first-line drugs for the treatment of gastric and duodenal ulcers.[1] These drugs are commonly available in the market as delayed release formulations.[2] Because of their instability in the gastric acidic fluids, these drugs are made into delayed release products by enteric coating over core tablets or capsules containing the PPIs.[3] But, the simple enteric coated products delay the drug release until the tablet reaches the small intestine only i.e. up to around 1-2 hr after administration. These formulations are generally administered twice daily. Nocturnal Acid Breakthrough (NAB) is a common characteristic symptom that occurs in around 70% of the patients suffered from *Helicobacter pylori*-negative ulcers.[4-6] The NAB results in gastric pH<4 for minimum of 1 hr continuously during post-midnight and early hours of morning. This causes severe discomfort to the patients during the sleep time. This chronophysiology necessitates the need of special drug delivery systems which can maintain effective plasma drug concentrations during the period of possible NAB.[7,8]

Pulsatile drug delivery systems are the formulations which release each dose of the contained drug as a pulse with a predetermined gap between the doses to match the circadian rhythm of the particular disease condition.[9,10] Many physiological conditions like NAB are chronological and their effective management requires effective plasma levels of drugs in synchronization with the symptoms. The applicability of PDDS for obtaining chronomodulated drug delivery is well supported by considerable extent of research. Rashid R *et al.*[11] reported compression-coated aceclofenac tablets were developed for chronomodulated drug delivery for the treatment of arthritis condition. Predetermined lag time of 5-6 hr was achieved using the combined matrix of Eudragit and HPMC. Mahalakshmi P *et al.*[12] also developed coated tablets by compression coating with Eudragit RSPO for achieving desired lag time before the release of patoprazole. Garg

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AK *et al.*[13] and Kharwade R *et al.*[14] reported combined HPMC, EC and xanthum gum matrix provided desired lag-time and timely release of the contained drugs from the tablets made by compression coating. This literature suggests that desired lag-time before the drug release can be effectively achieved using a combined matrix of water swellable and water insoluble polymer. But, single unit dosage forms have a drawback of possible failure of the dosage form due to rupture of the coating.[15] On the other hand; multi-particulate systems avoid this potential drawback and provide desired chronomodulated delivery effectively. Tekade AR *et al.*[16] developed microspheres using a novel swellable polymer for the pulsatile release of theophylline in the colon for the treatment of asthma. Dhurke R and Hua S[3] describe possible applications of microparticles for pulsatile drug delivery.

Considering the extensive literature survey, there is still a large scope to explore possible technologies for the development of pulsatile microparticulate systems to achieve chronomodulated delivery[17] for the suitable drugs like PPIs. Currently available Proton Pump Inhibitor (PPI) formulations (both Immediate release and Delayed release) given twice daily cannot control secretion of gastric acids adequately overnight. Hence, in this work esomeprazole was taken as the PPI and for this PDDS was aimed to be developed. The planned PDDS contain two doses of the PPI which were made into Delayed Immediate Release (DIR) portion and Delayed Extended Release (DER) portion. The planned hypothesis is that when this PDDS capsule is administered just before the night meal at around 8-9 PM, first dose has to be released after emptying of the capsule contents into small intestine from the DIR portion. Then after a lag of total 6 hr, second dose of the drug has to be released from the DER portion at around 3-4 am when the nocturnal acid breakthrough generally occurs.[4-6] One dose equivalent drug powder was prepared directly as enteric coated microcapsules (DIR portion) by emulsion solvent evaporation technique. These microcapsules were assumed to release the drug immediately once they were emptied from the stomach after administration. Another dose of the drug was first made into matrix microspheres using a combination of a water insoluble polymer and a water swellable/soluble polymer. These microspheres were finally subjected to enteric coating (DER portion). These microspheres were assumed to prevent the drug release for a predetermined extended period of time after they were emptied into small intestine. After a predetermined lag time (the time for sufficient dissolution of the matrix to allow the drug release), these microspheres were aimed to release the drug within a span of 2 hr. Both these portions of the drug were filled into a hard gelatin capsule such that the capsule produces two pulses of drug release.

Development of the matrix microspheres for the DER portion is the critical aspect in achieving the objective of the present research work. Hence, Quality by Design (QbD) approach[18,19] was implemented using Stat Ease Design Expert software to optimize DER portion microparticles. Type of swellable polymer, amount of swellable polymer, amount of insoluble polymer and stirring speed were taken as the independent variables (factors); particle size and drug released at various time intervals were taken as dependent variables (responses). Central Composite Design (CCD) was employed as the experimental design to investigate the effects of the factors on the responses. Later, numerical optimization was performed to identify the design space and optimized formulation of the DER microparticles.

MATERIALS AND METHODS

Materials

Esomeprazole 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

The matrix microspheres for developing DER portion microspheres of esomeprazole were planned to develop using Quality by Design (QbD) approach.

Quality Target Product Profile (QTPP)

To delay the drug release by 4 hr in the intestinal conditions and then to complete the drug release in 1-2 hr.

Critical Quality Attributes (CQAs)

Particle size; and percent drug release at 4, 5 and 6 hr in the intestinal conditions as D4%, D5%, and D6% respectively.

Critical Process/Formulation Parameters (CPPs)

Upon thorough literature study and experimental trials, the following factors were selected as critical formulation factors, Factor A: Amount of Eudragit RSPO (0.33-0.67 g); Factor B: Amount of Hydrophilic polymer (0.33-0.67 g); Factor C: Stirring speed (400-550); and Factor D: Type of hydrophilic polymer (PEO N60K/HPC MF).

Based on the nature and levels of factors, the best suitable CCD was selected. The combination of the factors and their levels according to the selected design are shown in Table 1.

Preparation of matrix microspheres

Emulsion solvent evaporation method was adopted for the development of the microspheres.[20] Desired amounts of the hydrophilic and hydrophobic polymers as specified in the Table 1 were dissolved in 15 mL of 1:1 mixture of methanol and dichloromethane. After obtaining clear solution, 1 g of

esomeprazole was dissolved in the above polymer solution using cylcomixer (Remi CM 101). Separately 80 mL of liquid paraffin was taken in a 250 mL beaker which was kept on a hot plate (Metalab) with set temperature of 45°C. Into this beaker, paddle type mechanical stirrer blade (Remi RQ-5 Plus) was dipped. The stirrer was set at a speed of 550 rpm. Then the drug-polymer dispersion was added slowly as drops into the liquid paraffin under constant stirring. This produced fine droplets of volatile solvent containing drug-polymers monolith in the liquid paraffin as an emulsion. The stirring was continued for 4-5 hr until the volatile solvent was completely evaporated and the formed droplets were rigidized as matrix microspheres. Then the dispersion was subjected to filtration to separate microspheres followed by washing them with petroleum ether to eliminate any adhered paraffin. Finally, the microspheres were washed with distilled water and subjected to drying immediately. The dried free-flowing microspheres were collected and stored appropriately for further use.

Characterization studies on the matrix microspheres

Percentage yield

The microspheres after drying were weighed precisely. Then the yield was computed as the ratio of the weight of the obtained microspheres to the combined weight of the drug and the polymer taken multiplied by 100.

Table 1: Combinations of the selected factors with the levels according to the CCD.

Formulation code	Std. order	Run order	Factor A	Factor B	Factor C	Factor D
MSF1	13	26	0.50	0.50	348.87	PEO N60K
MSF2	1	29	0.33	0.33	400.00	PEO N60K
MSF3	2	2	0.67	0.33	400.00	PEO N60K
MSF4	3	19	0.33	0.67	400.00	PEO N60K
MSF5	4	12	0.67	0.67	400.00	PEO N60K
MSF6	11	11	0.50	0.21	475.00	PEO N60K
MSF7	9	5	0.21	0.50	475.00	PEO N60K
MSF8	15	17	0.50	0.50	475.00	PEO N60K
MSF9	10	25	0.79	0.50	475.00	PEO N60K
MSF10	12	23	0.50	0.79	475.00	PEO N60K
MSF11	5	7	0.33	0.33	550.00	PEO N60K
MSF12	6	27	0.67	0.33	550.00	PEO N60K
MSF13	7	21	0.33	0.67	550.00	PEO N60K
MSF14	8	16	0.67	0.67	550.00	PEO N60K
MSF15	14	6	0.50	0.50	601.13	PEO N60K
MSF16	28	14	0.50	0.50	348.87	HPC MF
MSF17	16	15	0.33	0.33	400.00	HPC MF
MSF18	17	24	0.67	0.33	400.00	HPC MF
MSF19	18	3	0.33	0.67	400.00	HPC MF
MSF20	19	8	0.67	0.67	400.00	HPC MF
MSF21	26	4	0.50	0.21	475.00	HPC MF
MSF22	24	18	0.21	0.50	475.00	HPC MF
MSF23	30	28	0.50	0.50	475.00	HPC MF
MSF24	25	1	0.79	0.50	475.00	HPC MF
MSF25	27	30	0.50	0.79	475.00	HPC MF
MSF26	20	10	0.33	0.33	550.00	HPC MF
MSF27	21	20	0.67	0.33	550.00	HPC MF
MSF28	22	22	0.33	0.67	550.00	HPC MF
MSF29	23	9	0.67	0.67	550.00	HPC MF
MSF30	29	13	0.50	0.50	601.13	HPC MF

Entrapment Efficiency (EE)

100 mg drug equivalent weight of the microspheres were taken, grinded and added to water and then subjected to stirring on a rotary shaker. At regular intervals samples were withdrawn and analyzed for absorbance spectrophotometrically (Thermo Scientific Evolution One) until constant absorbance was obtained. The final absorbance was used to estimate the amount of drug present in the taken microspheres. Then the below formula was used to get the EE.

$$\text{Entrapment efficiency} = \frac{Estimated\ drug\ content}{Theoretical\ drug\ content} \times 100$$

Particle size

Microscopy technique[21,22] was employed to measure the size of the microspheres. Small amount of microspheres was spread on a glass slide and focused under an optical microscope. Using a pre-calibrated eye piece micrometer, Feret's diameter was measured for 200 particles. Arithmetic mean diameter was calculated using the obtained data.

Drug release studies

These were executed in USP type 2 apparatus (Lab India Disso 8000) maintained at 100 rpm. 1000 mL of phosphate buffer pH 6.8 was used as the medium.[23,24] 20 mg drug equivalent microspheres were added into the vessel and the test was initiated. At regular time intervals, samples of 5 mL were withdrawn and transferred into stoppered test tubes containing 1 mL of 0.25 M NaOH solution and were kept in a dark place until further analysis. The analysis for quantification of esomeprazole was done spectrophotometrically at its maximum wavelength of 302 nm.

Design validation and Optimization

Design of Experiments (DoE) analysis was performed using Design Expert software.[25] Sequential model sum of squares was performed for every response to elucidate the regression model of influence of the factors on every response. Then ANOVA was done to identify whether the selected model and the model parameters were significant or not so that the whether the adopted design and the model were checked for its suitability to proceed for optimization. Graphical optimization was done by desirability functions approach. The goal of optimization was set to achieve the drug release in a desired period of time i.e. the drug release should be minimum for first 4 hr and then the release should be completed within the next 2 hr.

Scanning Electron Microscopy (SEM)

Morphological characterization of microspheres was studied using SEM (ZEOL JSM-5610) according to the procedure reported by Srikar G *et al.*[18] The SEM images were also taken for the remaining microspheres after their drug release studies and compared with previous images so as to elucidate the possible mode of drug release.

Enteric coating of the optimized matrix microspheres and their characterization

This step was to convert the optimized matrix microspheres into DER portion microspheres. In order to avoid the drug release in the gastric region, the optimized microspheres were subjected to enteric coating. In this step, Eudragit S 100 was used as the enteric film forming material. Three different coating solution formulations as shown in Table 2 containing variable amounts of the film former and at variable viscosities were developed and verified for the best result. 100 g of the optimized microspheres were taken in the coating pan which was made to revolve at 50 rpm. Drying hot air, adjusted at 40°C temperature was allowed into the coating pan. The coating solution was sprayed onto the solid bed at a pre-optimized rate of 5 mL/min. After drying, the coated microspheres were taken and checked visually for any sticking.

The obtained enteric coated matrix microspheres (DER microspheres) were subjected to yield, drug content and particle size determination as per the above mentioned procedures. Dissolution studies were performed using paddle apparatus rotated at 100 rpm in acid and buffer stages.[23,24] 300 mL of 0.1 N HCl was taken as the medium for the acid stage (2 hr) and then, 1000 mL of phosphate buffer pH 6.8 was taken as the medium for the buffer stage.

Preparation of DIR Portion Microcapsules and their Characterization

This portion of the microcapsules has to avoid the drug in the gastric region and allow its release immediately once the microcapsules reach small intestine. Emulsion solvent evaporation method with core material dispersed in the solution of polymer[26] was used to coat the plain esomeprazole by the enteric polymer Eudragit S100. Desired quantity of Eudragit S100 (as shown in the Table 3) was dissolved in ethanol in which the drug was dispersed. Liquid paraffin containing 0.2% v/v of span 20 was taken in a beaker and kept under stirring at 550 rpm (Remi RQ-5 Plus) and maintained at a 45°C temperature. Into this beaker under these conditions, the dispersion of Esomeprazole in the polymer solution was transferred drop-wise. Then stirring was

Table 2: Composition of enteric coating solution formulations.

Sl. 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.	50mL	50mL	40mL

continued until the solvent was evaporated (took approximately 3.5 hr). The resulted microcapsules were collected from the liquid paraffin by filtration, followed by washing with petroleum ether to remove any adhered paraffin. Finally, the microcapsules were washed with water twice and kept in hot air oven for drying. The dried enteric coated microcapsules of esomeprazole were stored for further studies.

These microcapsules were also studied for their yield, EE and particle size using the same procedures as mentioned for the matrix microspheres. Dissolution studies were performed in the acid and buffer stages similar to enteric coated DER portion microspheres.

RESULTS

Matrix Microspheres

Yield and Entrapment efficiency

All the formulations of the microspheres were obtained with good yield ranging from 87.3-94.2% as shown in the Table 4. All the formulations of the microspheres exhibited the Entrapment Efficiency (EE) values in the range of 67.4-89.3% and are presented in the Table 4.

DoE analysis of the responses

Particle size

The results of particle size analysis are shown in the Table 4. The model type of the effect of the factors on this response was investigated by sequential sum of squares using the Design Expert software. The factors were found to influence the particle size linearly and the effect was shown in Figure 1. The model equation was obtained as

$$\text{Particle Size}=240.01+10.88*A+23.39*B-12.53*C+23.02*D$$

The influences of all the four factors were significant at $p<0.05$ by ANOVA (presented in the Table 5 and in Figure 1).

Drug release studies

The results of drug release in phosphate buffer pH 6.8 after 4, 5 and 6 hr are given in the Table 4. The percent drug release after 4 hr (D4%), after 5 hr (D5%) and after 6 hr (D6%) was considered as the other response so as to represent the QTPP. Effects of all the four factors on these three responses were analyzed by sequential sum of squares to understand the regression model between the factors and every response. The factors were found to have linear influence on the D4% and D5% whereas it was quadratic effect on the D6% as shown in Figure 2 (a)-(f). The model equations were obtained as

$$D4=+10.08-2.70*A-1.11*B-0.084*C-1.49*D$$

$$D5=+49.06-7.12*A-4.20*B+0.97*C-3.66*D$$

$$D6=+91.91-9.66*A-7.00*B+0.98*C-3.97*D-3.50*AB+0.18*AC-1.91*AD+0.050*BC-0.22*BD+0.32*CD-3.65*A2-3.34*B2+1.97*C2$$

On all these three responses, the influences of the factors A, B and D only were significant at $p<0.05$ by ANOVA which are shown in Table 5 for D4% and D5%, and in Table 6 for D6%; and are further illustrated in Figure 2.

Design validation and Optimization

The sequential sum of squares analysis performed using Design Expert showed that the factors had linear effect on the First Three Responses (R1, R2 and R3) and, quadratic effect on the Fourth Response (R4). All the responses were analyzed with the respective models. The results of ANOVA test are shown in Tables 5 and 6. The normal plots of the residuals of all the responses are illustrated in Figure 3. Graphical optimization was performed by setting desirability criteria or constraints for the responses so as to achieve the defined QTPP. Hence, D4 was set to minimum with a maximum limit of 10%; D5 was set be in the range of 45-55%; and D6 was set to be in the range of 90-99%. Particle size was set to minimum with a maximum limit of 250 µm. under these constraints, the graphical optimization was performed and the obtained overlay plot was shown in the Figure 4. One best optimized combination suggested by the software and the predicted values of the responses by the software are given in Table 7. Matrix microspheres with the suggested optimized combination was prepared and evaluated for the response values. The obtained responses are shown in Table 7.

Table 3: Formulation composition of enteric coating microcapsules of esomeprazole.

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

Table 4: Results* of various characterization studies including the responses of the matrix microspheres.

Formulation code	Yield (%)	EE (%)	Particle size (μm) (R1)	D4 (%) (R2)	D5 (%) (R3)	D6 (%) (R4)
MSF1	91.5±2.3	83.3±1.9	246.2±12.7	13.2±1.4	55.4±3.3	96.5±2.1
MSF2	93.6±1.9	73.6±0.7	187.9±10.5	14.3±0.8	58.9±1.7	100±4.6
MSF3	89.7±3.2	75.1±3.1	218.5±17.3	8.7±1.1	45.1±2.2	95.6±3.4
MSF4	94.2±1.4	85.9±2.4	234.6±14.8	12.4±0.6	53.7±3.1	97.8±2.9
MSF5	88.7±2.7	87.6±1.5	270.5±21.4	5.9±0.9	38.6±2.6	80.2±4.3
MSF6	91.2±4.3	67.4±5.1	173.7±9.6	18.4±1.2	66.7±4.7	100±2.8
MSF7	90.8±5.7	70.3±3.4	211.4±20.5	15.1±0.4	68.3±5.6	100±1.9
MSF8	92.5±2.9	71.7±2.2	206.9±15.6	16.5±0.7	58.4±2.9	98.1±2.7
MSF9	88.3±6.7	73.9±1.5	224.8±18.9	4.6±0.9	34.8±1.5	61.3±4.2
MSF10	91.1±3.8	77.2±2.9	253.7±23.1	5.2±1.3	40.5±3.2	62.7±2.3
MSF11	94.6±2.5	70.5±4.2	166.3±12.4	15.1±0.8	60.2±5.1	100±1.4
MSF12	90.7±4.1	71.6±3.1	203.5±18.2	7.9±0.5	47.5±3.7	95.4±3.5
MSF13	87.4±3.6	76.2±2.7	219.1±23.5	13.7±1.2	58.1±4.8	98.6±2.1
MSF14	91.5±5.3	77.1±1.5	243.2±21.7	7.2±1.1	43.9±2.6	84.5±5.2
MSF15	90.4±4.4	72.9±0.9	194.6±16.4	15.4±0.6	60.7±5.4	98.9±2.4
MSF16	93.7±2.7	85.1±1.4	291.3±13.7	10.4±0.8	51.6±3.9	89.7±4.8
MSF17	91.9±1.8	76.9±2.6	226.5±18.2	12.1±1.3	53.7±2.5	93.4±1.7
MSF18	89.7±5.6	79.3±2.2	263.7±22.6	5.6±0.4	40.3±1.6	80.1±5.3
MSF19	94.2±1.5	84.3±1.8	272.9±26.3	12.3±2.1	45.9±3.2	92.8±1.6
MSF20	90.5±0.9	88.7±3.5	312.4±24.5	8.2±1.4	34.7±4.5	62.9±6.4
MSF21	91.8±3.1	74.5±2.9	238.6±8.8	9.5±0.5	51.6±2.4	95.5±2.9
MSF22	87.3±2.8	80.6±2.1	255.3±12.6	10.9±0.6	54.8±1.4	98.2±3.3
MSF23	89.4±1.4	81.4±1.7	261.7±28.3	8.3±1.4	49.1±3.6	87.6±4.5
MSF24	92.9±1.5	83.2±1.2	269.1±24.3	3.7±1.2	35.4±2.9	55.9±4.2
MSF25	90.8±2.6	89.3±2.3	320.8±16.9	6.1±0.5	33.5±4.8	60.7±5.8
MSF26	93.3±1.9	74.2±3.1	214.8±19.2	8.9±0.9	52.9±1.7	97.3±2.6
MSF27	92.7±4.1	72.5±0.9	247.5±20.6	7.2±1.6	41.3±2.6	83.8±1.9
MSF28	94.1±2.2	82.1±1.4	263.9±24.7	11.8±0.4	49.2±5.1	94.5±3.4
MSF29	92.6±1.3	83.9±2.5	278.1±19.3	5.9±1.2	35.6±4.3	64.3±5.5
MSF30	91.5±4.4	76.2±2.8	228.9±14.8	7.9±1.5	51.4±2.9	93.9±1.7

* Note: All the results were expressed as Average±Standard deviation for n=3.

SEM analysis

The matrix microspheres were studied for surface morphology before, during and after the drug release study and the images are presented in Figure 5. Before subjecting to the drug release, the microspheres surface is shown in the Figure 5(a). The microspheres surface after 4 hr of the drug release study is shown in the Figure 5(b). Again at the end of 6 hr of the drug release study, the obtained SEM image is shown in the Figure 5(c).

Characterization of the enteric coated optimized DER microspheres

Microspheres in the previous section were optimized so as to obtain a delay of 4 hr in the intestinal medium. As the microspheres should come across the gastric environment first upon administration, enteric coating is necessary to avoid release of the drug in the gastric conditions. Eudragit S 100 based three different coating solution formulations (EECF1-EECF3)

were studied to find the best composition. The results of the characterization studies after enteric coating of the optimized DER microspheres were shown in Table 8 and the drug release profiles are shown in Figure 6(a).

Characterization of the DIR Microcapsules

Esomeprazole is sparingly soluble in ethanol and hence it just dispersed in the ethanolic solution of the Eudragit S100. The emulsion solvent evaporation method in this case produced microcapsules as the polymer solution deposited on the drug particles during emulsification. Further evaporation of ethanol and rigidization of the Eudragit S100 over the esomeprazole particles yielded microcapsules. Poloxamer 188 was used as surfactant that could enhance dissolution of the drug once the enteric polymer was dissolved upon reaching small intestine after their oral administration and hence these were termed as delayed Immediate Release (DIR) microcapsules.[27] Four different formulations of the DIR microcapsules (IECF1-IECF4) were prepared and studied for various characterization studies and the results were shown in Table 9. The dissolution profiles of these DIR microcapsules are shown in Figure 6(b).

Drug release studies on the combined DIR microcapsules and DER microspheres

Finally, one dose (20 mg) equivalent DIR microcapsules and other 20 mg equivalent DER microspheres were combinely filled into a hard gelatin capsule to make the final pulsatile drug delivery system. This was subjected to drug release study in both acid and

Table 5: Results of ANOVA for response surface linear model for the responses R1, R2 and R3.

Source	SS[a]	Df[b]	MSS[c]	F value	*p*-value	Inference[d]
R1: Particle size						
Model	38359.94	4	9589.99	98.49	<0.0001	Significant
A- Amount of Eudragit RSPO	3232.63	1	3232.63	33.20	<0.0001	Significant
B- Amount of Hydrophilic Polymer	14939.33	1	14939.33	153.43	<0.0001	Significant
C-Stirring speed	4290.37	1	4290.37	44.06	<0.0001	Significant
D-Type of Hydrophilic polymer	15897.61	1	15897.61	163.27	<0.0001	Significant
Residual	2434.21	25	97.37			
Cor Total	40794.15	29				
R2: D4%						
Model	299.98	4	74.99	12.52	<0.0001	Significant
A- Amount of Eudragit RSPO	199.23	1	199.23	33.26	<0.0001	Significant
B- Amount of Hydrophilic Polymer	33.65	1	33.65	5.62	0.0258	Significant
C-Stirring speed	0.19	1	0.19	0.032	0.8585	Insignificant
D-Type of Hydrophilic polymer	66.90	1	66.90	11.17	0.0026	Significant
Residual	149.77	25	5.99			
Cor Total	449.75	29				
R3: D5%						
Model	2295.02	4	573.75	31.53	<0.0001	Significant
A- Amount of Eudragit RSPO	1385.98	1	1385.98	76.18	<0.0001	Significant
B- Amount of Hydrophilic Polymer	481.69	1	481.69	26.48	<0.0001	Significant
C-Stirring speed	25.47	1	25.47	1.40	0.2478	Insignificant
D-Type of Hydrophilic polymer	401.87	1	401.87	22.09	<0.0001	Significant
Residual	454.86	25	18.19			
Cor Total	2749.87	29				

Note: a-Sum of Squares; b-Degrees of Freedom; c-Mean Sum of Squares;d-p-value less than 0.05 indicates model terms are significant.

Table 6: Results of ANOVA test for the response surface quadratic model for D6% (R4).

Source	SS[a]	D_f[b]	MSS[c]	F value	*p*-Value	Inference[d]
Model	5370.78	13	413.14	10.03	< 0.0001	Significant
A- Amount of Eudragit RSPO	2548.31	1	2548.31	61.89	< 0.0001	Significant
B- Amount of Hydrophilic Polymer	1339.23	1	1339.23	32.53	< 0.0001	Significant
C-Stirring speed	26.10	1	26.10	0.63	0.4376	
D-Type of Hydrophilic polymer	472.03	1	472.03	11.46	0.0038	Significant
AB	196.00	1	196.00	4.76	0.0444	Significant
AC	0.49	1	0.49	0.012	0.9145	
AD	99.97	1	99.97	2.43	0.1387	
BC	0.040	1	0.040	9.715E-004	0.9755	
BD	1.32	1	1.32	0.032	0.8603	
CD	2.85	1	2.85	0.069	0.7957	
A^2	161.33	1	161.33	3.92	0.0652	
B^2	135.14	1	135.14	3.28	0.0888	
C^2	47.03	1	47.03	1.14	0.3010	
Residual	658.75	16	41.17			
Cor Total	6029.53	29				

Note: a-Sum of Squares; b-Degrees of Freedom; c-Mean Sum of Squares; d-p-value less than 0.05 indicates model terms are significant.

Table 7: Comparison of the predicted and observed values of the responses for the optimized inclusion complex formulation.

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: Eudragit RSPO-0.66 g	R1: Particle Size (µm)	191.56	183.09	200.03	197.5
B: Hydrophilic Polymer-0.33 g	R2: D4 (%)	9.99999	7.90	12.10	8.36
C: Stirring speed-550	R3: D5 (%)	51.0291	47.37	54.69	49.72
D: Type of Hydrophilic polymer-PEO N60K	R4: D6 (%)	94.5977	85.36	103.83	97.64

buffer stages as per the previously mentioned procedures. The obtained dug release profile is shown in the Figure 6(c).

DISCUSSION

Matrix Microspheres

Yield and Entrapment efficiency

The high yield values designate the suitability of the selected processing conditions to the composition of the materials taken. Hence, the selected method and conditions were proved to be better suitable to the microspheres development.

The Entrapment Efficiency (EE) results specified that an increase in the amounts of either hydrophilic or hydrophobic polymers, the EE was found to be increased. This could be due to the increased adhesion/entrapment of the drug at higher polymer amounts.[18,19] But, an increase in the stirring speed resulted in decreased entrapment efficiency. High speed mixing conditions could allow rapid drug diffusion out of the polymer matrix before rigidization of the microspheres. Hence, upon complete removal of the volatile solvent the rigidized polymer matrix thus forming microspheres would contain less amount of the drug. The obtained EE values were in correlation with those reported by Pavelkova MHJMKKVDSPM *et al.*[28] Type of hydrophilic polymer also influenced the EE. The microspheres prepared with HPC MF exhibited high EE values than their corresponding formulations prepared with PEO N60K. This could be owing to the higher viscosity of HPC MF (4000-65000 cps at 2% w/v in water) than that of the PEO N60K (2000-4000 cps at 2% w/v in water). Higher viscosity of the polymer matrix before its rigidization would

Table 8: Characteristics of the enteric coated optimized matrix DER microspheres of Esomeprazole.

Sl. No	Characteristic property	Result*/Observation		
		EECF1	EECF2	EECF3
1	Physical observation	Free flowing microspheres	Free flowing microspheres	Moderate extent of sticking
2	Weight (g)	105.6	108.3	107.4
	Drug content	97.1±2.5	96.4±1.7	95.6±2.1
3	Particle size (μm)	218.2±10.7	226.9±14.5	233.6±22.1
4	% Drug released in acid stage after 2 hr (AD2%).	7.3±1.2	4.2±0.7	--
5	% Drug released in buffer stage after 4 hr (D4%).	12.1±0.9	8.9±1.0	--
6	% Drug released in buffer stage after 5 hr (D5%).	52.3±2.1	51.1±3.1	--
7	% Drug released in buffer stage after 6 hr (D6%).	95.6±1.7	97.9±2.8	--

* Note: The results were expressed as the Average±Standard deviation for n=3.

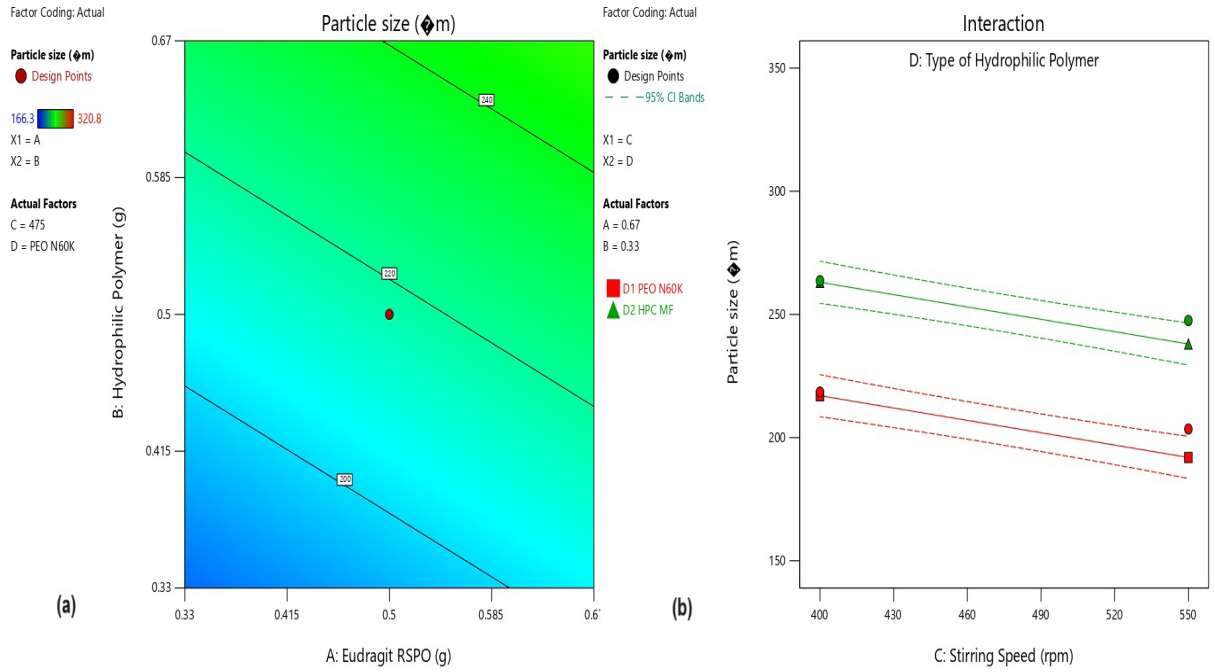


Figure 1: The effects of the factors on the Response 1, particle size (a) Contour plot showing the effect of the factors A and B; and (b) Interaction plot showing the effect of the factors C and D.

decrease the diffusion of the drug out of its matrix and hence the EE could be higher.[18,19]

DoE analysis of the responses

Particle size

Both the factors A and B were observed to have positive influence i.e. upon increase in amounts of any of the polymers, the size was also increased. This could be due to the increased viscosity of the polymer solution that could resist breakdown of the globules during emulsification. This would result in increased particle size upon solvent evaporation and this hypothesis was supported by the research results reported by Pavelkova MHJMKKVDSPM *et al.*[28] and Dashora K *et al.*[29] The factor C was observed to have negative influence on the particle size i.e. the size was decreased upon increasing the stirring rate. The high speed stirring produced more energy during emulsification which caused more size reduction of the dispersed phase globules which ultimately solidify into smaller microspheres upon solvent evaporation.[29,30] The factor D had positive effect i.e. the size was increased upon changing the polymer from PEO to HPC. This might be because of the viscosities of those polymers. The viscosities of the 2%

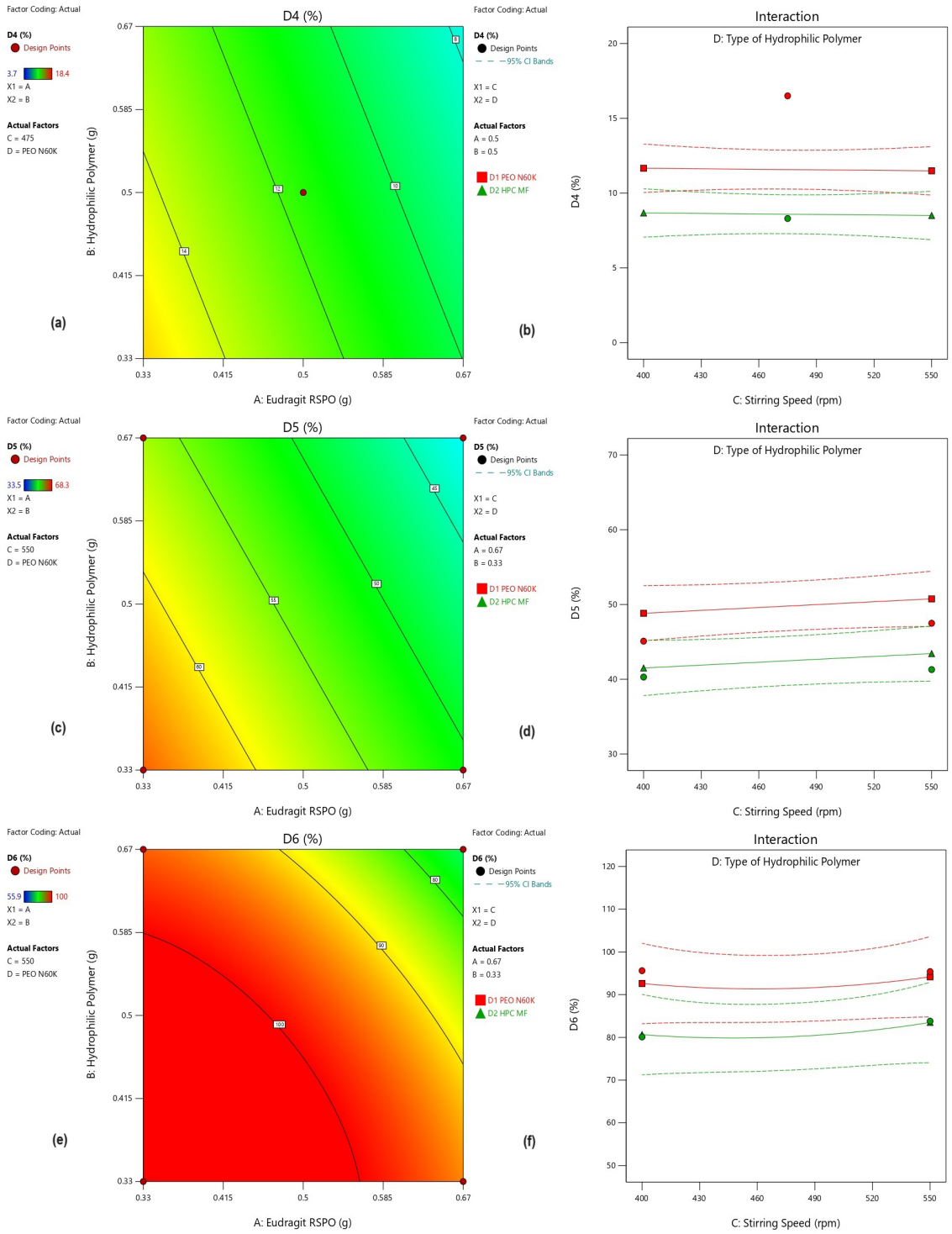


Figure 2: The effects of the factors on the Responses (a) Contour plot showing the effect of the factors A and B on D4%; (b) Interaction plot showing the effect of the factors C and D on D4%; (c) Contour plot showing the effect of the factors A and B on D5%; (d) Interaction plot showing the effect of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

w/v aqueous solutions of the PEO N60K and the HPC MF are 2000-4000 cps and 4000-6500 cps respectively.[31] High viscosity of the formulations containing HPC MF might resist breakdown of the globule size at same stirring speed and hence could result in bigger particle size.

Drug release studies

Both the factors A and B were found to have negative effect on the amount of drug release at all these time points i.e. upon increase in amounts of any of the polymers, the drug release got declined. This could be owing to the strong binding of the drug to the

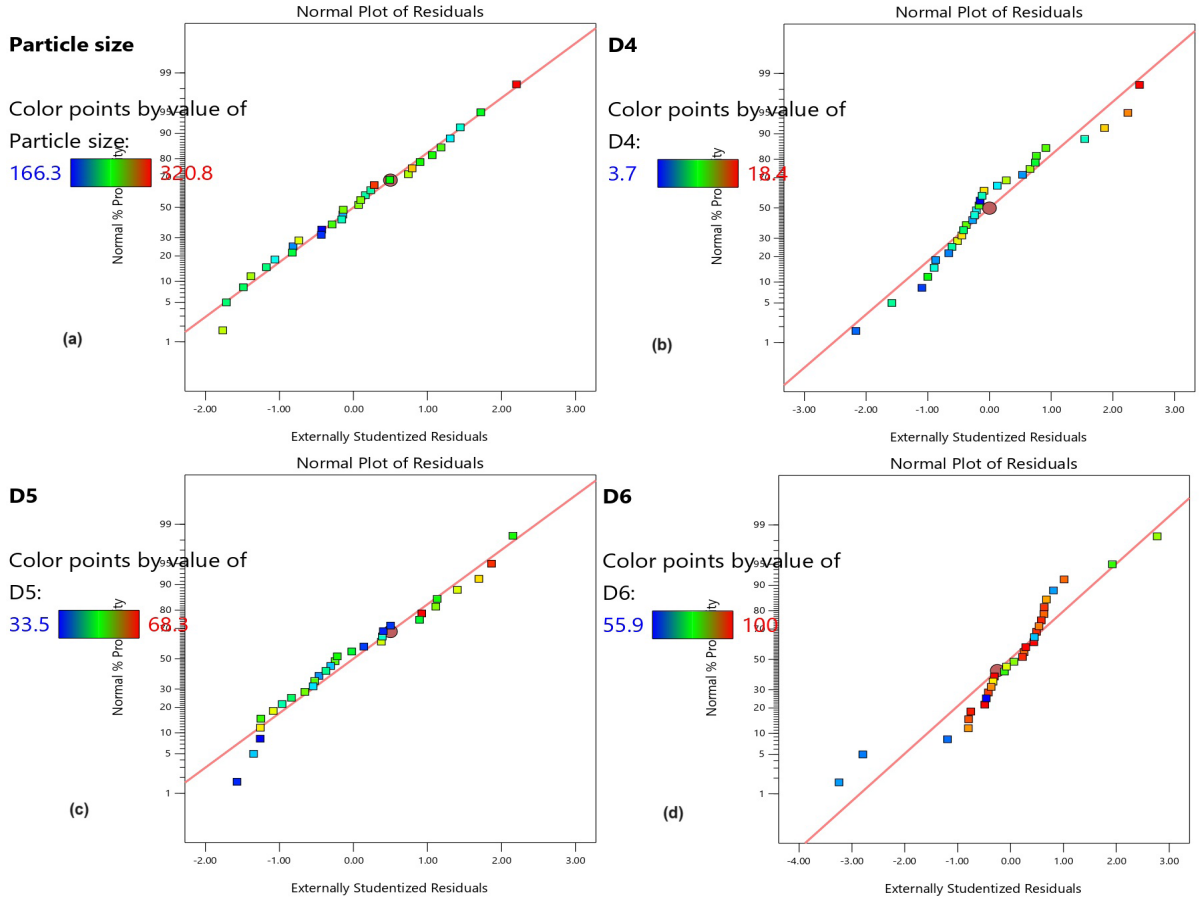


Figure 3: Normal plot of residuals for the responses (a) R1-Particles size; (b) R2-D4%; (c) R3-D5%; and (d) R4-D6%.

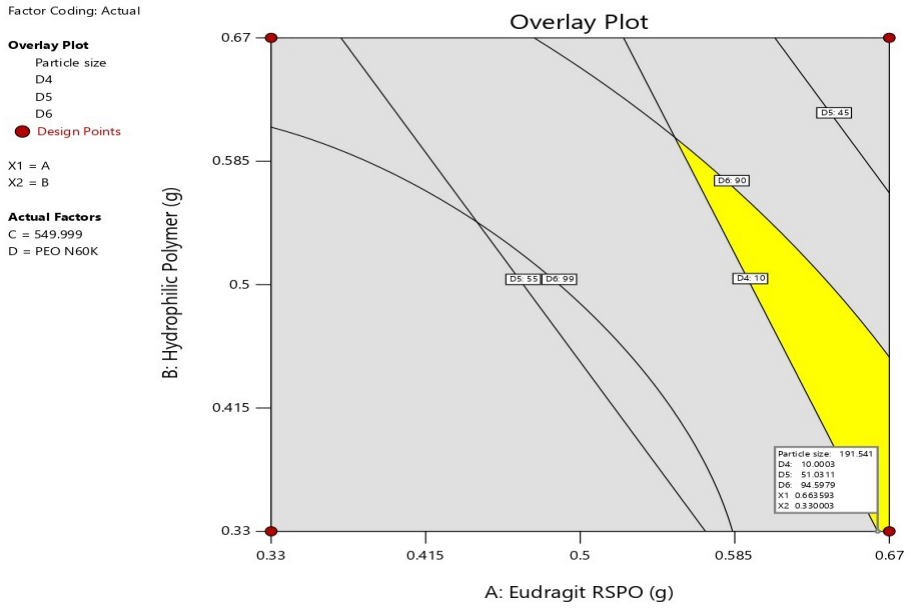
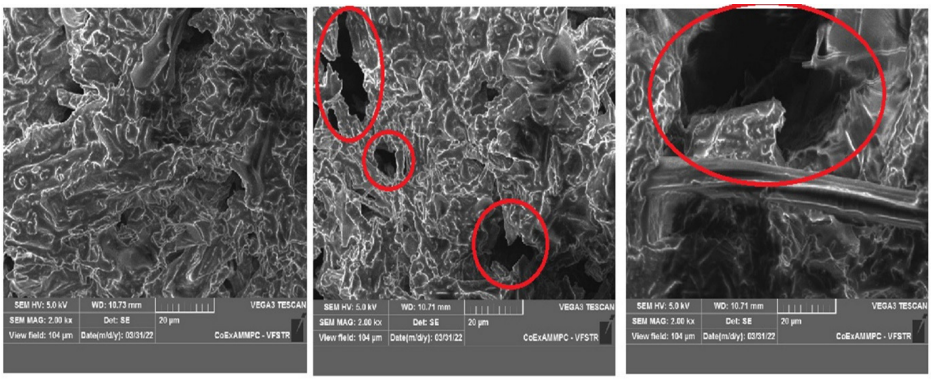


Figure 4: Overlay plot as a result of graphical optimization indicating the design space (Yellow color region).

polymer matrix at higher polymer amounts.[32] Further, increased amounts of hydrophobic polymer could cause more resistance on dissolution of the hydrophilic polymer and so the drug release.[33] And the increased amount of hydrophilic polymer might require more time to swell and dissolve and hence could cause more delay in the drug release. Further, the drug release in case of



(a) (b) (c)

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

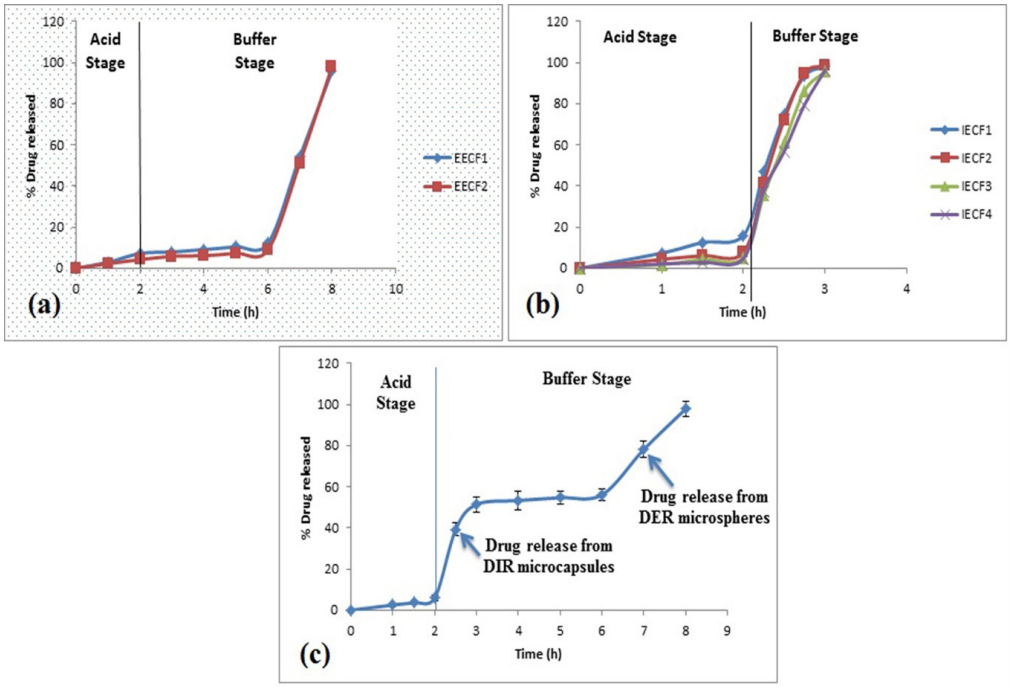


Figure 6: Overall drug release profiles of (a) DER portion microspheres; (b) DIR portion microcapsules; and (c) Pulsatile drug delivery capsule containing both DIR and DER portions.

PEO containing formulations was rapid than the corresponding HPC containing polymers. This could be because of the higher viscosity and molecular complexity of the HPC that would take more time for their swelling and dissolution.[34] And hence, HPC MF caused more delay in drug release than PEO 60K.

Design validation and optimization

The results of ANOVA test indicated that all the models of the four responses were significant. The normal plots of the residuals of all the responses demonstrated that the residual values formed almost straight line without any sigmoidal shape for every response. Besides, the adjusted and predicted R^2 values were found to be 0.9308 and 0.9136 respectively for the response R1; 0.6137 and 0.5305 for the response R2; 0.8081 and 0.7639 for the response R3; 0.8020 and 0.6278 for the response R4. As the difference between these R^2 values was below 0.2 for every response, it could be inferred that the selected models were significant. Hence, these results indicated that the selected models were suitably fitted in drawing the influences of the factors on the responses and can further be preceded for optimization.[19]

Graphical optimization was performed by setting desirability criteria or constraints for the responses so as to achieve the defined QTPP. This portion of the matrix microspheres should release the contained dose after a lag time of 4 hr after reaching the small intestine. And after 4 hr, the drug should release immediately and

Table 9: Characteristics of the DIR microcapsules of esomeprazole.

Sl. 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 (%)	85.2±1.6	82.8±2.4	87.3±2.8	78.7±4.1
3	EE (%)	82.7±2.5	88.5±1.9	89.2±2.1	90.4±3.6
4	Particle size (μm)	126.6±8.4	129.4±11.5	131.3±10.7	142.5±16.9
5	% Drug released in acid stage after 2 hr (AD2%)	15.9±2.1	7.6±1.3	5.1±0.7	4.7±1.5

* Note: The results were expressed as the Average±Standard deviation for *n*=3.

complete within 2 hr such that effective plasma concentrations can be maintained in the desired point of time. Hence, D4 was set to minimum with an upper limit of 10%; D5 was set be in the range of 45-55%; and D6 was set to be in the range of 90-99%. Particle size was set to minimum with an upper limit of 250 μm. Under these constraints, the graphical optimization was performed and the obtained overlay plot is shown in the Figure 4. Any point of combination of all the factors within the design space region (yellow color region) would yield a microsphere formulation with desired response values. One such best optimized combination and the predicted values of the responses by the software are given in Table 7. Matrix microspheres with the suggested optimized combination was prepared and evaluated for the response values. The obtained responses are shown in Table 7 and were found to be within the 95% confidence interval of the predicted values. This demonstrated that optimization of the matrix microspheres was successfully achieved with the desired drug release characteristics and a significantly high entrapment efficiency of 87.4%.

SEM analysis

The matrix microspheres were studied for surface morphology before, during and after the drug release study and the images are presented in Figure 5. Before subjecting to the drug release, the microspheres surface was continuous with the polymer matrix as shown in the Figure 5(a). The microspheres were taken out of the dissolution vessel, dried with filter paper to eliminate the water and then subjected to SEM studies.[35] The SEM images of the microspheres at this stage as shown in the Figure 5(b) exposed some small pores here and there which indicated the initiation of dissolution of the hydrophilic polymer. Again at the end of 6 hr of the drug release study, the obtained SEM images shown in the Figure 5(c) exhibited large pores which might be because of the possible complete dissolution of the hydrophilic polymer that allowed complete release of the drug from the matrix. These SEM images clearly exhibited the role of combined Eudragit RSPO and PEO in providing desired delay in the initiation of the drug release. Eudragit RSPO polymer prevented the early dissolution of the PEO and hence provided the delay in drug release. The amount of Eudragit RSPO was optimized to delay the drug release for 4 hr. These SEM images also exhibited that small pores on the matrix due to the dissolution of the PEO were observed after 4 hr of the initiation of drug release test. This confirmed that the optimized amount of the Eudragit RSPO could sufficiently delay the dissolution of PEO and the drug release. Further, the optimized amount of PEO dissolved and allowed the drug release simultaneously and completed in 2 hr. Results of the drug release studies along with these SEM images designated that the optimized amounts of the Eudragit RSPO and PEO successfully provided the desired delay in the release of Esomeprazole from the matrix microspheres.

Characterization of the enteric coated optimized DER microspheres

The EECF1 and EECF2 formulations yielded free flowing microspheres after coating. The EECF3 coated microspheres exhibited sticking which might be because of the high viscosity of the coating solution.[27] These microspheres were omitted from drug release study. Both the remaining two formulations restricted the drug release in acid stage to less than 10% and further exhibited a delay of 4 more hours in the buffer stage (shown in Figure 6 (a)). Later, the drug release was completed within 2 hr and hence a total delay of 6 hr was obtained from these DER microspheres. The EECF2 coated DER microspheres restricted the drug release to only 4.2% whereas the EECF1 coated DER microspheres exhibited 7.3% of drug release in the acid stage. This could be because of the greater amount of the enteric polymer in the ECEF2 that could provide more resistance to drug release.[36] Hence, the EECF2 coated microspheres were taken as the optimized DER portion microspheres.

Characterization of the DIR Microcapsules

The yield was found to be good in the range of 78.7-87.3% thus indicating the process conditions were acceptable enough to prepare microcapsules. The entrapment efficiency and particle size values (shown in Table 9) were found to be increased from IECF1 to IECF 4 which might be due to the increased amount of the polymer. The greater amount of polymer can encapsulate

more amount of drug and hence can improve the entrapment efficiency. Besides, higher amount of polymer increases the viscosity of the dispersed phase which resists the size reduction of their globules during emulsification under same experimental conditions and hence the particle size is increased at higher amounts of the polymer.[28,29]

Dissolution study results (shown in Table 9 and Figure 6(b)) from the acid stage indicated that IECF1 failed to restrict the drug release below the maximum limit of 10%. All the remaining three formulations successfully restricted the drug release to below 10% in the acid stage after 2 hr. In the buffer stage, the drug dissolution was found to be almost similar in all the formulations the entire dose was dissolved within 1 hr. Though all the IECF2, IECF3 and IECF4 passed the dissolution criteria of the delayed release formulations, IECF2 was chosen as the optimized formulation as it provided similar effectiveness with containing least amount of the enteric polymer which can reduce the final weight of the formulation.

Drug release studies on the combined DIR microcapsules and DER microspheres

Based on the entrapment efficiencies of the DIR and DER microspheres and also the enteric coat weight of the DER microspheres, 36.2 mg of the DIR microcapsules (equivalent to 20 mg of the drug) and 50.3 mg of the DER microspheres (equivalent to 20 mg of the drug) were together placed in hard gelatin capsules of Size 4. These capsules were studied for drug release. The obtained drug release profile shown in Figure 6(c) indicated that the drug release was well below 5.9% of the overall dose during the first 2 hr in the acid stage. This indicated that the developed systems could effectively prevent drug release in the stomach conditions. After one hour in the buffer stage, the drug release was found observed to be 51.4% which could be from the DIR microcapsules. This indicating one among the two doses was released completely as one pulse within one hour after reaching the small intestine. Further, after a predetermined lag of around 4 hr in the buffer stage, release of the second dose from the DER microspheres was started as the second pulse and completed in 2 hr. Therefore, the developed pulsatile drug delivery system of esomeprazole could effectively produce the drug release as two pulses that is one immediately after reaching small intestine and the other after a lag of 4 hr after reaching the small intestine.

CONCLUSION

PDDS for esomeprazole was developed as a capsule dosage form containing two doses of the drug to be released as two different pulses. One dose of the drug was developed as DIR microcapsules by microencapsulation of the esomeprazole with Eudragit S100 by solvent evaporation method. These microcapsules prevent the drug release in the stomach owing to their enteric coat and release the drug immediately once they reach small intestine. The second dose was made to release as another pulse after 4 hr after reaching the small intestine. This was achieved by developing esomeprazole as hydrophilic-hydrophobic matrix microspheres followed by enteric coating which were termed as DER microspheres. The outer enteric coat prevented drug release in the gastric acidic medium and the optimized quantities of the Eudragit RSPO and PEO provided desired lag of 4 hr before initiation of the matrix dissolution and hence the drug release. So, when this PDDS capsule is administered just before the night meal at around 8-9 PM, first dose will be released after emptying of the capsule contents into small intestine from the DIR portion. Then after a lag of total 6 hr, second dose of the drug will be released from the DER portion at around 3-4 am when the nocturnal acid breakthrough generally occurs. So, this pulsatile drug delivery capsule dosage form containing one dose of DIR microcapsules and one dose of DER microcapsules with chronomodulated drug release properties can effectively prevent the nocturnal acid breakthrough by releasing the drug at the desired time period. Therefore, the objective of the current research work was successfully achieved by employing quality by design approach.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of Variance; **CCD:** Central Composite Design; **CPPs:** Critical Process/Formulation Parameters; **CQAs:** Critical Quality Attributes; **DER:** Delayed Extended Release; **DIR:** Delayed Immediate Release; **DoE:** Design of Experiments; **EE:** Entrapment Efficiency; **HPC:** Hydroxypropyl Cellulose; **NAB:** Nocturnal Acid Breakthrough; **PDDS:** Pulsatile Drug Delivery Systems; **PEO:** Polyethylene Oxide; **PPIs:** Proton Pump Inhibitors; **QbD:** Quality by Design; **QTPP:** Quality Target Product Profile; **SEM:** Scanning Electron Microscopy.

SUMMARY

The major objective of the current work was to provide patient convenience in the form of developing Pulsatile Drug Delivery Systems (PDDS) with chronomodulated drug release for Esomeprazole. Wherein, one dose should be released immediately upon reaching small intestine (after gastric transit), and another dose should release after a lag of 6 hr (including gastric transit). In order to achieve the target, one dose of drug was developed

in the form of enteric coated immediate release drug particles (DIR portion) and another dose was developed as enteric coated extended release polymeric microspheres (DER portion). Various formulation parameters were optimized particularly to optimize lag time and the release from the extended release portion of the formulation using by employing QbD approach. The experimental design and the optimization were successfully given a combination of the formulation variables to develop the PDDS of Esomeprazole with the set objective of desired lag time between two doses. Administration of this product after dinner and before bed-time, would thus provide chronomodulated delivery as one dose would be released immediately and the other dose in the early morning so that the nocturnal asthma can be controlled effectively.

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 GenetMedMed. 2004;6(4):11. PMID PMID, PMCID 1480544.
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, *et al.* Press-coated aceclofenac tablets for pulsatile drug delivery: formulation and *in vitro* evaluations. Pharmaceuticals. 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 PharmSci 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 tenofovirdisoproxil 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. Rai SY, Ravikumar P. Development and evaluation of microsphere based topical formulation using design of experiments. Indian J Pharm Sci. 2016; 78(2): 182-92. doi: 10.4172/pharmaceutical-sciences.1000102.
21. Sinko PJ, Singh Y, editors. Martin's physical pharmacy and pharmaceutical sciences. New York. Walter Kluwer-Lippincott. Williams & Wilkins; 2011.
22. Allen T. Particle size analysis by image analysis. In: Allen T, editor. Powder sampling and particle size determination. Elsevier; 2003; 3(1): 142-207.
23. Halder S, Shuma ML, Kabir AKL, Rouf ASS. *In vitro* release kinetics study of esomeprazole magnesium trihydrate tablet available in Bangladesh and comparison with the originator brand (Nexium®). SJ Pharm Sci. 2011; 4(1): 79-83. doi: 10.3329/sjps.v4i1.8875.
24. Sultana S, Shohag Hosen M. *In vitro* comparative quality evaluation of different brands of esomeprazole tablets available in selected community pharmacies in Dhaka, Bangladesh. BMC Res Notes. 2018; 11(1): 1-5.
25. Srikar G, Rani AP. Aqueous-core nanocapsules of lamivudine: optimization by design of experiments. Asian J Pharm. 2019; 13(4): 318-27.
26. 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.
27. Bodratti AM, Alexandridis P. Formulation of poloxamers for drug delivery. J Funct Biomater. 2018; 9(1): 11. doi: 10.3390/jfb9010011, PMID 29346330.
28. Pavelková MHJMKKVDSPM, Vysloužil JMDVJ. Influence of emulsification mode, stirring speed and volume on ibuprofen-loaded PLGA microparticles. Cesk Slov Farm. 2021; 70(1): 32-40. PMID 34237951.
29. Dashora K, Saraf S, Saraf S. Effect of processing variables and *in vitro* study of microparticulate system of nimesulide. Rev Bras Cienc Farm. 2007; 43(4): 555-62. doi: 10.1590/S1516-93322007000400008.
30. 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.
31. Rowe RC, Sheskey PJ, Owen SC, editors. Handbook of pharmaceutical excipients. London: Pharmaceutical Press and American Pharmacists Association; 2006.
32. Wan B, Andhariya JV, Bao Q, Wang Y, Zou Y, Burgess DJ. Effect of polymer source on *in vitro* drug release from PLGA microspheres. Int J Pharm. 2021; 607: 120907. doi: 10.1016/j.ijpharm.2021.120907, PMID 34332059.
33. Rokhade AP, Patil SA, Belhekar AA, Halligudi SB, Aminabhavi TM. Preparation and evaluation of cellulose acetate butyrate and poly (ethylene oxide) blend microspheres for gastroretentive floating delivery of repaglinide. J Appl Polym Sci. 2007; 105(5): 2764-71. doi: 10.1002/app.26456.
34. 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.
35. 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.
36. 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, PMCID 7057410.

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