

Formulation Development and Evaluation of Colonspecific Esomeprazole Microspheres

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The development of Esomeprazole microspheres using enteric coated polymers, such as Eudragit L100, by antisolvent precipitation method has been the focus of recent research in order to achieve the best drug concentration along with improved stability and bioavailability without any adverse effects in an acidic environment. For the development of the Esomeprazole microsphere, a 32 factorial design was used, with polymer amount and rpm being the variables. According to the results of the factorial design investigation, it was found that polymer concentration has a substantial impact on drug release while stirring speed has a large impact on entrapment efficiency. Increased Eudragit L100 concentration caused the medication release to increase from 81.06 to 95.71%. The optimized formulation was then assessed for additional research on surface morphology, particle size, PDI, Zeta potential, percent entrapment efficiency, and in-vitro investigations drug release studies and stability of prepared formulation. According to the results of scanning electron microscopy for the optimised batch, developed microparticles with smooth surfaces and spherical shapes were found. 93.40% drug release and 70.63% drug entrapment efficiency were found in the optimised batch. As a result, the current study's findings indicated that microencapsulation had significant benefits for enhancing Esomeprazole's acid stability. By providing a delayed release effect for a duration of 10 hours and a higher patient compliance rate, developed microspheres were demonstrated to be efficient for protecting drugs in acidic media.

Keywords: Colon Targeted Drug Delivery; Esomeprazole; Eudragit L100; Microspheres.

The colon targeted drug delivery system (CDDS) is designed to deliver drugs to the large intestine in the lower GI tract. Irritable bowel syndrome (IBS), colorectal cancer, and inflammatory bowel disorders (IBD), which include ulcerative colitis and Chron's disease, have gained a lot of attention for use as prospective routes of treatment for the local treatment of a number of colonic diseases^{1,2}. The principal site of organ for the digestion of food eaten orally is the

stomach. The biological control of acid secretion by the parietal cell is significant for the use of various medicines to lower gastric acidity¹.

Colon targeted drug delivery system(CDDS) targets administration of medications into the lower GI tract, particularly in the large intestine. Colon specific targeted drug delivery have attracted a great deal of attention for application as potential route of treatment for the local treatment of a variety of colonic diseases

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such as irritable bowel syndrome (IBS), colorectal cancer and inflammatory bowel diseases (IBD) which includes ulcerative colitis and Chron's disease^{1,2}. Stomach is the primary site of organ for the digestion of orally consumed food. The parietal cell secretes acid and biological regulation of acid secretion has significance for the use of different agents to reduce gastric acidity¹. Massive amounts of excess gastric acid and pepsinogen are secreted, which damages the gastroduodenal mucosa and causes fatal ulcerations²⁻⁴. As a result, antacids, proton pump inhibitors, and other types of stomach acid secretion inhibitors can be used as a form of treatment⁵. To ensure a controlled and precise release of the medication, microencapsulation can offer protection to the gastric mucosa^{6,30}.

Inflammatory bowel disorders are one of the numerous diseases that can be treated using the GIT microflora of the colon. The prime reason behind the development of colon-targeted medication delivery systems is the modification of pH at various locations throughout the GIT tract⁷⁻⁹. The effective development of various CDDS is greatly aided by the promising modulation of pH difference in GIT. Utilizing a pH-sensitive polymer, enteric coating can be created for solid dosage forms such capsules, tablets, and pellets to protect cells from acidic pH¹⁰. pH-sensitive polymers for colon can be applied for the formulation development which can be specific to gastric pH of the digestive & proximal part of the small bowel whereas disintegrate selectively at intestinal pH¹¹. Eudragit L & S are copolymers of methacrylic acid & methyl methacrylate. Eudragit L is water-soluble at pH 6 & can be used to deliver medications to the small and large intestine¹²⁻¹⁴. The current study focussed on development and evaluation of colon-specific Esomeprazole microcapsules.

MATERIALS AND METHODS

Esomeprazole was obtained from Cadila Healthcare Ltd, Ahmedabad, India. Eudragit L100 was from Evonik Industries, India. All chemicals and reagents used were of analytical grades.

Formulations Development using 3² Factorial Design

Specified quantity of Eudragit L100 and Esomeprazole was accurately weighed and dissolved in methanol. As per Table 1, polymer

and drug solution was added drop wise to liquid paraffin containing tween 80 as an emulsifying agent. The resultant o/o emulsion was stirred for 3 hours. For three hours, the resulting o/o emulsion was swirled. Vacuum filters were used to filter the developed formulation, which was then washed in petroleum ether and dried overnight.¹⁵⁻¹⁷ Developed microspheres were evaluated for various characteristics such as particle size distribution, entrapment efficiency, surface morphology, and percent yield. *In vitro* release studies were performed to evaluate release kinetics and further investigated for the stability of Esomeprazole in UV and solar light.

Determination of Mean Particle Size

A glycerine based suspension of developed microsphere was subjected for particle size determination using an optical microscope. The size of developed microspheres was detected at 25 °C^{18,19}.

Entrapment Efficiency (EE)

Drug entrapment efficiency was investigated by carefully solubilising 100 mg of microspheres in 100 mL of methanol, followed by filtration of the solution. The amount of Esomeprazole in the filtrate was determined spectrophotometrically at 305 nm (Shimadzu 1201). The following equation was used to calculate % entrapment efficiency²⁰.

$$\text{Entrapment efficiency} = \frac{\text{Actual drug}}{\text{Theoretical drug content}} \times 100 \quad \dots(1)$$

Production Yield

The weight ratio of microspheres to loading quantity of the polymer and drug was used to calculate the production yield of microspheres containing a medicament. The production yield was calculated using following equation.

$$\% \text{Production yield} = \frac{\text{Total mass of microspheres}}{\text{Total mass of raw material}} \times 100 \quad \dots(2)$$

Micromeritic Characteristics of Microspheres

The developed microspheres were evaluated for Micromeritical properties such as particle size, tapped density, true density, compressibility index etc.

Scanning Electron Microscopy (SEM)

Morphological characteristics of the samples were tested using a scanning electron

microscope (Model JSM -5600, JEOL), Japan. Air dried microspheres were covered with platinum for 5-10 minutes using an auto fine covered ion sputter (JEOL-JFC-1600, JEOL, and Japan) and analysed. SEM was operated at a low frequency voltage of approximately 15 KV and load power of approximately 80 MA^{21,22}.

Fourier Transform Infrared Spectroscopy (FTIR)

IR spectra of pure drug as well as mixtures of drug and polymer were investigated to determine compatibility of drug with various types of polymers. Spectra of pure drug, polymer mixture, and polymer mixture containing pure drug was compared for compatibility evaluation²³.

Differential Scanning Calorimetry (DSC)

API and developed formulation were subjected for analysis to DSC (Shimadzu, DSC 60), which was equipped with a thermal analyzer. Approximately precisely weighed 3 mg of sample was placed in a aluminium pan and hermetically sealed with an aluminium lid. The system was subjected to liquefied nitrogen gas at a flow rate of 50 mL/min, and temperature was raised from 50°C to 400°C at a rate of 10 ° C/min²⁴.

In-vitro Dissolution Studies

USP XXII basket-type dissolution apparatus was used to determine entrapment efficiency of developed microspheres. Phosphate buffer with a pH of 1.2 (900 mL) was maintained at 37°C with a cycle speed of 100 rpm. A sample volume of 10 mL was taken at one-hour intervals and subjected to spectrophotometric analysis at 290 nm to determine quantity of drug which released from formulation in the dissolution media. The volume of dissolution media was maintained by adding 10 mL of fresh dissolution media after each sampling for a period of two hours to evaluate drug release in an acidic medium. Next to that, phosphate buffer pH 7.4 was applied to study release of drug from formulation in the dissolution media. The results of every test were recorded in triplicate.²⁵

Kinetics Modeling of Drug Dissolution Profiles

The zero order, first order, Higuchi and Korsmeyer-Peppas were used to determine the kinetic expression of the drug release with dissolution profile of all batches²⁶.

a. Zero Order Treatment

The equation for zero order treatment is represented

as,

$$Q_t = Q_0 + K_0t \quad \dots(3)$$

Where,

Q_t -Quantity of drug released in time (t)

Q_0 -Initial amount of drug in solution

K_0 -Zero order release constant

b. First Order Treatment

The equation for the first order treatment is demonstrated as-

$$\text{Log } c = \frac{\text{Log } c_0 - Kt}{2.303} \quad \dots(4)$$

Where,

c -Quantity of drug remaining unreleased at time (t)

c_0 - Initial amount of drug in solution

k - First order rate constant

c. Higuchi's Model

The Higuchi equation is denoted as-

$$Q_t = kt^{1/2} \quad \dots(5)$$

Where,

Q_t -Quantity of drug released in time t

k -Higuchi's constant

A linear association between quantity of drug released (Q) versus square root of time ($t^{1/2}$) is witnessed if the drug release from the matrix is diffusion controlled.

d. Korsmeyer- Peppas Model

The Korsmeyer-Peppas model communicates drug release exponentially to time. It is expressed by the below equation-

$$\frac{M_t}{M_{inf}} = at^n \quad \dots(6)$$

Where,

M_t / M_{inf} -fractional release of drug

a - Constant dependent on structural and linear characteristics of the drug dosageform

n - Release exponent

The value of n designates the drug release mechanism.

For a slab the value $n = 0.5$ designated Fickian diffusion and values of n between 0.5 and 1.0 or $n = 1.0$ indicated non-Fickian mechanism.

Stability Study

The optimized formulations were successfully subjected to stability studies. The

stability study was carried out at a temperature of 45 ± 2 C and a relative humidity of $75 \% \pm 5 \%$ RH for a period of one month. Evaluations of the formulation's drug content and *in vitro* drug release were performed at intervals of 15 days^{27, 28}.

RESULT AND DISCUSSION

By using a solvent evaporation approach, Esomeprazole microspheres were developed and subjected to several evaluation criteria. Eudragit L 100 was used to create Esomeprazole microspheres. For maximal drug entrapment and consistent particle size distribution, formulations were prepared using various amounts of polymer in various ratios. With formulation batches containing 1:1 and 1:2 ratios of drug and polymer, uniform microspheres did not developed. Optimum rpm of 800 was found to be suitable with respect to morphology, yield and percent drug entrapment efficiency for formulations developed using drug: polymer ratios of 1:3 and 1:4. 50 mL of liquid paraffin for 1:3 and 1:4 formulations was detected appropriate for desired result.

Statistical Data Analysis

Statistical analysis covers a set of experiment and makes certain accurate and persuasive interpretation of results of a study which determine the response variables, conducting appropriate statistical tests to select best possible model, fitting mathematical models to the data, and determining the values of independent formulation variables to produce optimum response. The present study applied a factorial design which is one of the popular statistical experimental design to optimise the formulation as well as to discover the interactions, if any, between the factors chosen

To study the effects of independent variables on its attributes and performance, a 3² factorial design was applied. The independent variables such as concentration of polymer and rpm at three levels were evaluated for their effect on % drug release, % entrapment efficiency, particle size and % yield. The responses obtained are given in Table 2.

Entrapment efficiency (actual factors)

$$EE = -1109.27796 + 6.35186 \times \text{EudragitL100} + 0.64970 \times \text{RPM} \dots(7)$$

Table 1. Formulation Development of Esomeprazole Microspheres

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eudragit L100 (mg)	251.71	260	260	280	280	280	300	300	308.28
Esomeprazole(mg)	70	70	70	70	70	70	70	70	70
Liquid paraffin(mL)	50	50	50	50	50	50	50	50	50
Tween 80(mL)	35	35	35	35	35	35	35	35	35
Methanol(mL)	10	10	10	10	10	10	10	10	10
RPM	800	700	900	650	800	950	700	900	800

Table 2. Experimental Design of the Optimization of Formulations

S No.	Batch	Factor 1 Eudragit L100(mg)	Factor 2 (RPM)	Mean particle size(im)	Entrapment efficiency (%)	Response 2 Drug Release (%)	Practical yield (%)
1	F1	251.71	800	132.91±0.056	60.12±0.034	94.42	87.81±0.03
2	F2	260	700	172.61±0.056	65.09±0.043	95.71	86.36±0.04
3	F3	260	900	143.05±0.034	63.41±0.054	94.81	95.33±0.05
4	F4	280	650	102.29±0.095	71.12±0.067	93.30	90.71±0.23
5	F5	280	800	210.34±0.043	75.79±0.054	94.40	95.64±0.23
6	F6	280	950	185.34±0.076	67.21±0.40	92.13	94.54±0.04
7	F7	300	700	163.19±0.073	72.41±0.08	86.69	93.16±0.8
8	F8	300	900	165.76±0.04	73.9±0.02	87.09	94.26±0.06
9	F9	308.28	800	195.27±0.069	72.21±0.056	81.01	92.29±0.09

The 3D response surface plots of the factorial model were plotted to demonstrate the effect of the variables on the entrapment efficiency. Figure 1(A) showed the effect of the amount of Eudragit L100 and rpm on the entrapment

efficiency. It was demonstrated that the entrapment efficiency depends upon Eudragit L100 and rpm. Meanwhile, it was found to be the most important element affecting the effectiveness of entrapment.

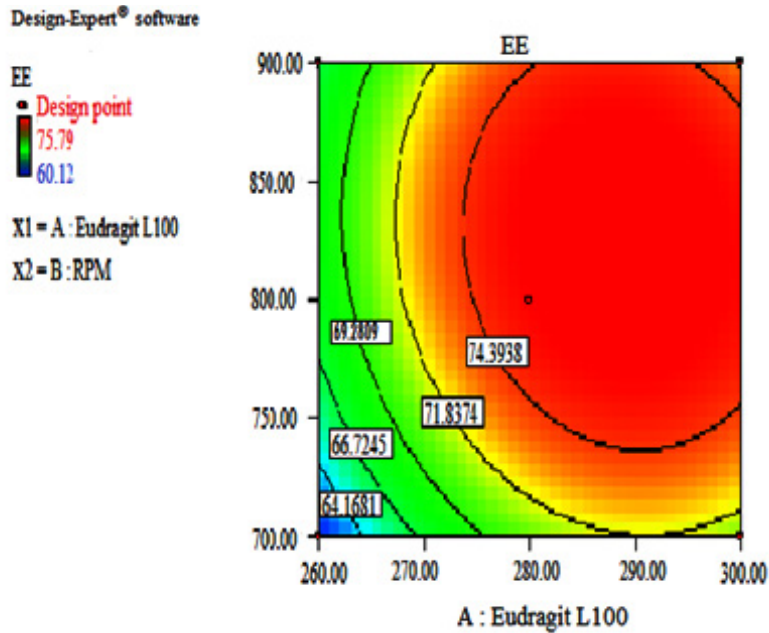


Fig. 1(A). Contour plot curve depicting the effect of factorial variables (a) Eudragit L100 and (b)rpm on the entrapment efficiency

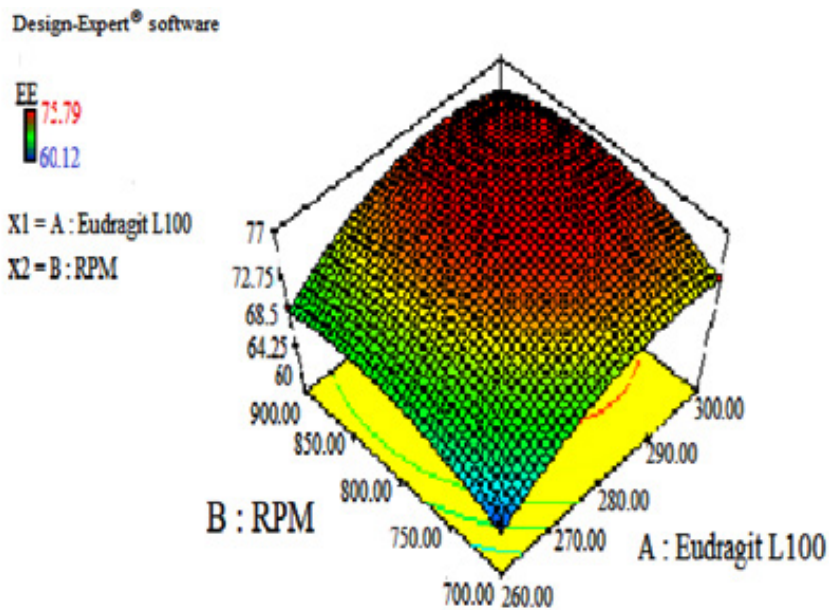


Fig. 1(B). Three-dimensional view of entrapment efficiency with respect to Eudragit L100 and rpm obtained by D.E.7.0 related to given data

Influence of two variables such as polymer concentration and rpm were selected at two levels. According to the surface response graph Figure 1(B), the highest amount of drug entrapment was obtained when both the polymer concentration and the rpm were remained high (280 mg and 800 rpm).

On the other hand, very less entrapment was detected when both the variables were applied at low level. Thus from current outcome, it was concluded that both variables had positive effect on percent drug entrapment (Table 2).

With increased concentration of polymer, viscosity also increases whereas high energy was required for formation of uniform microspheres. Low stirring rate was not sufficient to break emulsion into droplets which was resulted in lump formation. Hence from the above studies it was detected that when polymer concentration increased, stirring rate should be increased for production of uniform and optimum size microspheres.

Drug release (actual factors)

$$RE = +451.06239 - 4.01897 \times \text{EudragitL100} + 0.037304 \times \text{RPM}$$

...(8)

It was concluded that Eudragit L100 (factor A) and rpm (factor B) had individual effect on drug release.

Conferring to the achieved results, the established models are statistically precise and can be used for next examination.

The obtained graph (Figure 2) showed as the concentration of Eudragit L100(variable A) increases upto medium level and at that level reduction in drug release was detected. It can be concluded that factor B has significant effect on drug release.

Effect of interaction between Eudragit L100 and rpm on the drug release was obtained. Factor A and B have its individual significant effects. Eudragit L100 and rpm at medium level showed significant impact on drug release.

The 3D response surface plots of the factorial model were plotted to demonstrate the effect of the variables on the drug release. Figure 3(A and B) showed the effect of the amount of Eudragit L100 and rpm on the drug release. It was showed that dependency of drug release on Eudragit L100 and rpm. Meanwhile, selected factors showed most significant effect on the drug release.

Approaches For Optimized Solution

Based on acceptance criteria and desirability factor, Design Expert (Version 7.0)

Design-Expert software

%CDR

● Design Point

■ A- 260.000

▲ A+ 300.00

X1 = B : RPM

X2 = A : EudragitL 100

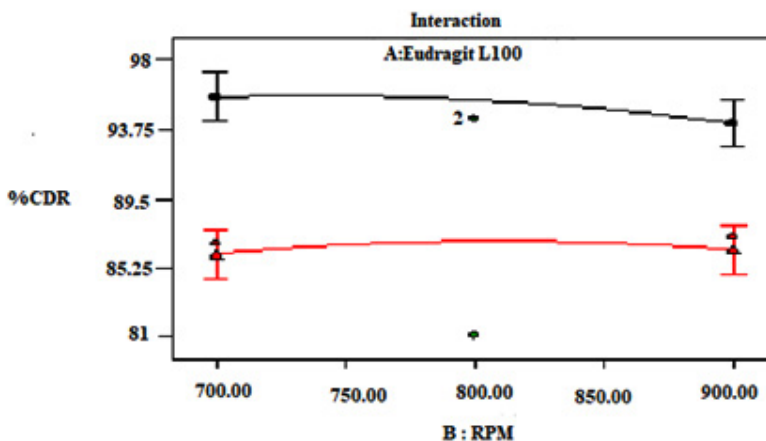


Fig. 2. Effect of interaction between Eudragit L100 and RPM on the drug release

Table 3. Suggested Formulations and their Responses by Design Expert7.0

Number	Eudragit L100	Rpm	Entrapment Efficiency	Drug release	Desirability
OB1	286.15	800	71.90	92.68	1.000

software suggested 30 optimum formulations. From the suggested formulations, selected optimized batch (OB1) indicated value of desirability closest to 1, which was considered as most favourable. As per Figure 4, *in vitro* release of optimized batch was detected with more than 80%. Therefore, from the results obtained for batch 1 were selected from

30 possible solutions as OB1, has been shown in table 3.

Particle Size

According to Figures 5A and 5B, developed microspheres with a uniform mean particle size distribution were obtained with a particle size distribution up to 210.34 μm .

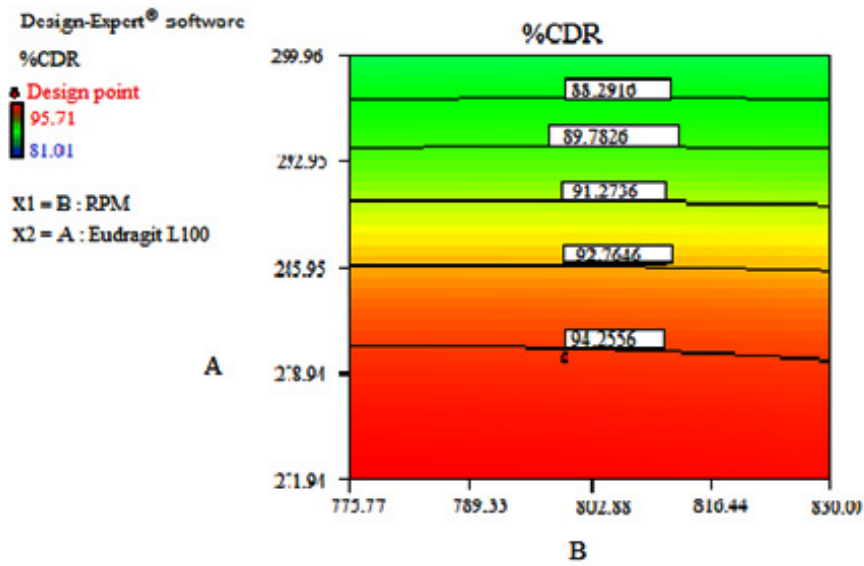


Fig. 3(A). Contour plot detected effect of Eudragit L100 and RPM on the drug release

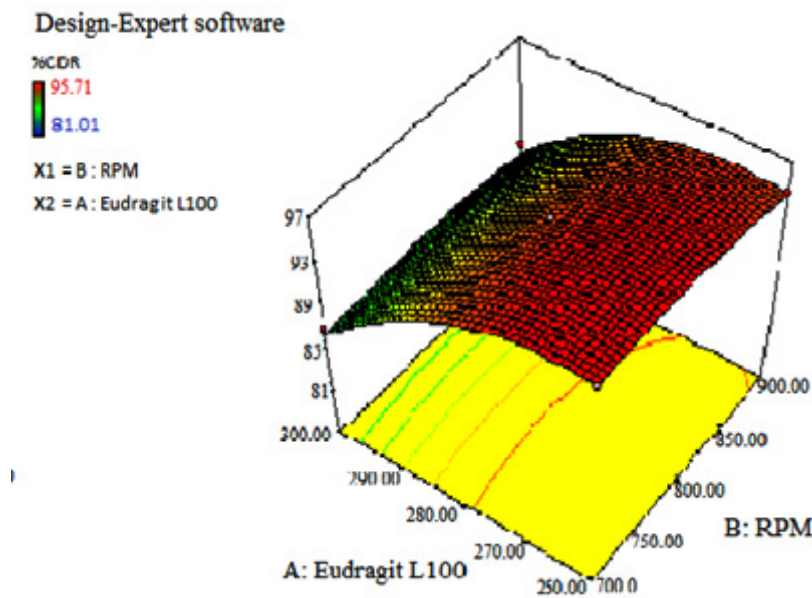


Fig. 3(B). Three-dimensional view of drug release with respect to Eudragit L100 and RPM obtained by D.E.7.0 related to the given data

Following Table 4 provided more information on optimised formulation.

Percentage Yield

It was found that the batches F3 and F5 simultaneously produced a maximum practical yield of approximately 95.33 and 95.64 percent respectively.³¹

Micrometric Properties of Microspheres

The flow characteristics of the developed formulations were determined by angle of repose. All the preparations indicated an angle of repose within the range which is 29.87° to 39.34° which was acceptable for free flowing characteristic²².

Fourier Transform Infrared Spectroscopy (FTIR)

The spectra of pure drug and polymer combination were matched with IR spectra of pure drug. The IR spectra of the drug, polymer and drug with polymer combination were shown in figure 6.

In the FTIR spectra of esomeprazole, the CH aromatic stretching was observed in the range of 3100 cm^{-1} to 3000 cm^{-1} , the CH aliphatic stretching was observed in the range of 2944 cm^{-1} to 2844 cm^{-1} , and the NH stretching was observed at 3414 cm^{-1} . CH aromatic stretching was observed at 3198 cm^{-1} , CH aliphatic stretching was observed

Graph of Cumulative % drug release vs Time

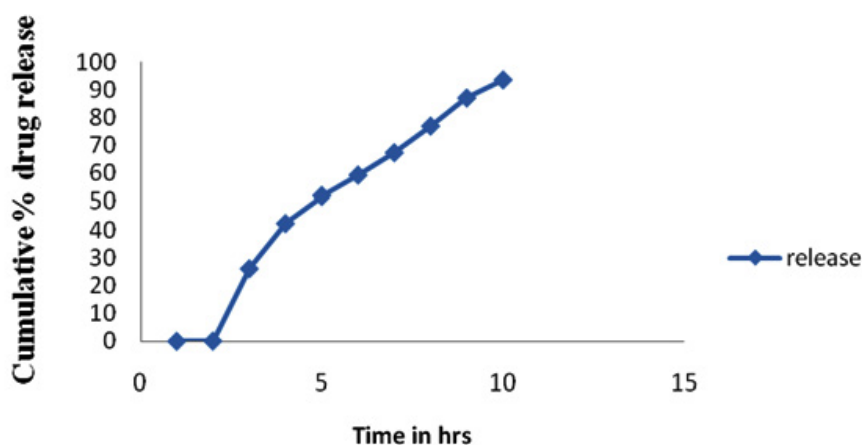


Fig. 4. *In-vitro* release profile of optimized Esomeprazole microspheres

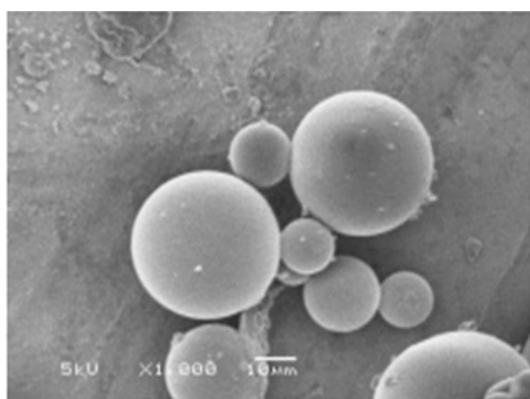


Fig. 5(A). Scanning Electron Microscopy of Esomeprazole microspheres prepared with Eudragit L100 at 1000X

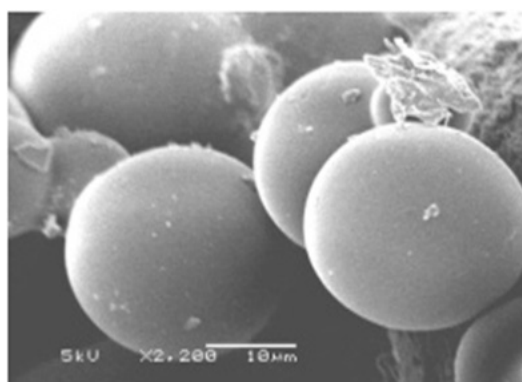


Fig. 5(B). Scanning Electron Microscopy of Esomeprazole microsphere prepared with Eudragit L100 at 2,200 X

at 2948 cm^{-1} , and NH stretching was observed at 3491 cm^{-1} when the polymer and medication were combined. The principle peaks that were accomplished for the combinations were almost identical to those that were achieved for the drug. There was no evidence of any variation in the IR spectrum of Drug-Eudragit L 100. Because there was no significant shift in the absorption bands of the drug and drug polymer combination, it was determined that there was no chance of an interaction taking place.

Table 4. Evaluation of Optimized formulations

No.	Parameter	OBI
1	Percentage Yield	95.12 \pm 1.12
2	Mean Particle size in μm (\pm SD)	220.1 \pm 3.51
3	Angle of Repose ($^\circ$)	35.55 \pm 0.01
4	Bulk Density (g/mL)	0.205 \pm 0.015
5	Tapped Density (g/mL)	0.240 \pm 0.034
6	Compressibility index (%)	25.2 \pm 2.23
7	% DEE	70.63 \pm 0.14

*OBI= Optimised Batch 1

Differential Scanning Calorimetry (DSC)

The assessment of the thermograms evidently exposed no physical interface between the polymer and the drug in the established formulation (Figure 7).

In-vitro Release

Theoretical Drug Release Profile of Esomeprazole

Theoretical release rate of Esomeprazole microspheres was considered by using pharmacokinetic parameters. From observation (Figure 8) it was observed that drug release

Table 5. FTIR spectra interpretation

No.	Wavenumber	Description
1.	3100 cm^{-1} to 3000 cm^{-1}	C-H aromatic stretching
2.	2944 cm^{-1} to 2844 cm^{-1}	C-H aliphatic stretching
3.	3414 cm^{-1}	N-H stretching
4.	3198 cm^{-1}	C-H aromatic stretching
5.	2948 cm^{-1}	C-H aliphatic stretching

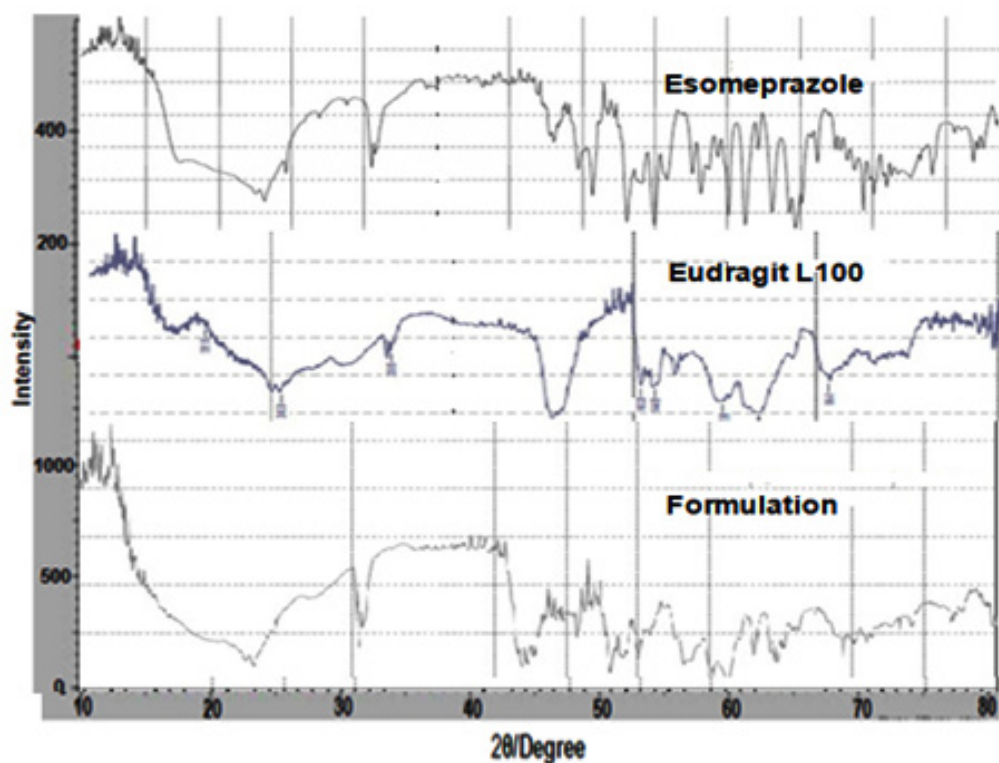


Fig. 6. FTIR spectra of Esomeprazole, Eudragit L 100 and developed formulation

rate of the formulation F5 was found greater in comparison to other formulation. *In vitro* release data showed that increase in polymer concentration decreases drug release. As the polymer concentration increases, the diffusion path for drug to get release from the matrix increases; that might be the reason for decrease in drug release with respect to enhanced in polymer concentration.

Within first hour, 16–25% of drug was released followed by a plateau pattern for 8 hours. The burst release was detected due to the untrapped drug presence on the surface of microspheres. This burst release was then followed by drug release from microspheres i.e. loaded or entrapped drug for a desired prolonged period of time.

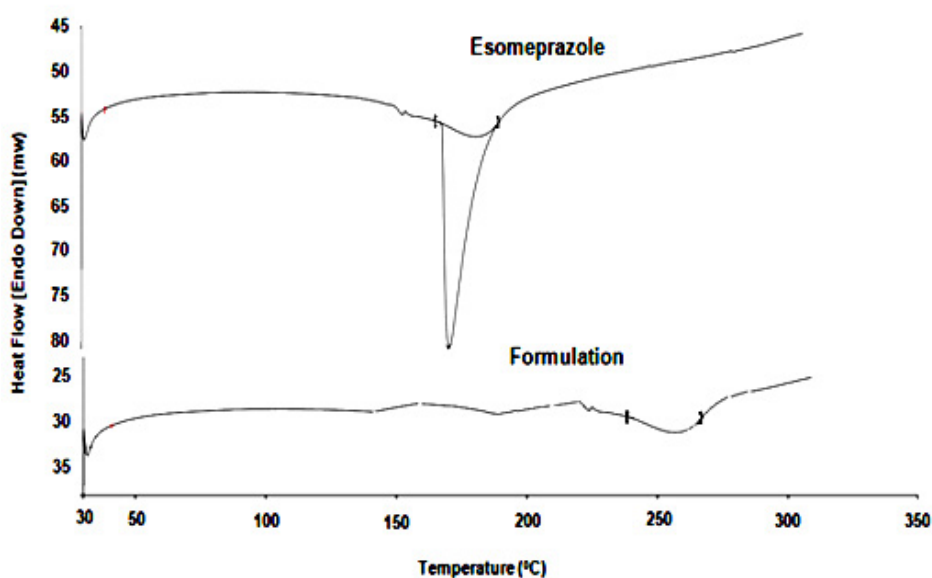


Fig. 7. DSC of Esomeprazole and developed formulation

Drug Release Profile of Esomeprazole loaded Microspheres

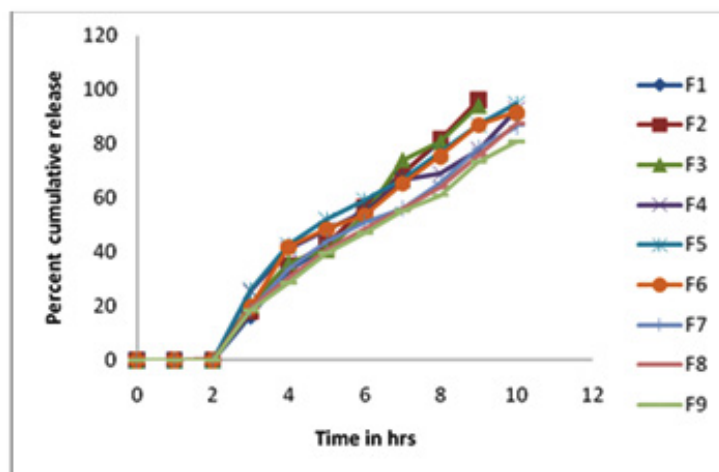


Fig. 8. Comparative dissolution profile of various batches of Esomeprazole loaded microsphere, mean ± SD, n=3

Table 6. *In-vitro* Release Data fitted in to Various Models

Model	Zero order Linearity (R2)	First order Linearity(R2)	Higuchi Linearity(R2)	Korsemeyer-Peppas Linearity (R2)
F1	0.9844	0.8059	0.9863	0.9684
F2	0.9776	0.8957	0.9846	0.9727
F3	0.9751	0.9126	0.9786	0.9836
F4	0.9616	0.8531	0.9781	0.9533
F5	0.9749	0.8281	0.9891	0.9582
F6	0.9692	0.8328	0.9807	0.9519
F7	0.9617	0.8949	0.9743	0.9663
F8	0.9721	0.9162	0.9832	0.9627
F9	0.9638	0.8093	0.9876	0.9711

Table 7. Stability Study of Optimized Formulation

Optimized Batch	Appearance	% EE (Mean \pm SD)	% Drug Release (Mean \pm SD)
OB1	White, spheres	70.14 \pm 0.42	89.25 \pm 1.35

*OB1= Optimised Batch 1

Kinetics Models of Drug Dissolution

In-vitro drug release data was subjected to different kinetic equations to witness the mechanism or kinetics of drug release from microspheres. Association measurements of separate batch with functional equation were given in Table 5. All batches displayed higher relationship with Higuchi plot and zero order as compared to first order and Hixon-Crowel whereas predominant drug release was detected by diffusion type of sustained release mechanism.

Stability Study

The short term accelerated stability study was accomplished at 40°C and 75% RH for 1 month. The optimized batch was verified for its physical presence, drug content and drug release. The optimized batch was found to be stable as per the outcome of data for the stability study after 1 month. Table 6 showed the results obtained in the stability study of the prepared formulation.

CONCLUSION

The various active pharmaceutical ingredients were compromised by the enormous excess stomach acid and pepsinogen secretion. Therefore, it is crucial to maintain safeguard such

pH and acid sensitive candidate in order to facilitate the medication administration. There are so many approaches to protect acid sensitive drug from the gastric environment, but microencapsulation approach provides the controlled drug delivery as well. Microencapsulation is utilised to achieve regulated release of the different drugs, to achieve targeted drug delivery, and to protect sensitive components from the stomach environment. In the recently published research, the solvent evaporation process was used to create Eudragit L100 microspheres that were loaded with esomeprazole. It was determined from the current investigation that the manufactured encapsulated esomeprazole-loaded microspheres proved to be a significant method for improving esomeprazole's stability in acid. In addition, it was claimed that the created microspheres were particularly effective at protecting the medicine in an acidic environment and made it acid resistant with sustained release activity for duration of 10 hours, increasing patient compliance.

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Conflict of Interest

The authors declare no conflicts of interest.

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