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Research Article

DESIGN OF GASTRORETENTIVE POLYMERIC LOW-DENSITY MICROBALLOONS OF MEBENDAZOLE USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Objective: The main objective of the present work is the development of polymeric low-density microballoons for prolonged gastroretentive delivery and optimization of promising formulation by central composite design of response surface methodology.

Methods: Mebendazole-loaded microballoons were prepared by emulsion solvent diffusion method using Eudragit S-100 and hydroxypropyl methylcellulose as release controlling polymers. All the formulations of mebendazole-loaded microballoons showed buoyancy up to 8 h. Percentage of Eudragit S-100 (X_1) in total amount of polymer and solvent ratio (X_2) was taken as two independent variables. The responses are evaluated to study the effect of independent variables and the optimum formulation was chosen based on numerical and graphical optimization.

Results: The optimized formulation MBZ_9 was composed of 100 mg of mebendazole, 75% of Eudragit S-100, and 25% of HPMC with DCM: ETH ratio of 1:1. The optimized formulation showed yield (81%), buoyancy (86.4%), entrapment efficiency (82.01%), and cumulative drug release for 12 h (79.99%). The optimized formulation was characterized by differential scanning calorimetry, and Fourier-transform infrared spectroscopy. It followed mixed order and the mechanism of drug release was diffusion as per R^2=0.905 in Higuchi model.

Conclusion: Microballoons of mebendazole produced with 75% Eudragit S-100, X_1 (750 mg), 25% of HPMC polymer, and 1:1 DCM: ETH solvent ratio X_2 (10:10 ml) optimized by response surface methodology are successful with enhanced gastroretentive effect and controlled release to elicit promising anthelminthic effect in the gastrointestinal tract.

Keywords: Microballoons, Mebendazole, Emulsion solvent diffusion, Response surface methodology.

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INTRODUCTION

Multiunit dosage forms represent controlled drug release systems which systematically deliver drug release at a pre-defined rate by which the drug's therapeutic effect can be increased with a lower dosing frequency. The gastrointestinal drug delivery is the most prominent drug delivery system for drugs to act locally in the stomach, short half-life, poor solubility at high pH, and narrow absorption window. Prolonged gastric residence time increases therapeutic efficacy, bioavailability, and target delivery. Various approaches of gastroretentive delivery such as high-density systems, mucoadhesive systems, raft forming systems, magnetic systems, low-density floating systems, and expanding systems have been investigated to extend the system's residence time in the upper part of the GIT [1].

Microballoons are the non-effervescent floating approach of gastroretentive drug delivery system. Microballoons are in a strict sense, spherical-shaped empty particles without core. These are free-flowing powders having a particle size <200 μm. These hollow microspheres are composed of proteins or synthetic polymers. The drug and polymers forms the outer shell of hollow microspheres. Microballoons are unique in possessing both multiple unit system properties and better floating buoyancy than other floating GRDDS due to their hollow space enclosed by outer shell of.microspheres [2].

The release of drug at desired rate and the gastric residence time of dosage form depend on the type of polymers, solvents, and plasticizers employed in preparation of microspheres. An optimized formulation of microballoons can be obtained by modulating the polymer and polymer-plasticizer concentrations. The polymers commonly used are polylactic acid, HPMC, cellulose acetate, and Eudragit polymers.

Mebendazole (MBZ) is a broad-spectrum benzimidazole anthelminthic drug prescribed for the treatment of intestinal infections of tapeworm, whipworm, hookworm, roundworm, and other nematode and trematode infections in humans. The antiparasitic action of mebendazole is due to degenerative alterations. It binds to colchicine sensitive site of tubulin and inhibits polymerization of microtubules [3,4]. MBZ is water insoluble with half-life of 2.5–5.5 h. However, its prolonged gastroretentive effect is expected to exhibit anthelmintic effect to treat intestinal infections. Hence, the present work is aimed at design gastroretentive polymeric low-density microballoons using emulsion solvent diffusion method. It shows only 10% absorption when administered orally and has extensive hepatic metabolism. Hence, gastric retention effervescent tablet formulations were developed to increase the bioavailability [5].

Eudragit S-100 is a methacrylate anionic polymer solubilizes at pH>7 hence helps in stabilization of formulation at gastric pH. Hydroxypropyl methylcellulose (HPMC) is low density and hydrophilic swellable polymer used for controlled release of drug [6-8]. PVA is added to the aqueous solvent which acts as emulsifying agent by decreasing the interfacial tension.

Response surface methodology is used in pharmaceutical research to study the effect of variables and their interaction effects on responses of formulations within few formulations. Optimization by response surface methodology using central composite design enables quick and efficient quantification and prediction of the effects of formulation changes on the considered critical responses [9,10].

MATERIALS AND METHODS

Materials

Mebendazole obtained as gift sample from Karnataka Antibiotics and Pharmaceuticals Ltd., Bengaluru. Eudragit S-100 and hydroxypropyl

methylcellulose cured from Merck Laboratories. Dichloromethane and polyvinyl alcohol were obtained from HiMedia Laboratories and ethanol from Loba Chemie Pvt. Ltd.

Methods

Preparation of mebendazole-loaded floating microballoons using response surface methodology

Gastroretentive microballoons containing 100 mg of mebendazole were prepared using emulsion solvent diffusion method. Drug and different percentages of polymers (HPMC and Eudragit S-100) shown in Table 1 were added to a mixture of organic solvents (DCM: ethanol) and sonicated for 15 min. The solution was added dropwise into 200 ml of 0.75% PVA aqueous solvent and stirred at 700 rpm by a mechanical stirrer with a three-blade propeller at 40°C for 2 h to allow the organic solvent to evaporate. The resulted microballoons were filtered, washed with distilled water, and dried at room temperature to complete the evaporation of water.

Multiple regression analysis was used to perform experimental analysis of the factorial design batches using Design-Expert® Software. The best-fitting mathematical model was selected based on the comparison of statistical parameters such as the standard deviation (SD), the multiple correlation coefficient (R^2), the adjusted multiple correlation coefficient (adjusted R^2), and the predicted residual sum of square (PRESS) (Table 2) [11].

Characterization of MBZ-loaded microballoons

Micromeritic properties

Angle of repose: Angle of repose is determined by fixed funnel method and calculated using the formula:

$$\theta = \text{Tan}^{-1} h/r \quad (1)$$

Where, h is height of pile and r is radius of pile.

Compressibility Index (CI): CI is calculated using the formula:

$$\text{Carr's index } (\%)=(T_d - B_d)\times 100/T_d \quad (2)$$

Hausner's ratio: It is an indirect index of powder flow. Calculated using the following equation:

$$\text{Hausner's ratio}=T_d/B_d \quad (3)$$

Where, T_d is tapped density and B_d bulk density.

Determination of particle size

Particle size was determined using optical microscope, eyepiece, and stage micrometer. Stage micrometer was mounted on the stage and eyepiece of microscope was calibrated by coinciding with stage micrometer scale. It was observed that the 6th division of eyepiece is equal to the 10th division of stage micrometer [12].

But, each division of stage micrometer=10 μ

Hence,

1 division of eye piece=100/6=16.66 μ

Yield of microballoons

The percentage yield of microballoons was calculated by dividing the dried weight of microspheres and actual weight of drug and polymers used to prepare microballoons.

$$\%\text{Yield} = \frac{\text{weight of dried microballoons}}{\text{weight of drug} + \text{weight of polymers}} \times 100$$

Drug entrapment efficiency

A 10 mg of hollow microspheres were dissolved in 10 ml of 1:1 mixture of DCM: ethanol, obtained clear solution was assayed spectrophotometrically for MBZ content at 291 nm using UV–visible spectrophotometer [13].

$$\%\text{ Entrapment Efficiency} = \frac{\text{Actual drug content}}{\text{Theoritical drug content}} \times 100$$

Buoyancy

A 100 mg of prepared microballoons were placed in 0.1 N HCL of pH-1.2 containing Tween 80 and stirred for 8 h at 100 rpm on magnetic stirrer. After 8 h, the floating and settled microballoons were filtered separately using a microporous filter paper and dried at 40°C. The fraction of dried microballoons was weighed separately. The buoyancy was determined by the following formula.

$$\%\text{ Buoyancy} = \frac{Wf}{Wf + Ws} \times 100$$

Where, W_f – Weight of floated microballoons and W_s – Weight of settled microballoons.

***In vitro* drug release studies**

The USP dissolution testing apparatus II (Paddle type) was used to determine the rate of drug release from formulations. The dissolution test was carried out by adding microballoons equivalent to 50 mg of drug in 900 ml of 0.1 N HCl of pH 1.2 at 37.5°C and 75 rpm. Aliquots of 5 ml were withdrawn at regular intervals for 12 h to maintain the sink condition, and the sample was replaced by its equivalent volume of fresh dissolution medium. The samples were analyzed spectrophotometrically at wavelength corresponding to mebendazole absorption maxima, that is, 291 nm [14].

Experimental design

Two-factor and two-level CCD was employed to optimize formulation. Based on the literature, percentage of polymer Eudragit S 100 in total

Table 1: Composition of MBZ-loaded microballoons prepared by central composite design

Formulation code	Percentage of Eudragit S 100 (X_1)		Amount of HPMC (mg)	Total amount of polymer (mg)	Solvent ratio (X_2) DCM: ETH	Volume of solvents (ml)	
	%	Mg				DCM	ETH
MBZ_1	50	500	500	1000	0.5:1	5	10
MBZ_2	100	1000	-	1000	0.5:1	5	10
MBZ_3	50	500	500	1000	1.5:1	15	10
MBZ_4	100	1000	-	1000	1.5:1	15	10
MBZ_5	39.644	396.447	603.553	1000	1:1	10	10
MBZ_6	110.355	1103.55	-	1000	1:1	10	10
MBZ_7	75	750	250	1000	0.2928:1	2.928	10
MBZ_8	75	750	250	1000	1.7071:1	17.071	10
MBZ_9	75	750	250	1000	1:1	10	10

Table 2: Levels of variables used for preparing MBZ-loaded low-density polymeric microballoons

Independent variable	Coded levels				
	−α	−1	0	+1	+α
Percentage of Eudragit S 100 (%) X_1	39.6447	50	75	100	110
Solvent ratio DCM: ethanol X_2	0.2928:1	05.:1	1:1	1.5:1	1.7071:1

polymer (X_1) and solvent ratio (DCM: ETH) was preferred as factors (independent variables). Each factor was put at high and low levels and designated as −1 and +1 as follows: % of Eudragit S 100, 50% (−1) and 100% (+1), solvent ratio (DCM: ETH) 0.5:1 (−1) and 1.5: 1 (+1). Yield (R1), buoyancy (R2), entrapment efficiency (R3), and CDR (R4) were used as responses (dependent variables).

Design-Expert Software (Version 13, Stat Ease Inc.) was employed for generation and evaluation of the CCD and constructed design layout with 13 experimental runs with four repeated runs. Hence, the nine experimental runs were performed and the obtained microballoons from each experimental batch were further evaluated for yield (R1), buoyancy (R2), entrapment efficiency (R3), and CDR (R4). All other process parameters were kept constant during the formulation of mebendazole-loaded microballoons. Response surface methodology was applied to study the effects of independent variables on responses to obtain the microballoons of mebendazole with enhanced retention in GI fluids and controlled release elicit very promising anthelmintic effect in the gastrointestinal tract with optimized values.

Statistical analysis

Using Design-Expert software, the effect of any factor on a specific response can be statistically analyzed (Version 13.Trial, Stat Ease Inc.). Multiple regression analysis was used in this study to investigate the effect of independent variables and their interactions on observed responses using polynomial models.

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2 \quad (4)$$

The Equation (4) represents a second-order polynomial model, where β_0 is an intercept defines the arithmetic mean response of all experimental runs. Coefficients with one factor (β_1 and β_2) describing main effects (X_1 and X_2) symbolizing the average result of change indicating one factor at a time from its low and high value. Coefficients with more than 1 factor term (β_3) describe interaction term (X_1X_2) indicates change in responses when two factors are simultaneously altered. The polynomial terms (β_4, β_5) are coefficients to study the non-linearity of the model. Conclusions are drawn from the polynomial model based on the magnitude and mathematical sign of the coefficients. A +ve sign indicates synergistic effect, whereas −ve sign indicates antagonistic effect. The best-fit model was selected based on statistical results from the Design-Expert. ANOVA for each response was performed to determine the significance of each factor parameter selected for the study using the p value. A response term with $p<0.05$ was considered to be statistically significant and for a response term with $p>0.05$ was considered to be statistically insignificant. The F-test and p values were also calculated using the software. The relationship between the factors and responses was further studied using contour plots and response surface plots. Response surface and contour plots were analyzed to elucidate the effect of the factors and their interactions on responses [15].

Numerical and graphical optimization

Numerical and graphical optimization technique using desirability approach was used to develop optimized formulation. After statistical analysis of appropriate models for individual responses, the simultaneous optimization of multiple responses was carried out using Design-Expert software to find a combination of factor levels that provide desired responses. For calculating desirability, required goals (minimum, maximum, target, or in range) were chosen for all response variables. Desirability plot was obtained with optimized conditions. Using the desirability plot and ramp solutions, an optimized formulation was selected [16].

Characterization of optimized formulation

Drug-excipient incompatibility studies

Compatibility of drug and polymer of optimized formulation was studied by performing FTIR and DSC.

FTIR of drug and mebendazole loaded microballoons

The functional groups of mebendazole were investigated by FTIR spectroscopy. Samples and KBr were taken in the ratio of 1:100 in a mortar and triturated. A transparent pellet of triturate was prepared by compressing at 10 Kg/cm^2 using hydraulic press. The pellet was placed in a sample holder and scanned at from 4000 cm-1 to 400 cm-1 in FT-IR spectrophotometer.

Differential scanning calorimetry

The thermal characteristics of pure drug and optimized formulation were determined using differential scanning calorimeter, Mettler Toledo, Switzerland. Approximately 10 mg of samples were taken into aluminum crucible and heat flow rate was 10°C/min and samples were heated under nitrogen atmosphere over the temperature range of 50–350°C temperature.

Phase contrast microscopy

Optimized formulation was examined under a high magnification optical microscope and spherical shape of formulation is compared with phase contrast microscopic images of pure mebendazole drug.

Kinetic models for the drug release

The kinetics of release from formulations was determined by finding the best fit of the release data to zero order, first order, matrix (Higuchi), and Korsmeyer–Peppas plots.

Zero-order equation

It describes the systems where the release rate is independent of the concentration of the dissolved species. The data are fitted into the zero-order equation.

$$Q=Q_0+K_0t$$

Q=Amount of drug released at a time "t"
Q_0=Amount of drug released initially (often considered zero)
K_0=Zero-order rate constant
Zero-order plot is derived from plotting the cumulative percent drug release versus time.

First-order equation

The first-order equation describes the release from systems in which the rate of dissolution is proportional to the concentration. All formulation dissolution data were plotted using the first-order equation, that is, the logarithm of the percent drug retained as a function of time.

$$\text{Log } C=\text{Log } C_0-K_1t/2.303$$

C=Amount of drug released at a time "t"
C_0=Amount of drug released initially
K_1=First-order rate constant

Higuchi equation

The Higuchi square root describes drug release from an insoluble matrix as a square root of a time-dependent process based on drug diffusion rate.

$$Q=Kt^{1/2}$$

K=Constant reflecting design parameters of the system
t=Time in hours

Higuchi plot is derived from plotting the cumulative percent drug release versus square root of time.

Korsmeyer–Peppas plot

To evaluate the mechanism of drug release, the dissolution data are fitted into the Korsmeyer–Peppas equation.

$$M_t/M^{\alpha}=K_tn$$

M_t/M^{α}=Fractional release of the drug
n=Release exponent indication of mechanism
K=Kinetic constant characteristics of the drug/polymer system

To determine the mechanism of drug release, log cumulative % drug release is plotted versus log time.

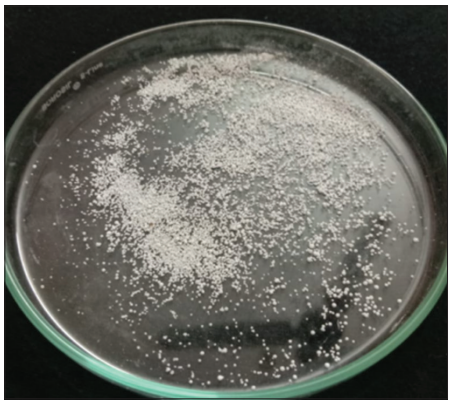
RESULTS AND DISCUSSION

In this study, low-density polymers and different solvent ratios were investigated for their ability to form successful mebendazole microballoons.

DCM and ethanol, the organic solvents used in the preparation, have low toxicity in comparison to many other solvents and have no harmful effects on the body because they evaporate during the process.

To control release of the drug, the polymers Eudragit S-100 and HPMC were chosen as hydrophobic and hydrophilic polymers, respectively. Microballoons were made using an emulsion solvent diffusion method which is more efficient compared to other solvent techniques.

When the polymer and drug solution mixture was added to the aqueous medium, ETH diffused from the droplets into the medium, causing coprecipitation of the polymer and drug on the outer surfaces of the droplets due to the higher solubility of ETH in water. Simultaneous diffusion of water within the spheres reduced the ETH concentration even further, and thus, the polymer precipitated, resulting in the formation of MBs. Due to its low water solubility, the remaining DCM diffused slowly within the droplet, which was surrounded by a film-like shell of coprecipitated polymer and drug as the central core. Water could not effectively invade the DCM-rich core due to its poor miscibility. As a result, DCM diffusion began late, after the initial solidification, and resulted in a central hollow structure. The polymer was pulled outward during solvent diffusion as a result of the solvents' dragging force, and thus, the central void space emerged. Because of the reduced internal pressure, the central cavity created by the solvents gradually filled with water**.** During the drying process, water escaped from the cavity, resulting in hollow microballoons of mebendazole, as shown in the figure.



Evaluation of mebendazole-loaded microballoons.

Micromeritic properties

Angle of repose

The flow property of the hollow microspheres can be studied by calculating the angle of repose (θ). From the data shown in Table 3, it was found that the obtained results were in the range of 24–32°, indicating that the obtained values were well within the limits for powder to have good-passable flow properties. This result clearly showed that the prepared hollow microspheres have reasonably good-passable flow property.

Compressibility index and Hausner's ratio:

The results of Carr's compressibility index and Hausner's ratio were mentioned in Table 3. The values of C.I were found to be in the range of 11.5–16.74%, the values of Hausner's ratio were in the range of 0.165–1.174.

The results of Hausner's ratio and Carr's index indicated that the prepared microballoons had good-fair flow properties.

Particle size

The particle size of all the prepared polymeric low-density microballoons of mebendazole was determined by optical microscopy and results are shown in Table 4. The particle size range of microballoons from 164.26±2.277 μm to 185.59±1.66 μm indicated that the present method used for production of microballoons is successful in ideal range of size in micrometers.

From the results, it was observed that solvent ratio affects the particle size, the formulations having 1:1 ratio had a larger size, this can be attributed to a greater chance for the polymer to precipitate as result of ETH evaporation droplets during formation of microballoons. At higher solvent ratio, where the concentration of DCM is higher than ethanol, large internal core is formed with outer precipitated polymer coat.

Experimental design

In response surface methodology, central composite design is the most commonly used method to determine the effect of independent

Table 3: Results of evaluation of micromeritic properties of microballoon formulations

Formulation code	Angle of repose (θ)	Hausner's Ratio	Carr's index (CI)
MBZ_1	27.021°	1.174	14.79
MBZ_2	31.38°	1.14	12.56
MBZ_3	27.021°	1.15	12.74
MBZ_4	32.00°	1.13	11.59
MBZ_5	28.146°	1.17	14.8
MBZ_6	23.025°	1.20	16.74
MBZ_7	29.24°	0.165	14.1
MBZ_8	24°	1.136	11.9
MBZ_9	29.24°	1.144	12.55

Table 4: Particle size of microballoon formulations of mebendazole

Formulation code	Particle size (μm)*
MBZ_1	183.26±1.884
MBZ_2	171.26±2.277
MBZ_3	164.26±1.906
MBZ_4	175.59±1.248
MBZ_5	185.59±1.66
MBZ_6	172.93±1.323
MBZ_7	172.26±2.172
MBZ_8	180.224±1.870
MBZ_9	181.26±1.692

*Mean±S.D, n=100

variable, their interaction, and quadratic effects from the data of responses that fit in suitable mathematical model (Linear or 2FI – two-factor interaction model or quadratic or cubic model) and to obtain the optimized formulation values.

Table 5 represents the results of responses of various batches of mebendazole-loaded microballoons designed by CCD, analyzed to determine the optimized formulation.

Statistical analysis of response

Statistically significant relationship between the independent and dependent variables was revealed by the ANOVA using Design-Expert software.

The best-fit model suggested was quadratic for all the responses considering the statistical parameters correlation coefficient (R^2), predicted R^2, adjusted R^2, adequate precision, and predicted residual sum of squares (PRESS) obtained, as shown in Table 6. In case of all responses, $p<0.001$ represents the quadratic model which is statistically significant. To be the significant model, the difference of adjusted and predicted R^2 values should be <2 or in case difference is >2 then the adequate precision value <4 indicates that the model is significant.

Effect of factors on % yield (R1)

The effect of variables can be explained based on contour, response surface, and coefficients of quadratic model shown in Fig. 1 and Equation 1, respectively.

Table 5: Responses (R_1, R_2, R_3, and R_4) of all the formulation batches designed by CCD

Formulation code	% Yield (R_1)	% Buoyancy (R_2)	%EE (R_3)	%CDR (R_4)
MBZ_1	81.09	83.4	74.01	75.8
MBZ_2	81.90	78.9	77.98	62.9
MBZ_3	81.36	84.1	77.83	82.97
MBZ_4	86.63	81.5	79.17	69.9
MBZ_5	80.81	83.9	79.82	82.09
MBZ_6	83.36	81.99	88.99	68.07
MBZ_7	80.63	79.1	75.91	70.04
MBZ_8	83.90	79.4	76.44	80.91
MBZ_9	81	86.4	82.01	79.99

The mathematical modeling of microballoons yield was represented by the following equation.

$$R1=81+1.21078\ X_1+1.20306\ X_2+1.115\ X_1X_2+0.685\ X_1^2+0.775\ X_2^2 \quad (5)$$

Where, X_1 is the percentage of Eudragit S 100 and X_2 is the solvent ratio. The positive value of the factors represents an effect that favors optimization. It can be seen that an increase of variables increases yield of microballoons. The interaction between factors X_1 and X_2 is synergistic and statistically significant. The synergistic effect was observed for X_1^2 and X_2^2.

From the results of ANOVA test as shown in Table 7, the model F=34.08 implies that the model is significant and both the Eudragit concentration and solvent ratio had significant effect on yield of microballoons ($p<0.05$).

Buoyancy (R_2)

The buoyancy of the microballoons was studied for up to 8 h. Table 5 shows the values of buoyancy of drug-loaded microballoons. Buoyancy of all formations was found to be in the range of 78.9–86.4 from table with MBZ_2 and MBZ_4 showing the lowest and highest values, respectively.

Effect of variables on buoyancy (R_2)

The effect of independent factors percentage of Eudragit polymer (X_1) and solvent ratio (X_2) on buoyancy was evaluated by response surface methodology.

The mathematical modeling of microballoons yield was represented by the following equation:

$$R_2=86.4-1.28764\ X_1+0.498492\ X_2+0.6\ X_1X_2-1.51063\ X_1^2-3.22313\ X_2^2 \quad (6)$$

From the contour, 3D response surface graphs and polynomial equation the effect of variables are explained. The negative value of coefficient of percentage of Eudragit S-100 indicates that with the increase of percentage of Eudragit polymer decreases the buoyancy which may be due to the decrease of percentage of the other polymer HPMC that is hydrophilic and responsible for swelling and buoyancy. The positive effect of solvent ratio (X_2) indicates that buoyancy increases with increase of DCM.

Table 6: Suggested model based on statistical parameters

Response	Suggested model	p-value	R^2	Adjusted R^2	Predicted R^2	Adequate precision	PRESS
R1	Quadratic	0.0024	0.9605	0.9324	0.7194	17.3476	10.19
R2	Quadratic	<0.0001	0.9527	0.9190	0.6639	12.6725	34.35
R3	Quadratic	0.0049	0.8401	0.7259	−0.1371	9.1659	199.92
R4	Quadratic	0.0008	0.9671	0.9436	0.7662	18.183	115.73

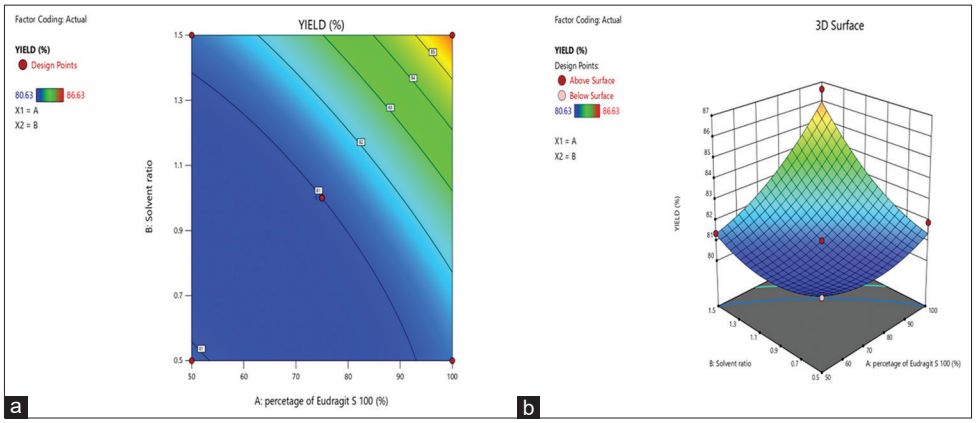


Fig. 1: (a) Contour plots of yield (R_1) and (b) 3D response surface plot of yield (R_1)

The interaction between factors X_1 and X_2 showed a synergistic effect but statistically insignificant. Antagonistic effect was observed with the terms X_1^2 and X_2^2.

However, from the results of ANOVA test shown in Table 7, the model F=28.22 implies that the model is significant. However, only the percentage of polymer showed a significant effect as the coefficient of variable X_1 is greater than the variable X_2 and also P value of X_1 is <0.05.

Entrapment efficiency (R_3)

Entrapment efficiency of mebendazole-loaded low-density microballoons is shown in Table 6. Entrapment efficiency of all formulations was in the range of 74.01–88.99% with MBZ_1 and MBZ_7 showing the lowest and highest values, respectively. High drug loading enables more effective delivery of high doses of drug with less amount of carrier.

Table 7: Summary of ANOVA for response variables

Response	Source	F-value	p-value
% yield (R1)	Model	34.08	<0.0001 (s)
	X_1-percetage of Eudragit S-100	57.31	0.0001 (s)
	X_2-solvent ratio	56.59	0.0001 (s)
	X_1X_2	24.30	0.0017 (s)
	X_1^2	15.95	0.0052 (s)
	X_2^2	20.42	0.0027 (s)
% buoyancy (R2)	Model	28.22	0.0002 (s)
	X_1-percetage of Eudragit S-100	19.22	0.0032 (s)
	X_2-solvent ratio	2.88	0.1334 (NS)
	X_1X_2	2.09	0.1918 (NS)
	X_1^2	23.01	0.0020 (s)
	X_2^2	104.74	<0.0001 (s)
% entrapment efficiency	Model	7.35	0.0104 (s)
	X_1-percetage of Eudragit S-100	10.40	0.0146 (s)
	X_2-solvent ratio	1.03	0.3434 (NS)
	X_1X_2	0.4306	0.5327 (NS)
	X_1^2	0.3306	0.5833 (NS)
	X_2^2	23.43	0.0019 (s)
% CDR	Model	41.18	<0.0001 (s)
	X_1-percetage of Eudragit S-100	112.77	<0.0001 (s)
	X_2-solvent ratio	46.93	0.0002 (s)
	X_1X_2	0.0031	0.9571 (NS)
	X_1^2	27.86	0.0012 (s)
	X_2^2	24.37	0.0017 (s)

R1, R2, R3, and R4=% yield, buoyancy, entrapment efficiency, and CDR, respectively. X_1 and X_2=% of Eudragit S 100 and solvent ratio (DCM: ETH)

Effect of variables on Entrapment Efficiency (R_3)

Contour, response surface, and coefficients of quadratic model are given in Figs. 2-4 and Equation 3, respectively.

The mathematical modeling of microballoons yield was represented by the following equation:

$$R_3=82.01+2.28479\ X_1+0.719942\ X_2-0.6575\ X_1X_2+0.436875\ X_1^2-3.67813\ X_2^2 \quad (7)$$

From the contour plots, 3D response surface plots, and polynomial equation, it can be seen that entrapment efficiency increases with the increase of percentage of Eudragit S-100 and DCM. Increased entrapment efficiency with increase of percentage of ES-100 might be attributed due to the increase of viscosity of solution. From the previous equation, it was observed that the coefficient of solvent ratio is much lower than the coefficient of percentage of Eudragit S-100; hence, the solvent ratio has less effect on entrapment efficiency.

The interaction terms X_1 and X_2 showed an antagonistic effect and statistically insignificant. It was observed that X_1^2 showed synergistic effect and X_2^2 showed antagonistic effect.

From the ANOVA test results in Table 7, the model F=7.35 implies that the model is significant. However, the effect of solvent ratio was statistically not significant (p>0.01).

Drug release (R_4)

Drug release of all the formulations was evaluated for 12 h. The percentage cumulative drug release was calculated and the results are shown in Table 8. The % CDR ranges from 62.9% to 82.97%.

Effect of variables on drug release (R_4)

The drug release data values were ranging from 62.9% to 82.97%. The wide variation among the formulations proves that selected independent variables strongly influence the drug release behavior. MBZ_5 attained maximum drug release behavior.

The mathematical modeling of drug release from MBs is represented by the following equation:

$$R_4=79.99-5.72466\ X_1+3.69281\ X_2-0.0425\ X_1X_2-3.05125\ X_1^2-2.85375\ X_2^2 \quad (8)$$

From the polynomial equation, contour plots, and 3D plots, we can deduce that the factors percentage of Eudragit S-100 and solvent ratio significantly affect the drug release. The negative coefficient value of polymer indicates with increase of ES 100 polymer concentration drug release decreases, it may be due to hydrophobic and insoluble nature of ES 100 polymer which retards the drug release [12]. The positive coefficient values indicate that with increase of solvent ratio, the drug release increases. Increase of DCM content in the organic mixture phase led to a significant increase in the drug release. This might be attributed

Table 8: *In vitro* dissolution data of mebendazole microballoon formulations

Time (h)	% drug release (n=3 ± s.d)								
	MBZ1	MBZ2	MBZ3	MBZ4	MBZ5	MBZ6	MBZ7	MBZ8	MBZ9
0	0	0	0	0	0	0	0	0	0
0.5	1.75	0.1	4.62	2.1	3.6	1.19	1.59	3.01	2.9
1	9.4	4	10.99	6.98	10.98	6.56	7.86	9.07	8.59
2	12.63	7.98	17.03	10.01	16.87	9.09	9.99	15.9	13.78
3	19.88	15.97	28.86	17.02	25.76	16.75	17.63	27.81	24.89
4	26.79	24.5	31.98	26.9	30.45	25.05	25.9	30	27.09
5	39.5	30.9	44.09	33.05	42.97	32.09	36.03	42.33	39.07
6	47.8	35.4	51.99	37.04	51.86	36.99	40.11	49.87	48.06
8	57.03	46.72	79.09	49.01	73.07	48.93	51.23	71.77	67.46
10	65.9	53.5	80.01	56.01	78.78	55.05	59.7	78.9	77.06
12	75.8	62.9	82.97	69.9	82.09	68.07	70.04	80.91	79.99

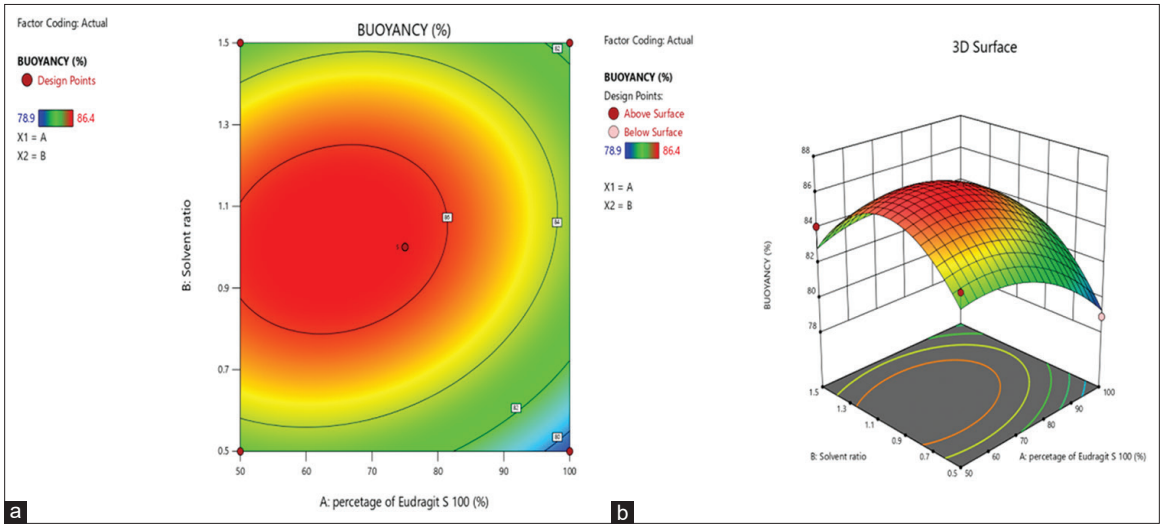


Fig. 2: (a) Contour plots of buoyancy (R_2) and (b) 3D response surface plot of buoyancy (R_2)

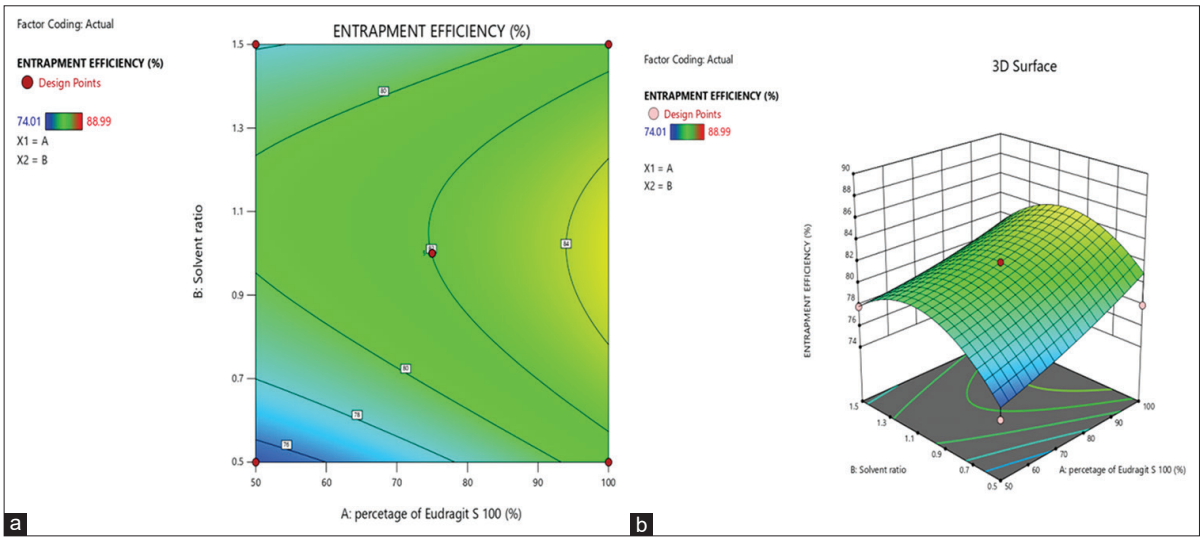


Fig. 3: (a) Contour plots of entrapment efficiency (R_3) and (b) 3D response surface plot of entrapment efficiency (R_3)

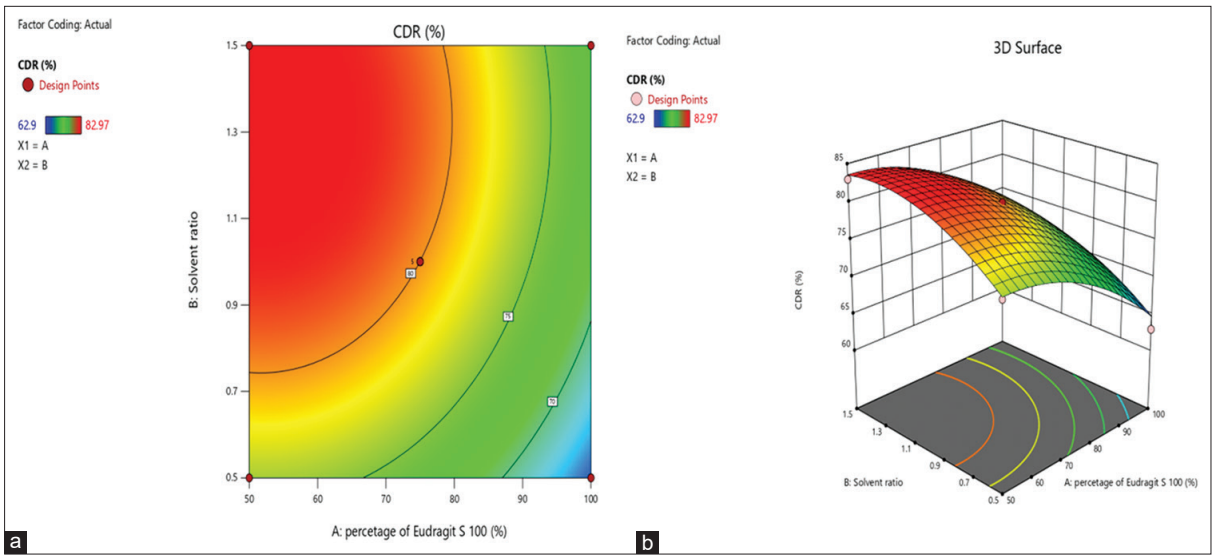


Fig. 4: (a) Contour plots of % CDR (Y_4) and (b) 3D response surface plot of % CDR (Y_4)

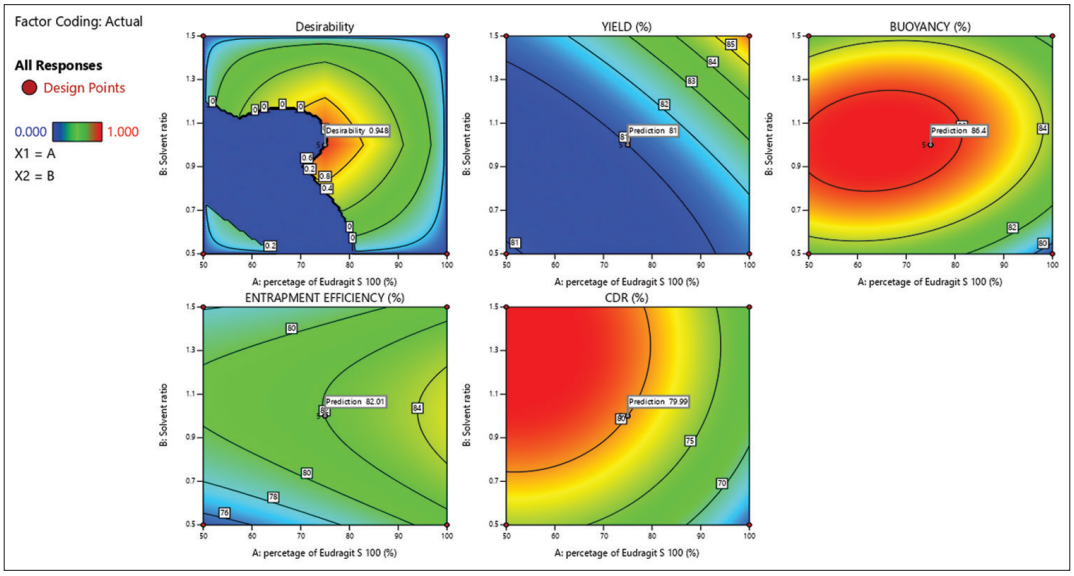


Fig. 5: Contour plots obtained by desirability approach for optimized formulation

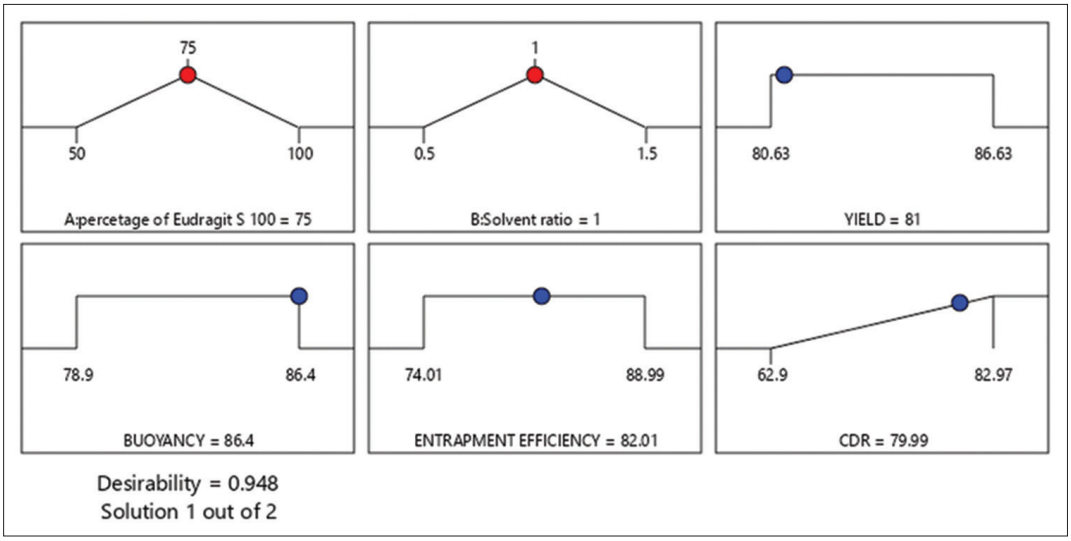


Fig. 6: Ramp solutions for optimization of mebendazole-loaded microballoons

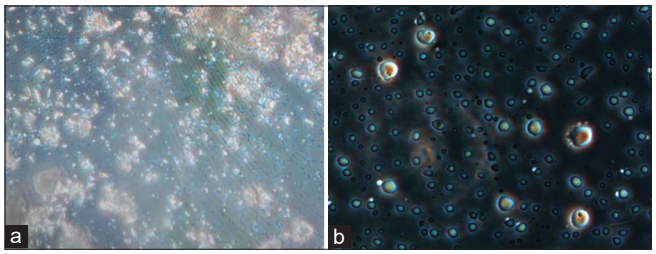


Fig. 7: Phase contrast microscopic images (a) pure mebendazole and (b) optimized formulation MBZ_9

to a shorter distance traveled by the drug through the polymer matrix due to presence of large pores generated from DCM evaporation.

The interaction terms X_1 and X_2 showed an antagonistic effect but statistically insignificant. Synergistic effect was shown by X_1^2 and X_2^2.

From the ANOVA test results in Table 7, the model F-value of 41.18 implies that the model is significant. In this case, the factors percentage of ES 100 (X1) and solvent ratio (X2) has $p<0.05$ which indicates that the model terms are significant.

Numerical optimization

Solution was obtained by the software from two different sets with highest desirability (0.948) to develop an optimized formulation for mebendazole-loaded microballoons with desired characteristics, as shown in Figs. 5 and 6. As per the goals set, actual values are compared with the predicted values obtained from the desirability function and formulation (MBZ_9) was found to be optimized formulation.

Characterization of optimized formulation

Phase contrast microscopy

The phase contrast microscopy images of pure drug mebendazole and promising microballoon formulation MBZ_9 are represented in Fig. 7a and, respectively. The spherical and hollow nature of the formed microballoons in Fig. 7b revealed smooth and spherical-shaped microballoons.

FTIR spectroscopy

FTIR spectrum of pure mebendazole and optimized MBZ_9 formulation is obtained, as shown in Fig. 8a and b. From the above FTIR studies, it was found that stretching peaks are within the actual ranges which are represented in Table 9. Hence, from the data, it was revealed that there is no interaction between polymers and drug.

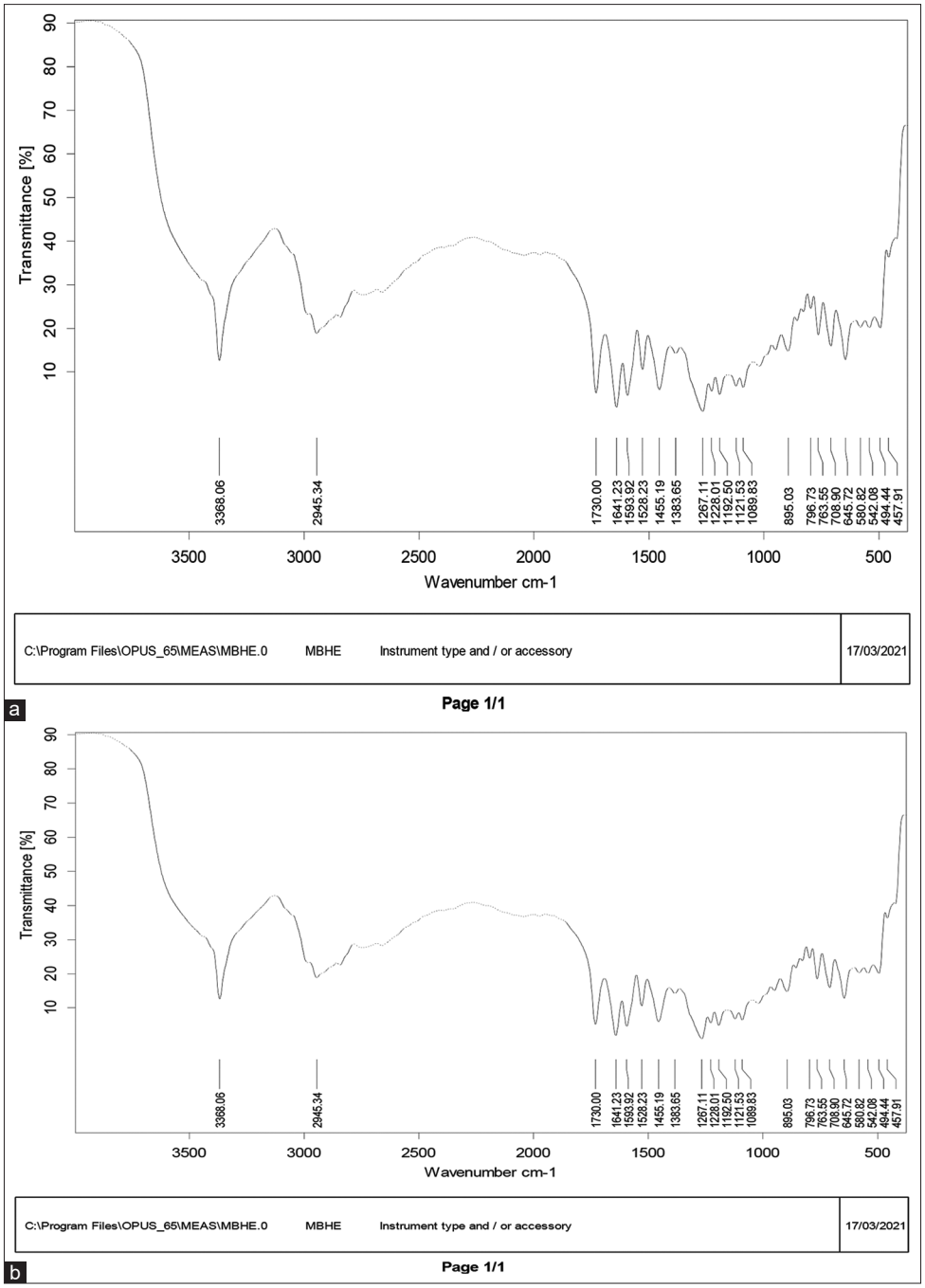


Fig. 8: IR spectrum of (a) pure mebendazole and (b) optimized formulation MBZ_9

Table 9: Characteristic peaks of pure drug and optimized formulation (MBZ_9)

Functional group	Peak positions in pure drug (cm^{-1})	Peak positions in optimized formulation (cm^{-1})
N-H	3367.44	3368.06
CH_3 stretching	2845.57	2945.34
Amide I band	1729.55	1730.00
Benzoyl (C=O)	1640.61	1641.23
C=C	1593.30	1593.92
Amide II band C=N	1527.87	1528.23
Amide III band C-N	1265.38	1267.11
Amide IV band C-N	1227.34	1228.01
CH_2 wagging	1192.36	1192.50
C-O	1088.67	1089.63

DSC

The DSC thermogram of pure drug and mebendazole-loaded hollow microballoon formulation is shown in Fig. 9. Results of pure mebendazole present a wide endothermic peak at 256.68°C followed by a final peak at 315.4°C corresponding to the melting point of mebendazole (288.5°C) and in thermogram of optimized formulation, small peaks were observed at 208.29°C followed by a final peak at 261.64°C. Thus, there was not a significant shift in endothermic peak of drug as that obtained from individual drug sample, it can be concluded that there was no interaction occurred between the excipients and drug mebendazole. Thus, mebendazole was found to be compatible with the selected excipients.

Drug release Kinetics of optimized formulation

The *in vitro* release studies data were fitted into various mathematical models to determine the best-fit model. The results of the best-fit model

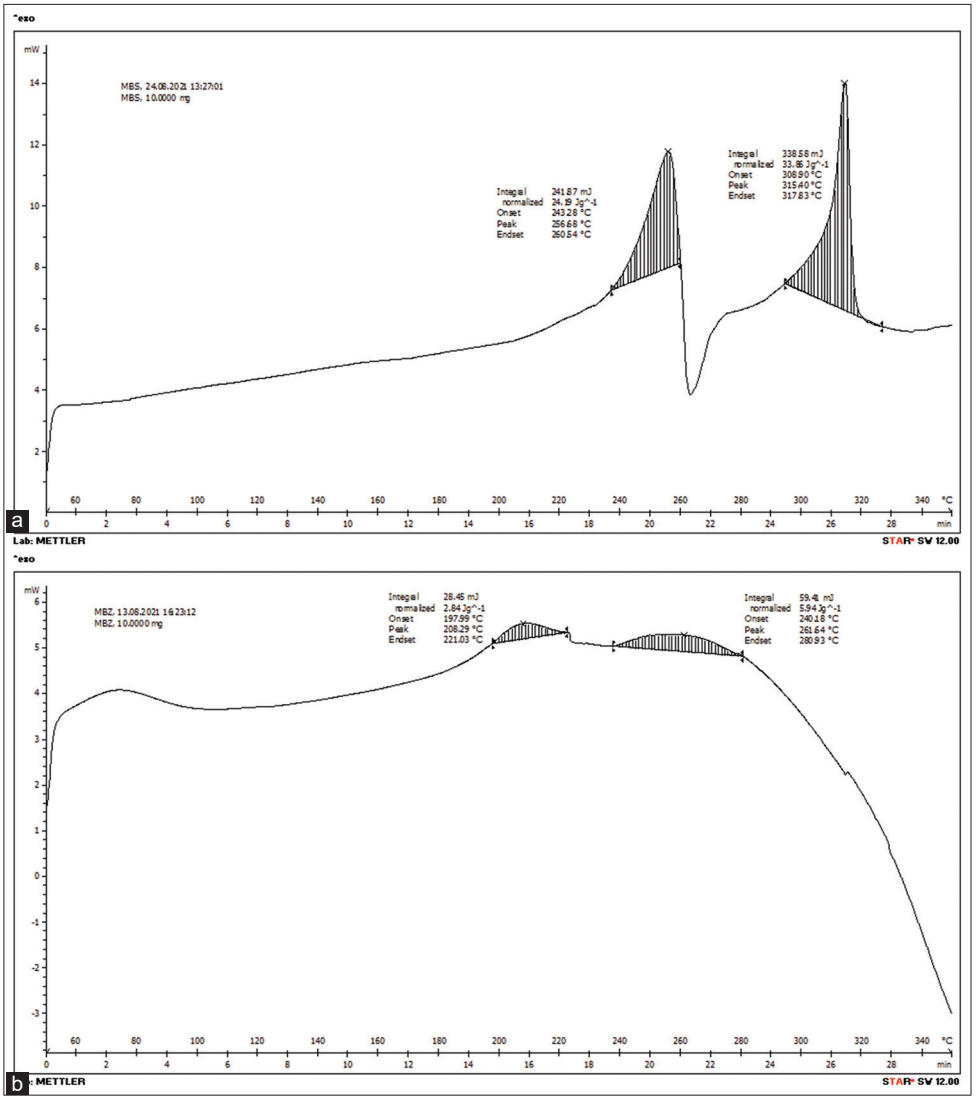


Fig. 9: DSC thermograms of (a) pure mebendazole and (b) optimized formulation MBZ_9

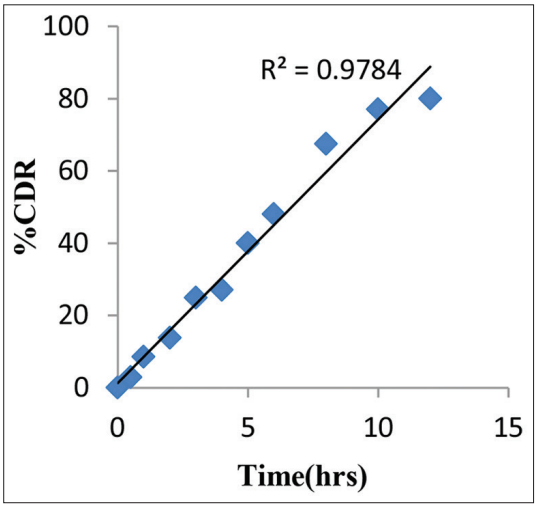


Fig. 10: Zero-order plot of MBZ_9

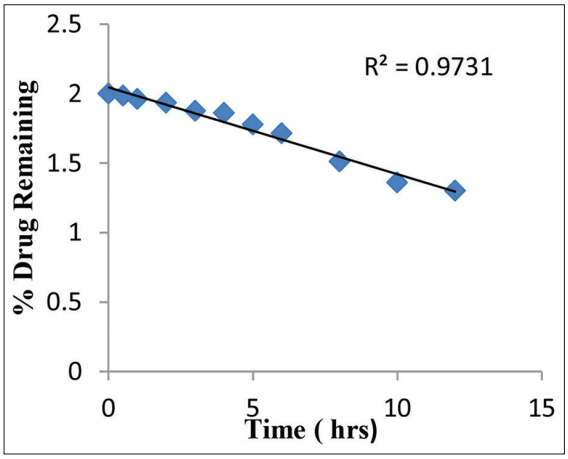


Fig. 11: First-order plot of MBZ_9

data are given in Table 10 and are given in Figs. 10-13. As per the plots and coefficient of R^2 values given in Table 10, optimized formulation is following the mixed mechanisms of zero and first order. The mechanism of drug release is diffusion as per R^2=0.905 in Higuchi model.

Table 10: Regression coefficients of optimized formulation MBZ_9

Release kinetic model	Zero order	First order	Higuchi	Korsmeyer–Peppas
R^2 value	0.978	0.973	0.905	0.880

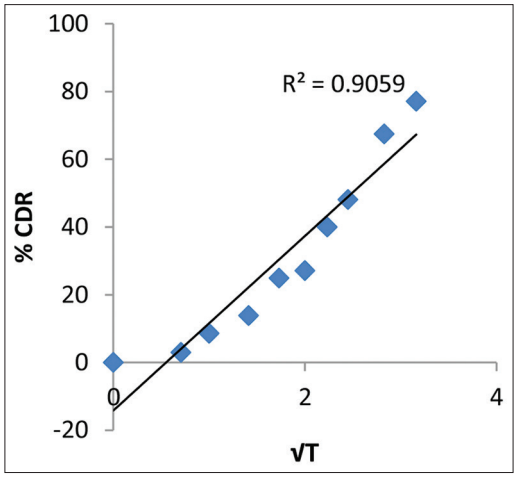


Fig. 12: Higuchi plot of MBZ_9

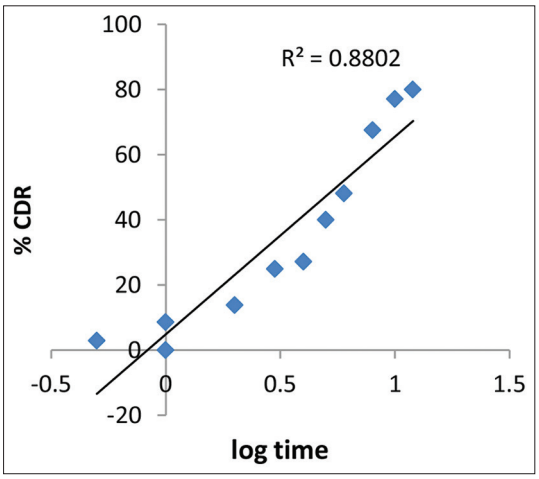


Fig. 13: Korsmeyer–Peppas plot of MBZ_9

CONCLUSION

Microballoons of mebendazole produced with 75% Eudragit S-100 polymer X_1 (750 mg), 25% of HPMC polymer, and 1:1 DCM: ETH solvent ratio X_2 (10:10 ml) optimized by response surface methodology are successful with enhanced retention in GI fluids with buoyancy of 8 h and controlled release for 12 h to elicit very promising anthelminthic effect in the gastrointestinal tract.

CONFLICTS OF INTEREST

The author, Dr. B. Jeevana Jyothi, is responsible for design of this novel work and preparation of manuscript and Ms. Sailaja PB has performed all the experiments involved in the present research work.

AUTHORS' CONTRIBUTIONS

The authors have no conflicts of interest

AUTHORS' FUNDING

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