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BIOAVAILABILITY ENHANCEMENT AND OPTIMIZATION OF TINIDAZOLE LOADED MICROSPONGES USING BOX- BEHNKEN DESIGN

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Abstract

The majority of available drug formulations are designed for oral administration, despite facing various pharmaceutical challenges. This is because oral delivery is the most convenient method, even though it requires careful dosage optimization to minimize toxic effects and dosing frequency. Therefore, our study aimed to develop gastro-retentive microsponges containing Tinidazole. These microsponges were fabricated and optimized using box- behnken design. The microsponges were formulated using the different ratios of the drug and Eudragit, with the goal of improving Tinidazole's absorption in the stomach, prolonging its retention time, ensuring sustained pharmacological activity and thus increased bioavailability in the GIT.

1. INTRODUCTION

Oral administration of drugs is considered the most convenient and preferred method for delivering drugs into the systemic circulation of the body. There has been a growing curiosity in the pharmaceutical business in terms of oral drug delivery systems. This interest stems from the desire to maximize the therapeutic benefits of drugs, including ease of dosing, patient compliance, and flexibility in drug formulation. Some types of medications are simply absorbed through the digestive system, but they have a small shelf-life, which leads to fast clearing from body. As a result, regular dosing of these drugs is necessary to achieve the desired therapeutic effects.[1] A microsponge is an innovative drug delivery device composed of microparticles with extremely porous microsponges and their size ranging from 5 to 300 micrometers in diameter. This system may be developed for numerous uses, includes oral and topical administration, delivering both systemic as well as limited effects. In the context of oral application, floating microsponges give various advantages. They enable effective medication targeting, extend drug release, decreasing toxicity and allergic responses. They also possess more drug loading capacity, feature self-sterilization qualities, are cheap, increase drug solubility and bioavailability, and employ biocompatible and cost-efficient polymers. Additionally, they promote patient quality of life and compliance. These microsponges are commonly manufactured using polymers like polyethyl acrylate), methyl methacrylate, and trimethyl ammonium-ethyl methacrylate chloride, in particular ratios, generating cationic polymers known as Eudragit RL (EGT). The presence of quaternary ammonium groups in these polymers influences their hydrophilicity, with a larger number of quaternary ammonium groups leading to enhanced hydrophilicity. Eudragit RL polymers, particularly Eudragit RL 100, are among the few that possess these hydrophilic groups in their chemical structures. Consequently, Eudragit RL polymers are practically insoluble in stomach acid but are permeable, allowing them to expand and generate a matrix structure irrespective of the pH of the surrounding environment. However, Eudragit RL 100, due to its low density, enables the microsponge to float on the surface of stomach gastric fluid.

These properties make Eudragit RS polymers good for generating sustained-release, floating matrix-type microsponge compositions.[2]

2. MATERIAL AND METHODS

2.1 Material

Tinidazole was obtained as a gift sample from Anant Pharmaceuticals Pvt. Ltd. Polyvinyl Alcohol, Eudragit RL100, Dibutyl phthalate was obtained From CDH Private Limited.

2.2 Methods

2.2.1 Solubility Studies

Tinidazole's solubility study was tested in a variety of solvents, including ethanol, dichloromethane, chloroform, phosphate buffer with a pH of 7.4, and filtered water. A certain amount of the drug was transferred to a flask holding 100ml of the solvent. To improve mixing, the flask was put on an orbital shaker. The tinidazole-containing solution was then filtered using Whatman filter paper and evaluated with a UV spectrophotometer.

2.2.2 Preparation of Calibration curve of Tinidazole

A sample weighing 100 mg was put into a 200 ml volumetric flask, then add 50 ml of ethanol. It is mixed with 10 ml ethanol under sonication for 10 mins. This resulted in a concentration of 1000 µg/ml, and a stock solution was created. 1 ml of the sample from the stock solution was extracted and put into another volumetric flask. Ethanol was then added to attain the appropriate volume in the flask. The absorbance of the solution was measured at 278 nm using a UV spectrophotometer against a blank.

2.2.3 Preparation of Tinidazole Microsponge:

Tinidazole microsponges are created by utilising the quasi-emulsion solvent diffusion method. For the internal phase, Tinidazole was dissolved in a mixture of dichloromethane and ethanol (in a 1:1 ratio) with a total volume of 20 ml. This solution contained both the medication and the polymer (Eudragit RL 100), and 0.5 ml of dibutyl phthalate was added as a plasticizer. The exterior phase comprised of 100 mg of polyvinyl alcohol (PVA) dissolved in water. The exterior phase was placed in a vessel equipped with a propeller stirrer, revolving at varied speeds. The internal phase was gently introduced to the external phase during mixing. The agitation phase, which lasted up to 30 minutes, enabled for the production of microsponges. Stirring was continued for a further 8 hours to generate the necessary stiff microsponges. After 8 hours, the stirring was stopped, and the stiff microsponges were filtered using Whatman filter paper (0.45 µm). The microsponges were then rinsed with distilled water and dried at room temperature.[3]

3. OPTIMIZATION OF TINIDAZOLE LOADED MICROSPONGES BY DESIGN OF EXPERT SOFTWARE (VERSİON 12.0.3.0)

The design was implemented using Design-Expert® software (trial version 12.0.3.0, Stat-Ease), and a total of 15 runs were created. For the final optimisation of floating Tinidazole microsponges, a response surface approach, three-factor, three-level Box-Behnken design[4] was used. The drug and polymer ratio, were taken as independent factors, whereas, % entrapment efficiency, % buoyancy, % Yield and Particle Size

were considered as dependent responses. The factors, levels and summary are given in Table 1 and 2:

Table 1: Factors and Their Levels Used in Box–Behnken Design

Factors	Lower Level (-1) (mg)	Upper Level (+1) (mg)
A	400	600
B	50	100
C	50	100

A = Eudragit RL100 (mg), B = Tinidazole TNZ (mg), C = Polyvinyl Alcohol PVA (mg)

Table 2: Summary by Design of Expert Software (version 12.0.3.0)

Study Type	Response Surface
Design Type	Box – Behnken
Sub Type	Randomized
Runs	15
Design Model	Quadratic

4. CHARACTERIZATION OF MICROSPONGES

4.1 Particle Size

The size of the microsponges were assessed using the dynamic light scattering (DLS) approach and average particle size was reported.[7]

4.2 Surface Morphology

SEM (scanning electron microscopy) was utilised to assess the surface morphology of the newly produced TNZ-loaded microsponges.

4.3 Drug Entrapment Efficiency

Tinidazole-loaded microsponges weighing 10 mg were, crushed, and vortexes with 5 mL ethanol. The sample was centrifuged by centrifugation machine at 2000 rpm for 10 minutes following dilution with methanol, filtered, and analysed by UV spectrophotometer at 278 nm. The amount of drug practically entrapped was divided by the overall amount of drug to ascertain entrapment efficiency.[5].

4.4 In-vitro Drug Release

The release of Tinidazole from microsponge has been studied via a USP type I apparatus in a pH 7.4 phosphate buffer as the dissolving medium, with a volume of 900 ml. A sample of microsponge containing 100 mg of Tinidazole has been laid in the basket. The experiment took place at a constant speed of 52 rpm at a temperature of 37± 0.5 °C. At defined various time intervals, 5 ml aliquots were removed and replaced with freshly prepared dissolving medium. The concentration of the released drug at different time intervals was measured by measuring its absorbance at 278 nm using a Double beam UV spectrophotometer against a blank. The full inquiry was done in duplicate.[6]

5. RESULT AND DISCUSSION

5.1 Solubility

The solubility was tested in Dichloromethane and Ethanol using UV spectroscopy at 278 nm wavelength. The maximum solubility was reported in dichloromethane.

5.2 Particle Size

The average particle size of the microsponges were determined using dynamic light scattering (DLS) technique. The mean sizes of these microsponges were reported as 244.2 µm minimum, while 296.3 µm maximum value.

5.3 Drug Entrapment Efficiency

The drug entrapment efficiency ranges from 72.25 to 97.8% for all microsponges, with 97.8% being the greatest loading efficiency.

All the reported results by Box-behnken design are mentioned in Table No.3:

Table 3: Reported result of TNZ loaded Microsponges using Box–behnken design

Run	Factor 1	Factor 2	Factor 3	Result			
	A: Eudragit RL100	B: TNZ	C: PVA	Particle Size	EE%	DR%	% Yield
	(mg)	(mg)	(mg)	(µm)	(%)	(%)	(%)
1.	400	50	75	244.25	72.35	92	71
2.	400	75	50	249.36	83.25	89	82.65
3.	400	100	75	259.11	82.14	81	84.57
4.	400	75	100	256.12	93.5	84	92
5.	500	75	75	265.41	72.15	77	74
6.	500	50	50	279.15	73.5	72.5	78
7.	500	100	100	261	84.15	79	88
8.	500	75	75	276.14	87.6	73.8	88.96
9.	500	50	100	287.25	89.6	70	88.97
10.	500	100	50	271.25	92.5	74	97.8
11.	500	75	75	283.63	92.4	71	97.9
12.	600	50	75	288	76.4	69	82
13.	600	100	75	293.25	92.5	65	88.99
14.	600	75	100	296.31	97.5	61	90.25
15.	600	75	50	291.21	93.4	69	98

Total 15 runs were given by Box-behnken design software. Three factors were taken as variables. Results were reported as particle size, entrapment efficiency (EE), and drug release (DR) and % yield. The **Run 14** was selected best run by software. Run 14 suggests that the particle size should be maximum for microsponge, so that it cannot be pass through the pylorus of GIT. The EE and % yield was reported as more than 90%, suggests that maximum drug has been entrapped with good yield.

5.4 In vitro Drug Release

Tinidazole-loaded microsponges were put through an in-vitro dissolution test, and the microsponges released the drug up to 14 hours, which were compared with the marketed preparation of tinidazole. The amount of drug release increased with respect of time, which suggests that drug will remain in GIT for longer period of time with continuous release. The drug release graph is shown in Fig1.

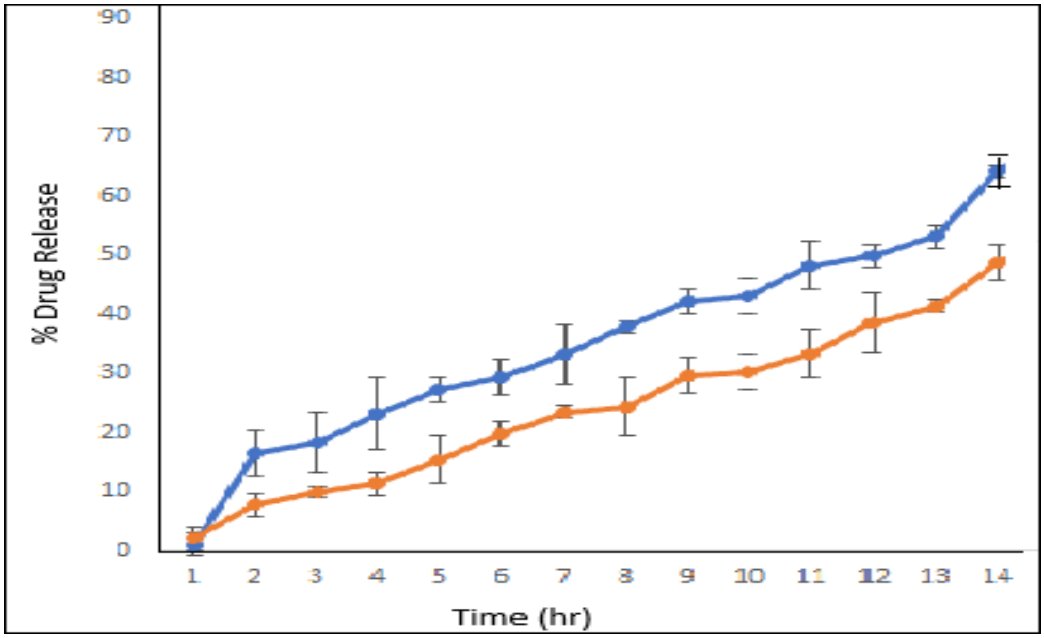


Figure 1: *In Vitro* Drug Release of Tinidazole Microsponge

5.5 Percentage Yield

The percentage yield was reported as more than 90%, suggests that formulation consists excellent drug amount.

6. MODEL ANALYSIS

The following model analysis was done by software. It was found that it is significant.

Source	Sum Of Squares	df	Mean Square	F- Value	p-Value	
Model	**3584.69**	**9**	**398.3**	**5.62**	**0.0359**	**Significant**
A-EUDRAGIT RL100	3197.2	1	3197.2	45.13	0.0011	
B-TNZ	80.65	1	80.65	1.14	0.3348	
C-PVA	8.1	1	8.1	0.1143	0.749	
AB	18.75	1	18.75	0.2646	0.6289	
AC	41.02	1	41.02	0.579	0.481	
BC	170.56	1	170.56	2.41	0.1815	
A^2	25.48	1	25.48	0.3597	0.5748	
B^2	19.1	1	19.1	0.2696	0.6257	
C^2	20.18	1	20.18	0.2848	0.6164	
Residual	354.25	5	70.85			
Lack of Fit	7.02	3	2.34	0.0135	0.9972	Not Significant
Pure Error	347.24	2	173.62			
Cor Total	3938.95	14				
Source	Sum Of Squares	df	Mean Square	F- Value	p-Value	
Model	957.51	9	106.39	7.01	0.0225	**significant**
A-EUDRAGIT RL100	101.96	1	101.96	6.72	0.0487	
B-TNZ	748.84	1	748.84	49.36	0.0009	
C-PVA	2.71	1	2.71	0.1789	0.6899	
AB	4.31	1	4.31	0.2838	0.617	

AC	3.78	1	3.78	0.2494	0.6387	
BC	0.3906	1	0.3906	0.0257	0.8788	
A²	8.34	1	8.34	0.5498	0.4918	
B²	81.81	1	81.81	5.39	0.0679	
C²	0.1918	1	0.1918	0.0126	0.9148	
Residual	75.85	5	15.17			
Lack of Fit	60.65	3	20.22	2.66	0.285	Not Significant
Pure Error	15.2	2	7.6			
Cor Total	1033.36	14				

Fit statistics have given adjusted and predicted values in close range

Std. Dev.	8.42		**R^2**	**R^2**	0.9101
Mean	273.43		**Adjusted R^2**	**Adjusted R^2**	0.7482
C.V. %	3.08		**Predicted R^2**	**Predicted R^2**	0.7732
			Adeq Precision	**Adeq Precision**	7.5853

Std. Dev.	3.89		**R^2**	**R^2**	0.9266
Mean	85.53		**Adjusted R^2**	**Adjusted R^2**	0.7945
C.V. %	4.55		**Predicted R^2**	**Predicted R^2**	0.0278
			Adeq Precision	**Adeq Precision**	8.3297

7. MODEL GRAPHS

7.1 Particle Size

The model graphs have shown effect on particle size by varying the ratio of A and B variable. It is shown in Fig 2.

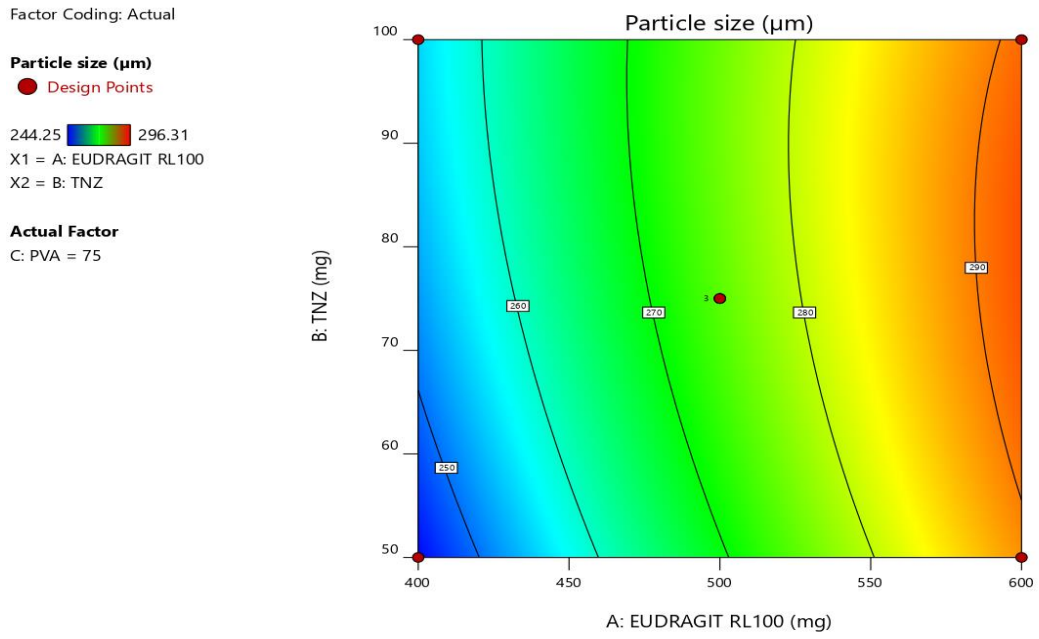


Figure 2: Effect on Particle Size by Varying the Ratio of A and B Variable

The values for predicted vs actual were reported by software. It is shown in Fig.3. it was found in close proximity.

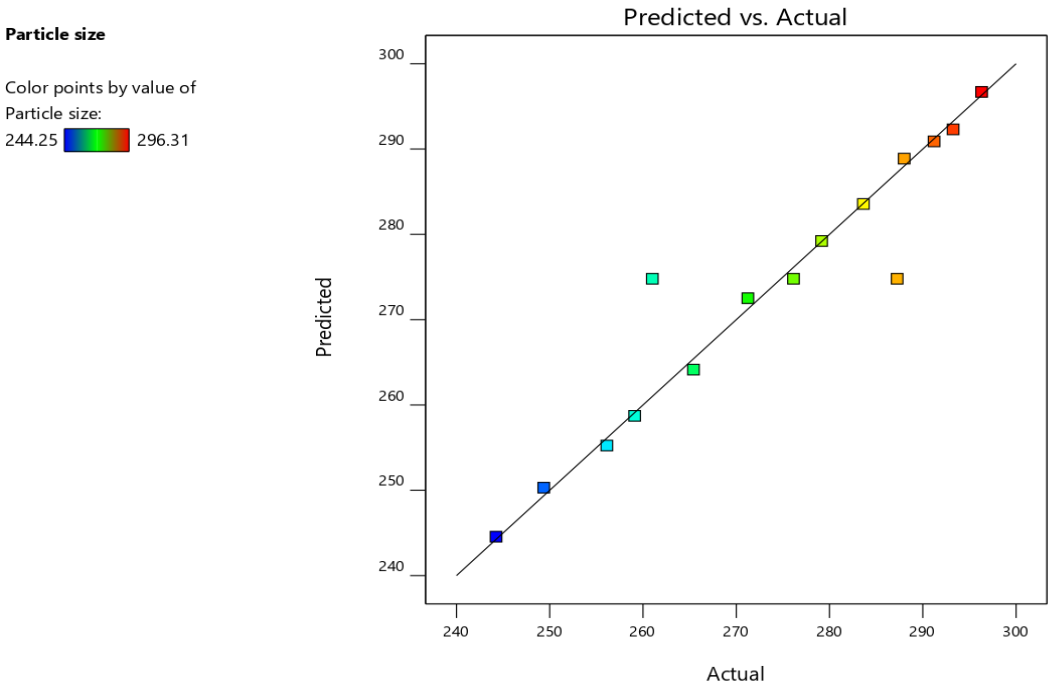


Figure 3

Following equation was reported by software:

Particle size = 274.797 + 19.9913A + 3.175B + 1.00625C + -2.165AB + -3.2025AC + -6.53BC + -2.62708A^2 + -2.27458B^2 + 2.33792C^2

The equation indicates that all the independent variables have a positive influence on the Particle Size of the microsponges. The thickness of the formulation grows as the polymer ratio increases, as does the size of the particles.

Analysis of variance (ANOVA) was performed to analyse the model employed to evaluate the influence on particle size. The ANOVA suggested that the utilised model was suitable and acceptable, with an F-value of 5.62 and a p-value of 0.0359 (P0.0500). The fit statistical analysis revealed a sufficient accuracy (ratio of signal to noise) value of 7.585 (higher than 4) for the model to explore the design space. Using diagnostic plots, the goodness of fit of the suggested model applied to examine the effect on Particle Size was tested.

7.2 Drug entrapment Efficiency

The model graphs have shown effect on drug entrapment by varying the ratio of A and B variable. It is shown in Fig 3.

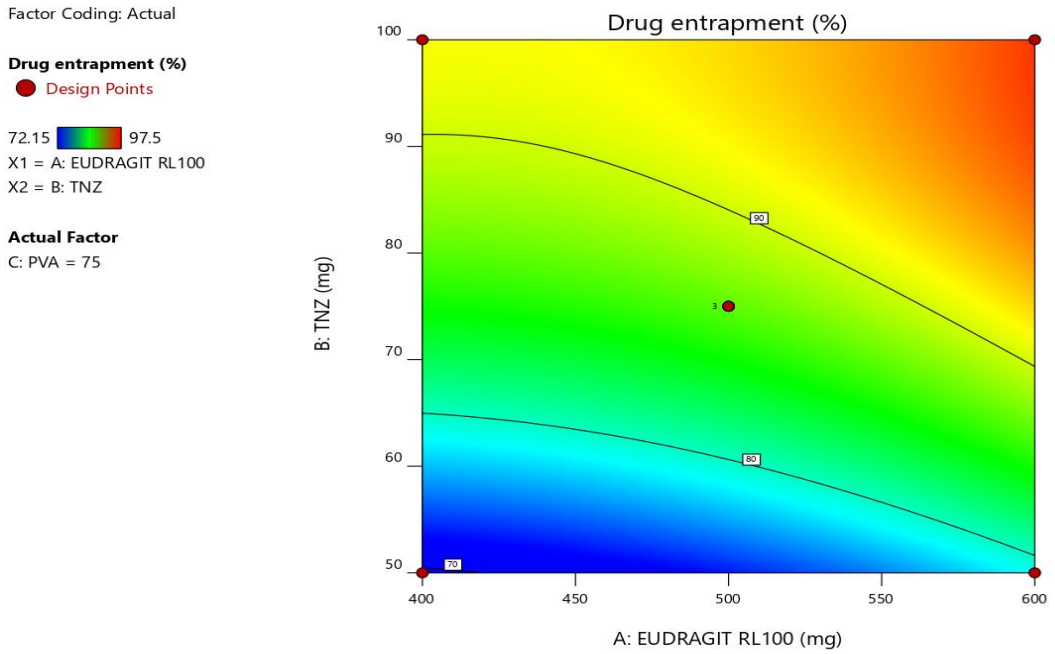


Figure 3: Effect on Drug Entrapment by Varying the Ratio of A and B Variable

The values for predicted vs actual were reported by software. It is shown in Fig.4. it was found in close proximity.

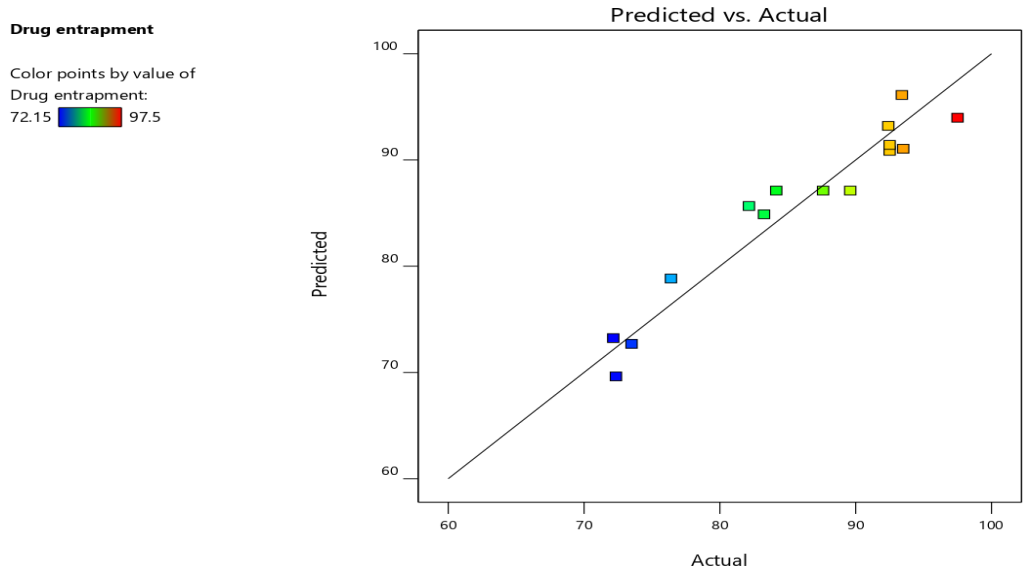


Figure 4

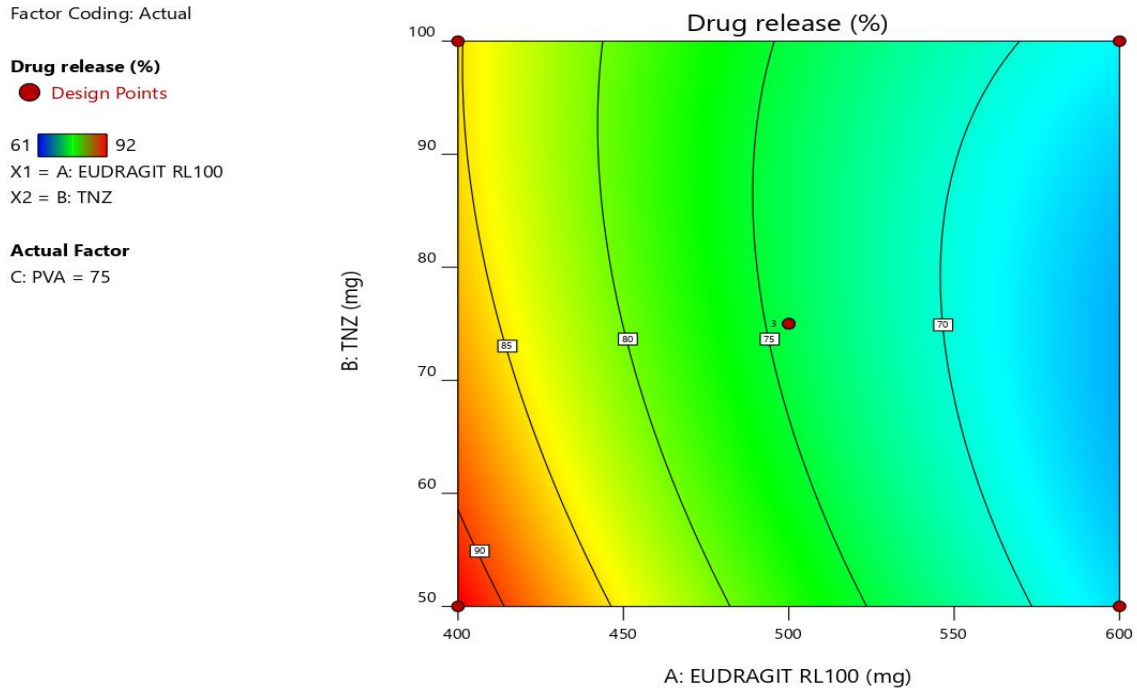
Following equation was reported by software:

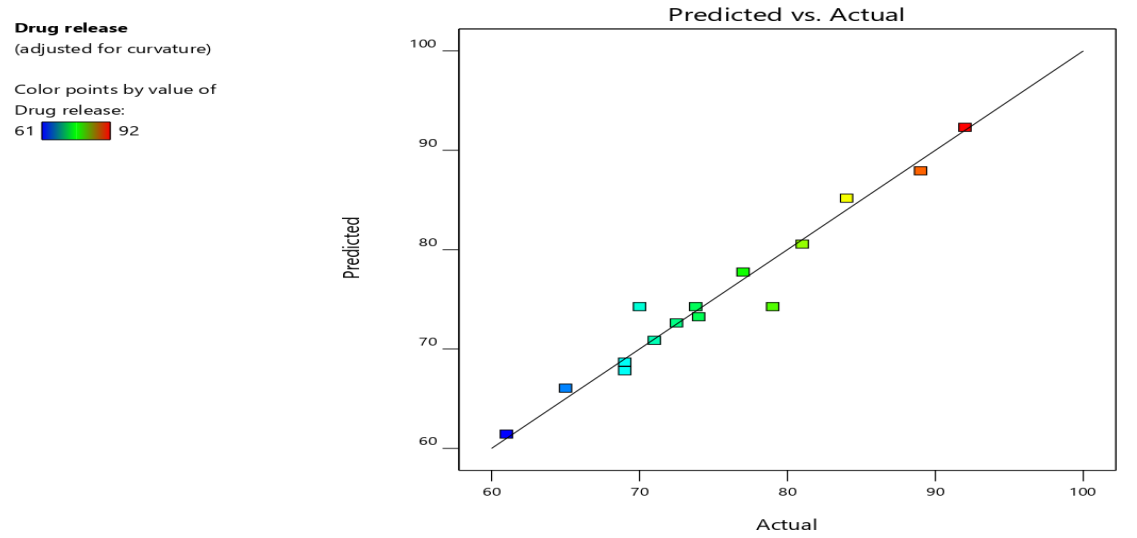
Drug Entrapment Efficiency = 87.1167 + 3.57A + 9.675B + -0.5825C + -1.0375AB + -0.9725AC + -0.3125BC + 1.50292A^2 + -4.70708B^2 + 0.227917C^2

Increase in the Drug entrapment efficiency grows with the concentration of Polymer ratio.The reduced quantity of polymer under the ideal level may not have been able to entrap the drug due to loss of polymer matrix formation, resulting in inadequate entrapment. In the event of a high polymer concentration, however, the matrix may be too hard to penetrate the drug.

Analysis of variance (ANOVA) was performed to analyse the model employed to evaluate the influence on% EE. The F-value (7.01) and p-value of 0.0225 (P0.0005) of the ANOVA demonstrated the adequacy and acceptability of the chosen model. The fit statistical analysis revealed a sufficient accuracy (ratio of signal to noise) value of 8.330 (higher than 4) for the model to explore the design space. Using diagnostic plots, the goodness of fit of the suggested model applied to examine the effect on %EE was tested.

7.3 Drug Release



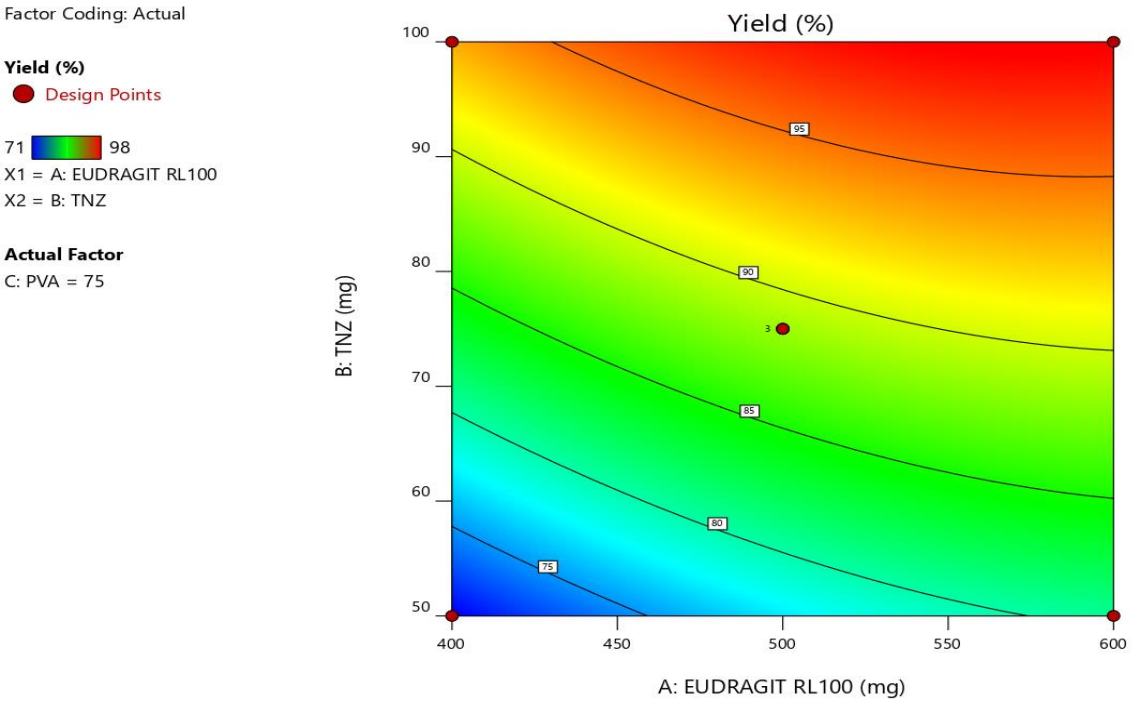


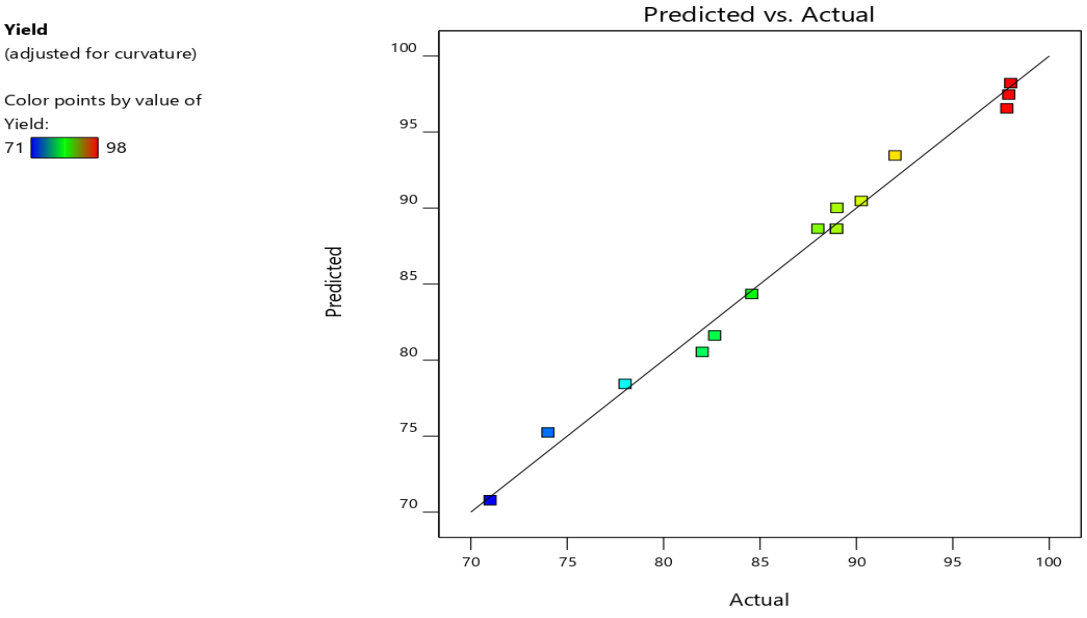
Drug release = 74.2667 + -10.25A + -1.5625B + -0.6875C + 2AB + AC + 1.875BC + 2.30417A^2 + 1.92917B^2 + -2.57083C^2

As the Polymer Ratio Increases the Drug Release Decreases. Analysis of variance (ANOVA) was performed to analyse the model employed to evaluate the influence on drug release. The ANOVA demonstrated the model's applicability and acceptance with

an F-value of 11.56 and a p-value of 0.0075 (P0.0500). The fit statistical analysis revealed a sufficient accuracy (ratio of signal to noise) value of 12.251 (higher than 4) for the model to explore the design space. Using diagnostic plots, the goodness of fit of the suggested model applied to examine the effect on drug release was tested.

7.4 Percentage Yield





Percentage yield = 88.6433 + 3.6275A + 10.0875B + 0.57C + -1.25AB + -0.795AC + -1.025BC + -1.60167A^2 + -1.29167B^2 + -0.426667C^2

All the independent variables shows significant effect on percentage yield. Analysis of variance (ANOVA) was performed to analyse the model employed to evaluate the influence on percentage yield. The ANOVA demonstrated the model's applicability and

acceptance with an F-value of 49.56 and a p-value of 0.0002 (P0.0500). The fit statistical analysis revealed a sufficient accuracy (ratio of signal to noise) value of 23.025 (higher than 4) for the model to explore the design space. Using diagnostic plots, the goodness of fit of the suggested model applied to examine the effect on drug yield was tested.

8. SURFACE MORPHOLOGY

The scanning electron microscopy study of important batches concentrated on evaluating the surface structure of microsponges. The results demonstrated that the microsponges had a round and smooth surface shown in Fig 5. They emerged as round cavities encircled by an exterior shell consisting of both the drug and polymer components of the reformulated formulation. This hollowness appears to originate from the quick evaporation of the volatile solvent from the polymer matrix. This particular hollowness trait was responsible for the microsponges' capacity to float in stomach juices.

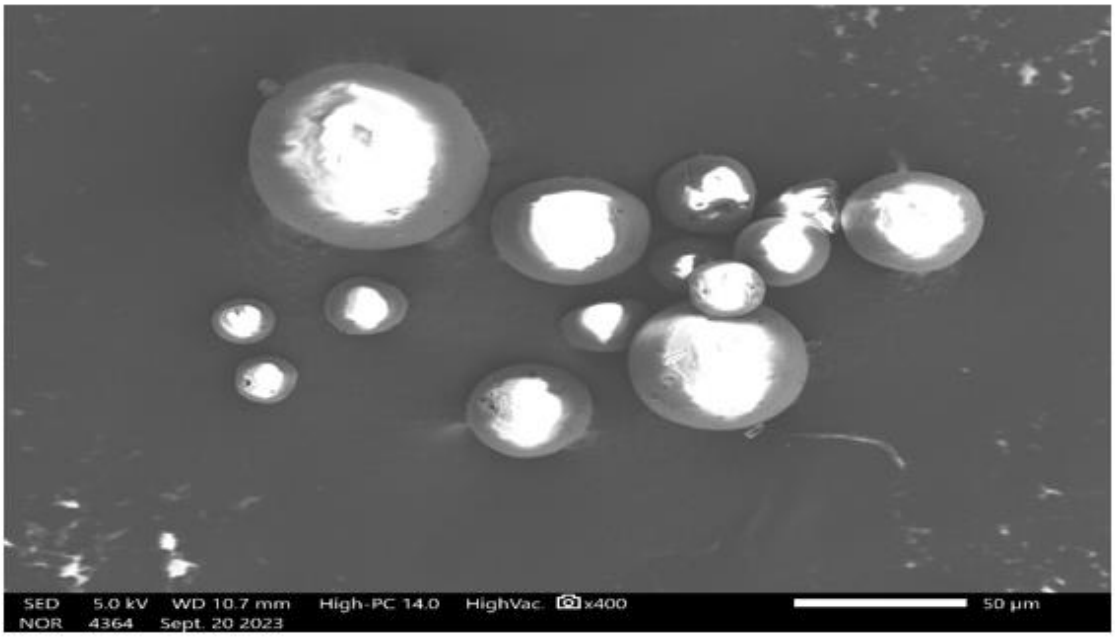


Figure 5: SEM images Tinidazole Microsponges

9. CONCLUSION

Utilizing experimental design proved to be a valuable tool in the development of tinidazole-loaded floating microsponges. Initial investigations revealed that numerous factors influenced the formulation process. However, relying on a trial-and-error approach to screen these formulation and process-related factors proved to be time-consuming and occasionally inaccurate. Consequently, these factors were incorporated into the final optimization process for Tinidazole floating microsponges, employing the Box-Behnken design. This optimization considered key parameters such as entrapment efficiency, particle size, drug release percentage, and yield. Statistical analysis of the results was conducted using Design Expert software.The physicochemical examination of the improved formulation indicated that there was no contact between the medication and polymer. It further validated the complete dispersion of the medication inside the polymeric matrix and the porous, spherical

character of the formulation. Ultimately, all the conclusions from *in-vitro* studies it is inferred that the tinidazole-loaded microspheres that were produced have the ability to efficiently serve as a sustained-release dosage form suited for longer retention in the stomach.

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