Application of Box-Behnken Design for Development and Evaluation of Gastric Floating Tablets of Riboflavin

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ABSTRACT

Sintering refers to bonding adjacent powder particles together or bonding particles in a compact using heat or solvents. This study applied sintering to develop controlled-release systems for vitamin B_2 (riboflavin) using response surface methodology. Pre-compression and post-compression parameters were evaluated and found to be satisfactory. Ethylene vinyl acetate (EVA) 15 was used as the release-controlling polymer. Among three models, a quadratic model was suggested for the T_{90}, floating lag time, and floating time responses, which were found to be 115.24, 101.09 and 86.28 respectively, indicating the models were significant. The ideal values for the selected variables as recommended by the Design Expert® 12 software were 33.78 mg of EVA 15, 7.92% by weight of sodium bicarbonate based on tablet weight, a sintering temperature of 60.47^{O}C, and a sintering exposure time of 4.5 hours. The optimized formulation showed 103 seconds floating lag time, 11.8 hours floating time, and 10.9 hours T_{90}, following zero-order release kinetics with a non-Fickian diffusion mechanism.

Keywords: Sintering, Controlled release, Riboflavin, Response surface methodology, Ethylene vinyl acetate, Quadratic model

INTRODUCTION

Sintering refers to "the bonding of adjacent particle surfaces in a mass of powder, or in a compact, by the application of heat or by exposing to solvents".[1] Sintering means fusing particles together or forming welds between polymer particle surfaces. In other words, sintering enhances cross-linking across particles in a polymer. There are limited reports applying sintering to design controlled drug delivery systems using different drugs and polymers.[2] Using sintering to develop gastroretentive drug delivery systems also shows promise but there are few reports on its use with a few polymers.[3]

Riboflavin is composed of d-ribitol with the hydroxy group at the 5 position replaced by a 7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl substituent. Riboflavin belongs to the category of vitamins, enzyme cofactors and nutrients.[4, 5]

Riboflavin is used to treat vitamin deficiency due to poor diet or nutrition. Riboflavin is indicated for conditions like intestinal issues, stomach problems, infections, liver disease, alcoholism, cancer and more - which can cause riboflavin deficiency. It is used to maintain eye health, skin health, nerve health, and red blood cells. It promotes normal cell growth and function. Riboflavin is used for slow aging, canker sores, multiple sclerosis, Alzheimer's disease, burns, liver disease, high blood pressure, sickle cell anemia, migraines, several cancers with low riboflavin, acne, muscle cramps, carpal tunnel syndrome, burning feet syndrome, congenital methemoglobinemia, and red blood cell aplasia. It is also used for eye issues like cataracts, eye fatigue, and glaucoma [4,6].

In this study, riboflavin gastric floating tablets (GFT) were prepared using thermal sintering. Box-Behnken design was used to optimize selected independent variables. Initial trials evaluated the drug-polymer ratio (1:1 to 1:2) and sodium bicarbonate weight/tablet. Based on results, the drug-polymer ratio and sodium bicarbonate weight/tablet were selected for experimental design.

2. MATERIALS AND METHODS

Riboflavin, EVA 15, sodium bicarbonate, microcrystalline cellulose and magnesium stearate are used of analytical grade. All other chemicals used are also of analytical grade.

Experimental design

Total four factors (two are formulation related and two are process related) at three levels were used to design the experiments for optimization. The formulation related independent variables are drug-polymer ratio and weight

of gas generating agent/tablet (%w/w) and thatof process related are sintering temperature and sintering time. Box-Behnken design was used for optimizing the independent variables selected. The dependent variables were: T90 (time taken to release 90% of the drug), Floating lag time (time for the tablet to float on the fluid surface), Floating time (duration the tablet floats on the liquid surface) [7,8]. The independent variables and levels are shown in Table 1.

Table 1: Independent variables and their levels used in Box-Behnken design

Code	Independent variable	EVA 15		
		Low (-1)	Medium (0)	High (+1)
X1	Drug-polymer ratio	1:0.4	1:0.6	1.08
X2	Weight of gas generating agent/tablet (%w/w)	5	10	15
X3	Sintering temperature (°C)	60	70	80
X4	Sintering time (hours)	1.5	3	4.5

The Design Expert software version 12 forecasted the critical values required to achieve the intended response and potential interactions between the chosen predictor variables on the responses. Based on a Box-Behnken design with four predictor variables at three levels, 29 trials comprising 5 replicate center points were performed [9,10]. Table 2 shows the independent variables and levels used.

Table 2: Experimental design codes for 4 factors at 3 levels

Standard run	X1	X2	X3	X4
1	-1	-1	0	0
2	-1	0	0	-1
3	-1	0	0	+1
4	-1	0	-1	0
5	-1	0	+1	0
6	-1	+1	0	0
7	0	-1	-1	0
8	0	-1	+1	0
9	0	-1	0	-1
10	0	-1	0	+1
11	0	0	-1	-1
12	0	0	+1	-1
13	0	0	-1	+1
14	0	0	+1	+1
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	+1	-1	0
21	0	+1	+1	0
22	0	+1	0	-1
23	0	+1	0	+1
24	+1	-1	0	0
25	+1	0	0	-1
26	+1	0	0	+1
27	+1	0	-1	0
28	+1	0	+1	0
29	+1	+1	0	0

Formulation of riboflavin GFT

A total of 9 formulations were predicted using the two formulation independent variable i.e., drug-polymer ratio and weight of gas generating agent/tablet (%w/w) as per Box-Behnken design [11]. The formulae are shown in **Table 3 and 4** for tablets. These are coded as unsintered tablets. Initially these tablets were prepared and further subjected to process related independent variables i.e. sintering temperature and sintering time. These unsintered tablets were subjected to the 29 runs as shown in **Table 2** containing 50 mg of riboflavin.

Table 3: Formulae of riboflavin unsinteredGFT using EVA 15

Ingredient	RE1U	RE2U	RE6U	RE7U	RE11U
Riboflavin	50	50	50	50	50
EVA 15	20	20	20	30	30
Sodium bicarbonate	6	12	18	7	13
Microcrystalline cellulose	50	50	50	50	50
Magnesium stearate	2	2	2	2	2
Total weight (mg)	128	134	140	139	145

Table 4: Formulae of riboflavin unsintered GFT using EVA 15

Ingredient	RE20U	RE24U	RE25U	RE29U
Riboflavin	50	50	50	50
EVA 15	30	40	40	40
Sodium bicarbonate	20	7	14	21
Microcrystalline cellulose	50	50	50	50
Magnesium stearate	2.5	2.5	2.5	2.5
Total weight (mg)	152.5	149.5	156.5	163.5

Evaluation of precompression parameters

The flow characteristics of the pre-compression blend were assessed using angle of repose, Carr's compressibility index and Hausner's ratio.

Evaluation of riboflavin GFT

The prepared gastric floating tablets (GFT) containing riboflavin were evaluated for various post manufacturing

parameters including tablet thickness, diameter, hardness, fragility, weight consistency, drug content, in vitro buoyancy properties, and in vitro drug release [12–14].

***In vitro* drug release studies**

In vitro drug release for the prepared riboflavin formulations was evaluated using a USP type-II dissolution rate test apparatus (paddle method). The equipment, referred to as lab India DS-8000, utilized 900 ml of 0.1N HCl as the dissolution medium kept at 37°C ± 0.5°C and a shaft spun at 50 rpm. The experiment lasted 12 hours, taking out 5 mL specimens at fixed intervals employing a syringe with a prefilter. [13,15]. Each 5 mL specimen was substituted with 5 mL fresh medium at 37°C ± 0.5°C, washing particles back into the dissolution medium on the prefilter. The collected specimens measured the riboflavin content through absorbance at 444 nm after dilution as required versus 0.1N HCl as a blank. The in vitro drug release studies were performed in triplicate and mean values reported. This evaluated how much riboflavin released from tablets over time. Higher, prolonged release indicated an improved gastroretentive delivery system. Release profiles showed whether formulations met standards for administration and targeting/extending release in the stomach and intestines. Adjustments to optimize release could be made, but analysis provided comprehensive feedback to develop high quality formulations. Key details included using a USP type-II paddle method, 900 ml 0.1N HCl medium at 37°C, 50 rpm, 12 hour study withdrawing/ replacing 5 mL samples, measuring absorbance at 444 nm after dilution versus a 0.1N HCl blank, and triplicate studies averaging results [16].

3. RESULTS AND DISCUSSION

Pre-compression studies

Drug and excipient powder blends were prepared according to formulas in Tables 3 and 4. Their flow properties were evaluated, including angle of repose, Carr's index and Hausner's ratio. Bulk and tapped density provided additional characteristics. Angle of repose, 26.62-31.93°, indicated good to excellent flow [17]. Carr's index, 10.94-14.92, showed compressibility. Hausner's ratio, 1.13-1.17, supported good flow for prepared blends. Bulk density reflected loose powder density while tapped density considered density after consolidation. Differences showed how well powder flowed. Lower differences meant better flow.

Ideal values for good flow were an angle of repose <40°, Carr's index <15% and Hausner's ratio <1.25. Flow evaluations ensured blends compressed properly into tablets with desired properties. Poor flowing blends can cause issues like segregation, lamination and blockage impacting final tablet quality. Adjustments improved poor flow, e.g. removing fines, adding a glidant or increasing binder. Once ideal flow was achieved, blends compressed into tablets with good characteristics [18].

Post- compression studies

Post-compression studies evaluated riboflavin gastric floating tablets (GFT) prepared with EVA 15. Results showed uniform tablet thickness, diameter, hardness (4.2-5.1 kg/cm2), friability (0.39-0.71%) and weight (within ±7.5% average) meeting standards. Drug content was 90-110%, complying with official compendia tests for tablets [19]. Findings indicated all formulations floated with a lag time of 65-134 seconds and floating time of 3.5-14 hours, extending release. Evaluations ensured tablets met standards for administration and targeting/extending release in the stomach and intestines. Measures included uniformity of size, hardness, friability, weight and drug content along with floating properties. Minor adjustments may improve quality but analysis provided comprehensive feedback to develop high quality, gastroretentive EVA 15 GFT.

Drug release from unsintered riboflavin tablets containing EVA 15 showed 100% release within 5-8 hours. Sintered tablets extended release to 6-16 hours with varied drug-polymer ratio, sintering temperature and duration. Results were optimized statistically as discussed. All formulations except RE2U and RE6U followed zero order release kinetics. All except RE2U, RE6U and RE20U followed diffusion release based on 'r' values. All followed non-Fickian diffusion, a mechanism of release [20].

Quadratic models were suggested for T_{90} (time for 90% release), floating lag time and floating time responses. F values of 115.24, 101.09 and 86.28 showed model significance. Optimized values included 33.78 mg EVA 15, 7.92% w/w sodium bicarbonate (to tablet weight), 60.47°C sintering temperature and 4.5 hours sintering exposure. The optimized formulation showed 103 seconds floating lag time, 11.8 hours floating time and 10.9 hours T90 following zero order release with non-Fickian diffusion [16]. Evaluations determined how much and how quickly riboflavin released from tablets with varied EVA 15 amounts, sodium bicarbonate levels, sintering temperatures and durations. Higher, prolonged release indicated an improved gastroretentive delivery system. Release profiles showed whether formulations met standards for administration and targeting/extending release in the stomach and intestines. Minor adjustments to optimize release could improve quality but analysis provided comprehensive feedback to develop high quality, gastroretentive riboflavin tablets. Statistical optimization interpreted results. Key details included release kinetics (zero order), mechanism (diffusion, non-Fickian), models (quadratic) for T90, floating lag and float time, optimized variable values and results for optimized formulation.

Response surface methodology (RSM) yielded regression equations relating logarithmic T90 (time for 90% release), floating lag time and floating time values [14]. Equations provide an empirical relationship between test variables in coded units: Drug-polymer ratio (A), Sodium bicarbonate weight/tablet (%w/w) (B), Sintering temperature (C) and Sintering time (D).

T_{90}= *11.00 + 3.81 A + 0.2500 B + 1.12 C + 0.6875 D - 0.1250 AB + 0.3750 AC + 0.0000 AD - 0.1250 BC + 0.2500 BD + 0.1250 CD – 0.1250 A² - 0.3633 B² - 0.7500 C² - 0.3750 D²*

Floating lag time = 100.33 – 24.00 A - 20.75 B + 3.50 C + 2.25 D + 7.00 AB – 0.0000 AC – 2.00 AD - 0.7500 BC + 0.0000 BD + 0.500 CD + 1.33 A² - 3.72 B² - 3.33 C² - 6.33 D²

Floating time = 9.60 + 4.00 A + 0.2500 B + 0.9375 C + 0.9378 D

Contour plots, response surface plots for T90 vs formulation factors (Figs. 1) and desirability plots (Fig. 2) showed relationships. The response plots illustrated the response surface as a function of two factors simultaneously, keeping the rest at set levels. This helped to comprehend the primary and joint effects. Regression equations provided empirical relationships between formulation factors and responses (T_{90}, floating lag time, floating time) in coded units. Optimization evaluated these relationships to determine ideal factor levels for a targeted response. Key insights included regression equations, contour/response surface plots showing relationships, desirability plots for optimization, and understanding main and interaction effects of factors [21].

Optimization

An optimized formulation attained 90% release in 11-12 hours, minimal floating lag time and 12 hours floating time. These constraints were common across all formulations. Feasibility and grid searches identified the optimized formulation. Design Expert 12 software calculated independent variable concentrations indicating suitable desirability, close to 1.0.

Optimized values of selected variables included: 33.78 mg EVA 15, 7.92% w/w sodium bicarbonate (to tablet weight), 60.47°C sintering temperature and 4.5 hours sintering exposure time. The working formula for the statistically optimized formulation (TEopt) is in Table 5. The optimized formulation met strict criteria for release (90% in 11-12 hours), floating lag time (minimal) and floating time (12 hours). Multiple searches identified factor levels providing the best results and highest desirability for these critical responses. Software calculated ideal concentrations for EVA 15, sodium bicarbonate, sintering temperature and duration based on comprehensively evaluating relationships and optimizing key parameters [22]. The optimized formula (TEopt, Table 5) used these values, developed to have prolonged, higher release for improved riboflavin supplementation.

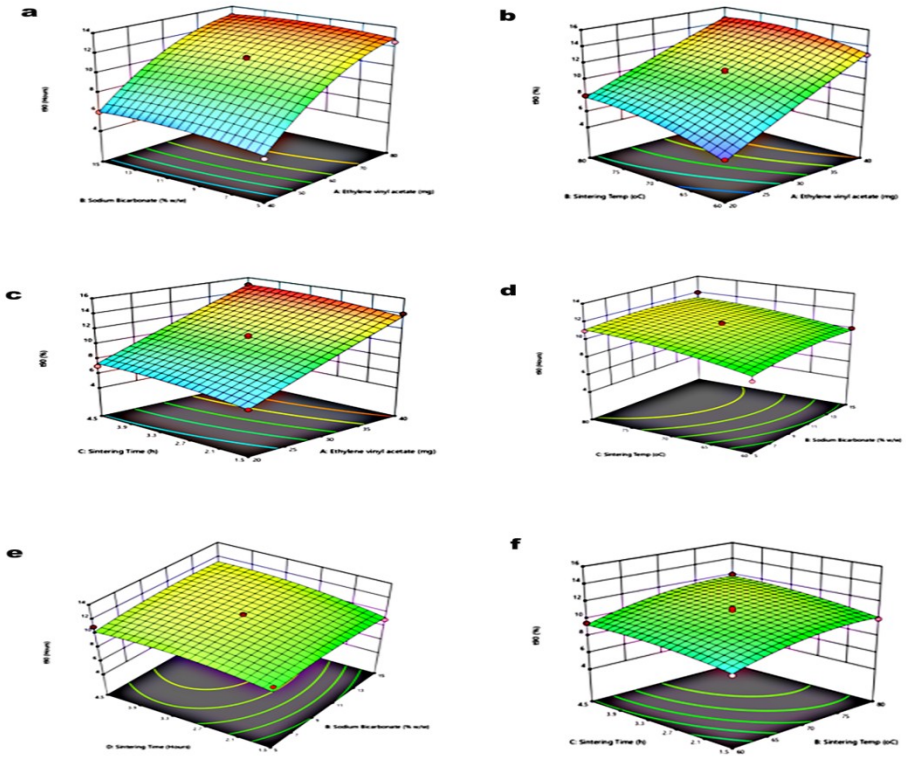

Fig.1: Contour plots for the effect of various independent variables on T_{90} (quadratic model) a) EVA 15 and sodium bicarbonate (b) EVA 15 and sintering temperature(c) EVA 15 and sintering time (d) Sodium bicarbonate and sintering temperature (e) Sodium bicarbonate and sintering time (f) Sintering temperature and sintering time

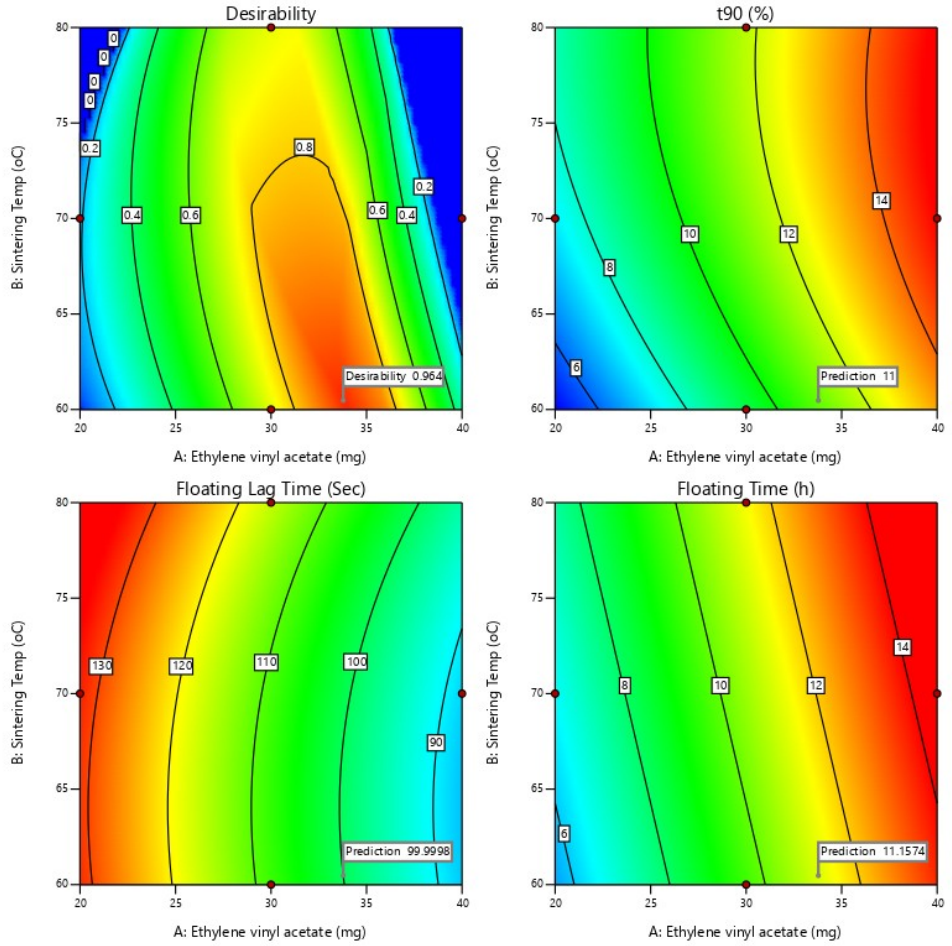

Fig.3: Desirability plots for responses

Cross validation of optimized formulation

The statistically optimized riboflavin formulation met all physicochemical property criteria. In vitro dissolution verified theoretical predictions. Experimental findings closely matched model predictions (Table 6). Physicochemical evaluations ensured the optimized formulation and laboratory processing produced tablets meeting standards. Key properties included hardness, friability, weight variation, drug content and floating characteristics. Optimized values from statistical optimization were evaluated to confirm product quality before further testing [11]. Dissolution studies evaluated how quickly and completely the optimized formulation released riboflavin in simulated stomach fluid. Experimental release profiles were compared to model predictions. Close agreement showed the optimized factor levels successfully targeted prolonged, controlled release. Dissolution testing provided critical feedback on real-world performance to develop an effective, high-quality delivery system. Refining and optimizing at each step helped ensure the final formulation achieved the goal of improved riboflavin supplementation through controlled, extended release.

Table 5: Formula of statistically optimized formulation, TE_{opt}

S. No.	Ingredient	Quantity (mg)
1	Riboflavin	50
2	EVA 15	33.78
3	Sodium bicarbonate	11.72
4	Microcrystalline cellulose	50
5	Magnesium stearate	1.5
	Total	147

Sintering temperature: 60.47° C; Sintering time: 4.5hours

Table 6: Comparison of predicted and observed responses of statistically optimized formulation, TE_{opt}

Response	Observed	Predicted	% Relative error
T_{90}	10.9	11	0.91
Floating lag time	103	99.99	3.01
Floating time	11.8	11.51	2.52

The optimized riboflavin formulation had a 103 second floating lag time and 11.8 hour floating time. The time for 90% release (T90) was 10.9 hours, following zero order kinetics with non-Fickian diffusion release. The

percentage relative error between predicted and experimental response values was <5%. Experimental and predicted values agreed, confirming model predictability and validity.

The optimized formulation met targets for key releasing parameters:

Floating lag time: Minimize floating lag time (103 seconds)
Floating time: Maintain floating for extended period (11.8 hours)
Release time: 90% release within 11-12 hours (T90 10.9 hours)
Release kinetics: Zero order
Release mechanism: Non-Fickian diffusion

Low relative error (<5%) and agreement between predicted and experimental values validated the optimized model. The model accurately predicted how the formulation would release riboflavin, confirming the optimized factor levels were effective and the approach was valid.

CONCLUSION

Gastric floating tablets of riboflavin with EVA 15 were developed and optimized to obtain 12 hours of drug release meeting all other tableting characteristics and floating properties as per the objectives of present investigation using EVA 15 as polymer and following thermal sintering technique. Box-Behnken design was successfully applied for the optimization of formulation. Pre-compression and post-compression evaluation parameters revealed the quality characteristics of prepared formulation mixtures and tablets respectively are within acceptable criteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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