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FULL FACTORIAL DESIGN IN FORMULATION OF LAMOTRIGINE SUSPENSION USING LOCUST BEAN GUM

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ABSTRACT

Lamotrigine, a weakly basic, anticonvulsant drug have high permeability but poor solubility belonging to BCS Class II. The aim of this work was to formulate suspension in order to improve the bioavailability and patient compliance. In the present investigation, a perfect suspension was made using Locust bean gum, sodium alginate as viscosity enhancers and sodium citrate as anti-flocculant. Using 2 level 3 factor full factorial design, amount of ingredients were selected as independent variables while viscosity and sedimentation volume as response or dependent variables. The responses of data were recorded and analyzed for ANOVA, the individual parameters were evaluated by polynomial equation for each response generated using multiple linear regression analysis. The relationship between dependent and independent variables was further elucidated using contour plots finally optimized for desired response. From the results the eight formulations were made by factorial design, later optimized batch was prepared after desirability analysis and validated the obtained value with predicted showing good reliability of the design, highest r^2 and lowest sum of squares of residual was observed for the responses. Thus the prepared suspension showed high sedimentation volume, high redispersibility and optimum viscosity following non-Newtonian type behavior.

Key words: Lamotrigine, Full factorial design, Locust bean gum, Suspension.

INTRODUCTION

Lamotrigine (LMT) is a novel antiepileptic agent belonging to Biopharmaceutical Classification System II with oral bioavailability of about 98%[1]. It is available as tablet with various strengths such as 25 mg, 50 mg and 100 mg. In the present work, LMT was designed with a view to enhance patient compliance and thus formulated into suspension. The oral route of drug administration is the most important method of administering drugs for systemic effect. Among all the forms liquid form is preferred class of dosage forms for the

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reasons that high rate of bioavailability, better organoleptic properties and ease of administration[2]. Based on electrokinetic properties, suspensions are of flocculated and deflocculated type[3]. In this study, carob gum was used as viscosity enhancer which can also act as stabilizing agent and suspending agent. It is a yellowish-green, odorless powder and was 5 times effective than starch where there was no ADI as per WHO[4]. Much of the work was focused on controlled release liquid formuation[5], nanosuspension[6].

From different optimization methodologies such as simultaneous where experimentation is completed before optimization was important like factorial design where target value of objective can be measured by optimization[7]. Factorial design was a first degree model because they have maximum efficiency in estimating main effects. The obtained mathematical model from these designs describe factor-response relationship as quadratic equation regardless the results were validated by contour plots and further the results are typically interpreted from ANOVA[8].

The main purpose of this work was only to improve patient compliance without compromising the therapeutic efficacy. The objective of this study was also to investigate the combined effect of viscosity enhancers and anti-flocculant using full factorial design.

EXPERIMENTAL

Materials and methods

Lamotrigine was obtained as gift sample from Dr Reddy's Labs, Hyderabad, India. Locust bean gum (LBG) was gifted from Rhodia organic fine Ltd, UK. Sodium alginate (SA), Sodim Citrate (SC) was obtained as gift sample from FMC biopolymer, USA and Fischer Scientific, UK. Remaining all other excepients and chemicals were obtained from local suppliers.

Experimental design

A selected two level, three factor experimental design describe the proportion in which the independent variables LBG, SA and SC were used in formulation of LMT suspension. The viscosity and sedimentation volume were selected as dependent variables. All eight formulations were prepared in three sets and analyzed individually for viscosity and sedimentation volume in triplicate. The linear regression model was derived from systat version 12.02.00, statistical software. Significance terms were chosen at 95% confidence interval ($p < 0.05$) for final equations. In addition, contour plots were obtained to represent the effect of the independent variables graphically.

Formulation of LMT suspension

LMT was formulated into suspension with various inactive ingredients such as LBG, SA as viscosity enhancers, SC as anti-flocculant, sodium benzoate as preservative and sodium saccharin as sweetener. All ingredients were weighed and passed through 40 #, firstly LBG, SA were dissolved in water before 24 h. Later SC, sodium benzoate was added and dissolved. Then drug was added to the solution and dispersed, finally sodium saccharin and flavorant were added.

Formulation optimization

From the computation for optimized formulation, the response variables considered for optimization were sedimentation volume and viscosity. The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors).

Determination of viscosity

Brookfield viscometer (Spindle LV model) was used to measure viscosity. 1 mL of fluid was taken in cup and bob was allowed to completely immerse in the cup. Viscometer was switch on, run it till indicator shifted from red zone to green zone. Viscometer was allowed to run in both ascending and descending mode (10-100 rpm) at 5 min. interval and 1 min. data collection interval time.

Determination of sedimentation volume

20 mL of solution was taken in 50 mL glass measuring cylinder. The suspension was dispersed thoroughly by moving upside down for three times and volume of sedimentation was noted (V_0). Later suspension was allowed to settle for 24 h and volume was read (V_u).

$$F = \frac{V_0}{V_u}$$

Assessment of re-dispersibility

Suspension was allowed to settle in measuring cylinder. Mouth of cylinder was closed and was inverted through 180° and number of inversions required for restoration was noted. If uniformity attained in one inversion, then it has 100% redispersibility. Every additional inversion decreases the percentage of ease of redispersibility by 5%.

RESULTS AND DISCUSSION

Preparation and characterization of suspension

The different suspension formulations were prepared at various coded values for locust bean gum, sodium alginate and sodium citrate (Table 1 and 2).

Table 1: 2^3 full factorial design for suspension formulation

Formulation	Locust bean gum		Sodium alginate		Sodium citrate	
	Percentage	Actual amount (mg)	Percentage	Actual amount (mg)	Percentage	Actual amount (mg)
F1	1	1.5	2	3	2	3
F2	1	1.5	2	3	4	6
F3	1	1.5	4	6	2	3
F4	1	1.5	4	6	4	6
F5	4	6	2	3	2	3
F6	4	6	2	3	4	6
F7	4	6	4	6	2	3
F8	4	6	4	6	4	6

Table 2: Formulation table of lamotrigine suspension

Formulation code	Drug (mg)	Locust bean gum (mg)	Sodium alginate (mg)	Sodium citrate (mg)	Sodium benzoate (mg)	Sodium saccharin (mg)	Flavor	Distilled water (mL)
A1	250	7.5	15	15	3.75	458.75	qs	20
A2	250	7.5	15	30	3.75	443.75	qs	20
A3	250	7.5	30	15	3.75	443.75	qs	20
A4	250	7.5	30	30	3.75	428.75	qs	20
A5	250	30	15	15	3.75	436.25	qs	20
A6	250	30	15	30	3.75	421.25	qs	20
A7	250	30	30	15	3.75	421.25	qs	20
A8	250	30	30	30	3.75	406.25	qs	20
20 mL of suspension was prepared containing 50 mg of drug in 4 mL of suspension								

Assessment of viscosity and sedimentation volume

As per the prescribed specifications, the concentrations were selected for LBG, SA and SC. The obtained results for viscosity and sedimentation volume (Table 3) explains that with high ratios of locust bean gum and sodium alginate, the viscosity increases drastically and with increase in sodium citrate, the sedimentation volume in increases and in order to obtain the desired value, experimental design was used.

Table 3: Suspension evaluation responses (mean ± standard deviation)

Formulation	A1	A2	A3	A4	A5	A6	A7	A8
Appearance	**White color**	**White color**	**White color**	**White color**	**White color**	**White color**	**White color**	**White color**
Vis. (cP)	60	82	121	103	172	180	210	181
SV (24 h)	0.23	0.74	0.76	0.95	0.44	0.79	0.90	0.95
Redispersibility (%)	90	95	90	90	90	95	95	90

Experimental designing and analysis of variance

Full factorial design was used to investigate the effect of 3 factors; the effects of independent variables upon responses were modeled from polynomial equation for viscosity and sedimentation volume as represented in Table 4.

Table 4: Regression equations for the responses

YSV = -1.246C + 0.138LBG + 0.433SA + 0.428SC – 0.025LBG*SA – 0.078SA*SC-0.010LBG*SC
YVisc = -108.04C + 48.417LBG + 31.958SA + 52.958SC – 2.083LBG*SA – 9.625SA*SC-3.583LBG*SC
SV-Sedimentation volume; Visc-Viscosity; LBG-Locust bean gum; SA-Sodium alginate; SC-Sodium citrate

These fitted equations relate the responses, showed good correlation coefficient of 0.999. The results reveal the good reproducibility of the system. The regression equations draw conclusions after considering the magnitude of the coefficient and the mathematical equation carries positive sign in the polynomial equation indicating that the response increases with increase in the value and negative sign represents the decrease in response with increase in the value. The interaction terms showed how the response changes when two factors were simultaneously changed. From contour plots only, the formulation

optimization for required response was predicted graphically. The criteria for selecting the most appropriate model are lowest sum of square of residuals (SSR) and highest r^2 value indicates linearity of data (Table 5). Residual values of predicted and observed data were used to calculate sum of squares of residuals in ANOVA.

Table 5: Analysis of Variance (ANOVA) of dependent variables

Dependent variables	Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio calculated (tabular)	P-value
Sedimentation volume (Y_{SV})	Regression residuals total R^2	6 1 7 1.000	0.464 0.0 0.464	0.077 0.0	1545.2 (234)	0.019
Viscosity (Y_{VISC})	Regression residuals total R^2	6 1 7 0.999	20682.7 1.125 20683.9	3447.12 1.125	3064.1 (234)	0.014

Constraints for optimization as desirability analysis (Table 6) were used and exact amount of LBG, SA and SC for achieving desired response was found out from optimization; desirability 1.0 indicated optimum formulation was achieved at 29 mg of LBG, 17 mg of SA and 29 mg of SC (Table 7). Validation of optimization technique done by preparing optimized batch, response was evaluated and compared to predicted response.

Table 6: Constraints for optimization

Response	Lower value	Target	Upper value
Sedimentation volume	0.23	0.94	0.95
Viscosity	60	200	210

Table 7: Optimization through desirability approach

Factor	Stationary point		
	Coded	Uncoded (%)	Amount (mg)
Taragum	0.919	3.878	29.085
Sodium alginate	-0.667	2.333	17.497
Sodium citrate	0.889	3.889	29.167

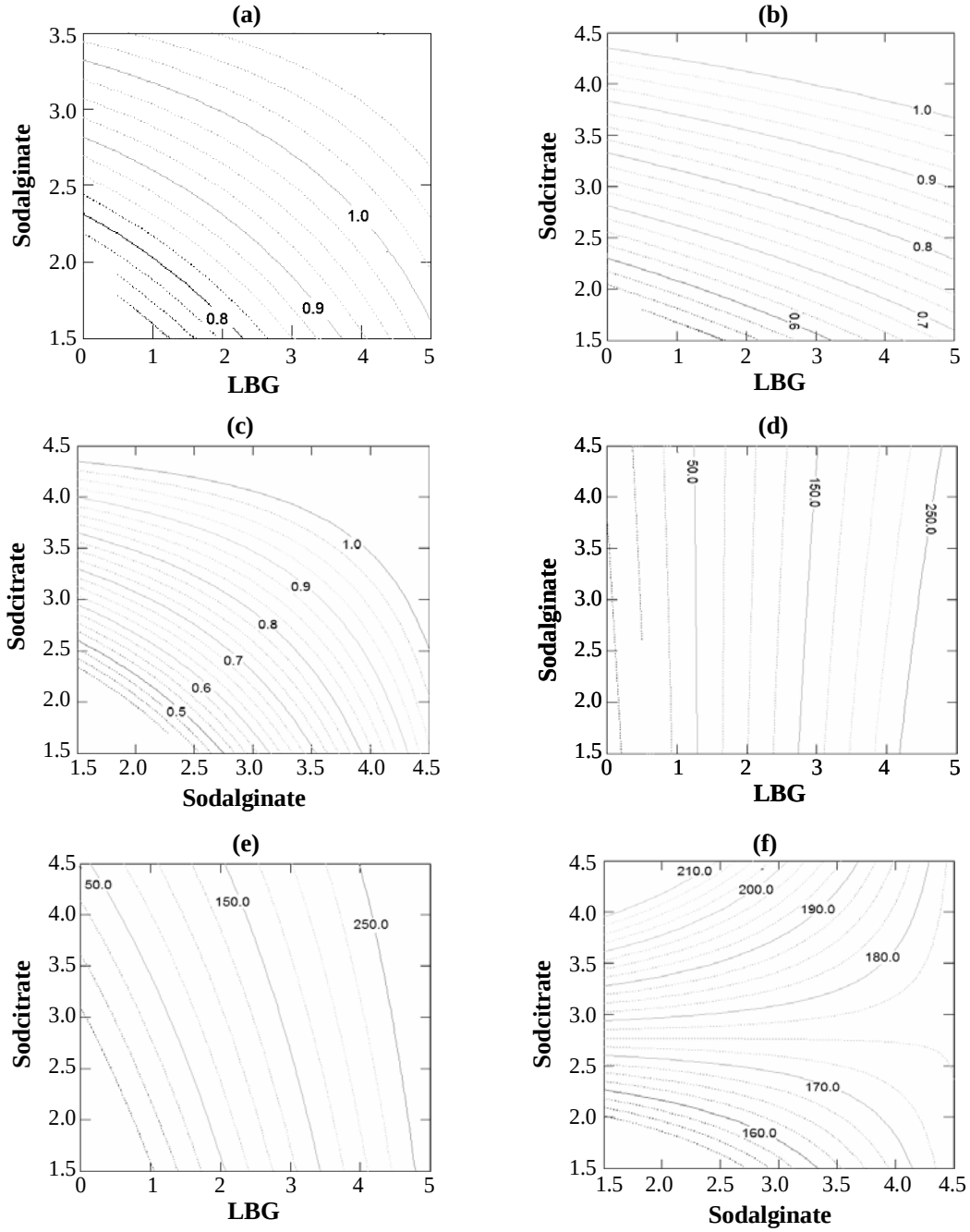

Fig. 1: Contour plots by the effect of LBG, SA and SC on (a), (b), (c) sedimentation volume and (d), (e), (f) viscosity

Thus finally the prepared formulation was observed its viscosity in order to state the type of flow behavior. From the Fig. 2, at different rates of speed, viscosity decreases with rate of shear (Table 8) indicating the pseudo-plastic flow behavior of the fluid[9]. In non-Newtonian systems, one point determination is virtually useless in characterizing the flow properties of the system. Thus multipoint instrument that operates at a variety of rates of shear was opted where tackiness, slip and spreadability are easy to measure. The Fig. 3 clearly represents that it follows non-Newtonian pseudo-plastic flow behavior. Under several types of behavior observed when the rate of shear progressively increased and plotted against the resultant shear stress.

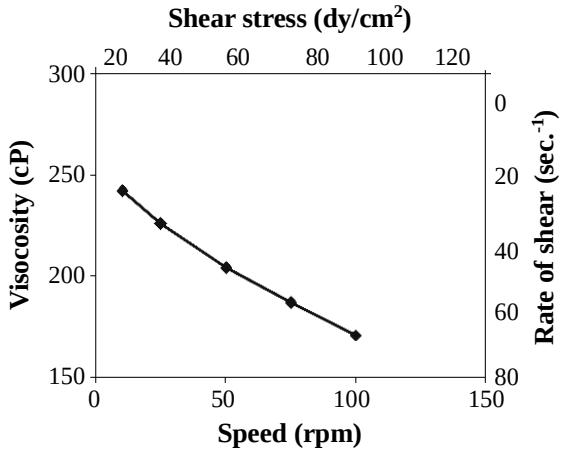

Fig. 2: Influence of rotational speed, shear stress and rate of shear on viscosity of optimized formulation

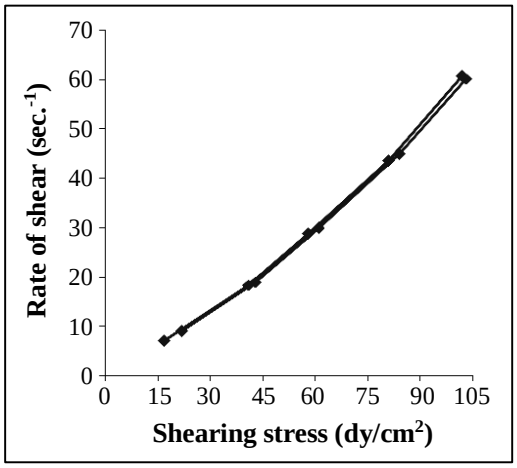

Fig. 3: Relationship between shearing stress *vs* rate of shear

Table 8: Viscosity parameters of optimized formulation

Rotational speed (rpm)	Viscosity (cP)	Shearing stress (dy/cm^2)	Rate of shear (sec^{-1})	Torque (%)
10	242	22	9.09	11.83
25	226	43	19.03	23.12
50	204	61	29.90	32.79
75	187	84	44.92	45.16
100	171	103	60.23	55.38
100	168	102	60.71	54.84
75	186	81	43.55	43.55
50	202	58	28.71	31.18
25	223	41	18.38	22.10
10	241	17	7.05	9.14

In the non-Newtonian system with shear-thinning systems, the down-curve is frequently displaced to the left of the up-curve showing that the material has a lower consistency at any one rate of shear on the down-curve than it had on the up-curve. This indicates a break down of the structure that does-not reform immediately when the stress is removed or reduced which is known as thixotropy that is most commonly exhibited by shear-thinning systems.

CONCLUSION

From above work, LMT suspension formulation was made using locust bean gum and other ingredients in order to enhance the patient compliance and this method may minimize the number of trials in final optimization there by improving formulation development process.

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