Box Behnken Design in Optimization and Evaluation of Olanzapine Loaded Guar Gum Microspheres

Chitra Singh[a], Suresh Purohit[a], B.L.Pandey[a] and Priyanka[b]*

[a] Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005
[b] Department of Pharmaceutical Sciences, Guru Jambeshwar University of Science and Technology, Hisar, Haryana-125001

ABSTRACT

The purpose of this research was to formulate and systematically evaluate in vitro performances of biodegradable microspheres of Olanzapine, an atypical antipsychotic agent for treatment of schizophrenia,bipolar disorder. The present study consisted of a three-level three-factorial (3^3) design- Box Behnken design for experimentation. A 3- -factor, 3-level Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. Response surface graphics were used to show the factor interaction between the considered variables. Selected independent variables studied were the Drug: Polymer Ratio (X_1); Agitation Speed (rpm) (X_2); and Surfactant Ratio (X_3) added to the formulation. The selected dependent variables investigated were particle size (µm), percentage drug entrapment efficiency and percentage of drug released at 50 hrs. Batch P1 exhibited excellent results in terms of particle size (32.78µm), percentage drug entrapment efficiency (75.25) and percentage of drug released (82.24) at 50 hrs.

INTRODUCTION

Guar gum (GG) is mainly consisting of the high molecular weight (approximately 50,000-8,000,000) polysaccharides composed of galactomannans and is obtained from the endosperm of the seed of the guar plant, *Cyamopsis tetragonaloba* (L) Taub. (syn. *Cyamopsis psoraloides)*. It is used as thickener, stabilizer and emulsifier, and approved in most areas of the world. GG is a non-ionic, water soluble polysaccharide that is found abundantly in nature and has many properties desirable for drug delivery applications. Guar gum can be modified by derivatization, grafting and network formation to improve its property profile for a wide spectrum of biomedical applications.[1]

Guar gum is a dietary fiber advocated for use in lowering serum total cholesterol levels in patients with hypercholesterolemia.

Olanzapine is an atypical antipsychotic drug. Olanzapine is used to treat the manifestations of psychotic disorders such as schizophrenia, hallucinations, delusions, hostility and other bipolar disorder. starting dose: 5-10 mg once daily. To reduce the dosing frequency ; once in three days, it is needed to formulate in long acting dosage forms.[2,3]

Various methods have been used for the manufacture of biodegradable microspheres. Among these, the most popular method has been the Oil/Water (O/W) type emulsion solvent evaporation method. Although this procedure is simple, halogenated solvents (e.g., methylene chloride) that are commonly used for the preparation of the dispersed phase are considered harmful for the body and the environment. Therefore, their use is being regulated.

A new preparation method for microspheres based on an Oil/Water type emulsion solvent evaporation method using non-halogenated

solvents. This method is based on phase separation between acetone and aqueous glycerol. A Box-Behnken experimental design was employed to statistically optimize the formulation parameters of Olanzapine microsphere preparation for maximum entrapment, optimum diameter and controlled drug release and evaluates the main effects, interaction effects and quadratic effects of the formulation ingredients on the % drug entrapment efficiency, particle size and % drug release of microsphere.[4-9]

MATERIALS AND METHODS

Materials

Olanzapine (Drug) and Guar gum (Polymer) were provided as gift samples from Akumbs Pharmaceuticals Ltd. Rorkee, India and Dinesh Enterprises, Jodhpur, India respectively. Acetone and Liquid paraffin were purchased from Loba Chemie Pvt Ltd (Mumbai, India) respectively.

Methods

Experimental Design

Use of experimental design allows for testing a large number of factors simultaneously and precludes the use of a huge number of independent runs when the traditional step-by-step approach is used. Systematic optimization procedures are carried out by selecting an objective function, finding the most important or contributing factors and investigating the relationship between responses and factors by the so-called response surface methodology. Objective function for the present study was selected as maximizing the % Drug entrapment efficiency while minimizing the particle size.

A Box-Behnken experimental design was employed to statistically optimize the formulation parameters of (drug) microsphere preparation for maximum entrapment, optimum diameter and controlled drug release and evaluates the main effects, interaction effects and quadratic effects of the formulation ingredients on the % encapsulation efficiency, particle size and % drug release of microsphere. The Box-Behnken design was specifically selected since it requires fewer treatment combinations than other design in cases involving three or four factors. The Box- Behnken design is also rotable and contains statistical "missing corners" which may be useful when the experimenter is trying to avoid combined factor extremes. This property prevents a potential loss of data in those cases. This property prevents a potential loss of data in those cases. A design matrix comprising of 15 experimental runs was constructed, for which the non-linear computer generated quadratic model is defined as;

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y from experimental runs; and X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_1X_2 and X_{2i} (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively.

Independent variables studied were the Drug: Polymer Ratio (X_1), Agitation Speed (rpm) (X_2) and Surfactant Ratio (X_3). The dependent variables were the % yield (Y_1), Drug entrapment efficiency (Y_2), and amount of drug release after 50 hrs (Y_3).

The concentration range of independent variables under study is shown in Table 1and Table 2 along with their low, medium and high levels, which were selected based on the results from preliminary experimentation.

Table 1: Factors and their corresponding levels implemented for the construction of Box Behnken design

Factor	Factor Level		
	-1	0	+1
Drug: Polymer Ratio (X_1)	1:1 (1)	1:2 (0.5)	1:3 (0.33)
Agitation Speed (rpm) (X_2)	10000	15000	20000
Surfactant Ratio (X_3)[#]	-1	0	1

[#] **-1 = PVA, O= Tween 80: PVA, +1 = Tween 80**

Table 2: Composition of 15 batches prepared using Box Behnken design with measured response

Batch No.	Drug: Polymer Ratio (X_1)	Agitation Speed (rpm) (X_2)	Surfactant Ratio (X_3)	Particle size (µm)	Drug entrapment efficiency (%)	$t_{\%}$ release (50 hrs)
1	-1	-1	0	32.78	60.94	76.48
2	0	1	-1	20.57	71.23	68.34
3	1	0	1	53.00	55.43	59.23
4	-1	0	1	78.89	61.45	57.78
5	1	-1	0	28.84	55.69	64.93
6	0	0	0	28.57	58.45	65.34
7	0	-1	1	67.85	62.78	71.27
8	-1	1	0	102.58	66.67	67.65
9	0	0	0	65.00	53.78	67.23
10	1	0	-1	68.59	69.46	57.45
11	0	0	0	36.87	57.89	66.46
12	0	1	1	105.59	53.78	73.21
13	1	1	0	45.52	73.74	69.32
14	-1	0	-1	47.59	68.89	70.43
15	-1	-1	0	46.58	65.47	62.34

Preparation of Microspheres

Olanzapine microspheres were prepared by emulsification and solvent evaporation technique employing guar gum as polymer and using Polyvinyl alcohol (PVA) and Tween 80 as the emulsifying agent in varying concentration.[10-14]

Guar gum and Olanzapine was dispersed in acetone to obtain the dispersed phase. Then emulsification and solvent evaporation processes were performed. The dispersed phase was emulsified with aqueous glycerol containing 0.5% (Tween 80: PVA) using a homogenizer (IKA-T 10 Basic, China) for 2 hrs at desired agitation speed at 27ºC. The resulting solution was added to 250 ml of 50/50 (w/w %) of glycerol-water and stirred for 6 hrs. Next 50 ml of water was added and the solution was stirred for 3hrs to extract acetone from emulsion.

Assay of Olanzapine

Olanzapine was estimated by ultraviolet visible (UV/Vis) spectrophotometric method (Shimadzu UV-1601 UV/Vis double beam spectrophotometer, Kyoto, Japan). Aqueous solutions of Olanzapine were

prepared in phosphate buffer (pH 6.6) and absorbance was measured on UV/Vis spectrophotometer at 258 nm. The method was validated for linearity, accuracy, and precision. The method obeys Beer's Law in the concentration range of 2 to 10 µg/ml.

Drug Entrapment Efficiency

Microspheres (50 mg) were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 10 ml of phosphate buffer (pH 6.6). After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content using Shimadzu UV-1601 UV/Vis double beam spectrophotometer, Kyoto, Japan. The drug entrapment efficiency was calculated using the following formula: Practical drug content/ Theoretical drug content X 100.

Yield (%) = (Weight of microspheres/ Total expected weight of drug and polymer) × 100

Morphology and particle size[15-18]

A scanning electron photomicrograph of drug-loaded guar gum microspheres was taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 20 kV, chamber pressure of 0. 6 mm Hg, original magnification 3800. The particle size of the microspheres was determined by using optical microscopy method. Approximately 100 microspheres were counted for particle size using a calibrated optical microscope (Labomed CX RIII, Ambala, India).

Micromeritic Properties of Microspheres

The microspheres were characterized by their micromeritic properties such as particle size, tapped density, Carr's index, and flow property.

In Vitro Wash-off Test for Microspheres

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test .A 1-cm by 1-cm piece of goat nasal mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (350) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the phosphate buffer (pH 6.6). At the end of 30 minutes, 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted.

In vitro drug release studies

The drug release experiments employed **Small Wonder -Lyzer™**, multifunctional membrane device-membrane sac with graduated container and lid, since this model would allow microspheres to hydrate slowly in a humid environment using phosphate buffer (pH 6.6).

Drug Release Kinetics

To investigate the mechanism of drug release from the microspheres, the release data were analyzed using zero-order kinetic, Higuchi, Korsmeyer–Peppas models etc. Modeling was performed using GraphPad Prism Software Version 4.0 (GraphPad Prism Software, San Diego, CA, USA). The software estimates the parameters of a nonlinear function that provides the closest fit between experimental observations and the non-linear function. The best-fit solution was identified by evaluating the

coefficient of determination (R^2), where the highest R^2 value indicates the best fit.

RESULTS AND DISCUSSION

Optimization data analysis and model-validation

ANOVA provision available in the software was used to establish the statistical validation of the polynomial equations generated by Design Expert. A total of 15 runs with triplicate center points were generated by Box-Behnken design. All the responses observed were simultaneously fitted to first order-, second order- and quadratic-models and were evaluated in terms of statistically significant coefficients and R^2 values.

Various feasibility and grid searches were conducted over the experimental domain to find the compositions of the optimized microsphere formulations. Three dimensional response surface plots were provided by the Design Expert software, where by intensive grid search performed over the whole experimental region, five optimum checkpoint formulations were selected to validate the chosen experimental domain and polynomial equations. The optimized checkpoint formulations were prepared and evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with that of the predicted values to calculate the percentage prediction error. Also, linear regression plots between actual and predicted values of the responses were produced using MS-Excel.

For the response surface methodology involving Box-Behnken design, a total of 15 experiments were performed for three factors at three levels each. Table 1 summarizes the experimental runs, their factor combinations and the levels of experimental units used in the study as well as the entrapment, mean diameter and % drug released after 50 hrs for each factor combination.

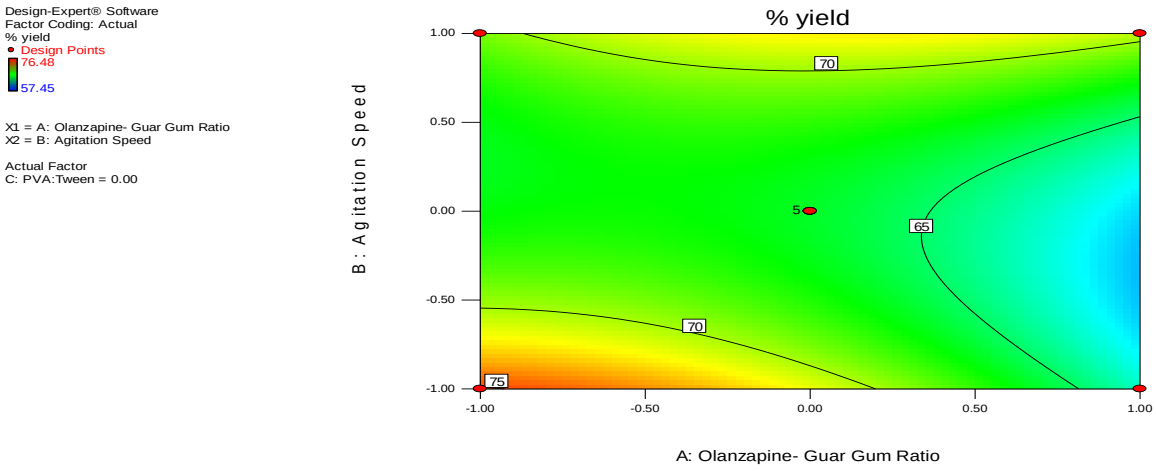
In order to determine the levels of factors which yielded maximum entrapment, mathematical relationships were generated between the dependent and independent variables. For estimation of coefficients in the approximating polynomial function applying uncoded values of factor levels, the least square regression method was used. A suitable polynomial equation involving the individual main effects and interaction factors was selected based on the estimation of several statistical parameters such as the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) .The resultant equations for both responses Y_1, Y_2 and Y_3 presented below (full model):

Y_1(%yield) = $+66.25-2.68X_1-0.44X_2+0.37X_3+3.31X_1X_2+3.61X_1X_3-1.02X_2X_3-2.11X_1^2+5.45X_2^2-2.92X_3^2$

Y_2 (Drug entrapment efficiency) $=+56.69-0.45X_1+2.57X_2-5.20X_3+3.08X_1X_2-1.65X_1X_3-3.69X_2X_3+4.03X_1^2+3.45X_2^2+3.08X_3^2$

Y_3 (% drug release after 50 hrs.) = $+65.01-0.13X_1-1.35X_2-0.63X_3-3.58X_1X_2-1.44X_1X_3-2.13X_2X_3-3.87X_1^2+1.84X_2^2+0.50X_3^2$

The relationship between the dependent and independent variables were further elucidated using contour plots and response surface plots. In Figure 1 are the contour plots showing the effect of factors X1 X2, and X3 on the response Y1, where the small circles indicate levels at which maximum response would be observed. As the drug polymer ratio and agitation speed increases %yield increases while X3 has negative effect on % yield.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% yield
Design Points
76.48
57.45
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X2 = B: Agitation Speed
Actual Factor
C: PVA:Tween = 0.00
% yield
B: Agitation Speed
A: Olanzapine- Guar Gum Ratio
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75
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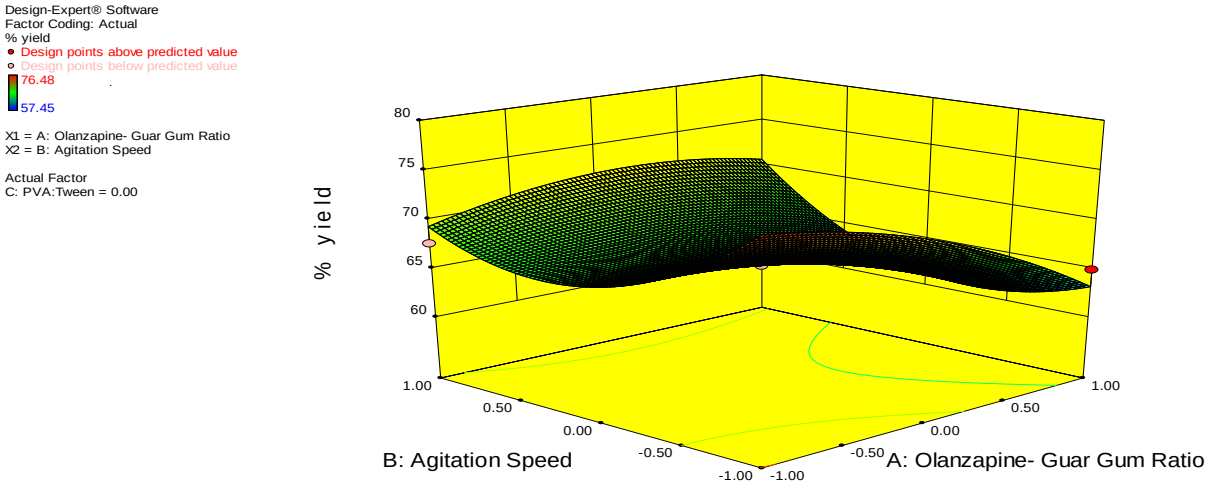
-1.00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Factor Coding: Actual
% yield
Design points above predicted value
Design points below predicted value
76.48
57.45
X1 = A: Olanzapine- Guar Gum Ratio
X2 = B: Agitation Speed
Actual Factor
C: PVA:Tween = 0.00
% yield
80
75
70
65
60
B: Agitation Speed
A: Olanzapine- Guar Gum Ratio
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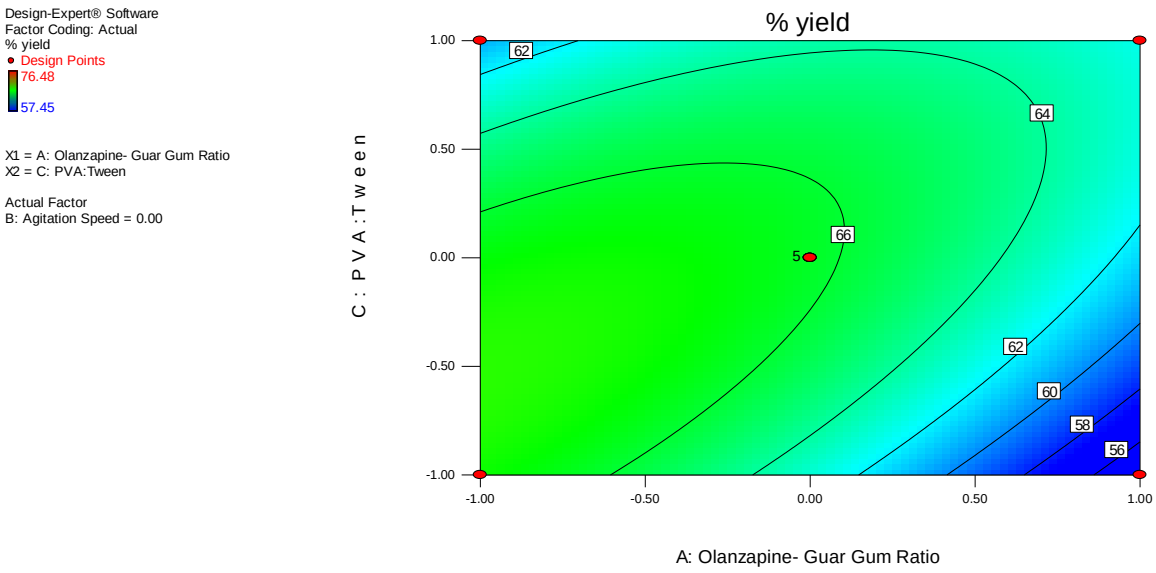
-0.50
-1.00


Design-Expert® Software
Factor Coding: Actual
% yield
Design Points
76.48
57.45
X1 = A: Olanzapine- Guar Gum Ratio
X2 = C: PVA:Tween
Actual Factor
B: Agitation Speed = 0.00
% yield
C : P V A : T w e e n
A: Olanzapine- Guar Gum Ratio
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66
62
60
58
56
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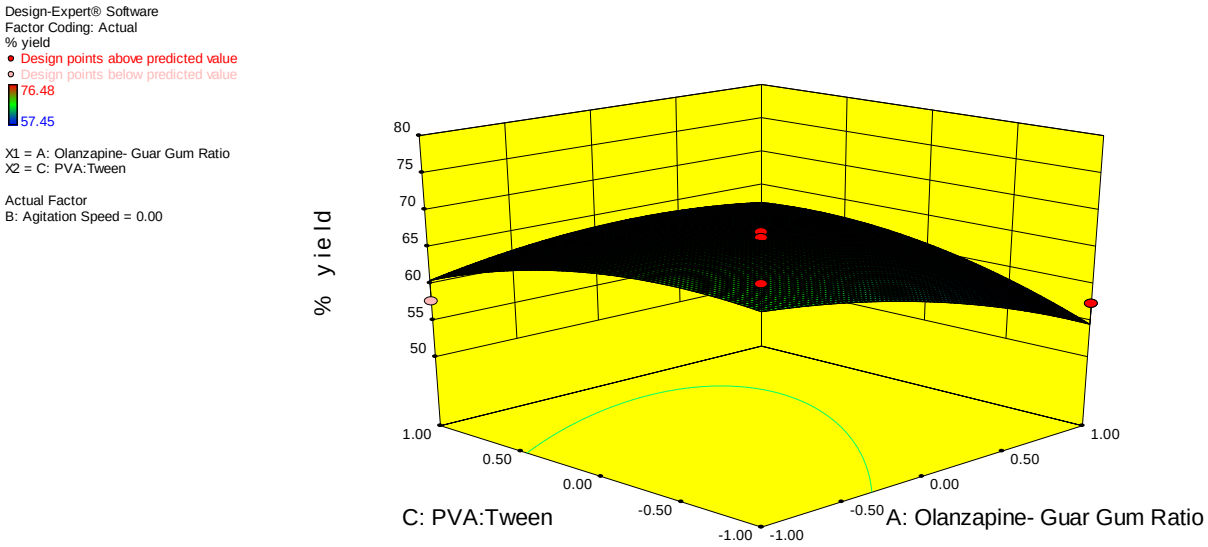
-1.00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% yield
Design points above predicted value
Design points below predicted value
76.48
57.45
X1 = A: Olanzapine- Guar Gum Ratio
X2 = C: PVA:Tween
Actual Factor
B: Agitation Speed = 0.00
% yield
C: PVA:Tween
A: Olanzapine- Guar Gum Ratio
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-1.00

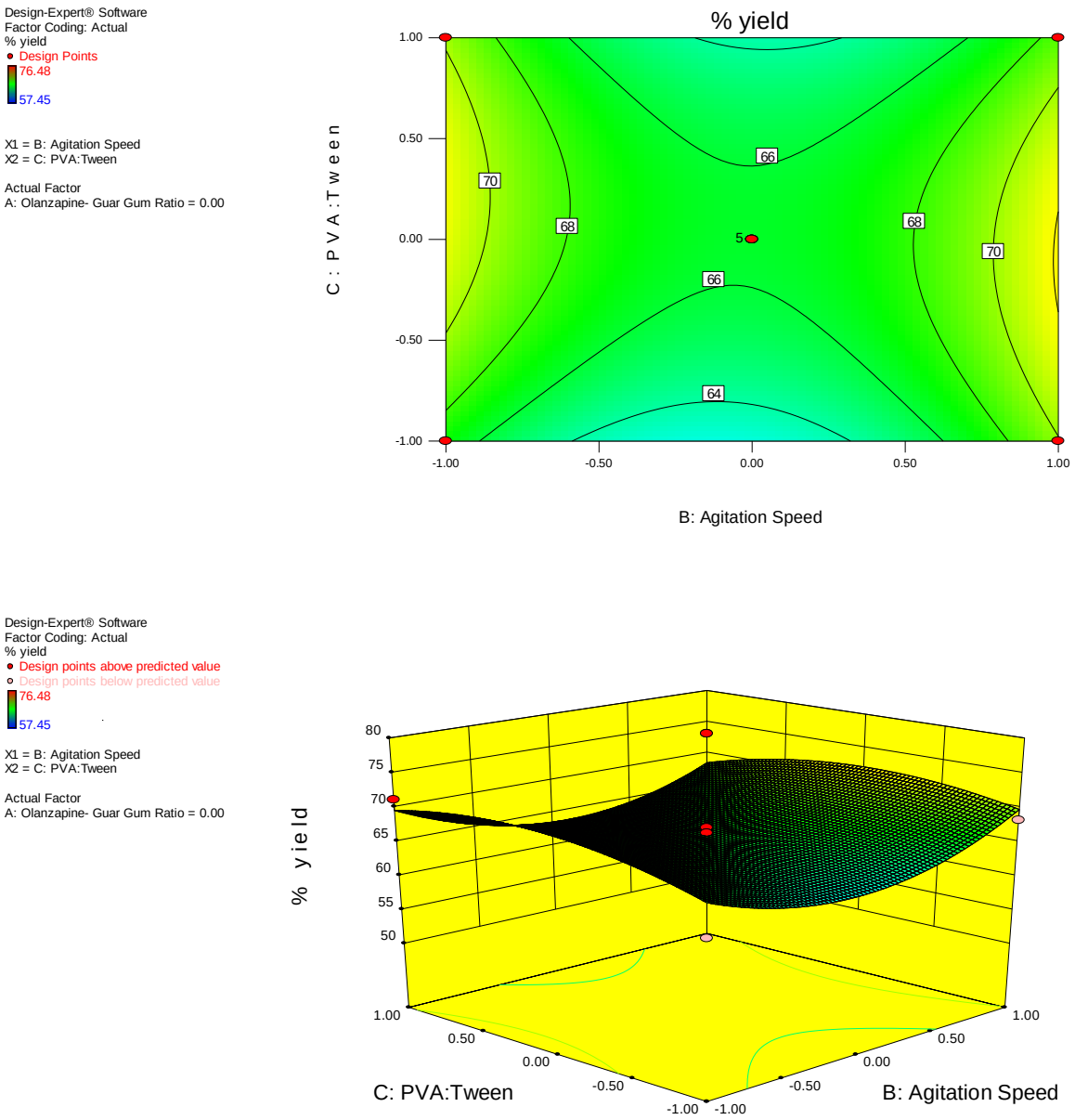
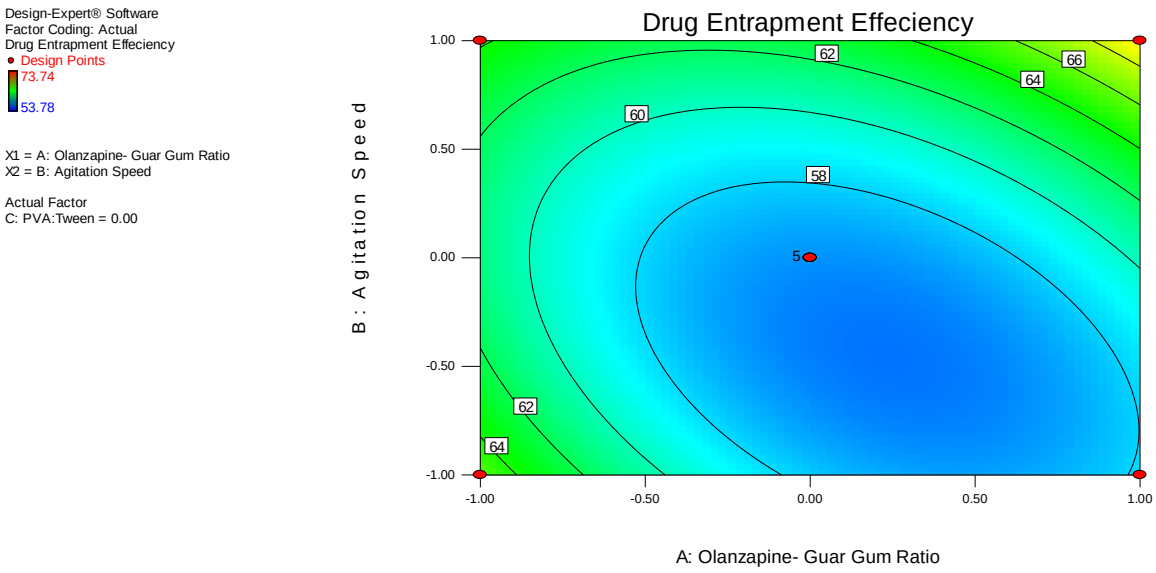


Figure. 1 Response surface plots showing the effect of different levels of independent variable (X) on percentage yield (Y1).

In Figure 2 are the contour plots showing the effect of factors X1 X2, and X3 on the response Y2, it indicates that factor X1,X2 and X3 has positive effect on drug entrapment efficiency. surfactant Tween 80 alone and as the concentration of polymer, agitation speed increases, drug entrapment into polymer matrices also increases.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Drug Entrapment Effeciency
Design Points
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53.78
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X2 = B: Agitation Speed
Actual Factor
C: PVA:Tween = 0.00
Drug Entrapment Effeciency
B : A g i t a t i o n S p e e d
A: Olanzapine- Guar Gum Ratio
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64
60
58
5
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0.50
0.00
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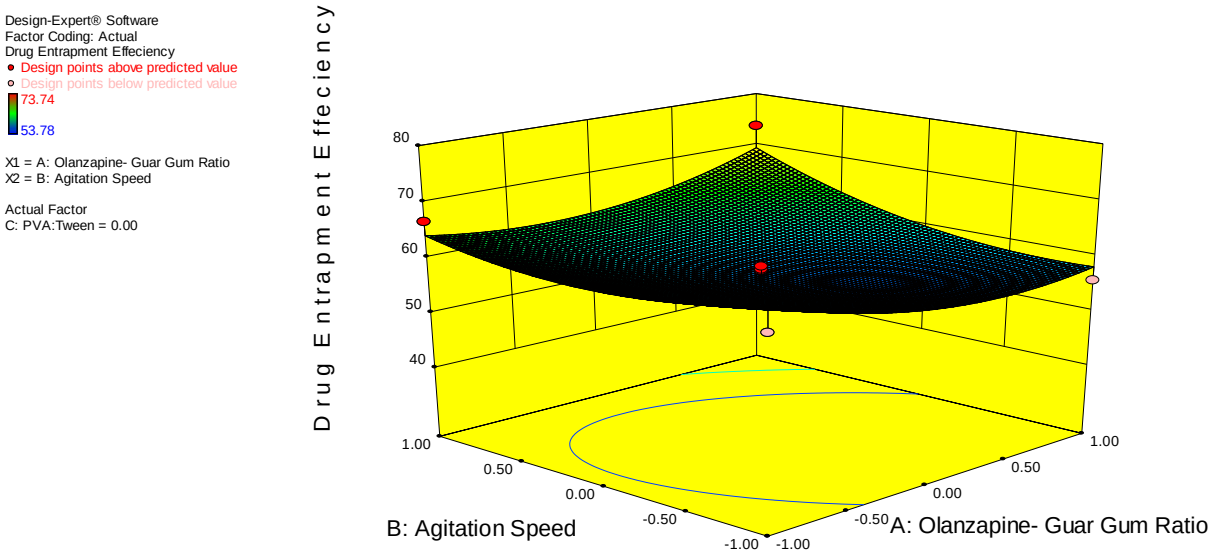
-1.00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Drug Entrapment Effeciency
Design points above predicted value
Design points below predicted value
73.74
53.78
X1 = A: Olanzapine- Guar Gum Ratio
X2 = B: Agitation Speed
Actual Factor
C: PVA:Tween = 0.00
D r u g E n t r a p m e n t E f f e c i e n c y
80
70
60
50
40
B: Agitation Speed
A: Olanzapine- Guar Gum Ratio
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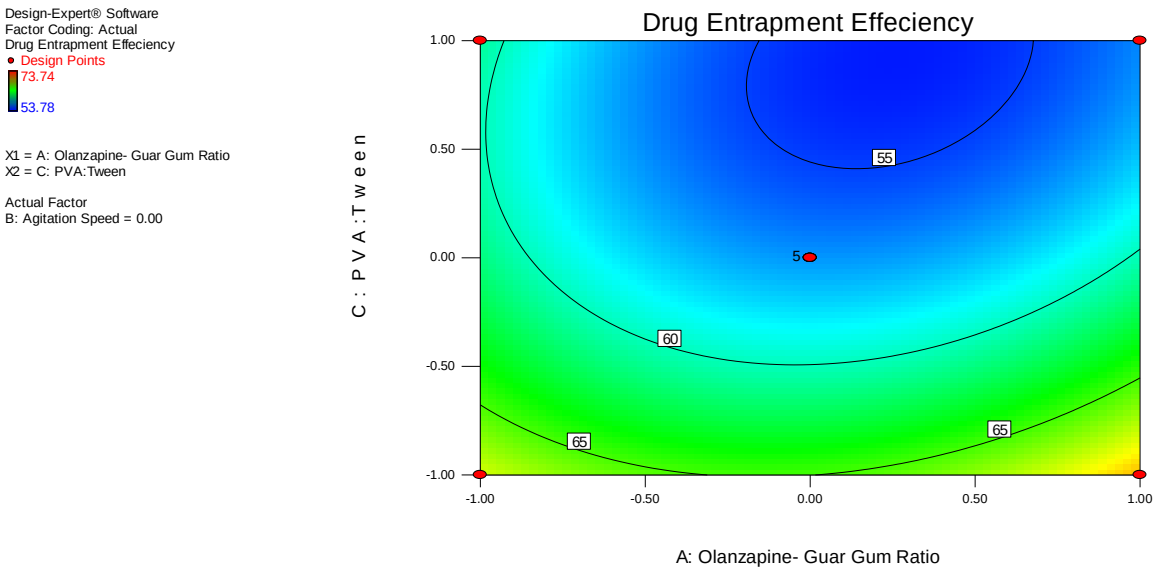
-1.00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Design Points
73.74
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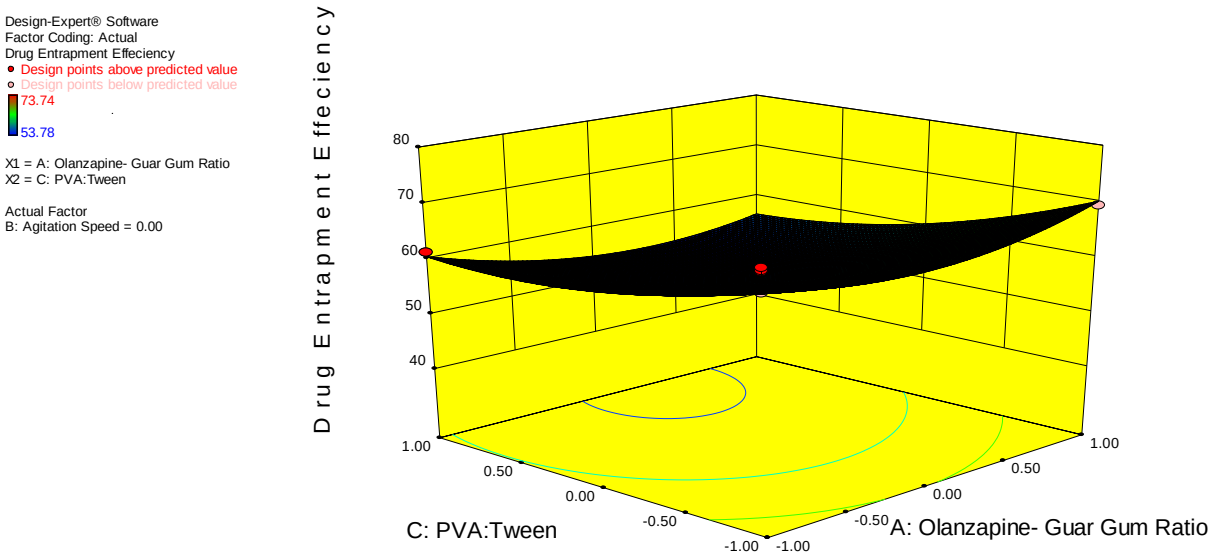
X1 = A: Olanzapine- Guar Gum Ratio
X2 = C: PVA:Tween
Actual Factor
B: Agitation Speed = 0.00
Drug Entrapment Effeciency
C : P V A : T w e e n
1.00
0.50
0.00
-0.50
-1.00
55
60
65
65
5
-1.00
-0.50
0.00
0.50
1.00
A: Olanzapine- Guar Gum Ratio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Drug Entrapment Effeciency
Design points above predicted value
Design points below predicted value
73.74
53.78
X1 = A: Olanzapine- Guar Gum Ratio
X2 = C: PVA:Tween
Actual Factor
B: Agitation Speed = 0.00
D r u g E n t r a p m e n t E f f e c i e n c y
80
70
60
50
40
1.00
0.50
0.00
-0.50
-1.00
C: PVA:Tween
-1.00
-0.50
0.00
0.50
1.00
A: Olanzapine- Guar Gum Ratio

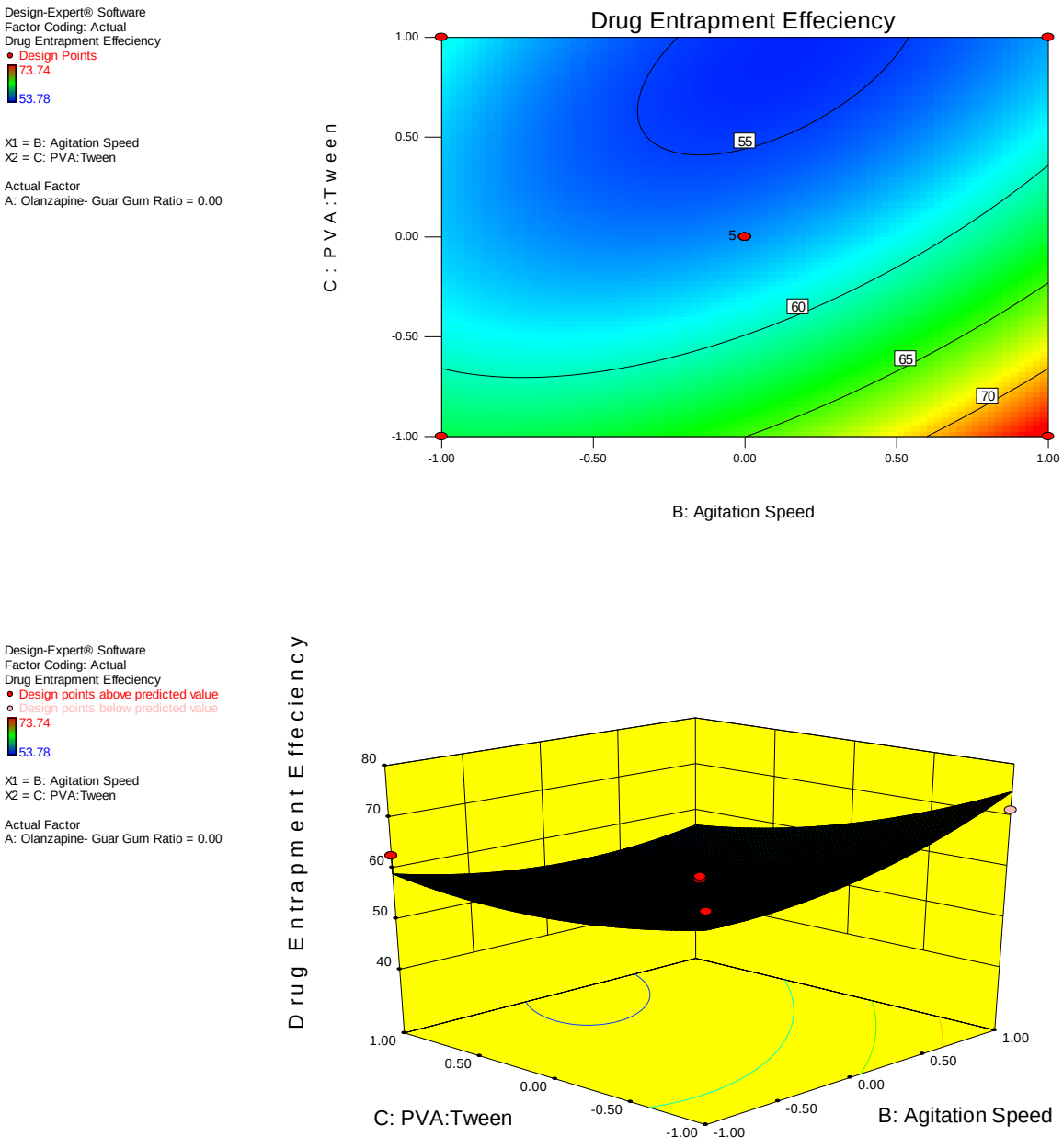
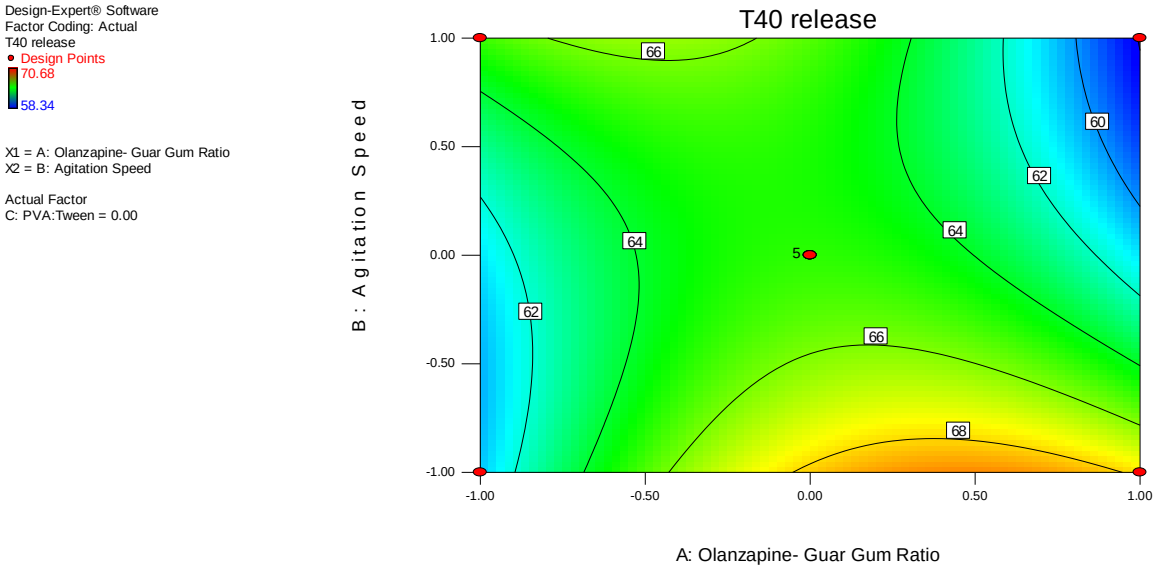


Figure. 2 Response surface plots showing the effect of different levels of independent variable (X) on entrapment efficiency(Y2).

In Figure 3 are the contour plots showing the effect of factors X1 X2, and X3 on the response Y3, it indicates that factor X2 and X3 has not major contribution to the response Y3, i.e. agitation speed and surfactant ratio has not major effect on drug release after 50 hrs. While factor X1 has negative effect on drug release so as the concentration of polymer increases, drug release from microsphere retards as formation of viscous layer of polymer around drug particle.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Factor Coding: Actual
T40 release
Design Points
70.68
58.34
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X2 = B: Agitation Speed
Actual Factor
C: PVA:Tween = 0.00
T40 release
B : A g i t a t i o n S p e e d
A: Olanzapine- Guar Gum Ratio
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-1.00
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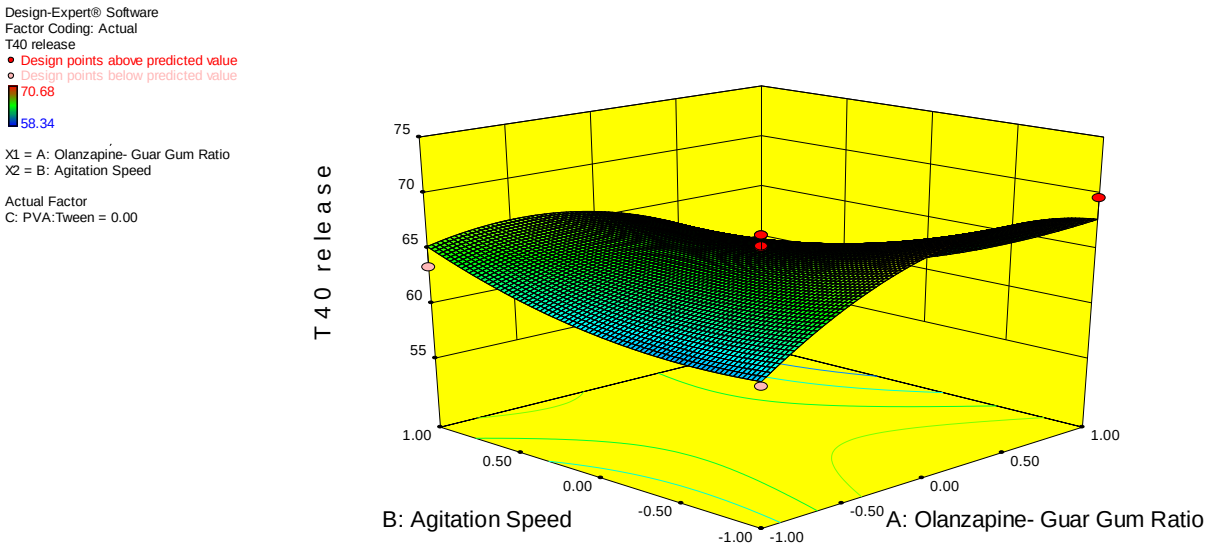
64
62
60
68
5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Design points below predicted value
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X2 = B: Agitation Speed
Actual Factor
C: PVA:Tween = 0.00
T 4 0 r e l e a s e
75
70
65
60
55
B: Agitation Speed
A: Olanzapine- Guar Gum Ratio
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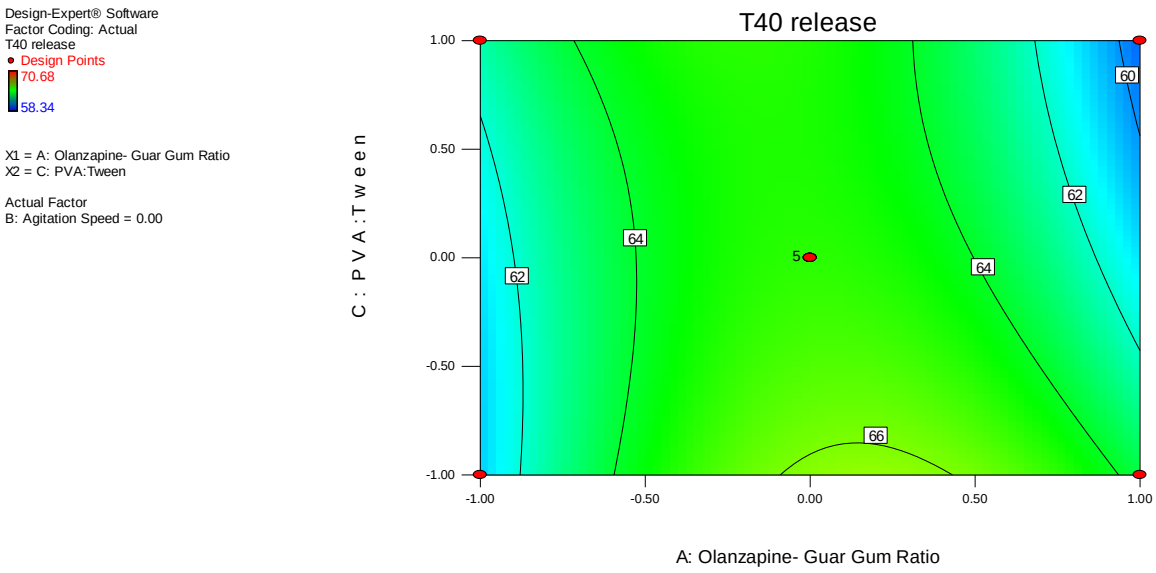
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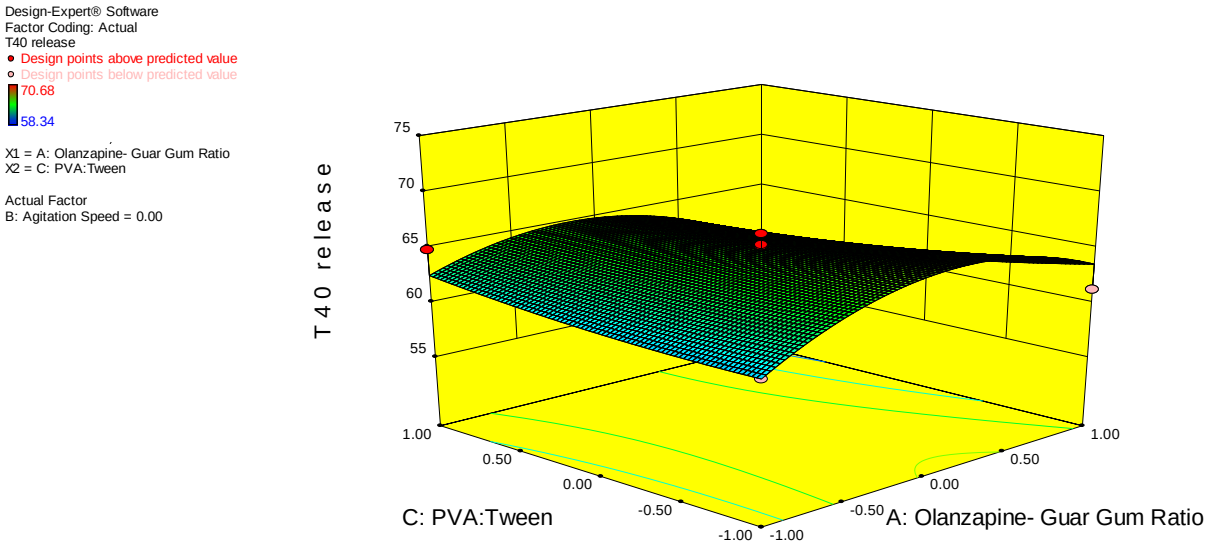
1.00
0.50
0.00
-0.50
-1.00
60
62
64
66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B: Agitation Speed = 0.00
T 4 0 r e l e a s e
75
70
65
60
55
C: PVA:Tween
A: Olanzapine- Guar Gum Ratio
1.00
0.50
0.00
-0.50
-1.00

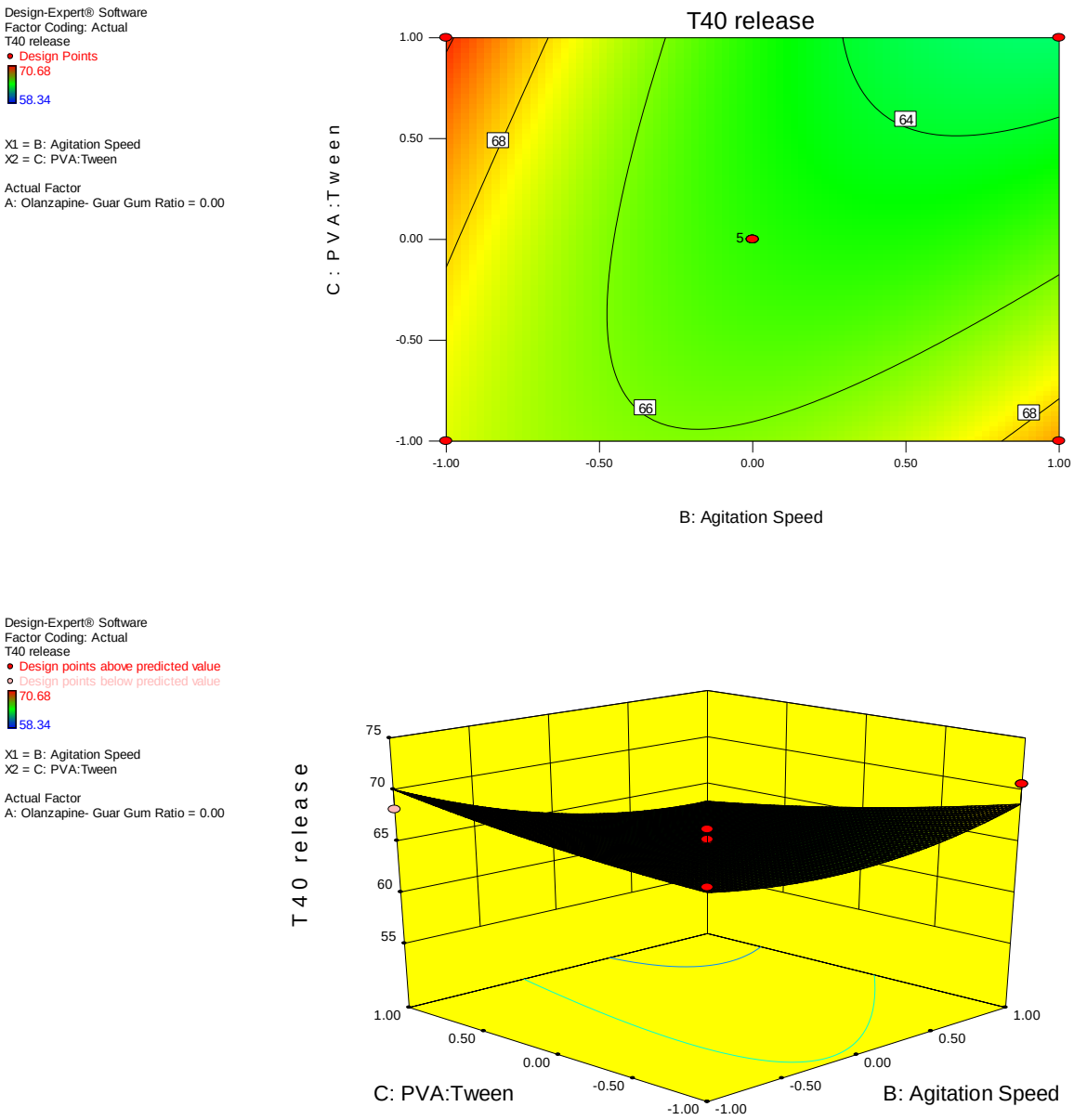

Figure 3. Response surface plots showing the effect of different levels of independent variable (X) on percent drug release (Y3).

Optimization

The optimum formulation of drug-loaded microsphere was selected based on the criteria of attaining the maximum value of encapsulation efficiency; minimizing the particle size and optimum % drug release. Upon 'trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with

Drug: Polymer Ratio (1:1), Agitation speed (20000) and alone PVA and Tween 80 surfactant in 1:1 ratio was found to fulfill requisites of an optimum formulation. The optimized formulation has the Drug entrapment efficiency of 71.23% with particle size

20.57 um and % drug release after 50hrs was 68.34% respectively.

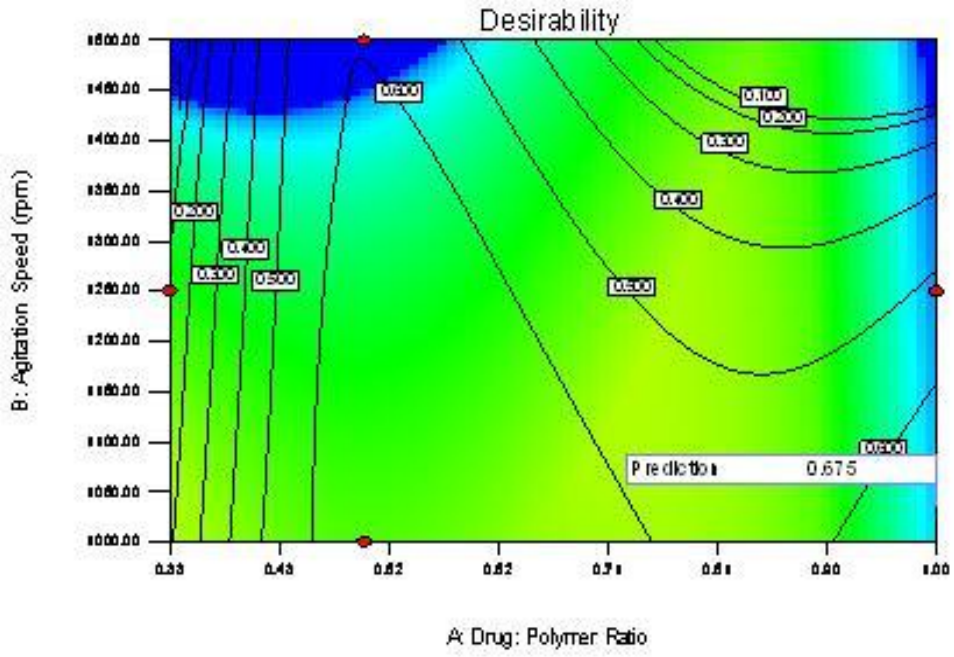

Figure. 4 Optimization of microsphere

Drug Entrapment Efficiency, % Yield and In Vitro Wash-off Test for Microspheres

The DEE of the prepared batches was found in the range 55 % to74 %. The production yield of the prepared batches was found to be in the range 58% to71% as shown in Table 3 for P1 to P15 respectively.

Table No. 3 Production yield, Drug Entrapment Efficiency and In Vitro Wash-off Test for Olanzapine Microspheres (In-vitro mucoadhesion studies)

Batch No.	% Yield	Drug entrapment efficiency (%)	In-vitro mucoadhesion (4 hrs.)
P1	60.45	60.94	78.24
P2	70.68	71.23	66.56
P3	59.45	55.43	52.89
P4	64.89	61.45	65.34
P5	69.67	55.69	68.58
P6	66.34	58.45	78.23
P7	68.26	62.78	75.78

P8	63.45	66.67	56.19
P9	64.26	53.78	67.74
P10	61.27	69.46	57.34
P11	65.35	57.89	64.45
P12	62.78	53.78	59.34
P13	58.34	73.74	72.86
P14	60.94	68.89	80.14
P15	67.65	65.47	68.28

Morphology and particle size

The microspheres were found to be discrete, spherical, free-flowing, and of the monolithic matrix type as shown in Figure 4.

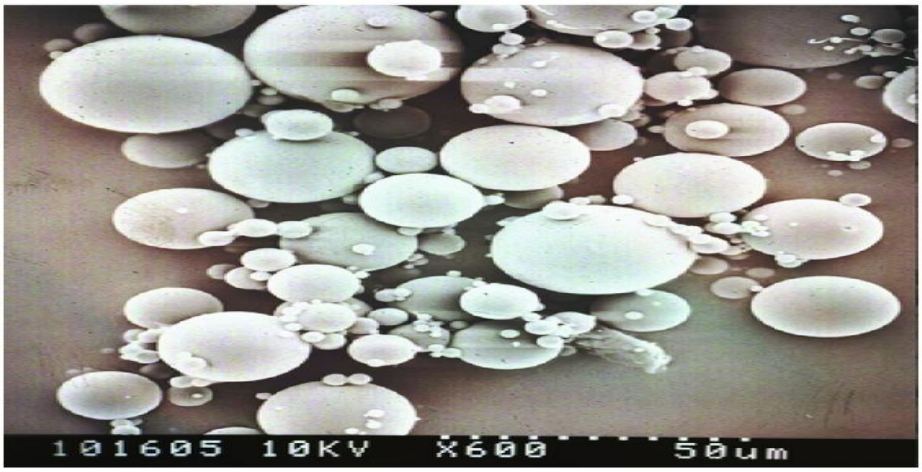

Figure 4. Photomicrograph (SEM) of Olanzapine Microspheres

Micromeritic Properties of Microspheres

The rheological parameters like angle of repose, tapped density, bulk density and packing properties (Table 4.) confirms better flow and packing properties of the prepared microspheres. All the formulation showed angle of repose within appreciable limit for microspheres to show flow property .The microspheres were characterized by their micromeritic properties such as particle size, tapped density, Carr's index, and flow property. The rheological parameters and packing properties confirms better flow and packing properties of the prepared microspheres.

Table 4. Micromeritic Properties of Microspheres

Batch No.	Avg.Particle Size (µm)	Carr's Index	Hausner's Ratio	Angle of repose
P1	32.78±4.6	10.1	1.16	26˚65´
P2	20.57±6.2	10.4	1.14	25˚16´
P3	53.00± 7.1	12.3	1.18	38˚21´
P4	78.89±8.4	11.7	1.13	34˚16´
P5	28.84±9.7	13.4	1.14	35˚40´
P6	28.57±10.3	16.4	1.19	40˚78´
P7	67.85±5.8	14.2	1.12	41˚18´
P8	88.26±7.5	13.9	1.13	31˚44´
P9	65.00±9.2	15.3	1.15	37˚92´
P10	68.89±8.4	10.6	1.16	44˚18´
P11	36.87±6.7	14.2	1.15	40˚16´
P12	90.26±7.9	18.6	1.18	38˚21´
P13	45.52±6.2	11.4	1.10	40˚18´
P14	47.59±4.3	12.6	1.07	36˚81´
P15	46.58±7.74	10.59	1.15	35˚78´

Drug Release Kinetics

Korsmeyer–Peppas model best described the sustained release phase (R^2 = 0.998) suggesting **non-Fickian** diffusion process. This is further supported by the fact that the sequential process of polymer hydration, solvent penetration, drug dissolution and/or polymer erosion determine the drug release from hydrophilic matrices.

CONCLUSION

All the **formulations** prepared with the experimental design yielded smooth spherical microspheres with

size in the range of 20.57 to 90.26 µm. .Drug polymer ratio at low level (X_1, -1), Agitation Speed at high level (X_2 , +1) and Surfactant Ratio at medium level (X_3, 0) yielded microspheres with 20.57 mean diameter, good drug entrapment (71.23%) and % drug release after 50hrs. (68.34%). Korsmeyer–Peppas model best described the sustained release phase (R^2 = 0.998) suggesting non-Fickian diffusion process. This is further supported by the fact that the sequential process of polymer hydration, solvent penetration, drug dissolution and/or polymer erosion determine the drug release from hydrophilic matrices.

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