

FORMULATION AND OPTIMIZATION OF MUCOADHESIVE MICROSPHERES OF VALSARTAN BY USING BOX-BEHNKEN DESIGN

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Received: 23 Apr 2019, Revised and Accepted: 01 Jun 2019

ABSTRACT

Objective: The purpose of this study was to formulate and optimize mucoadhesive microspheres of antihypertensive drug (valsartan) within ethyl cellulose as a carrier polymer and carbopol 934P as a mucoadhesive polymer for controlling the release of valsartan.

Methods: The emulsion solvent evaporation technique was used for preparation of microspheres of valsartan and the Box-Behnken design was employed with three independent variables that is amount of ethyl cellulose (X_1) and amount of carbopol 934P (X_2) and stirring speed (X_3) and evaluate four dependent variables such as percentage mucoadhesion, Q_1 h, $t_{90\%}$ and drug entrapment efficiency.

Results: The optimum conditions were found to be $X_1= 200$ mg, $X_2= 107$ mg and $X_3= 1200$ rpm. The optimized batch exhibited a high drug entrapment efficiency of $85.63 \pm 1.384\%$, percentage mucoadhesion was $66.76 \pm 0.986\%$ and drug release was also sustained for more than 12 h.

Conclusion: The analysis of variance showed a significant effect of independent variables. The scanning electron microscopy (SEM) analysis showed that the microspheres were spherical and free-flowing. The microspheres of valsartan were stable after three month stability study at accelerated condition.

Keywords: Mucoadhesive microspheres, Valsartan, Carbopol 934P, Ethylcellulose, Box-Behnken design

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DOI: <http://dx.doi.org/10.22159/ijap.2019v11i4.33710>

INTRODUCTION

Hypertension is one of the primary risk factors for heart disease and stroke, the leading causes of death due to its high prevalence all around the globe [1-4]. Approximately 7.5 million deaths worldwide occur due to hypertension and predicted to be increased to 1.56 billion adults with high blood pressure in 2025 [5, 6].

A number of different types of anti-hypertensive agents used to control elevated blood pressure [7]. Valsartan, a non-peptide, specific competitive angiotensin II type 1 receptor antagonist is a drug of choice in cardiovascular disorders, particularly in hypertension [8, 9].

The oral route is most convenient for the delivery of different dosage form as compared with other routes [10]. The absorption of valsartan is high ($t_{max} \approx 2$) through oral administration, but it has poor bioavailability (23%) due to slow absorption from the gastrointestinal tract. Therefore, improving its therapeutic efficacy through oral route alternative drug delivery systems and dosage forms is needed [11-13].

Various investigations have confirmed that a gastro-retentive mucoadhesive delivery system could remain in the gastric mucosa for a long period of time and it improves the bioavailability and reduce drug loss [14, 15]. Moreover, it could play an effective role in drug localization in the stomach [16, 17]. The high surface-to-volume ratio of this dosage form could improve drug absorption and drug bioavailability [18, 19].

In this work, mucoadhesive microspheres of valsartan were prepared with carbopol 934P as mucoadhesive and ethyl cellulose as carrier polymer by using the emulsion solvent evaporation technique. A Box-Behnken design was employed to study the effect of independent variables (amount of ethyl cellulose, amount of carbopol 934P and stirring speed) of mucoadhesive microspheres of valsartan on the physicochemical characteristics (drug entrapment efficiency, Q_1 h, $t_{90\%}$ and mucoadhesion).

MATERIALS AND METHODS

Materials

Valsartan was obtained from Macleods Pharmaceutical Ltd. Sarigam (Gujarat, India). Carbopol 934P was purchased from sigma Aldrich

Pvt. Ltd. Ethylcellulose, light liquid paraffin, Span 80, Ethanol 95%, petroleum ether, and methanol were purchased from Loba Chemie Pvt. Ltd. Mumbai. Hydrochloric acid (HCl) was purchased from Thermo Fisher Scientific India Pvt. Ltd. Mumbai. All other chemicals were of analytical grade.

Preparation of microspheres

Mucoadhesive microspheres of valsartan were prepared with an emulsion solvent evaporation technique. Ethyl cellulose was dissolved in 95% ethanol; carbopol 934P and valsartan powder were added in ethyl cellulose solution under magnetic stirring for 2 h. Then this suspension was dispersed dropwise in light liquid paraffin containing span 80 as surfactant with continuous stirring on Remi mechanical stirrer at 1000 rpm. After 5 h of stirring, ethanol was evaporated gradually and microspheres were produced. Prepared microspheres were washed with petroleum ether, filtered, dried at room temperature for 24 h and then stored in the desiccators until used.

Box-behnken optimization design

The box-behnken design was employed for optimizing the microspheres of valsartan and investigating the correlation between the experimental factors and responses.

The independent factors chosen in the Box-Behnken design were: the amount of ethyl cellulose (X_1), amount of carbopol 934P (X_2) and stirring speed (X_3). The levels of the factors are selected according to the literature [20, 21]. Twelve batches of microspheres and three center point batches were prepared according to this design.

In order to elucidate the effect of preparation conditions on dependent variables such as encapsulation efficiency, Q_1 h, $t_{90\%}$ and mucoadhesion, the Box-Behnken design was used with the three factors selected and their respective three levels (low, medium and high). To evaluate the response variables, a second-order polynomial model was utilized:

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 \dots \text{(Eq.1)}$$

Y is the predicted response; β_0 is model constant; x_1 , x_2 , and x_3 are independent variables; β_1 , β_2 , and β_3 are linear coefficients; β_{12} , β_{13} and β_{23} are cross-product coefficients; and β_{11} , β_{22} , and β_{33} are the

quadratic coefficients. The quality of fit of the polynomial model equation was expressed with the coefficient of determination R^2

[22]. The selection of independent and dependent variables and their levels are given in table 1.

Table 1: Independent and dependent variables

Independent variable	Variable level		
	Low (-1)	Medium (0)	High (1)
Amount of ethyl cellulose(X_1)	100 mg	150 mg	200 mg
Amount of carbopol 934P(X_2)	50 mg	100 mg	150 mg
Stirring speed(X_3)	800	1000	1200
Dependent Variables			

Y_1 = percent entrapment efficiency, Y_2 = Q_{1h} (percent cumulative drug release at 1h), Y_3 = $t_{90\%}$, Y_4 = percent mucoadhesion

Determination of percentage yield

The production yields of mucoadhesive microspheres of all formulation batches were evaluated using the weight of the final dried product (W1) with respect to the initial total weight of the raw materials used for the preparation of microspheres (W2) and percent production yields according to the equation 2 cited below [23-25].

$$\% \text{yield} = \frac{\text{Total amount of dried microspheres (W1)}}{\text{Total weight of raw material (W2)}} \times 100. \text{ (Eq. 2)}$$

Determination of drug entrapment efficiency

A precisely measured amount of mucoadhesive microspheres (50 mg) of valsartan was dissolved in methanol with stirring until complete dissolution. The solution was then filtered, an aliquot of the filtrate was appropriately diluted, and the content of valsartan was determined spectrophotometrically at 248 nm. The determinations were made in triplicate. The percent drug entrapment efficiency was determined according to the following equation [26-28].

$$\% \text{ Entrapment efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100 \dots \text{ (Eq. 3)}$$

Determination of particle size of microspheres

The microsphere was examined with an optical microscope and the size of the microspheres was calculated with a pre-calibrated ocular micrometer and stage micrometer. About 200-300 particles of each formulation were observed and counted [29-31].

In vitro release study

An appropriate amount of Microspheres (50 mg) was taken in a muslin cloth. Dissolution was performed using USP type II apparatus at $37 \pm 0.5^\circ\text{C}$, rotational speed of 50 rpm in 900 ml of 0.1N HCL (pH 1.2) for 12 h. Samples (5 ml) were withdrawn at fixed time intervals and equally (5 ml) replaced with fresh dissolution medium [32, 33], filtered, diluted suitably and analyzed spectrophotometrically at 249 nm. Concentrations

were calculated using calibration curves developed in respective media. Taking into account, the loss of the drug in aliquot replaced, the correction factor was used as shown in equation 4 [34-36].

$$C_i = A_i + \frac{V_s}{V_t} \sum_{i=1}^{n-1} A_i \left(\frac{V_t}{V_t - V_s} \right) \dots \dots \dots \text{ (Eq. 4)}$$

Where, C_i = Corrected absorbance, V_s = Sample of dissolution media withdrawn, V_t = Total volume of dissolution media.

Dissolution release profiles were plotted with percentage drug released at different time intervals. Q_1 h percent cumulative drug release (% CDR) and the average value of $t_{90\%}$ for all batches was calculated from the dissolution data.

In vitro wash-off test

Mucoadhesive property of Microspheres was evaluated by *In vitro* wash-off test. The rats were sacrificed by cervical dislocation. The abdomen was opened and the stomach excised [37]. A 1-cm by 1-cm piece of rat stomach mucosa was cut and tied on a glass slide (3-inch by 1-inch) with thread. Approximately 100 microspheres were spread onto the wet rinsed tissue specimen and hanging prepared slide onto the grooves of a USP tablet disintegrating test apparatus. After that, the apparatus was switched on and the tissue specimen was given up and down movements for 10 h in a beaker having simulated gastric fluid (pH 1.2). The microspheres left behind on the surface of the gastric mucosa were counted after 10 h and the % mucoadhesion was calculated by the formula shown in Equation 5 [38, 39].

$$\text{Percent mucoadhesion} = \frac{\text{Weight of adhered microspheres}}{\text{Weight of applied microspheres}} \times 100 \dots \text{ (Eq. 5)}$$

Release kinetics and mechanism

To determine the release mechanism and kinetics, optimized formulation was attempted to fit into different mathematical models like zero order, first order, Higuchi's Hixon-Crowndwell and Korsmeyer-Peppas models [40, 41].

Table 2: Experiment plan and observed responses of the model

Batch No.	Independent factors			Responses			
	Amount of ethyl cellulose (mg)	Amount of carbopol 934P(mg)	Stirring speed (rpm)	Entrapment efficiency* (%)	Q_1 h (%CDR)*	$t_{90\%}$ (min.)	Mucoadhesion (%)
V ₁	-1(100)	1(150)	0(1000)	56.18±1.12	34.76±0.83	498	74
V ₂	-1(100)	0(100)	1(1200)	57.54±0.25	25.34±1.02	538	67
V ₃	1(200)	-1(50)	0(1000)	74.66±0.68	22.24±0.95	725	51
V ₄	1(200)	0(100)	1(1200)	87.32±2.32	18.72±0.79	745	64
V ₅	1(200)	0(100)	-1(800)	78.86±1.62	26.86±0.13	636	65
V ₆	1(200)	1(150)	0(1000)	85.16±0.58	28.48±1.58	558	68
V ₇	-1(100)	-1(50)	0(1000)	51.68±0.74	33.56±0.90	612	54
V ₈	0(150)	-1(50)	1(1200)	62.54±3.62	23.26±1.03	716	52
V ₉	0(150)	1(150)	-1(800)	60.65±1.23	32.85±0.30	538	72
V ₁₀	0(150)	1(150)	1(1200)	64.94±2.22	24.98±0.72	568	69
V ₁₁	0(150)	0(100)	0(1000)	56.38±0.26	25.26±1.14	547	62
V ₁₂	-1(100)	0(100)	-1(800)	50.45±2.32	35.66±1.16	524	70
V ₁₃	0(150)	0(100)	0(1000)	58.76±2.47	23.64±1.07	556	60
V ₁₄	0(150)	0(100)	0(1000)	58.12±0.16	23.88±0.90	576	62
V ₁₅	0(150)	-1(50)	-1(800)	53.76±0.72	31.08±1.75	628	52

*mean±SD, n = 3

Determination of surface morphology

Scanning electron microscope (SEM) (JEOL JSM-6100 scanning microscope, Japan) was used to determine surface characteristics of microspheres. Sample of microspheres was mounted on a stub and coated with a layer of gold using a sputter coater (JFC-1100). The samples were scanned at 5kV and photomicrograph at different magnification ratio [42].

RESULTS AND DISCUSSION

Optimization

In the Box-Behnken design, a total 15 formulations were proposed by Design expert software for three factors such as the amount of ethyl cellulose (X_1), amount of carbopol 934P (X_2) and stirring speed (X_3), which were varied at three different levels (-1, 0 and 1). The

effects of independent variables (factors) on the drug entrapment efficiency (%), Q_1 h (%), $t_{90\%}$ and mucoadhesion (%) were examined as optimization response parameters in this study. Summarize of the experimental plan and observed responses were presented in table 2 and the percent yield, particle size and shape of microspheres were shown in table 3.

The polynomial equation for entrapment efficiency as follows, according to the model:

$$Y_1 = +57.75 + 13.77X_1 + 3.04X_2 + 3.58X_3 + 1.50 X_1 X_2 + 0.3425 X_1 X_3 - 1.12 X_2 X_3 + 8.62 X_1^2 + 0.5483 X_2^2 + 2.17 X_3^2$$

The F-value of 86.85 implies the model is significant ($p < 0.0001$) (table 4). So the X_1 , X_2 , X_3 , X_1^2 and X_3^2 are significant model terms. The 'Pred R-Squared' of 0.9191 is in rational agreement with the 'Adj R-Squared' of 0.9822.

Table 3: Results of percentage yield, particle size and shape of microspheres

Batch No.	Percent yield	Particle size* (um)	Shape of microspheres
V ₁	75.24	104.28±2.64	Spherical
V ₂	82.86	92.64±2.84	Spherical
V ₃	78.52	98.84±3.67	Spherical
V ₄	87.64	95.21±2.34	Spherical
V ₅	70.16	124.32±3.98	Spherical
V ₆	82.34	118.16±3.24	Spherical
V ₇	73.28	96.74±1.82	Spherical
V ₈	83.25	95.98±2.73	Spherical
V ₉	71.86	120.22±4.16	Spherical
V ₁₀	87.36	93.96±3.14	Spherical
V ₁₁	72.56	108.62±3.21	Spherical
V ₁₂	68.14	112.26±2.63	Spherical
V ₁₃	74.64	106.92±2.85	Spherical
V ₁₄	76.86	108.56±1.64	Spherical
V ₁₅	74.52	114.76±3.93	Spherical

*mean±SD, n = 3

Table 4: Analysis of variance calculated for responses

Batch No.	Entrapment efficiency	Q_1 h	$t_{90\%}$	Mucoadhesion
Regression sum of square	1990.15	360.32	80885.92	807.48
df	9	9	9	9
Mean square	221.13	40.04	8987.32	89.72
F-value	86.85	39.78	13.46	153.81
p-value	<0.0001	<0.0004	<0.0053	<0.0001
Lack of fit sum of square	9.70	3.50	2898.75	0.2500
df	3	3	3	3
Mean square	3.23	1.17	966.25	0.0833
F-value	2.13	1.53	4.39	0.0625
Correlation coefficient (R^2)	0.9936	0.9862	0.9604	0.9964

The polynomial equation for Q_1 h as follows, according to the model:

$$Y_1 = +24.26 - 4.13X_1 + 1.37X_2 - 4.27X_3 + 1.37 X_1 X_2 + 0.5450 X_1 X_3 - 0.0125 X_2 X_3 + 2.05 X_1^2 + 3.45 X_2^2 + 0.3337 X_3^2$$

The F-value of 39.78 implies the model is significant ($p < 0.0004$) (table 4). So the X_1 , X_2 , X_3 , X_1^2 , X_2^2 and X_3^2 are significant model terms. The 'Pred R-Squared' of 0.8372 is in rational agreement with the 'Adj R-Squared' of 0.9614.

The polynomial equation for $t_{90\%}$ as follows, according to the model:

$$Y_1 = +559.67 + 61.50X_1 - 64.88X_2 + 30.13X_3 - 13.25 X_1 X_2 + 23.75 X_1 X_3 - 14.50X_2 X_3 + 18.42 X_1^2 + 20.17 X_2^2 + 32.67 X_3^2$$

The F-value of 13.46 implies the model is significant ($p < 0.0053$) (table 4). So the X_1 , X_2 and X_3 are significant model terms. The 'Pred R-Squared' of 0.4376 is in rational agreement with the 'Adj R-Squared' of 0.8890.

The polynomial equation for mucoadhesion as follows, according to the model:

$$Y_1 = +61.33 - 2.13X_1 + 9.25X_2 - 0.8750X_3 - 0.7500 X_1 X_2 + 0.5000 X_1 X_3 - 0.7500X_2 X_3 + 2.83 X_1^2 - 2.42 X_2^2 + 2.33 X_3^2$$

The F-value of 153.81 implies the model is significant ($p < 0.0053$) (table 4). So the X_1 , X_2 , X_3 , X_1^2 , X_2^2 and X_3^2 are significant model terms. The 'Pred R-Squared' of 0.9877 is in rational agreement with the 'Adj R-Squared' of 0.9890.

Coefficients with more than one-factor term represent interaction terms and those with second order terms represent quadratic relationships. The coefficient's sign shows how independent factors influence the responses. The positive sign of the coefficient indicates the response increases (synergistic effect), and a negative sign indicates the response decreases (antagonist effect).

For all these formulations proposed by the Box-Behnken design, the results of examining responses (entrapment efficiency, Q_1 h, $t_{90\%}$ and mucoadhesion) were found within the limits. The actual and predicted responses for all response variables investigated in this study with the parentage of errors in prognosis are given in table 5.

Table 5: Actual and predicted responses of all response variables

Batch No.	Entrapment efficiency		Q ₁ h (%CDR)		T _{90%}		Percent mucoadhesion	
	Actual	Pred.	Actual	Pred.	Actual	Pred.	Actual	Pred.
V ₁	56.18	54.69	34.76	33.99	498.00	485.13	74.00	73.88
V ₂	57.54	58.01	25.34	25.96	538.00	555.63	67.00	67.25
V ₃	74.66	76.15	22.24	23.01	725.00	737.88	51.00	51.13
V ₄	87.32	86.23	18.72	18.79	745.00	726.13	64.00	64.00
V ₅	78.86	78.39	26.86	26.24	636.00	618.38	65.00	64.75
V ₆	85.16	85.22	28.48	28.26	558.00	581.63	68.00	68.13
V ₇	51.68	51.61	33.56	33.78	612.00	588.38	54.00	53.88
V ₈	62.54	62.14	23.26	22.42	716.00	722.00	52.00	51.88
V ₉	60.65	61.05	32.85	33.69	538.00	532.00	72.00	72.13
V ₁₀	64.94	65.96	24.98	25.13	568.00	563.25	69.00	68.88
V ₁₁	56.38	57.75	25.26	24.26	547.00	559.67	62.00	61.33
V ₁₂	50.45	51.54	35.66	35.59	524.00	542.88	70.00	70.00
V ₁₃	58.76	57.75	23.64	24.26	556.00	559.67	60.00	61.33
V ₁₄	58.12	57.75	23.88	24.26	576.00	559.67	62.00	61.33
V ₁₅	53.76	52.74	31.08	30.93	628.00	632.75	52.00	52.13

Linear correlation plots between the actual and predicted response variables are given in fig. 1, and their corresponding residual plots

showing the scatter of the residuals versus predicted values are given in fig. 2.

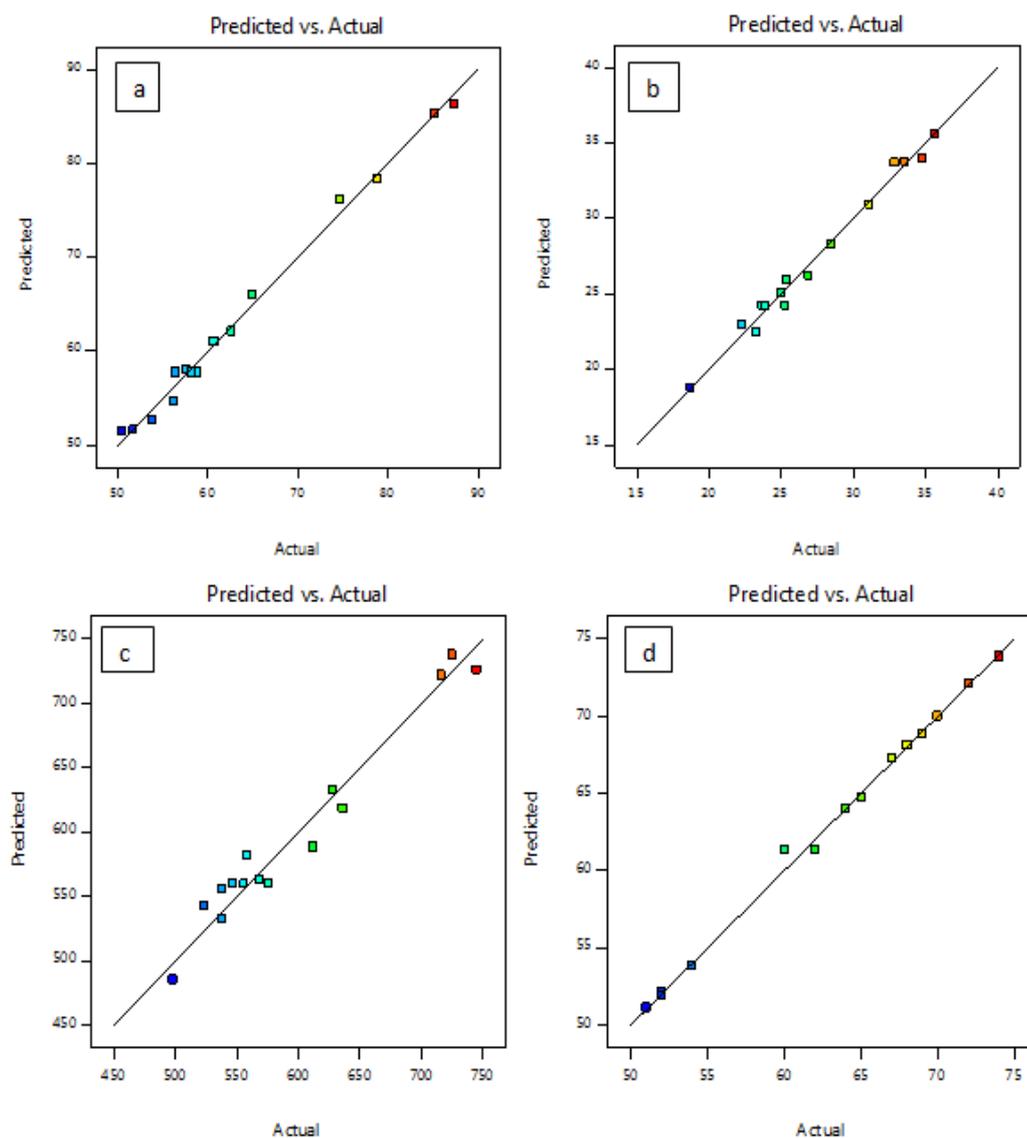


Fig. 1: Linear correlation plots between the actual vs predicted. a)-entrapment efficiency, b)-Q₁ h, c)-t_{90%} and d)-mucoadhesion

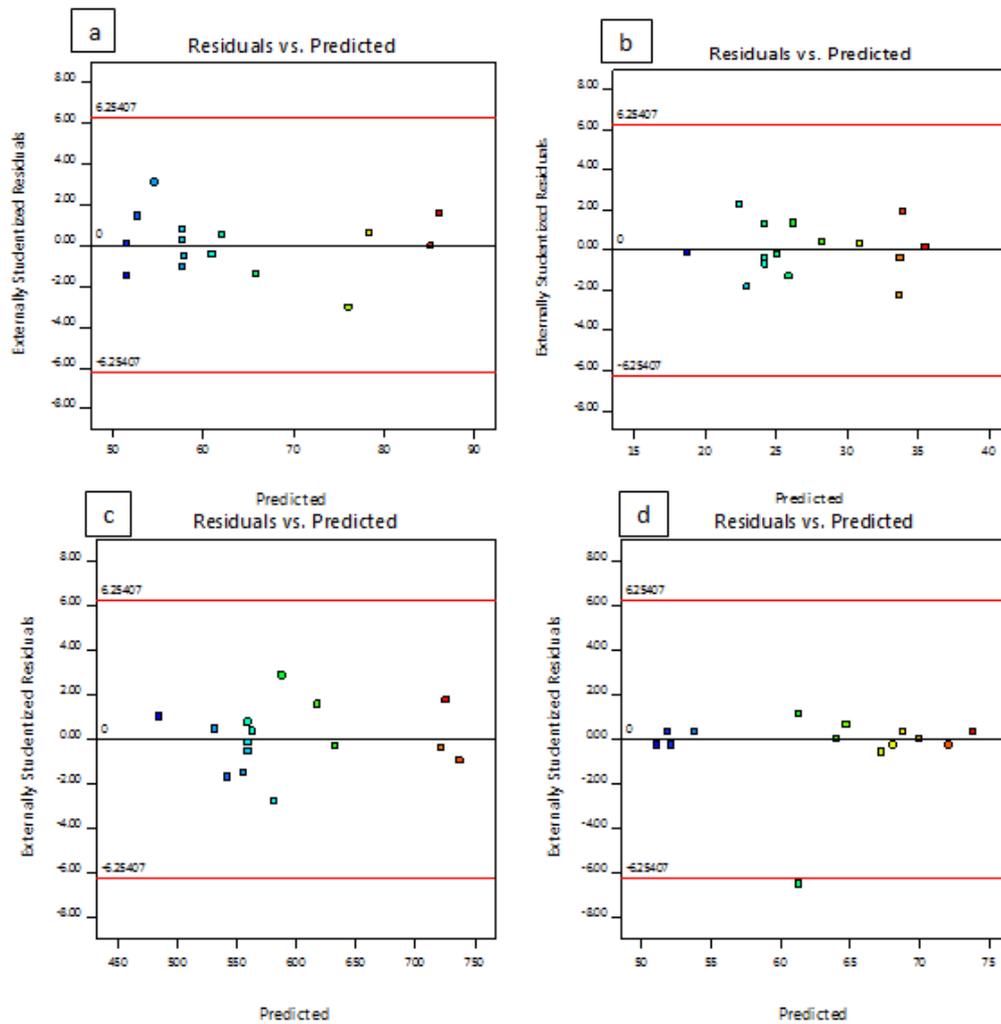


Fig. 2: Showing the scatter of residues vs predicted a)-entrapment efficiency, b)-Q₁ h, c)-t_{90%} and d)-mucoadhesion

Evaluating the main and interaction effects of the independent variables (factors), a three-dimensional response surface graph is very useful. The three-dimensional response surface graph relating drug entrapment efficiency (fig. 3) has shown that increase in drug entrapment with the increase in the amount of ethyl cellulose (X₁), amount of carbopol 934P (X₂) and stirring speed (X₃). The three-dimensional response surface graph relating Q₁ h (fig. 4) indicates

the increase in the amount of ethyl cellulose(X₁) and stirring speed (X₃) percent Q₁ h is decreasing, however the amount of carbopol 934P (X₂) increases Q₁ h and all the three factors opposes the effect in case of t_{90%} (fig. 5). In case of percent mucoadhesion (fig. 6), shown that increase the amount of carbopol 934P (X₂) mucoadhesion is also increasing, however, increases the amount of ethyl cellulose (X₁) and stirring speed (X₃) the mucoadhesion is decreased.

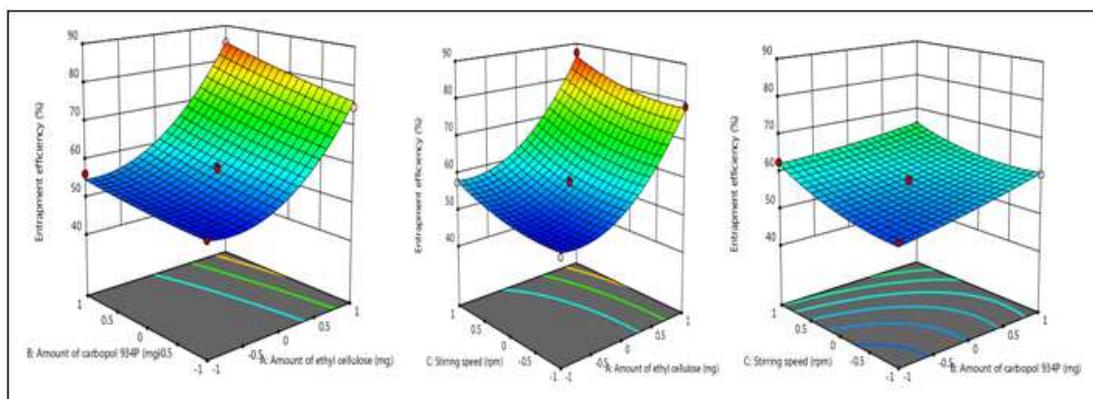


Fig. 3: Three-dimensional response surface graphs related to drug entrapment efficiency

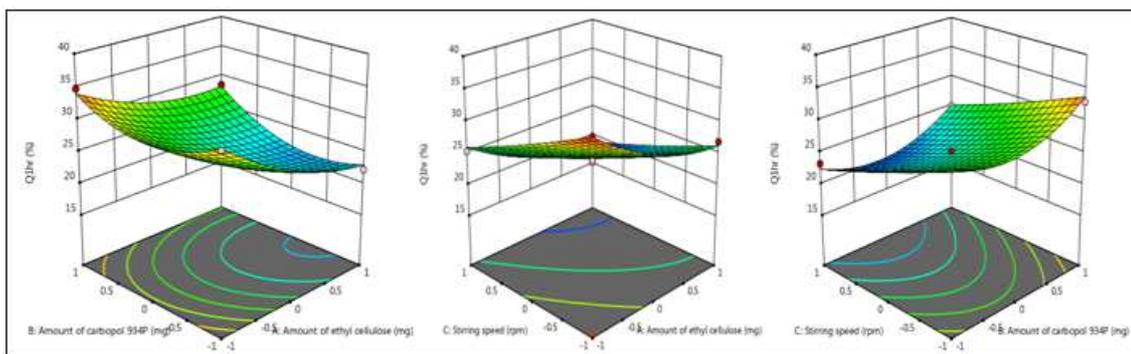


Fig. 4: Three-dimensional response surface graphs related to Q_1 h (%CDR)

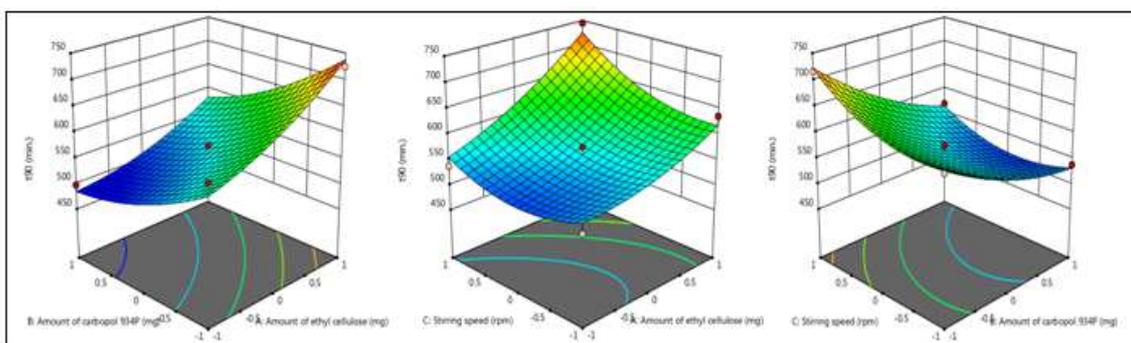


Fig. 5: Three-dimensional response surface graphs related to $t_{90\%}$

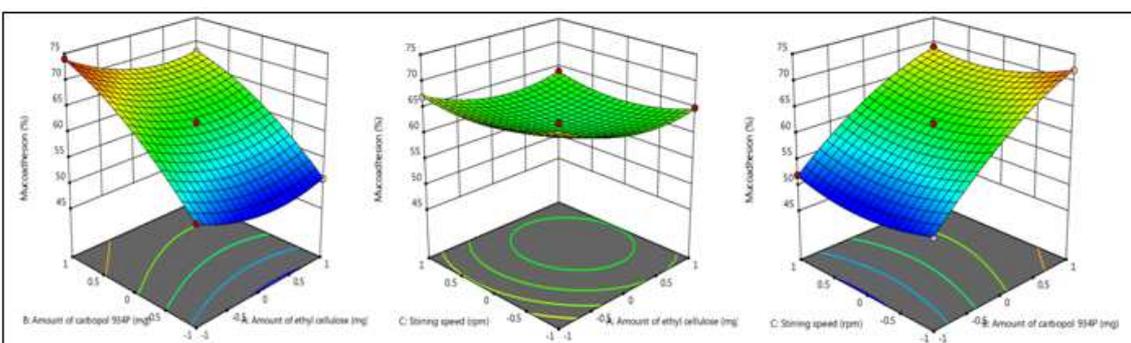


Fig. 6: Three-dimensional response surface graphs related to mucoadhesion

Percent yield and entrapment efficiency

The results of percent yield are given in table 3. It is shown that all the coded batches varies from 68.14 to 87.64% the minimum percentage yield was 68.14% of batch V_{12} whereas maximum of 87.64% of V_4 batch.

The entrapment efficiency of all batches was varied from 50.45 to 87.32% shown in table 2. The maximum entrapment efficiency was 87.32% of batch V_4 . It shows entrapment efficiency is increased due to an increase in the concentration of ethyl cellulose, carbopol 934P and increasing stirring speed. The entrapment efficiency is highly depending on the amount of ethylcellulose used, however other two factors such as the amount of carbopol 934P and stirring speed also increases entrapment efficiency, but in lesser extent as compared to the amount of ethyl cellulose. Similar results have been reported in the literature with other drugs [43, 44].

Particle size of microspheres

The microspheres of all the batches are spherical and free-flowing and the size varies from 92.64 to 124.32 μ m as shown in table 3. The

size of the microspheres also depends on the concentration of polymer solution and stirring speed. The size of the microspheres was increased by increasing the amount of polymers [45, 46], but decreases with increasing the stirring speed.

In vitro drug release study

In vitro drug release study carried on all the coded formulations and Q_1 h and $t_{90\%}$ were calculated. The values of Q_1 h of prepared formulations are 18.72 to 35.66% and $t_{90\%}$ in the range of 498 to 745 min as shown in table 2. The amount of ethyl cellulose and stirring speed inversely affects the Q_1 h, although increasing the concentration of carbopol 934P, Q_1 h is also increased.

Batch V_3 and V_4 shows drug release for a long time and batch V_1 and V_{12} shows 90% drug release in less than 525 min. It shows that by increasing the amount of ethyl cellulose and stirring speed the microspheres released drug for a long time while the amount of carbopol 934P reduced drug release. The result agrees with that of Patel J. *et al.* [21]. The drug release curves of all the batches were shown in fig. 7.

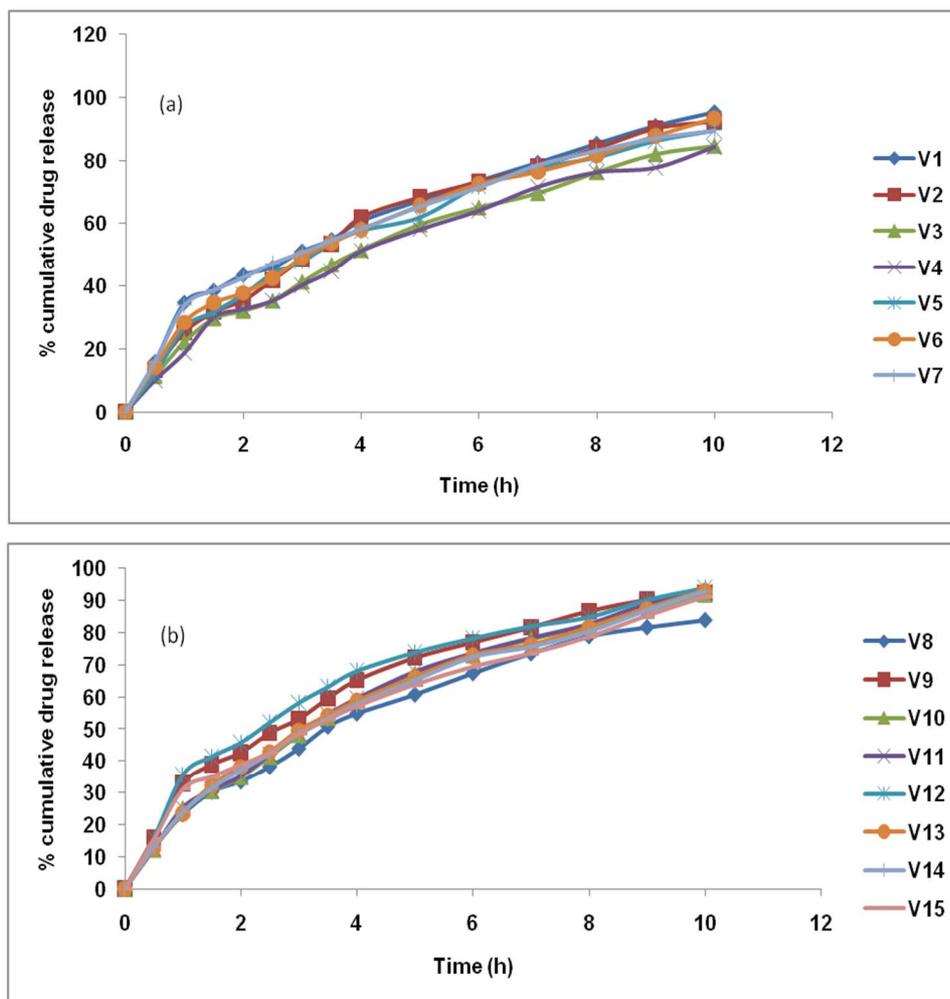


Fig. 7 Drug release profile of batches (a)-V1-V7 and (b)-V8-V15, mean \pm SD, n = 3

Percent mucoadhesion

In vitro wash-off test for percentage mucoadhesion of coded formulations were varied from 51 to 74%. Results of mucoadhesion indicated that the amount of ethyl cellulose and stirring speed had a negative effect on percent mucoadhesion. Mucoadhesion totally depends upon carbopol 934P because it is mucoadhesive polymer and the higher amount of polymer result in a greater amount of free-COOH (carboxyl group) [47] which are responsible for binding to the sialic acid group inside the mucous network that's why batch V₁ and V₉ show maximum mucoadhesion as shown in table 2.

Optimized formulation

Post analysis of the software was used to determine the optimum values of the factors for maximum entrapment, minimum Q₁ h, maximum t_{90%} and maximum percent mucoadhesion. Finally, the optimum levels of amount of ethyl cellulose (X₁ = 1), the amount of carbopol 934P (X₂ = 0.123) and stirring speed (X₃ = 1) were

obtained. These values predict 86.65% entrapment efficiency, 19.16% Q₁ h, 715 min t_{90%} and 64.91% mucoadhesion.

After the confirmation of the optimum batch and their predicted responses, all values were validated by further conducting *in vitro* studies of mucoadhesive microspheres of valsartan and an average of 85.63% entrapment efficiency, 20.14% Q₁ h, 730 min t_{90%} and 66.76% mucoadhesion. The predicted values and actual responses of the optimized formulation are shown in table 6 and the release profile is shown in fig. 8.

To determine the mechanism of drug release from the optimized formulation, the release profile fitted to zero order, first order, Higuchi equation and Hixon-Crowndwell, the 'R²' value was found to be 0.966, 0.974, 0.987 and 0.991 respectively as shown in table 7. The release profile fitted to Korsmeyer-Peppas equation, the "R²" value was found to be 0.998 and "n" value was 0.633 for the optimized batch. It confirmed that the mechanism of drug release was the nonfickian diffusion, may be owing to the swelling of the microspheres.

Table 6: Predicted values and actual responses of optimum formulation

Response variables	Predicted value	Actual responses*
Y ₁ = Entrapment efficiency	86.65	85.63 \pm 1.92
Y ₂ = Q ₁ h = % cumulative drug release after 1 h	19.16	20.32 \pm 0.63
Y ₃ = t _{90%}	715	730
Y ₄ = Percent mucoadhesion	64.91	66.76

*mean \pm SD, n = 3

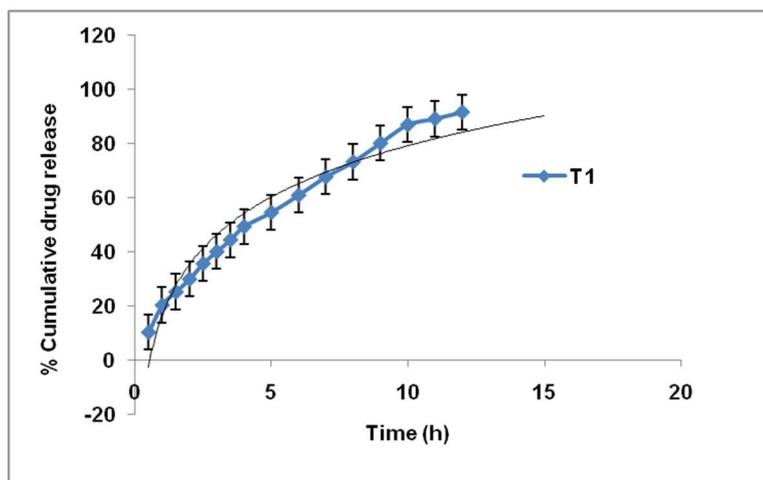


Fig. 8: Release profile of optimized formulation, mean±SD, n = 3

Table 7: Release kinetic data for the batch T₁

Batch No.	Zero-order kinetics R ²	First order kinetics R ²	Higuchi kinetics R ²	Hixon-crowell R ²	Korsmeyer-peppas
T ₁	0.966	0.974	0.987	0.991	0.633

Surface morphology

Scanning Electron Microscopic (SEM) analysis of the optimized batch (T₁) revealed discrete and spherical microspheres. It appeared to have smooth surfaces at higher magnification as shown in fig. 9.

Stability studies of optimized formulation (T₁)

The formulation stored for three months on 40±2 °C temperature and 75±5% relative humidity. It was found to be stable in between and after three months. The results are shown in table 8.

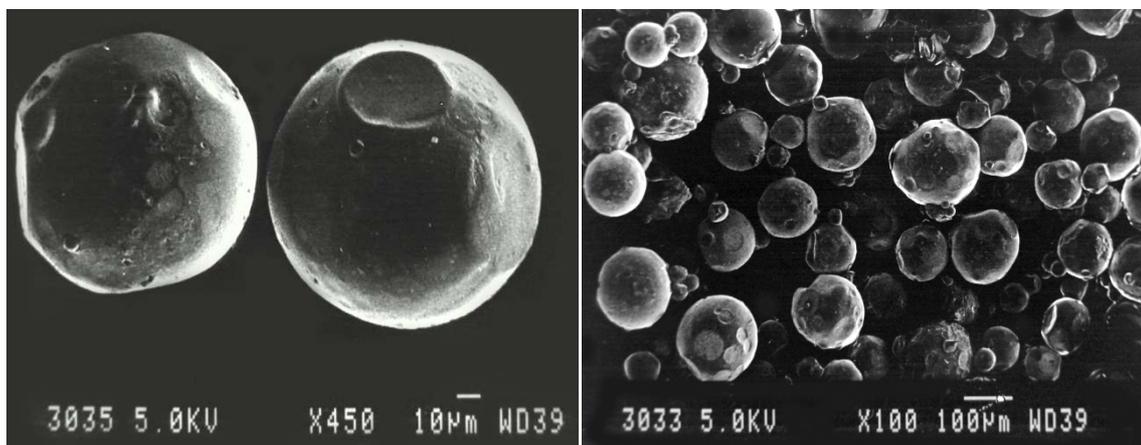


Fig. 9: Scanning electron microscopic images of optimized formulation

Table 8: Accelerated stability studies of optimized formulation

Response	Initial	After 3 mo*
Y ₁ = Entrapment efficiency	85.63±1.92	83.46±2.62
Y ₂ = Q _{1h} = % cumulative drug release after 1 h	20.32±0.63	22.04±2.86
Y ₃ = t _{90%}	732	724
Y ₄ = Percent mucoadhesion	66.76	65.96

*mean±SD, n = 3

CONCLUSION

The entrapment efficiency, Q_{1h}, t_{90%} and percent Mucoadhesion, analyzed through Box-Behnken design significantly affected by independent variables such as the amount of ethyl cellulose (X₁), amount of carbopol 934P (X₂) and stirring speed (X₃). The optimized

formulation exhibited drug release of the microspheres is more than 12 h, indicates the microspheres have sustained release. Moreover, it has shown high entrapment efficiency and good Mucoadhesion.

Actual responses of the optimized batch have close proximity with the predicted value. SEM analysis revealed that the microspheres of

spherical in shape and free-flowing and the stability studies of optimum formulation proved that the optimum formulation is stable for a long period of time. All these studies show that the mucoadhesive microspheres of valsartan are excellent alternative over the conventional delivery system.

AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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