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(RESEARCH ARTICLE)

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QbD - A novel approach for design, optimization and evaluation of sitagliptin mucoadhesive microspheres

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Abstract

Quality by Design (QbD) represents a highly systematic approach implementing the Design of Experiment (DoE) for finding the optimal product and process characteristics. Sitagliptin (SIT), a potent and selective dipeptidyl peptidase (DPP) IV inhibitor. Typically, it improves the glycaemic control by increasing the active level of incretin peptides particularly glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide. A response surface CCD studied by using Design Expert Stat-Ease software. The study includes two factors (independent variable) viz., Sodium alginate (SA) (X_1), Carbopol (CA) (X_2), 2 levels (-1, +1), three response (dependent variables) viz., Particle size (Y_1), % EE (Y_2), t50 (Y_3), 13 runs with 5 center point. The design trials of SIT loaded mucoadhesive microspheres (SIT-MS) were fabricated by ionotropic gelation method. The SIT-MS were characterized for design response and further CCD was applied for studying the influence of factors on response. The optimized formulas generated as per CCD was formulated, characterized and validated with predicted values. FTIR studies showed no interaction between drug and polymers. ANOVA suggest that few model terms are significant ($p < 0.05$) suggest significant first order linear and quadratic model. The R^2 values suggest significant correlation between the factors and response. The quadratic polynomial equations suggest linear and quadratic terms influence on stated response and justified by 2D contour, 3D surface plot. The experimental values of optimized formulations ratified with predicted values. The QbD through CCD results concludes that DoE can be carried out successfully for formulation, characterization and optimization of SIT-MS.

Keywords: Sitagliptin; QbD; CCD; ANOVA; SA; CA

1. Introduction

Delivery of a medication for an acute or chronic disease is carried out via various pharmaceutical dosage forms such as matrix tablets, capsules, suspensions etc. Therapy with the conventional dosage formulation shows variation in the concentration of the drug in plasma. After administering first dose, the drug concentration declines due to the effect of metabolism. As the concentration is decreased below the therapeutic range, there is a need for the administration of the second dose to maintain concentration in plasma[1]. In order to avoid the frequency of administration and to maintain the steady state concentration of drug in plasma the controlled release formulations were used during which the concentration is maintained constant within therapeutic range for long period of time with minimum unwanted effects and with more patient compliance[1]. A number of approaches have been developed to increase the residence time of the drug formulation. One of the approaches is the formulation of mucoadhesive microspheres (MS)[2-4]. These class of microspheres have the potential to be used for targeted and controlled drug delivery, but coupling of mucoadhesive properties as additional advantages such as effective absorption and enhanced bioavailability of the drugs, a much more intimate contact with the mucus layer, specific targeting of the drug to the absorption site[5-7].

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Microspheres fabricated using mucoadhesive polymer with a diameter of 1-1000 µm are well known as MS. These microspheres can be tailored to adhere any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs. Microspheres prepared with mucoadhesive and biodegradable polymers undergo selective uptake by the M cells of peyer patches in gastrointestinal (GI) mucosa. This uptake mechanism has been used for the delivery of protein and peptide drugs, antigens for vaccination and plasmid DNA for gene therapy[8-10]. MS offer more attention because of their advantages such as targeting the drugs to the specific sites, greater physical and chemical stability during sterilization and storage, entrap both hydrophilic and hydrophobic drugs, ease of transfer, distribution and dosing.

Quality by Design (QbD) regulatory initiative represents a highly systematic approach implementing the Design of Experiments (DoE) for finding the optimal product and process characteristics[11,12]. DoE, affords the remarkable and even more quantity of instructions as of the slightest number of experimental runs by methodical distinction of the factors and simultaneous evaluation of the effects of multiple variables[13]. Quality assurance (QA) has altered from the demand to elucidate that the ultimate product gets the predefined requirements and specifications to a novel circumstance where it needs to be confirmed that the product is controlled within a significant and organized design space[14]. The design space stated as a renowned technique enclosing multidimensional series of input variables (e.g., formulation factors) and process parameters, which detonated in order to insist typical quality assurance[15].

Sitagliptin (SIT)[16], a potent and selective dipeptidyl peptidase (DPP) IV inhibitor mainly used for the treatment of type 2 diabetes. Typically, it improves the glycaemic control by increasing the active level of incretin peptides particularly glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide. Administration of SIT in controlled release MS as once daily dose would be further enviable. Therefore, the present investigation was aimed to Design, formulate and characterize Sitagliptin loaded mucoadhesive microspheres using polymer blends viz., SA, CA by a novel DoE studies (CCD) using Design Expert software Trial Version 13.

2. Materials and methods

2.1. Materials: Sitagliptin (SIT) was procured from Caplin Point laboratories Ltd. Chennai, Tamilnadu. Sodium alginate (SA), Carbopol 934 (CA) Calcium chloride ($CaCl_2$) was purchased S.D Fine Chemicals Mumbai, Maharashtra, India. All other ingredients employed in the study were of pharmaceutical and analytical purity.

2.1. Methods

- Design of experiments: Optimization of SIT-MS were done as per DoE by selecting response surface design viz., Central Composite Design[17] (CCD) using Design Expert 13 Trial version software. A CCD with 2 factors, 2 levels and 13 runs were selected for the optimization study. The designs and possible formula trials were shown in tables 1, 2 and second order polynomial equation was generated as,

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + \ldots\ldots\ldots\ldots$$

Where Y_n- responses; b_0- intercept;

b_1 to b_{33}- regression coefficients; X_1, X_2, X_3- independent variables

Table 1 Selection of variables and levels as per CCD

Variables	**Levels used, actual (coded)**	
Independent variable	Low (-1)	High (+1)
X_1-SA in mg	150	200
X_2-CA in mg	50	100
Response variables		
Y_1 - Particle size in µm ; Y_2 - % EE; Y_3 - t_{50} hr		

Table 2 Possible design trial formulations as per CCD

Std	Runs	X1	X2	SIT
		A:SA (mg)	B:CA (mg)	mg
12	1	175	75	50
8	2	175	100	50
6	3	200	75	50
9	4	175	75	50
11	5	175	75	50
4	6	200	100	50
7	7	175	50	50
2	8	200	50	50
3	9	150	100	50
1	10	150	50	50
5	11	150	75	50
10	12	175	75	50
13	13	175	75	50

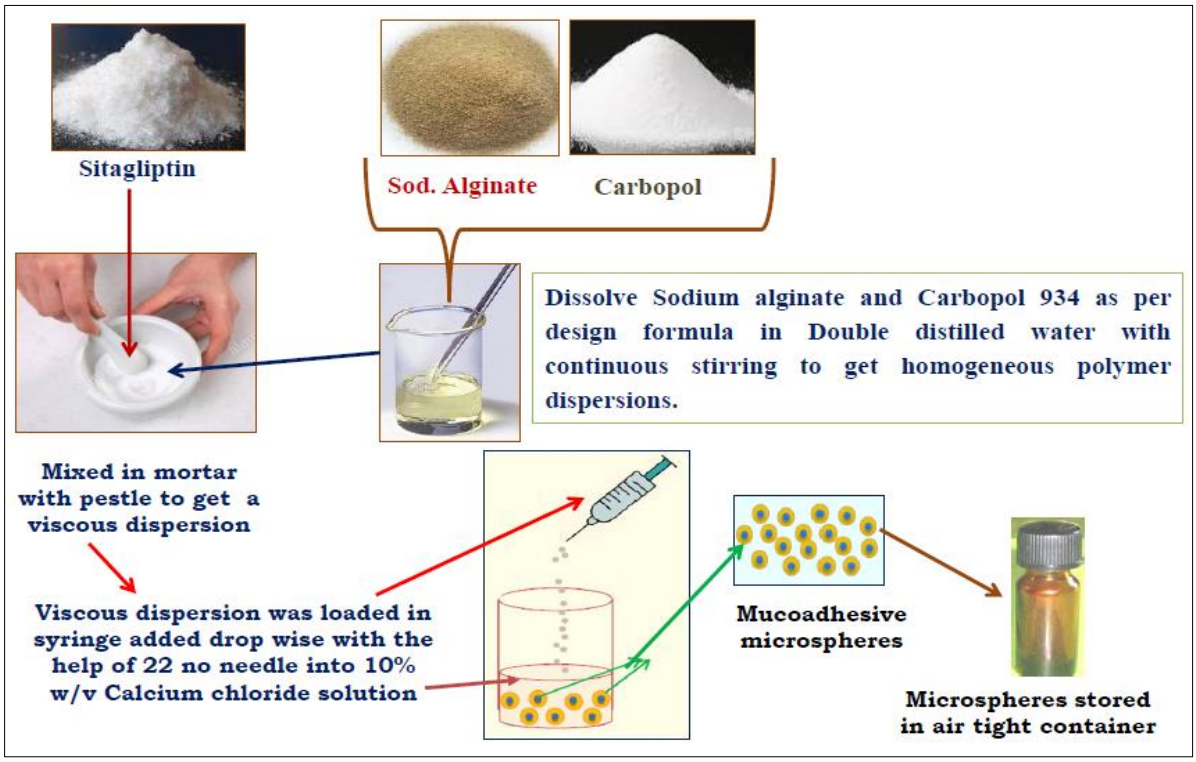

Figure 1 Fabrication scheme of SIT-MS by ionotropic gelation method

- Fabrication of SIT loaded mucoadhesive microspheres (SIT-MS): SIT-MS were fabricated using ionotropic gelation[18,19] method as shown in figure 1. To develop a homogeneous drug polymer solution, the necessary quantity of SA, CA and SIT were dissolved in double distilled water by continuously stirring over magnetic stirrer for 30 min. The homogeneous drug polymer solution was added dropwise into 10 % w/v $CaCl_2$ solution using a disposable syringe (needle size 22), and continuously stirred at 200 rpm on magnetic stirrer, to

generate a spherical and rigid microspheres. The content were filtered and collect rigid microspheres and wash thoroughly with distilled water to remove any unreacted calcium ions, further the MS were air dried for 24 hr. Dried SIT-MS were stored at room temperature for further evaluation.

2.2. Evaluation

- FTIR study: The drug-excipient interaction between SIT and added excipients was studied by comparing FTIR spectrums of SIT and OP-SIT-MS. The FTIR were recorded over the wave number of 4000 cm^{-1} to 500 cm^{-1} using BRUKER-FTIR spectrophotometer. During study ground, small amount of solid samples mixed with 100 times its weight of potassium bromide and compressed into a thin transparent pellet using hydraulic press. Transfer these pellets in to FTIR instrument and determine the spectrum.
- Encapsulation efficiency: The % EE of design trial batches of SIT-MS were determined by standard method. In each case transfer 100 mg of powdered SIT-MS into 100 ml volumetric flask, extract the SIT content with 100 ml of phosphate buffer pH 6.8 by shaking occasionally for 1hr, followed by sonication for 10 min. Filter the contents, dilute appropriately with phosphate buffer pH 6.8 and measure the absorbance at 267 nm. The percent encapsulation efficiency was determined by using below given formula,

$$\%\ \textbf{Encapsulation effeciency} = \frac{\textbf{Actual amount of drug encapsulated}}{\textbf{Theoretical drug content}} \times \textbf{100}$$

- Drug content: Drug content of SIT-MS design trials were determined. In each case, powdered SIT-MS equivalent to 50 mg of SIT was transferred into 100 ml volumetric flask, extract the SIT content with 100 ml of phosphate buffer pH 6.8 by shaking occasionally for 1h, followed by sonication for 10 min. The contents were filtered and dilute appropriately with phosphate buffer pH 6.8 and SIT content was determined by the absorbance at 267 nm.
- Particle size: Particle size of SIT-MS design trials were determined by sieve analysis method. In each case, weighed amount of SIT-MS were sieved through a set of standard sieves (viz., # 22, # 44 and # 60) arranged in descending order with respect to the aperture size by using mechanical sieve shaker. After shaking period, the microspheres retained on each sieve were weighed and determine the average particle size by using following equation,

$$\mathbf{D_{Avg}} = \frac{\sum \mathbf{Xifi}}{\mathbf{fi}}$$

Where, X_i Mean size range; f_i % of microspheres retained on the smaller sieve size range

- Surface morphology: Surface morphology studied by SEM to check surface topography, texture and to examine the morphology of fractured or sectioned surface of the OP-SIT-MS. The OP-SIT-MS was mounted using a double-sided sticking tape and coated with gold (200 Ao) on the scanning electron microscopy (SEM) sample stab, under reduced pressure (0.001 torr) for 5min using ion sputtering device (Jeol JFC-1100E, Tokyo, Japan). The gold-coated samples observed under the scanning electron microscopy (SEM-Jeol JSM-840A, Tokyo, Japan) and photomicrographs of suitable magnification were obtained.
- *In vitro* mucoadhesion study[20,21]: *In vitro* wash off for OP-SIT-MS was studied by modified paddle dissolution apparatus as shown in figure 2. During the study freshly cut everted sheep intestine (collected from the slaughter house) 8x3 cm was fixed on to the paddle. In each case, spread about 100 microspheres onto wet and rinsed tissue specimen. The tissue specimen was given a regular, slow movement in a vessel containing 900 ml of phosphate buffer pH 6.8 at 37°C by rotating the paddle at 50 rpm. Measure the number of microspheres adhering to tissue after 6 hr, from the data determine percentage mucoadhesion by using the formula,

$$\%\ \textbf{Mucoadhesion} = \frac{\textbf{Number of microsphere adhered}}{\textbf{Number of microsphere applied}} \times \textbf{100}$$

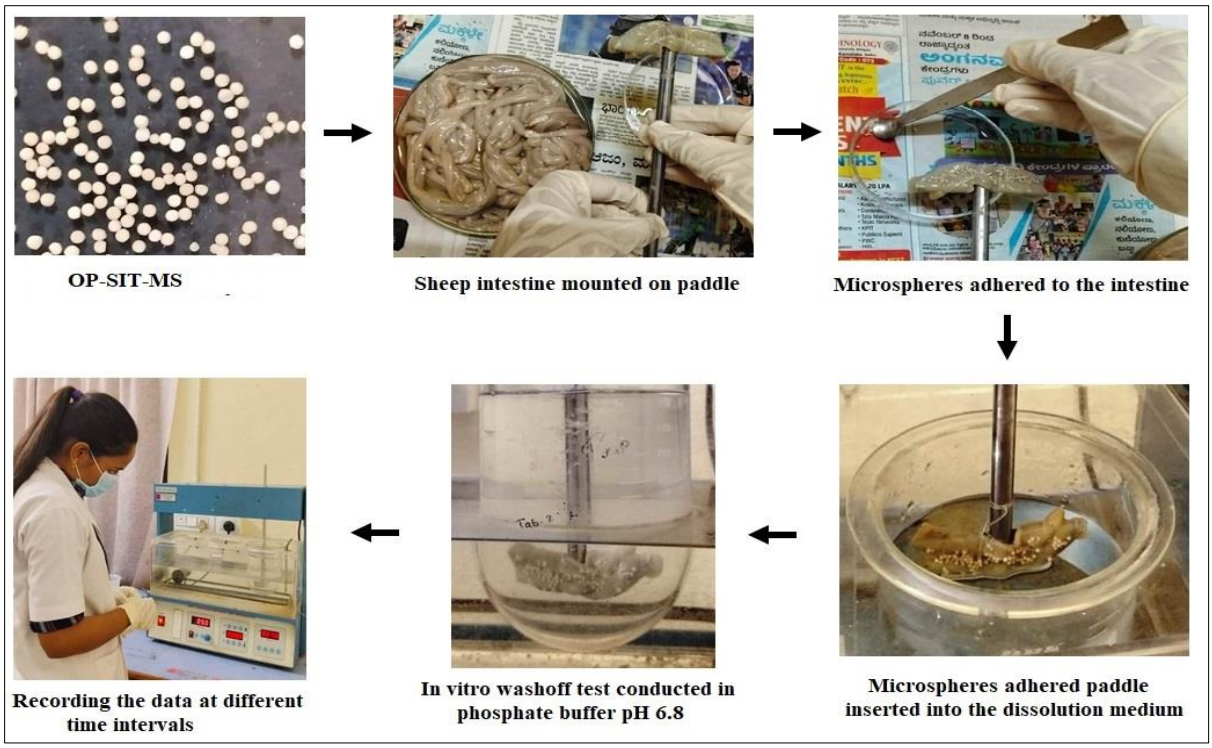

Figure 2 Schematic diagram of *in vitro* wash-off test

- *In vitro* dissolution: The *in vitro* drug release was studied for design trial batches of SIT-MS by using USP type I basket apparatus. In each case fill the SIT-MS design trials equivalent to 50 mg of SIT in hard gelatin capsules were used. The speed of 50 rpm and temperature of 37 ± 0.5^0 C was maintained throughout the study period. The dissolution was carried in two simulated medium, first 2 hr dissolution was carried out in 0.1N HCl, after 2 hr emptied the dissolution medium, replaced with phosphate buffer pH 6.8 and continue the dissolution for 12 hr. At different time intervals, 5 ml sample was withdraw and dilute appropriately with solvent medium and SIT content was determined by measuring the absorbance at 267 nm. The dissolution data was model fitted with different kinetic models using PCP Disso V3.

3. Results and discussion

3.1. Preformulation studies

The model drug SIT was subjected for preformulation studies such as solubility, melting point and partition coefficient. The solubility of SIT complies with the standard values. The melting point was found to be 215°C against standard 216-219^0C, the partition coefficient is 1.91 against 1.8 and pKa is 7.43 against 7.7 (log P). The results were complies with the standard values indicate the drug sample was stable and pure.

3.2. FTIR studies

FTIR spectra of SIT and OP-SIT-MS were shown in figure 3. FTIR spectra of SIT showed the characteristic peak at 1428.82 cm^{-1} is related to alkane stretching (C-H), 1741.80 cm^{-1} is associated with the C= O bond of carbonyl, 1667.96 -1631.22 cm^{-1} refers to the imine group (C=N), 3,331.31 cm^{-1} related to amine. The vibration at 1272.46 - 1059.06 cm^{-1} is related to fluoride (C-F) and 3211.72 - 3056.94 cm^{-1} is associated with amine groups (NH_2), N–H bending occurs around 1,555.91 cm^{-1} for primary amides, while for secondary amides it occurs around 1,512.53 cm^{-1}. The spectral data was ratified with literature indicate the SIT found to be pure and can be used for further studies[22-24]. The characteristic FTIR peaks of SIT were found in optimized batch of OP-SIT-MS indicates no interaction.

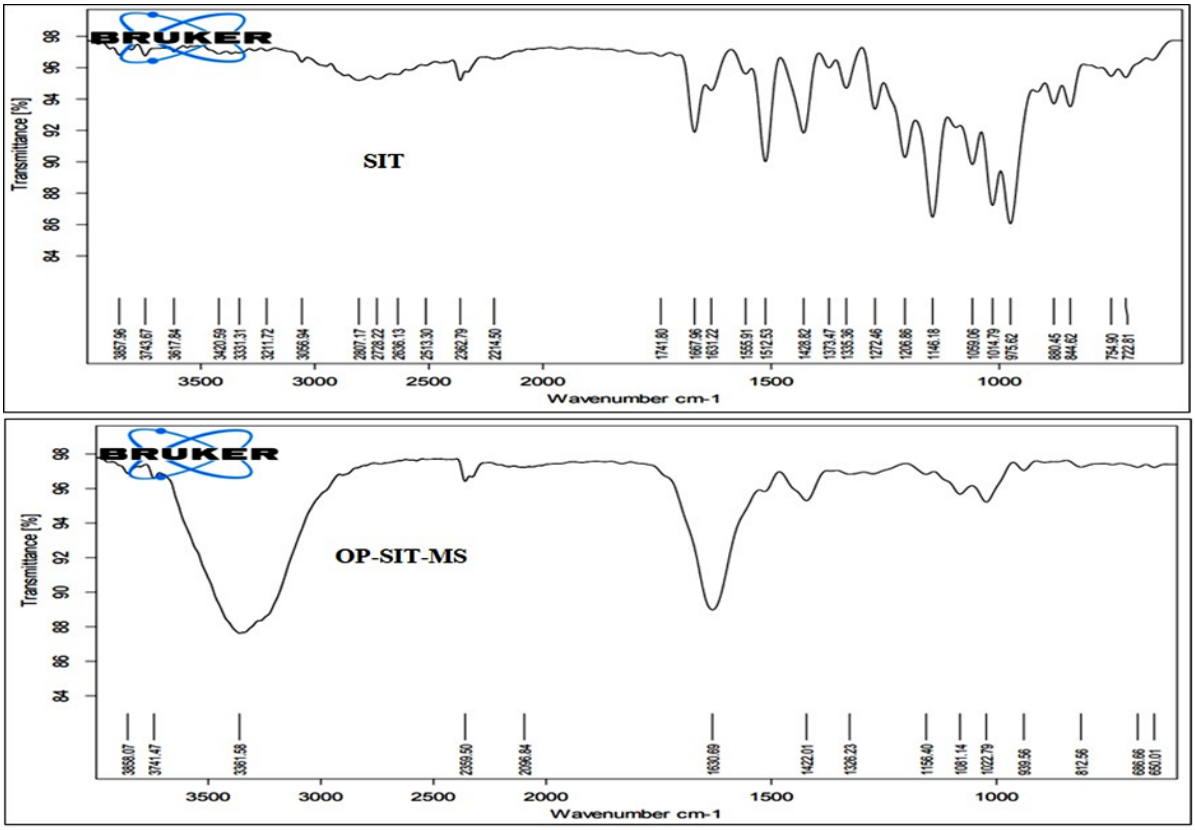

Figure 3 Comparative FTIR of SIT and OP-SIT-MS

3.3. Analysis of CCD

The relationships between independent variables viz., amount of SA (X_1), and amount of CA (X_2) at two levels (-1, +1), with dependent responses, such as % Particle size (Y1), % EE (Y_1) and t50 (Y3) were assessed by the CCD. The design trials were experimentally evaluated for stated responses and data were shown in table 3 and figure 4. The response data was further substituted in Design Expert Software and generate relative statistical data and identify the relative models as the optimum models for all the responses. The significance of the model was estimated by ANOVA, where, at p-value < 0.05, the model is considered significant. The p-value < 0.05 clarifies that, the models generated were statistically significant. Inter-relationship between the independent factors and the response variables through appropriate polynomial equations. Interpret the influence of factors on each response were done with supporting data.

Table 3 Design trial response data as per CCD

Design trial runs	**Batch code**	**X1**	**X2**	**Y1**	**Y2**	**Y3**
		A:SA mg	B:CA mg	Partical size µm	EE %	t50 hr
1	F1	175	75	960.45	90.12	4.59
2	F2	175	100	980.65	92.85	4.8
3	F3	200	75	1030.25	96.85	5.31
4	F4	175	75	943.8	91.12	4.66
5	F5	175	75	959.86	91.13	4.57
6	F6	200	100	1050.35	98.96	5.78
7	F7	175	50	946.56	88.56	4.1
8	F8	200	50	1000.34	92.45	5.12
9	F9	150	100	920.56	85.56	4.21

10	F10	150	50	880.45	78.98	3.82
11	F11	150	75	900.15	81.45	4.02
12	F12	175	75	964.25	90.98	4.45
13	F13	175	75	965.35	90.98	4.62

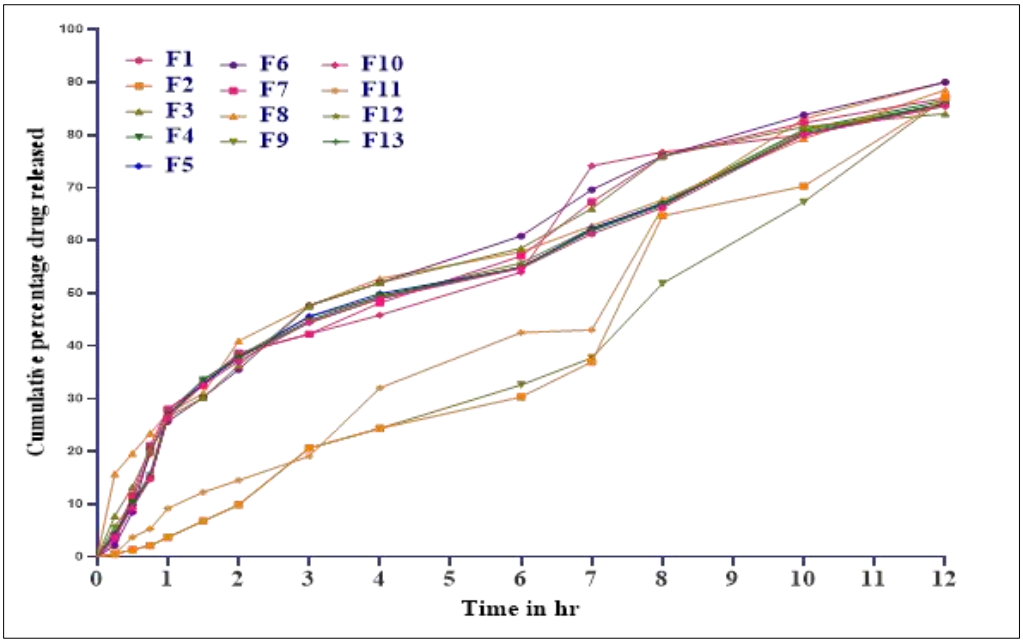

Figure 4 *In vitro* dissolution profile of SIT-MS design trial batches as per CCD

Table 4 ANOVA data of all response as per CCD

Response Y1-Particle size					
Linear Model	Sum of Squares	df	Mean Square	F-value	p-value
Significant	26610.16	2	13305.08	291.32	< 0.0001
A-SA	24038.81	1	24038.81	526.34	< 0.0001
B-CA	2571.35	1	2571.35	56.30	< 0.0001
Residual	456.72	10	45.67		
Lack of Fit-Not Significant	155.28	6	25.88	0.3434	0.8829
Pure Error	301.43	4	75.36		
Cor Total	27066.88	12			
Response Y2-%EE					
Quadratic Model	Sum of Squares	df	Mean Square	F-value	p-value
Significant	358.43	5	71.69	135.66	< 0.0001
A-SA	297.79	1	297.79	563.57	< 0.0001
B-CA	50.34	1	50.34	95.28	< 0.0001
AB	0.0012	1	0.0012	0.0023	0.9629
A^2	8.14	1	8.14	15.40	0.0057

B^2	0.0722	1	0.0722	0.1367	0.7225
Residual	3.70	7	0.5284		
Lack of Fit-Not Significant	2.98	3	0.9940	5.55	0.0657
Pure Error	0.7167	4	0.1792		
Cor Total	362.13	12			
Response Y3 - t50					
Quadratic model	Sum of Squares	df	Mean Square	F-value	p-value
Significant	3.51	5	0.7016	71.53	< 0.0001
A-SA	2.88	1	2.88	294.08	< 0.0001
B-CA	0.5104	1	0.5104	52.04	0.0002
AB	0.0182	1	0.0182	1.86	0.2151
A^2	0.0909	1	0.0909	9.26	0.0187
B^2	0.0031	1	0.0031	0.3183	0.5902
Residual	0.0687	7	0.0098		
Lack of Fit- Not Significant	0.0436	3	0.0145	2.32	0.2173
Pure Error	0.0251	4	0.0063		
Cor Total	3.58	12			

3.3.1. Effect of factors on Response Y_1 – Particle size

ANOVA suggested (table 4) Linear model and F-value of 291.32 implies the model is significant, there is only a 0.01% chance (< 0.0001) that an F-value large this could occur due to noise, P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms with low values of predicted residual sum of squares. The Lack of Fit F-value of 0.34 implies the Lack of Fit is not significant relative to the pure error, there is a 88.29% chance that a Lack of Fit F-value this large could occur due to noise, non-significant lack of fit is good so we want the model to fit. The linearity plot of predicted value versus actual value of model condition particle size was shown in figure 5a. The linearity plot had good correlation i.e., R^2 - 0.9831. The Predicted R^2 of 0.9798 is in reasonable agreement with the Adjusted R^2 of 0.9759 indicates prediction results from the Design-Expert® program had precision and reliability. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 51.7473 indicates an adequate signal, model used to navigate the design space. The observed value for Y1 (Particle size) for all 13 batches F1-F13 varied from 880.45 µm (F-10) to 1050.35 µm (F-6). The result clearly indicates that Y1 is strongly affected by the independent variables selected for the study. The F6, F3 and F8 had the higher value of Y1 (> 1000 µm). The response Y1 obtained at various levels of two independent variables were subjected to regression to give a linear polynomial equation,

Y1-Particle size = + 456.58910 + 2.53187*SA + 0.828067*CA

The polynomial equation was generated for actual factors, this equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor, coefficient with one factor term represents linear term and the 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). Here the linear term factors has synergistic effect on Y1, as the concentration of SA and CA increases the Y1 increases. These results were justified through Contour and 3D surface plots (figure 5b, 5c) which explains the relationship between factors vs response, the interaction plot (figure 5d) clearly suggest no interaction between the factors and stated response and was further justified in ANOVA data where only two significant model terms. The average particle size of the SIT-MS were increased as the concentration of SA and CA increased, this may be due to increase in the relative viscosity and increased coat thickness during addition of the polymer solution to the cross-linking agents.

3.3.2. Effect of factors on Response Y_2 – % EE

ANOVA suggested (table 4) Quadratic model, the F-value of 135.66 implies the model is significant, and there is only a 0.01% (< 0.0001) chance that an F-value this large could occur due to noise, P-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2 are significant model terms with low values of predicted residual sum of squares The Lack of Fit F-value of 5.55 implies the Lack of Fit is not significant relative to the pure error, there is a 6.57% chance that a Lack of Fit F-value this large could occur due to noise, non-significant lack of fit is good so we want the model to fit. The linearity plot of predicted value versus actual value of model condition % EE was shown in figure 6a. The linearity plot had good correlation i.e., R^2 - 0.9898. The Predicted R^2 of 0.9133 is in reasonable agreement with the Adjusted R^2 of 0.9825 indicates prediction results from the Design-Expert® program had precision and reliability. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 40.2628 indicates an adequate signal, model used to navigate the design space. The observed value for % EE for all 13 batches F1-F13 varied from 81.45 % (F-11) to 98.96 % (F-6). The result clearly indicates that Y2 is strongly affected by the independent variables selected for the study. The F6, F3 and F8 had the higher value of % (> 92 %). The response (Y2) obtained at various levels of two independent variables were subjected to regression to give a Quadratic second order polynomial equation,

$$\% \text{ EE} = - 53.08129 + 1.24527*SA + 0.159580*SA - 0.000028*SA*CA - 0.002747*SA^2 - 0.000259* CA^2$$

The polynomial equation was generated for actual factors, this equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. 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. Coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space. The positive sign of the coefficient indicates the response increases (synergistic effect), and a negative sign indicates the response decreases (antagonist effect). Here the linear terms has synergistic effect, interaction and quadratic term has antagonistic on % EE, as the concentration of SA and CA increases the % EE increases as shown in Contour and 3D surface plots (figure 6b, 6c). The interaction plot (figure 6d) clearly suggest no interaction between the factors and stated response and was further justified in ANOVA data. The entrapment efficiency increased progressively with increasing SA concentration, increase in the SA concentration resulted in the formation of larger microspheres entrapping greater amounts of the drug. This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and consequently, the greater degree of cross-linking as the quantity of sodium alginate increased[25].

3.3.3. Effect of factors on Response Y_3 – t50

ANOVA suggested (table 4) Quadratic model was suggested, the F-value of 71.53 implies the model is significant, and there is only a 0.01% chance that an F-value this large could occur due to noise, P-values less than 0.0500 (<0.0001) indicate model terms are significant. In this case A, B, A^2 are significant model terms with low values of predicted residual sum of squares The Lack of Fit F-value of 2.32 implies the Lack of Fit is not significant relative to the pure error, there is a 21.73 % chance that a Lack of Fit F-value this large could occur due to noise, non-significant lack of fit is good so we want the model to fit. The linearity plot of predicted value versus actual value of model condition t50 was shown in figure 7a. The linearity plot had good correlation i.e., R^2 - 0.9808. The Predicted R^2 of 0.8905 is in reasonable agreement with the Adjusted R^2 of 0.9671 indicates prediction results from the Design-Expert® program had precision and reliability. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 29.2802 indicates an adequate signal, model used to navigate the design space. The observed value for t50 for all 13 batches F1-F13 varied from 3.82 hr (F-10) to 5.78 hr % (F-6). The result clearly indicates that Y3 is strongly affected by the independent variables selected for the study. The F6, F3 and F8 had the higher value of t50 (> 5 hr). The response (Y3) obtained at various levels of two independent variables were subjected to regression to give a Quadratic polynomial equation,

$$t50 = + 8.82520 - 0.081939*SA + 0.000836*CA + 0.000108*SA*CA + 0.000290*SA^2 -0.000054* CA^2$$

The polynomial equation was generated for actual factors, this equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. 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. Coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space. The positive sign of the coefficient indicates the response increases (synergistic effect), and a negative sign indicates the response decreases (antagonist effect). Here the SA has antagonistic effect and CA has synergistic effect, interaction

and quadratic term has synergistic/antagonistic on % t50, as the concentration of SA increases the t50 value decreases but in presence of CA the t50 value increase significantly to achieve desired t50 value shown in Contour and 3D surface plots (figure 7b, 7c). The interaction plot (figure 7d) clearly suggest no interaction between the factors and stated response and was further justified in ANOVA data.

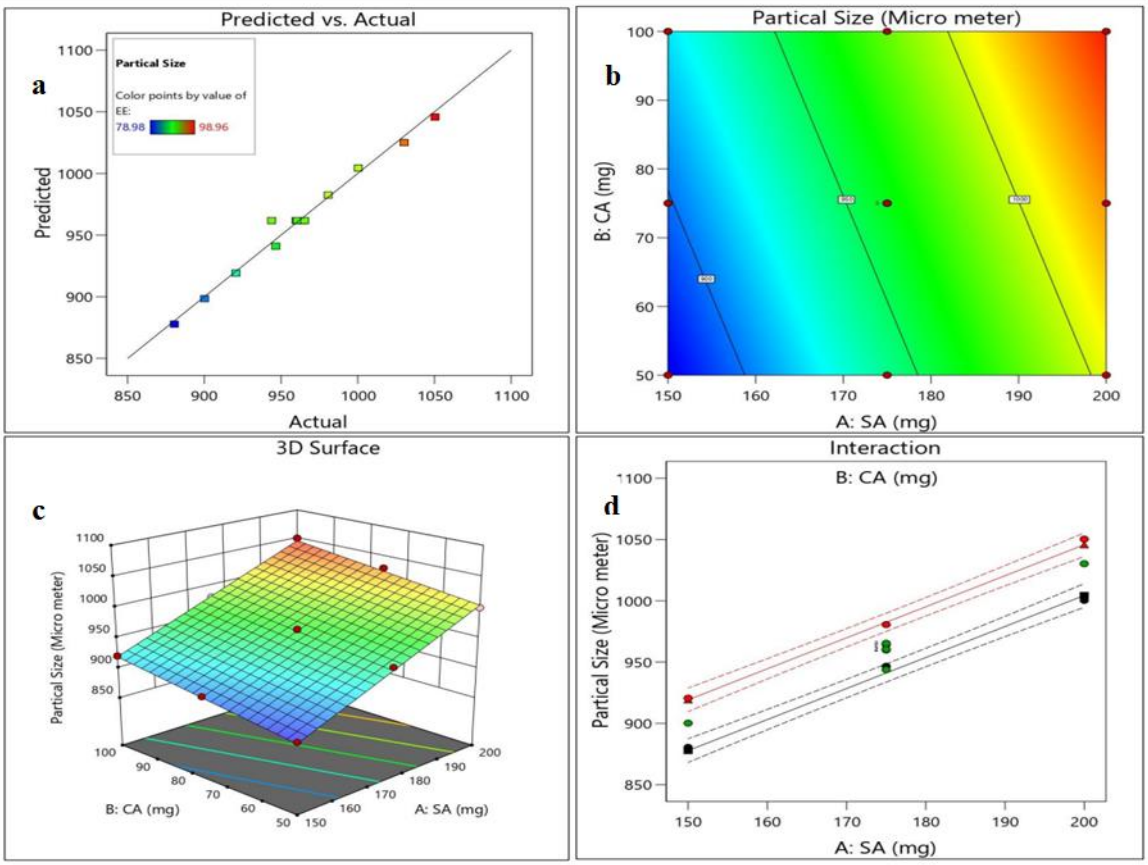

Figure 5 Diagnostic and Response surface plots a) Predicted vs Actual b) Contour c) 3D surface d) Interaction for Particle size.

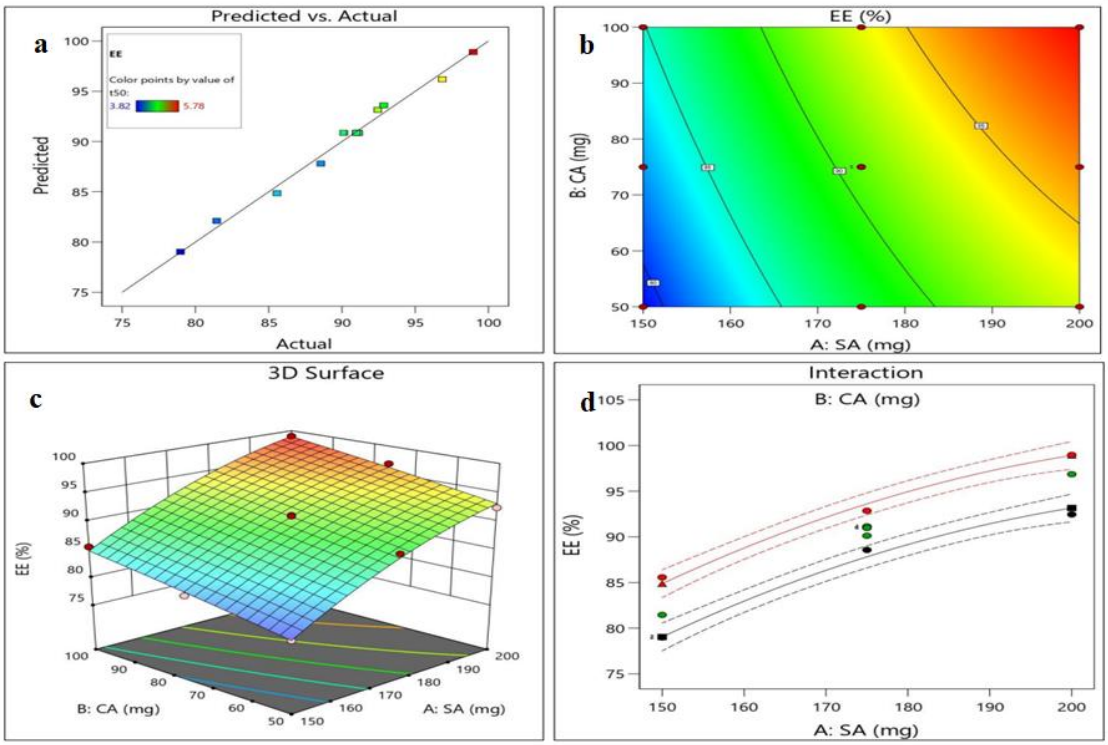

Figure 6 Diagnostic and Response surface plots a) Predicted vs Actual b) Contour c) 3D surface d) Interaction for % EE.

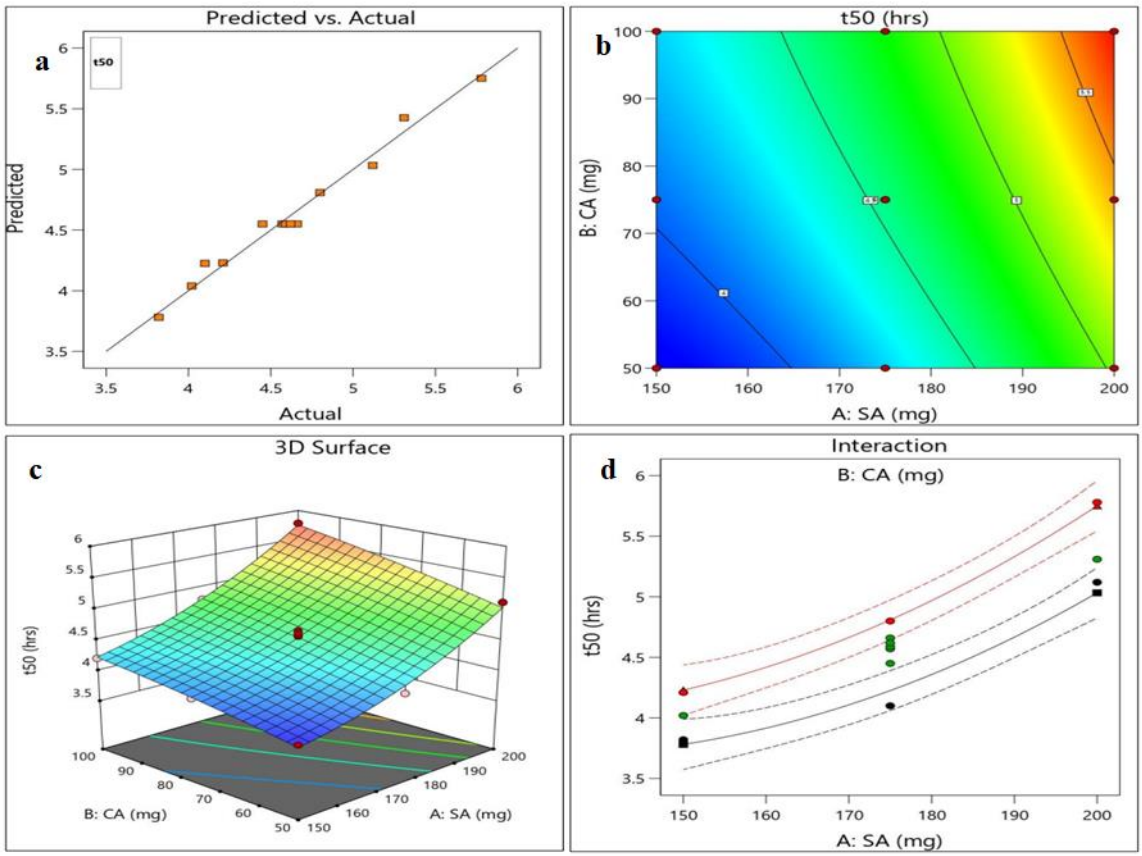

Figure 7 Diagnostic and Response surface plots a) Predicted vs Actual b) Contour c) 3D surface d) Interaction for t50

3.4. Numerical optimization

A numerical optimization technique using the desirability approach was employed to develop an optimized formulation with the desired responses. Fix the constraints for factors viz., maximize the X_1 (SA) in range X_2 (CA); for response, set in target for Y_1 (Particle size), none for Y2 (% EE) and Y3 (t_{50}). Optimize the constraints by using Deign Expert software to generate the possible solution with high degree of desirability and generate the possible overlay plot to explain the details of the optimized batch as shown in figure 8. The point prediction method confirms the concentrations of X_1, and X_2 as shown in the table and confirmed by predicted response mean with standard deviation (as per Two sided. Confidence interval = 95%) in table 5.

Table 5 Data of OP-SIT-MS with predicted response as per CCD and Experimental response

Optimized values for factors		
SA	184.611 mg	
CA	50 mg	
Predicted response mean ± SD		Experiments response Mean ±SD
Partical Size µm	965.402 ±6.75809	955.45±2.132
EE %	90.2691 ±0.726912	91.50±0.8132
t50 hr	4.49314 ±0.099035	4.62±0.08932

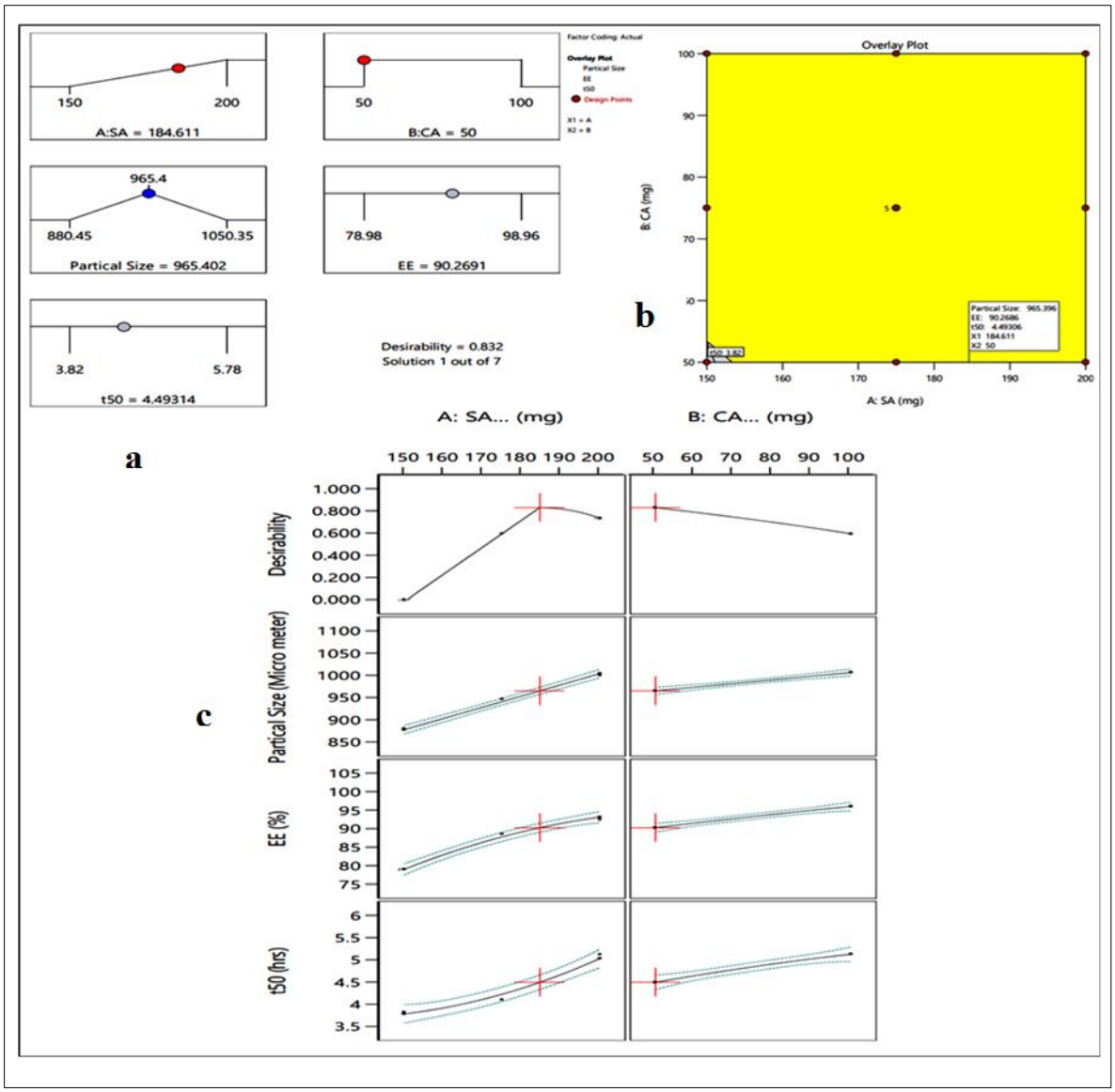

Figure 8 Plots representing a) Solutions for fixed constraints b) Over lay plot c) All factors and response for optimization

3.5. Validation

The OP-SIT-MS generated as per CCD was formulated experimentally by ionotropic gelation method. The formulated OP-SIT-MS was evaluated for responses under the design studies such as Particle size, % EE and t_{50} and all relative parameters such as interaction studies by FTIR, surface morphology, drug content, *in vitro* mucoadhesion, *in vitro* drug release. The experimental results were validated and ratified with predicted data, it clearly indicates the DoE studies can be used to study the influence of two factor on three responses. Validation of the predicted values of responses was performed by comparing with the experimental data, which indicated high degree closeness between the predicted and experimental values of the responses and confirmed excellent prognostic ability of the employed mathematical model. The FTIR study confirms all the characteristic bands of SIT appeared in OP-SIT-MS indicates no interaction between SIT and added polymers (figure 3). The microphotographs of OP-SIT-MS shows (figure 9) the fabricated microspheres were spherical and free flowing. The Scanning Electron Microscopy (SEM) at 27X, 75X and 500X magnification showed discrete, spherical microspheres and showed the presence of few drug crystals due to the presence of unentrapped drug on the surface of microspheres indicate some rough surface (figure 10). The drug content was found to be 98.96 ± 1.001 % with low SD values indicate the drug is uniformly distributed within the microspheres. The *in vitro* wash-off results (40% of microspheres adhered to the mucosal surface after 6 hr period) indicates the OP-SIT-MS exhibited good mucoadhesive property, the initial rapid-wash-off may be due to ionization and increasing solubility of polymers, these results were indicative of slow and spread drug release over extended period of time, further justified by *in vitro*

dissolution studies. Simulate the *in vitro* drug release of OP-SIT-MS in acidic medium and basic medium since the drug has an absorption window in stomach as well as intestine. The cumulative percent drug release (figure 11) was 14.556 ± 1.35 after 2 hr; 42.561 ± 1.11 after 6 hr and 84.036 ± 1.62 after 12 hr. Little higher, amount of drug release in stomach window due to adhered drug particles to the microspheres and improper formed microspheres. After 2 hr the drug release was steady due to the retaining of intact microspheres expected to be adhered to the intestinal absorption window for 6 hr, these results were justified by mucoadhesion test. The drug release was controlled for 12 hr. The R values for various models were 0.9901 (Zero order), 0.9290 (1^{st} order), 0.9070 (Matrix), 0.9883 (Peppas) and 0.9607 (Hix.Crow) suggest best fit model was zero order and exponential 'n' value was greater than 0.5 indicate the drug release mechanism follows fickanian i.e. swelling followed by diffusion controlled. The SA concentration in the formulation greatly influenced the steady state release of SIT from the microspheres. The principle of gelation or cross-linking of SA with $CaCl_2$ is based on the formation of tight junction between the glucuronic acid residues. The number of the apparent cross-linking points formed within the calcium alginate gel beads increased with increasing alginate concentration in the formulation. This increase in the apparent cross linking density delayed the alginate gel disintegration in phosphate buffer due to the retardation of Ca^{2+} exchange with Na^{+} and eventually increasing retention time. Increased alginate gel density per unit volume was also thought to affect the decreased pore size within the gels, and thus SIT release becomes slow.

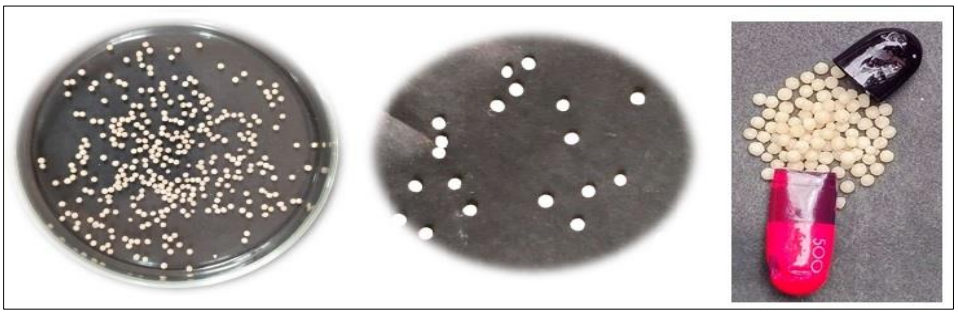

Figure 9 Microphotographs of OP-SIT-MS

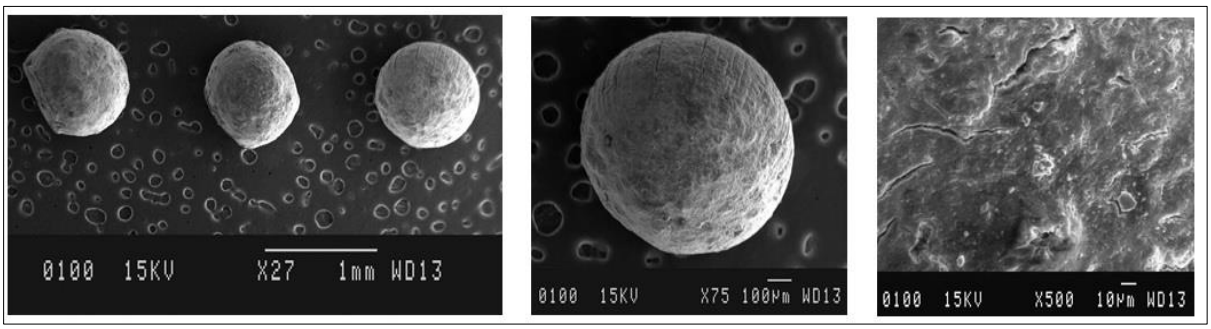

Figure 10 SEM images of OP-SIT-MS at different magnifications

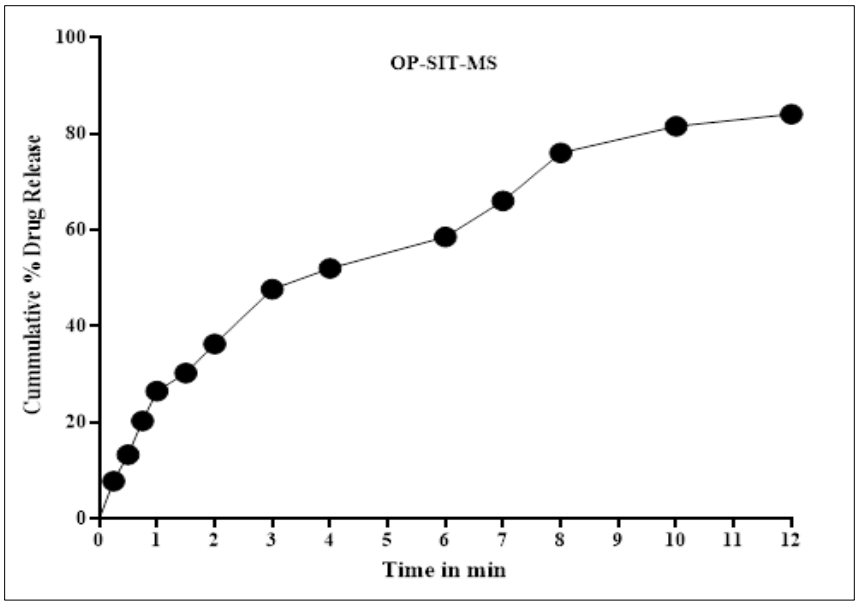

Figure 11 *In vitro* dissolution profile of OP-SIT-MS

4. Conclusion

The Sitagliptin Mucoadhesive Microspheres were successfully prepared by Ionotropic gelation method using polymers Sodium alginate and Carbopol. The results shows good entrapment efficiency, drug content and better mucoadhesion and confirmed good method for preparing mucoadhesive microspheres. Further the response surface design CCD studies suggest optimized formulation with significant relationship between factors on particle size, entrapment efficiency and *in vitro* drug release. Overall study concludes that QbD by DoE can be successfully applied to optimize and characterize Sitagliptin mucoadhesive microspheres.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest

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