GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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QbD- a novel approach for design, optimization and evaluation of Linagliptin mucoadhesive microspheres

G. Ragini, Bhumika.C and Y. Anand Kumar *

Department of Pharmaceutics, V. L. College of Pharmacy, Raichur, Karnataka, India.

GSC Advanced Research and Reviews, 2025, 25(01), 051-066

Publication history: Received on 25 August 2025; revised on 05 October 2025; accepted on 07 October 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.25.1.0300

Abstract

The quality by design (QbD) approach was applied for optimizing the formulation of model antidiabetic drug Linagliptin (LIN) mucoadhesive microspheres (MS) through design of experiment (DoE). To Optimize LIN-MS a quality target product profile (QTPP) was established in which critical quality attributes (CQAs) such as MVD, % encapsulation efficiency (% EE) and t_{50} drug release were quantified. As critical material attributes (CMA) viz., Keltone (KE), Carbopol (CA) and Pectin (PC) were chosen and evaluated their effect on CQAs. Response surface design (RSM) viz., Central Composite Design (CCD) was studied to evaluate effects of CMA on stated CQAs within the design space. The main effect was observed with varied levels of KE, CA and PC on CQAs. Numerical optimization by point prediction method was applied to generate optimized formula with predicted response, later the experimental responses were validated and ratified within the design space. The results suggest QbD through DoE appears to be a useful approach for the rational design, optimization and characterization of LIN-MS.

Keywords: Linagliptin; QbD; QTPP; CQAs; CMA; DoE

1. Introduction

Pharmaceutical industry is constantly searching the ways to ensure and enhance product safety, quality and efficacy. However, drug recalls, manufacturing failure cost, scale up issues and regulatory burden in recent past produce huge challenge for industry. In traditional, the product quality and performance are predominantly ensured by end product testing, with limited understanding of the process and critical process parameters. Regulatory bodies are therefore focusing on implementing quality by design (QbD), a science based approach that improves process understanding by reducing process variation and the enabling process control strategies.

QbD is a systematic approach to optimize pharmaceutical preparations and to improve the control over and the quality of the production process. The QbD approach consistently yields a product with desired characteristics and built in quality[1-4]. The preferred tool for strategic drug development using the QbD approach is the establishment of a quality target product profile (QTPP)[5,6]. A QTPP starts with defining the critical quality attributes (CQAs) for the final product. A CQAs can be defined as, physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality and thereby adequate performance and safety of the drug product when used[7]. A subsequent step of the QTTP is the identification of the critical material attributes (CMA) that influence the CQAs. By combining the CQAs and CMA a design space can be created. As long as the formulation and process variables remain within the design space, a product will be obtained that meets the quality requirements.

Delivery of a medication for an acute or chronic disease is carried out via conventional pharmaceutical dosage forms such as matrix tablets, capsules, suspensions etc. Therapy with the conventional dosage forms shows variation in the

* Corresponding author: Y. Anand Kumar.

concentration of the drug in plasma. After administering first dose, the drug concentration declines due to the effect of metabolism. 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 prolonged period of time with minimum unwanted effects and with more patient compliance[8]. 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)[9-11]. 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[12-14]. 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[15-17]. 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.

QbD regulatory initiative represents a highly systematic approach implementing the Design of Experiments (DoE) for finding the optimal product and process characteristics[18,19]. 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[20]. 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[21]. The design space stated as a renowned technique enclosing multidimensional series of input variables and process parameters, which detonated in order to insist typical quality assurance[22].

Linagliptin (LIN)[23], a potent and selective dipeptidyl peptidase (DPP) IV inhibitor mainly used for the treatment of type 2 diabetes. Typically, it improves the glyceamic control by increasing the active level of incretin peptides particularly glucagon like peptide-1 (GLP-1) and glucose-dependent insulin tropic peptide. The rationale of selecting LIN as a model drug for designing MS formulation is to administer once daily to avoid repetitive administration would be further enviable.

The aim of the present research was adapting QbD approach to design, optimize and characterize Linagliptin loaded mucoadhesive microspheres using novel mucoadhesive polymer blends viz., Keltone, Carbopol and Pectin by DoE studies.

2. Materials and methods

Materials: Linagliptin (LIN) was obtained as gift sample Magnus Pharma Ltd, Birgunj, Nepal. Keltone (KE), Carbopol 934(CA), Pectin (PC), Calcium chloride ($CaCl_2$) were purchased S.D Fine Chemicals Mumbai, Maharashtra, India. All other reagents employed in the study were of pharmaceutical and analytical purity.

Choice of design and experimental layout: The design space was calculated using Design Expert Software V13 trial, Stat Ease (DES). The CMA (KE, CA and PC) at varied levels were used for the study and Central Composite Design (CCD) was made for the RSM and the number of runs needed were calculated. The CMA were varied over two levels (-1 low, +1 High) resulting in a setup of 17 runs which were performed randomly to prevent bias. Table 1 shows the ranges of CMA applied and trial run generated and keeping other excipients kept constant. To each run different variables were assigned by the program resulting in different data visualization plots[24]. For each run a different percentage of KE (X_1), CA (X_2) and PC (X_3) were applied to assess the CQAs viz., Mean vesicle diameter (MVD-Y_1) % EE (Y_2), and t_{50} (Y3). The best fitted models were assigned by DES and were chosen based on their significance using an analysis of variance (ANOVA) F-test with appropriate polynomial equations,

$$Y_n = b_0 + b_1X_1 + b^2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + \ldots\ldots\ldots\ldots$$

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 CMA and levels as per CCD

Variables (CMA)	**Levels used, actual (coded)**	
Independent variable	Low (-1)	High (+1)
X_1-KE in mg	250	300
X_2-CA in mg	75	100
X_3-PC in mg	75	100
Dependent variables/Response (CQAs)		
Y_1 - MVD in µm ; Y_2 - % EE; Y_3 - t_{50}		

Preparation of LIN-MS[25-27]: LIN-MS were prepared by ionotropic gelation method (figure 1). First homogeneous polymer solution was prepared by mixing accurately weighed quantities (table 2) of KE, CA and PC with 10 ml of double distilled water, to this slowly disperse weighed amount of LIN with constant stirring until smooth dispersion was obtained. The smooth dispersion was loaded into syringe and fix needle size 22, further inject slowly the mixture into 10 % w/v $CaCl_2$ solution with constant stirring at 200 rpm for 1 hr on thermostat magnetic stirrer to produce viscous, rigid microspheres. The obtained LIN-MS were washed with distilled water to remove any unreacted calcium ions, and air dried for 24 hr. Dried LIN-MS were stored at room temperature for further evaluation.

Table 2 Possible formulation trials as per CCD

Trial Runs	**X_1**	**X_2**	**X_3**
	A:KE (mg)	B:CA (mg)	C:PC (mg)
1	275	87.5	100
2	275	100	87.5
3	300	75	100
4	250	100	75
5	250	100	100
6	300	75	75
7	275	75	87.5
8	300	100	75
9	250	87.5	87.5
10	275	87.5	87.5
11	250	100	100
12	275	75	75
13	250	75	75
14	300	100	100
15	275	87.5	87.5
16	300	87.5	87.5
17	275	87.5	87.5

2.1. Evaluation

FTIR study: The interaction between LIN and added excipients was studied by comparing FTIR spectrums of LIN and OP-LIN-MS. The FTIR of respective 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 trial batches of LIN-MS were determined by standard method. In each case transfer 50 mg of powdered microspheres into 25 ml volumetric flask, extract the LIN content with 25 ml of methanol by shaking occasionally for 1hr, followed by sonication for 10 min. Filter the contents, dilute appropriately with 0.1N HCl and measure the absorbance at 293 nm. The percent encapsulation efficiency was determined by using below given formula,

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

Drug content: The LIN content in LIN-MS trials were determined, in each case powdered microspheres equivalent to 5 mg of LIN was transfered into 25 ml volumetric flask, extract the LIN content with 25 ml of methanol by shaking occasionally for 1h, followed by sonication for 10 min. Filter the contents, dilute appropriately with 0.1N HCl and measure the absorbance at 293 nm. The % drug content was determined by using following equation,

$$\% \text{ Drug content} = \frac{\text{Experimenta drug content}}{\text{Theoretical drug content}} \times 100$$

Mean vesicle diameter (MVD): MVD of trial LIN-MS were determined by sieve analysis method. In each case, weighed amount of microspheres sieved through a set of standard sieves (viz., #16, #22 and #40) arranged in descending order with respect to the aperture size by using mechanical sieve shaker. After shaking period, microspheres which retained on each sieve were weighed and determine the MVD by using following equation,

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

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

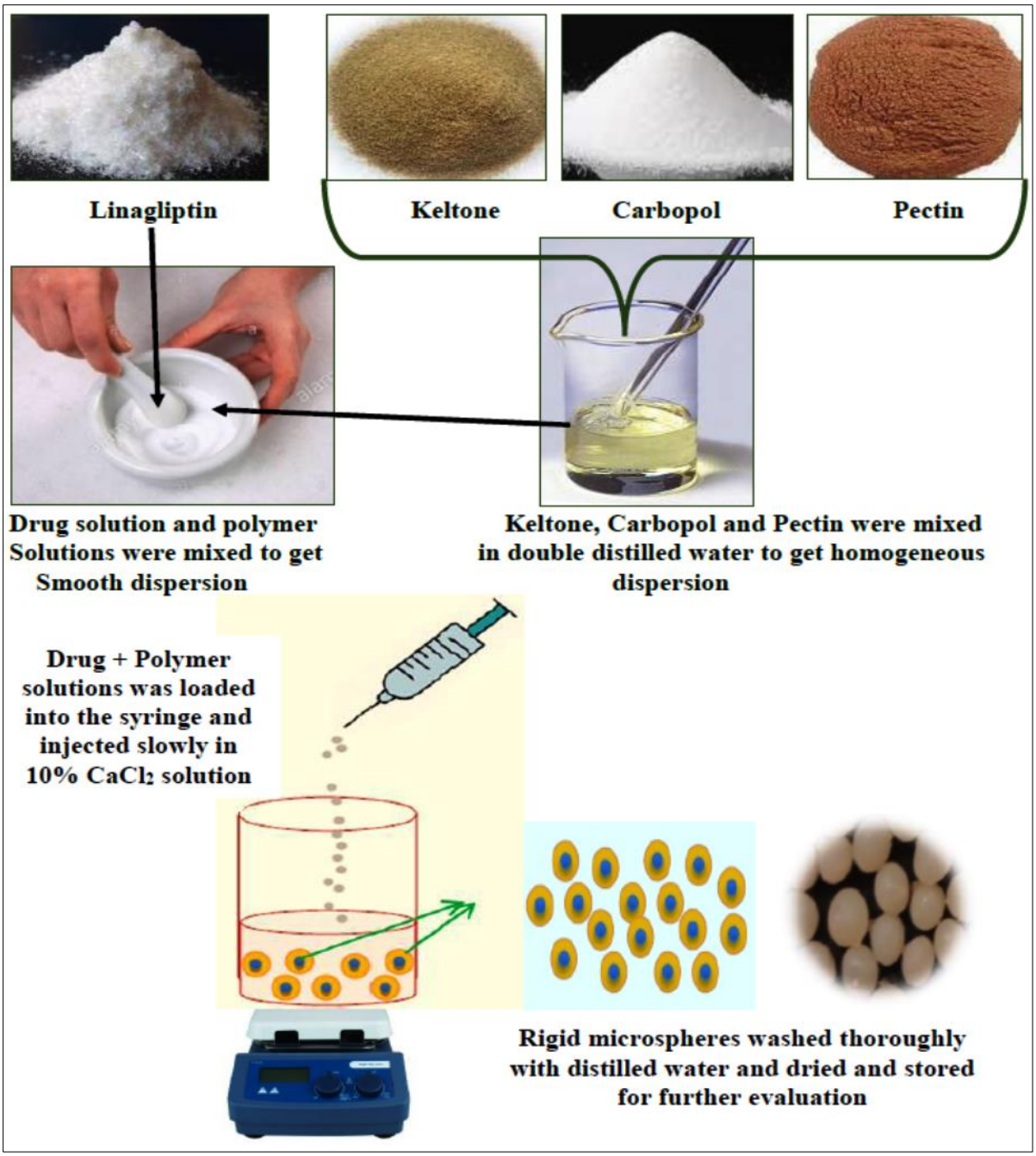

Figure 1 Scheme for preparation of LIN-MS by inotropic gelation method

Surface morphology: Surface morphology studied by scanning electron microscopy (SEM) to check surface topography, texture and to examine the morphology of fractured or sectioned surface of the OP-LIN-MS. The OP- LIN - MS was mounted using a double-sided sticking tape and coated with gold (200 Ao) on the SEM sample stab, under reduced pressure (0.001 torr) for 5 min using ion sputtering device (SEM-Jeol JFC-1100E, Tokyo, Japan). The gold-coated samples observed under the SEM and photomicrographs of suitable magnification were obtained.

***In vitro* mucoadhesion test:** *In vitro* mucoadhesion through wash-off test was studied for OP-LIN-MS to analyze extent of mucoadhesion using 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. Spread about 100 microspheres onto wet and rinsed tissue specimen, and the tissue specimen was given a regular, slow movement in a vessel containing 600 ml of 0.1N HCl at 37°C by rotating the paddle at 50 rpm. Measure the number of microspheres adhering to tissue at different intervals and continued for 6 hr, from the data percentage mucoadhesion was determined by using the formula,

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microsphered adhered}}{\text{Number of microspheres applied}} \times 100$$

***In vitro* dissolution:** The *in vitro* drug release was studied for trial LIN-MS and OP-LIN-MS using USP type I basket apparatus. In each case LIN-MS and OP-LIN-MS equivalent to 10 mg of LIN were filled in hard gelatin capsules and studied for drug release. The drug release was studied in two simulated fluids, for first 2 hr studied in 0.1N HCl followed by phosphate buffer pH 6.8 for 12 hr. During the dissolution studies, maintain a speed of 50 rpm and temperature of 37 ± 0.5^0 C throughout the study period. At different time intervals, 5 ml sample was withdrawn and replaced with fresh dissolution medium to maintain the sink condition. The LIN content at different time intervals was determined by using appropriate analytical method. The drug release data was model fitted with different kinetic models using PCP Disso V3.

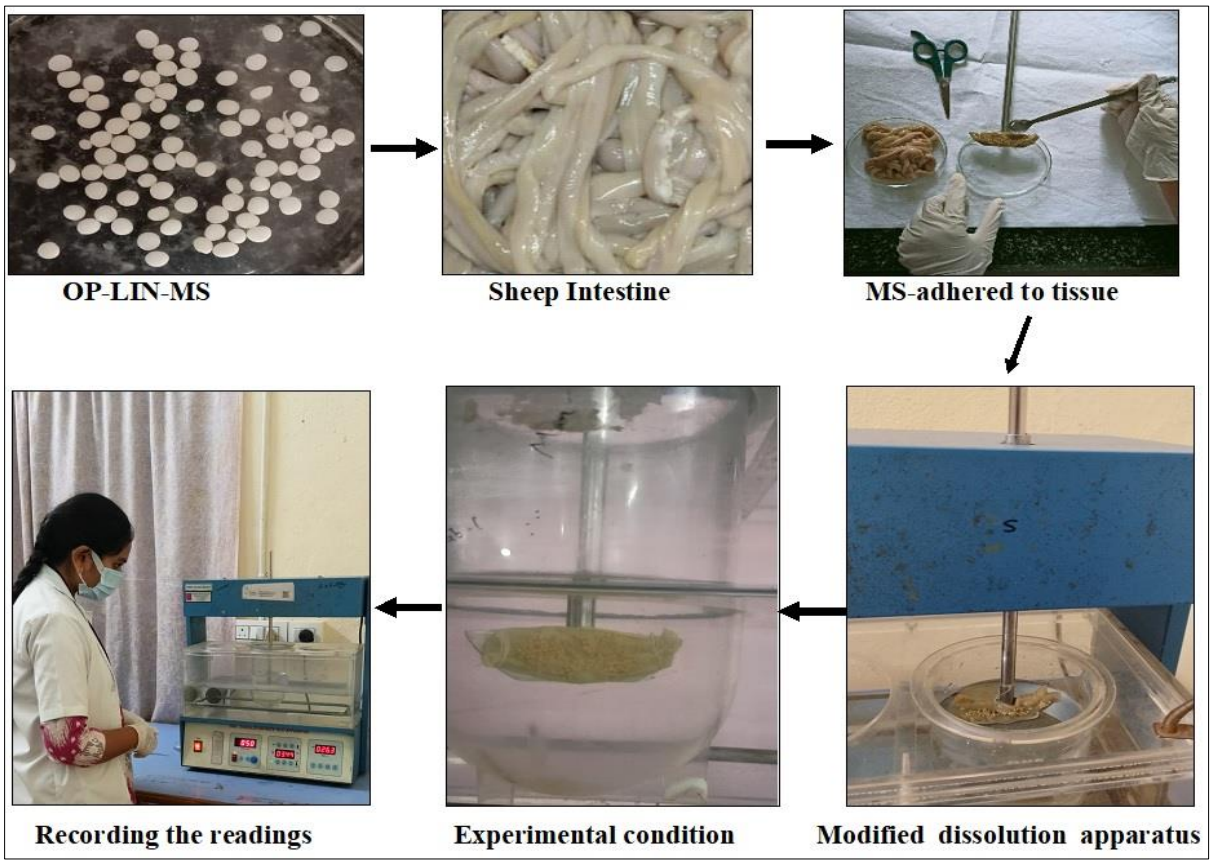

Figure 2 *In vitro* wash off test of OP-LIN-MS

3. Results and Discussion

The model drug LIN was subjected for preformulation studies such as solubility, melting point partition coefficient. The solubility of LIN complies with the standard values, melting point was found to be 202^oC against standard 190-202^0C, the partition coefficient is 1.7 against 1.8 and pKa is 8.6 against 7.4 (log P). The results were complies with the standard values indicate the drug sample was stable and pure. The trial LIN-MS appears as slightly pale yellow colour free flowing microspheres, % drug content was in the range of 98.71 ± 0.5590 to 99.11 ± 0.1206, low SD value indicate the drug is uniformly distributed throughout the microspheres. During characterization of trial LIN-MS two important CQAs were generated viz., mean vesicle diameter and % EE. The comparative *in vitro* dissolution profile was shown in figure 3, the profile suggest the release of drug was less in acidic pH, once it enters into basic pH, the drug release was increased and sustained for 12 hr, from this profile one important CQA viz., t50 was generated under the influence of CMA.

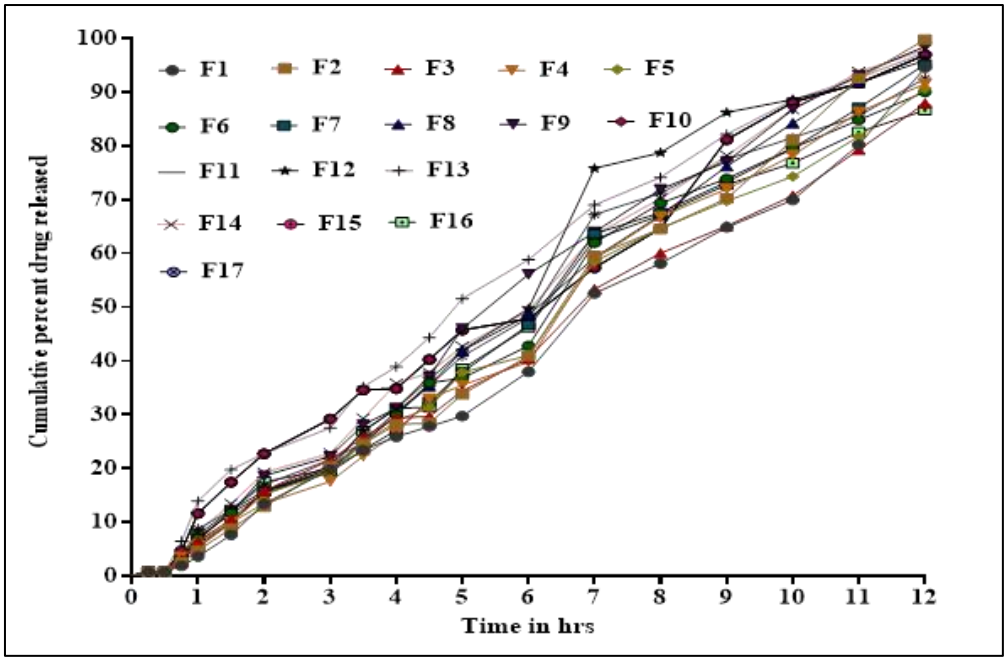

Figure 3 Comparative *in vitro* dissolution profile of trial LIN-MS as per CCD

Table 3 CQAs data of trial batches as per CCD under the influence of CMA

Design trials Batch code	CMA			CQAs		
	X_1	X_2	X_3	Y_1	Y_2	Y_3
	A:KE mg	B:CA mg	C:PC mg	MVD µm	% EE	t50 hr
F-1	275	87.5	100	835.52	70.12	5.8
F-2	275	100	87.5	837.23	69.78	5.81
F-3	300	75	100	895.65	78.56	6.52
F-4	250	100	75	800.12	55.65	4.85
F-5	250	100	100	798.56	56.21	4.78
F-6	300	75	75	885.63	79.56	6.61
F-7	275	75	87.5	832.21	68.95	5.79
F-8	300	100	75	891.26	80.12	6.58
F-9	250	87.5	87.5	801.01	58.12	4.81
F-10	275	87.5	87.5	831.24	70.1	5.81
F-11	250	75	100	800.11	56.1	4.79
F-12	275	87.5	75	829.32	71.02	5.77
F-13	250	75	75	799.12	55.62	4.81
F-14	300	100	100	890.21	80.21	6.61
F-15	275	87.5	87.5	830.01	76.56	5.79
F-16	300	87.5	87.5	889.25	79.56	6.64
F-17	275	87.5	87.5	829.98	71.65	5.82

CQAs studies within the design space: CQAs for LIN-MS set MVD (<1000 µm), % EE (> 50) and t50 (> 4hr) and these limits are based on earlier experiments on MS. The results of the CQAs under the study with stated levels of CMA was given in table 3. They were further analyzed with the DES yielding diagnostic and model surface response plots. All the measurements visualized with Normality, Predicted vs actual, Contour and 3D surface plots, which can be used interpret effect, main effect and interaction effects under the influence of varied levels of CMA and provide clear idea about which combination of the CMA give the preferred or unfavorable response for stated CQAs[22]. The influence of CMA on CQAs were further explained with relative statistical data viz., ANOVA, model fit statistics and polynomial equations generated from the DES, as given in tables 4, 5.

Table 4 ANOVA and model fit statistical data of CQAs under the study

CQAs / **Statistical data**	**MVD**	**% EE**	**t50**
ANOVA F-Value	371.81 P < 0.0001	68.60 P < 0.0001	860.91 P < 0.0001
MODEL Suggested	Quadratic Significant	Linear Significant	Quadratic Significant
Significant model terms	A- Keltone P < 0.0001	A- Keltone P < 0.0001	A- Keltone P < 0.0001
	A^2- Keltone^2 P < 0.0001		A^2- Keltone^2 P < 0.0039
Actual R^2	0.9979	0.9406	0.9991
Predicted R^2	0.9794	0.9130	0.9860
Adjusted R^2	0.9952	0.9269	0.9979
C.V %	0.3003	3.70	0.5598
Adequate precision	50.029	19.29	74.91

Table 5 Polynomial equations for CQAs

MVD Y_1	**Y_1 =** + 831.84 + 45.31*A + 0.4660*B + 1.46*C + 0.0925*AB + 1.19*AC -1.70*BC + 12.21*A^2 + 1.80*B^2 - 0.5011*C^2
% EE Y_2	**Y3** = + 69.30 + 11.62*A + 0.3070*B - 0.0660*C
t_{50} Y_3	**Y_3** = + 5.81 + 0.8920*A + 0.0110*B 0.0120*C + 0.0037*AB + 0.0037*AC + 0.0087*BC - 0.0828*A^2 - 0.0078*B^2- 0.0228*C^2

Effect of factors on CQAs Y_1 – MVD: ANOVA suggested Quadratic model with F-value 371.81 implies the model is significant. Here A and A^2 were significant model terms, which gives main effect on MVD. The Lack of Fit F-value 16.82 (p= 0.0571) implies non significant Lack of Fit which is good for model to fit. The R^2 value was greater than 0.9 (0.9979) explains a perfect positive correlation was existed between the CMA under the study with MVD, justifies with normality plot (figure 4a) where all the MVD values of trial LIN-MS were nearer to the model fit line. This explains linearity among the CMA under the study with CQAs (MVD).The adequate precision was greater than 4 which is desirable and can be used to navigate the relationship within the design space. The actual MVD values for trial LIN-MS was within the desirable range (>1000 µm) and ranges from 800.12 (Trial 4) to 895.65 (Trial 3). The polynomial equation (table 5) suggest second order impact of CMA on MVD with two significant model term viz., A-Keltone and A^2-Keltone^2, the Keltone and its exponential terms has main effect as indicated by the positive sign, as concentration of Keltone increases the MVD increases significantly, this could be attributed due to the swelling and hydrophilic nature of the Keltone. The actual MVD values were placed within the three ranges of design space and all were placed in and around of the

predicted fitted line as shown in predicted vs actual plot (figure 4b). The Keltone and its exponential terms has significant influence on the MVD as shown in the Contour and 3D surface plot (figure 4c, d), remaining model terms has less or negligible effect on the MVD.

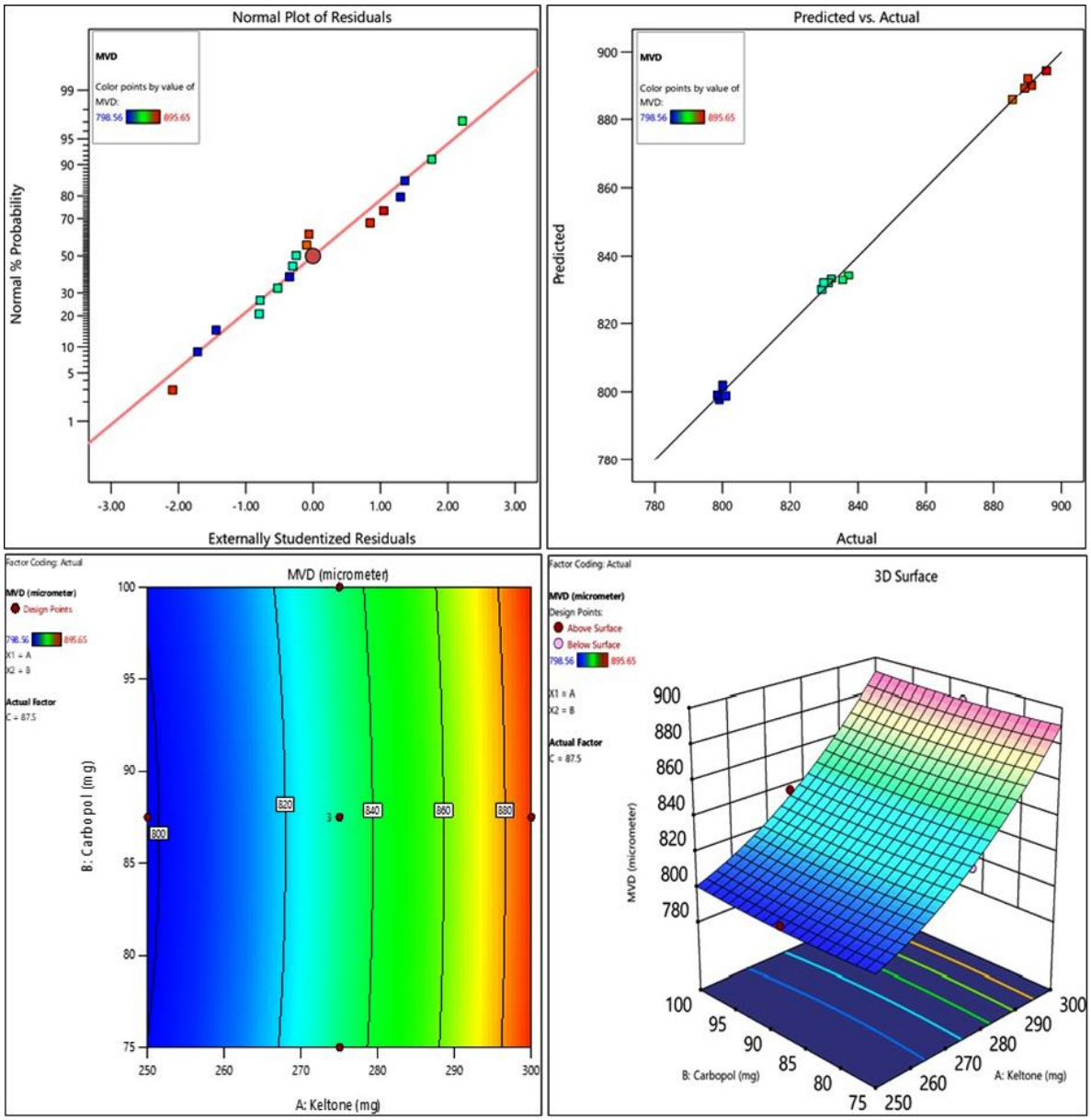

Figure 4 Data visualization of MVD under the influence of CMA a) Normality, b) Predicted vs Actual, c) Contour and d) 3D surface

Effect of factors on CQAs Y_2 – % EE: ANOVA suggested Linear model with F-value 68.60 implies the model is significant. Here A was significant model terms, which gives main effect on % EE. The Lack of Fit F-value was 0.5182 (p= 0.8087) implies non significant Lack of Fit which is good for model to fit. The R^2 value was greater than 0.9 (0.9406) explains a perfect positive correlation was existed between the CMA under the study with % EE, justifies with normality plot (figure 5a) where all the % EE values of trial LIN-MS were nearer to the model fit line. This explains linearity among the CMA under the study with CQAs (% EE).The adequate precision was greater than 4 which is desirable and can be used to navigate the relationship within the design space. The actual % EE values for trial LIN-MS was within the desirable range (< 50 %) and ranges from 55.62 (Trial 13) to 80.21 (Trial 14). The polynomial equation (table 5) suggest first order impact of CMA on % EE with only one significant model term viz., A-Keltone, has main effect as indicated by the positive sign, as concentration of Keltone increases the % EE increases significantly, this could be attributed due to the swelling and hydrophilic nature of the Keltone. The actual % EE values were placed within the three ranges of design space and all were placed in and around of the predicted fitted line as shown in predicted vs actual plot (figure 5b). The

Keltone term has significant influence on the % EE as shown in the Contour and 3D surface plot (figure 5 c, d), remaining model terms has less or negligible effect on the % EE.

Effect of factors on CQAs Y_3 – t_{50}: ANOVA suggested Quadratic model with F-value 860.91 implies the model is significant. Here A and A^2 were significant model terms, which gives main effect on t_{50}. The Lack of Fit F-value was 5.80 (p=0.1526) implies non significant Lack of Fit which is good for model to fit. The R^2 value was greater than 0.9 (0.9991) explains a perfect positive correlation was existed between the CMA under the study with t_{50}, justifies with normality plot (figure 6a) where all the t_{50} values of trial LIN-MS were nearer to the model fit line. This explains linearity among the CMA under the study with CQAs (t_{50}). The adequate precision was greater than 4 which is desirable and can be used to navigate the relationship within the design space. The actual t_{50} values for trial LIN-MS was within the desirable range (>1000 µm) and ranges from 4.79 hr (Trial 4) to 6.64 hr (Trial 3). The polynomial equation (table 5) suggest second order impact of CMA on t_{50} with two significant model term viz., A-Keltone and A^2- Keltone^2, the Keltone and its exponential terms has main effect as indicated by the positive sign, as concentration of Keltone increases the t_{50} increases significantly, this could be attributed due to the swelling nature of the Keltone which extended the drug release for prolonged period. The actual t_{50} values were placed within the three ranges of design space and all were placed in and around of the predicted fitted line as shown in predicted vs actual plot (figure 6b). The Keltone and its exponential terms has significant influence on the t_{50} as shown in the Contour and 3D surface plot (figure 6 c, d), remaining model terms has less or negligible effect on the t_{50}.

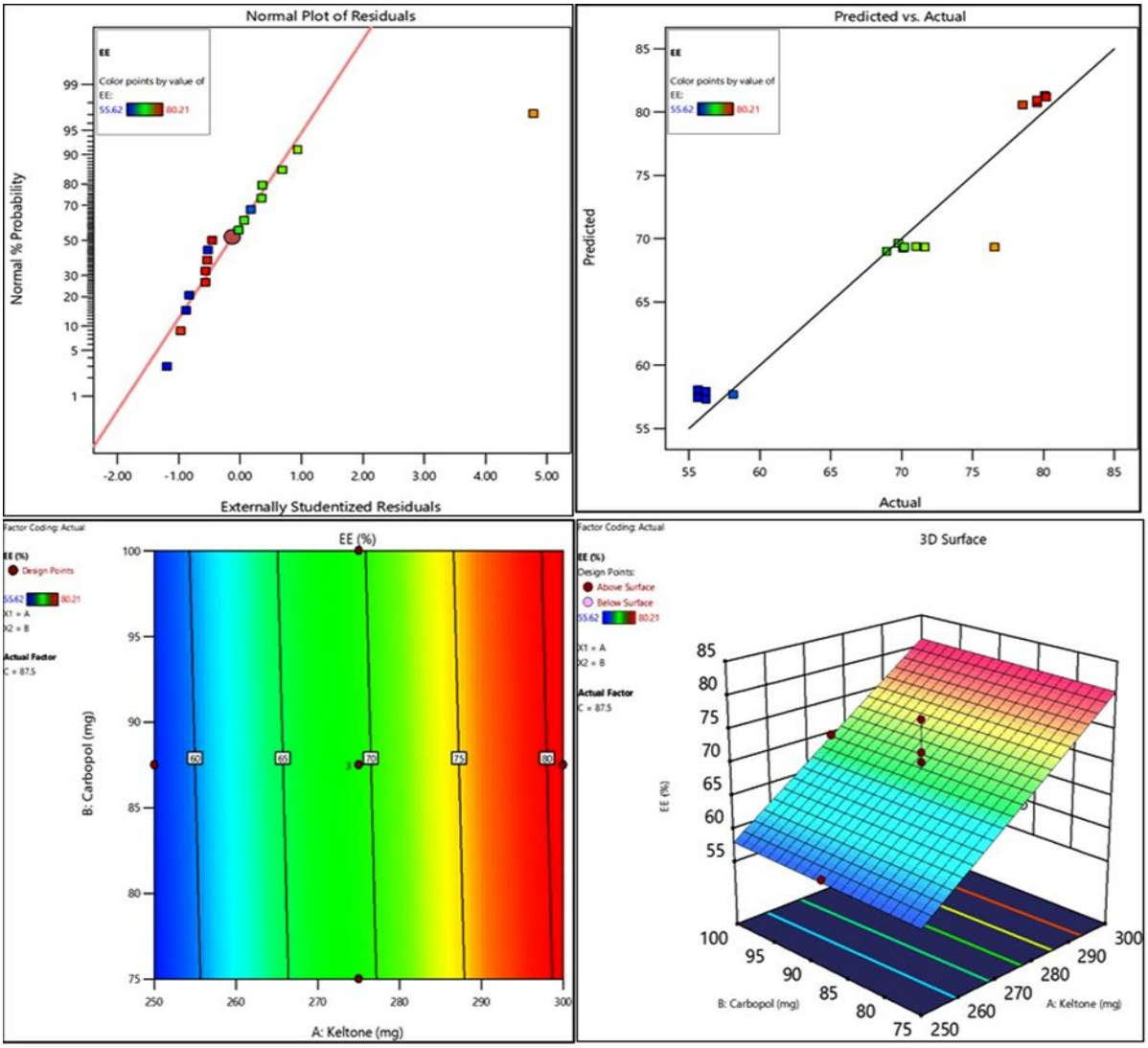

Figure 5 Data visualization of % EE under the influence of CMA a) Normality, b) Predicted vs Actual, c) Contour and d) 3D surface

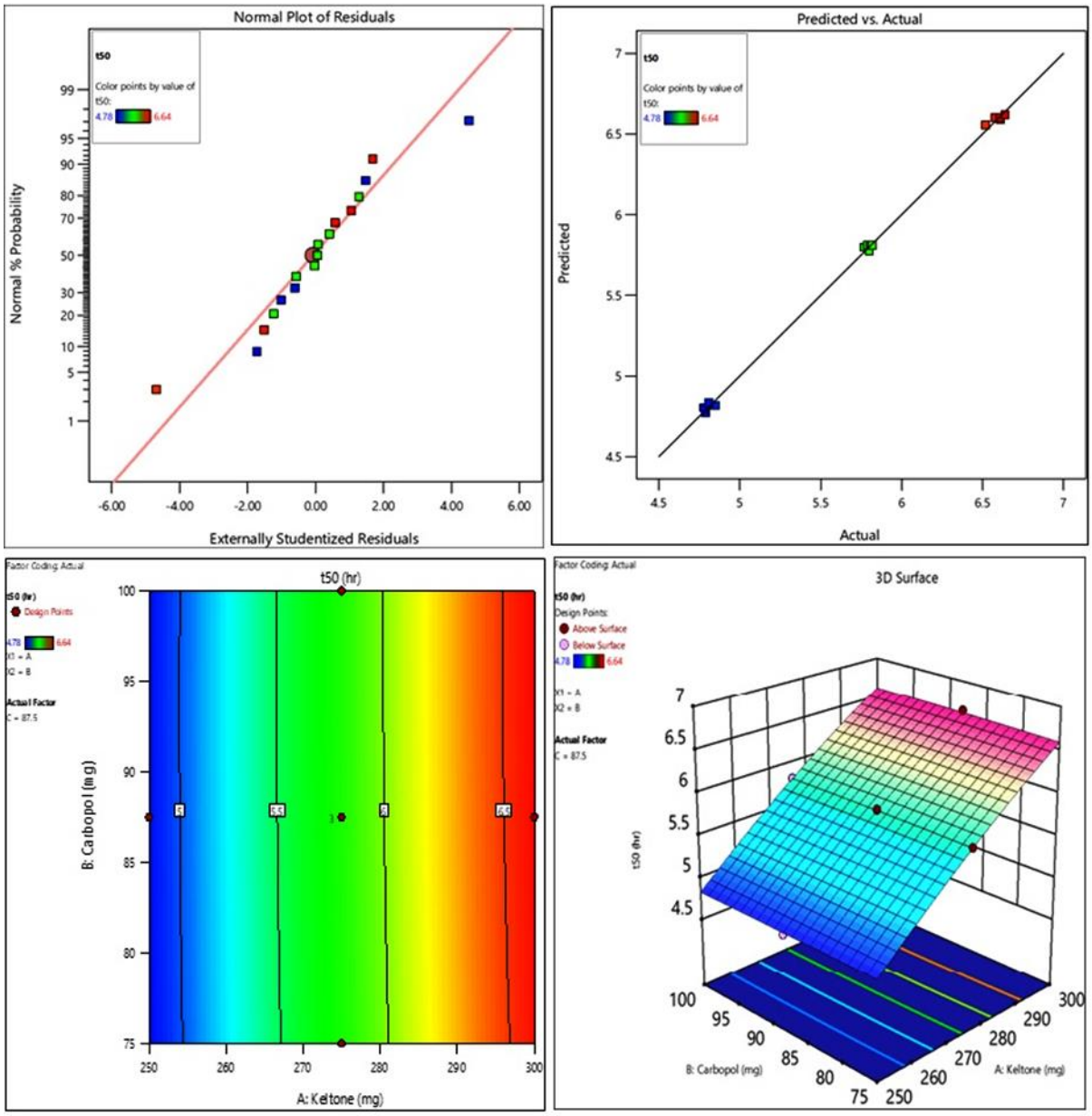

Figure 6 Data visualization of t50 under the influence of CMA a) Normality, b) Predicted vs Actual, c) Contour and d) 3D surface

Numerical optimization: A point prediction method using the desirability approach was employed to develop an optimized formula with predicted CQAs under the influence of CMA by using the CCD study. During the study constraints were fixed for CMA viz., maximize the X_1 (KE) in range X_2 (CA) in range X_3 (PC); for CQAs, set in range for Y_1 (MVD), maximize Y_2 (% EE) and maximize Y_3 (t_{50}). The DES generate desirability (figure 7) solution 0.997 (1 in 100) to predict the CQAs. The optimized formula with concentrations of CMA was shown in table 6 along with predicted CQAs.

Table 6 Data of OP-LIN -MS with predicted response as per CCD and Experimental response

Optimized CMA levels For OP-LIN-MS		**CQAs**	**Predicted response mean ± SD**	**Experiments response Mean ± SD**
KE	300 mg	MVD µm	891.496 ± 2.52163	821.2 ± 2.132
CA	99.3225 mg	EE %	81.2111 ± 2.56228	78.5 ± 0.8132
PC	87.459 mg	t50 hr	6.62346 ± 0.0321371	6.17 ± 0.08932

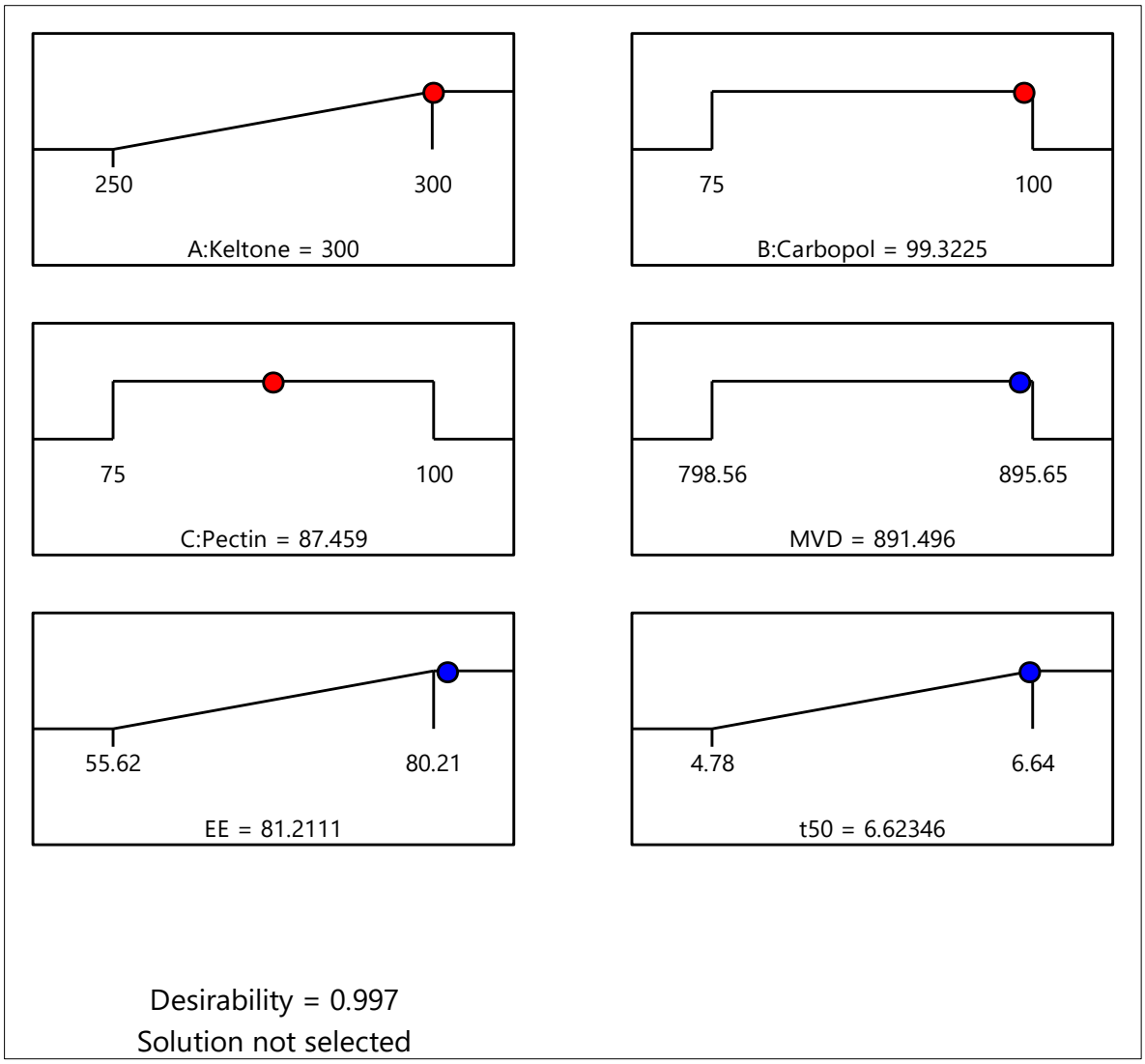

Figure 7 Desirability solutions for CQAs at generated CMA

3.1. Characterization of OP-LIN-MS

The OP-LIN-MS generated as per CCD was formulated experimentally by ionotropic gelation method and characterized for % yield, FTIR, % drug content, surface morphology, SEM, MVD, *in vitro* mucoadhesion (wash off test), % EE and *in vitro* drug release.

The production yield was 95.45 ± 1.05 % suggest the method was reproducible and % drug content 99.45 ± 1.15 with low SD value indicate the drug is uniformly distributed throughout the microspheres.

The FTIR spectra of LIN and OP-LIN-MS was shown in figure 8, FTIR spectra of LIN showed the characteristic peak at 1400.28 cm^{-1} is related to alkane stretching (C-N), 1696.36, 1653.04 cm^{-1} is associated with the C=O bond of carbonyl group in purine ring, 2911.45 cm^{-1} refers to $-NH_2$ primary amine, 3368.12 associated with Stretching the piperidine. The spectral data was ratified with literature indicate the LIN found to be pure and can be used for further. Major characteristic FTIR bands of LIN were found in optimized batch of OP- LIN –MS with slight shifting towards lower to higher wavelength indicates no interaction.

The digital photographs and SEM of OP-LIN-MS was shown in figure 9, the results suggest the microspheres were spherical and free flowing without any tailing. SEM at 75X 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.

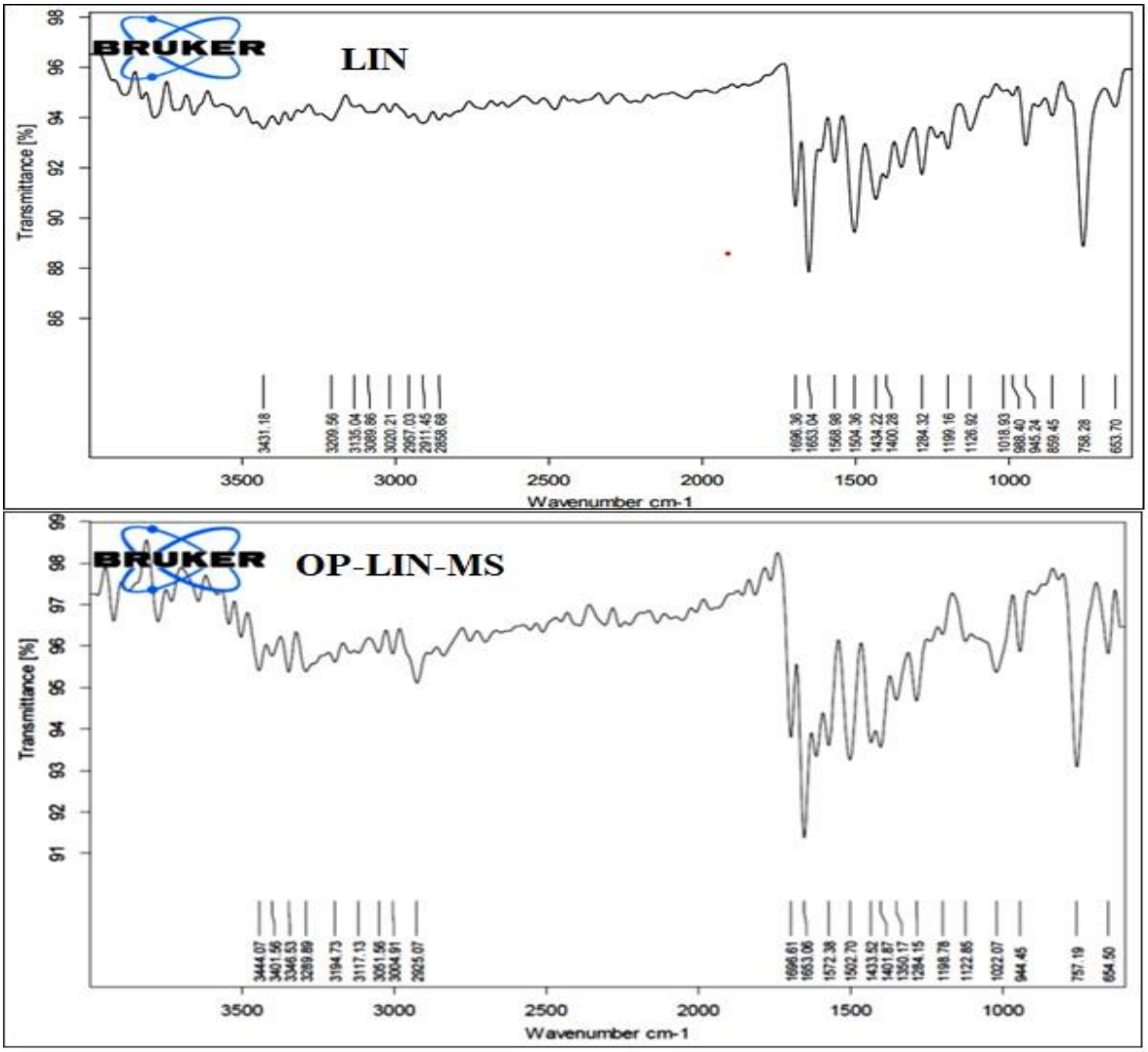

Figure 8 Comparative FTIR of LIN and OP-LIN-MS

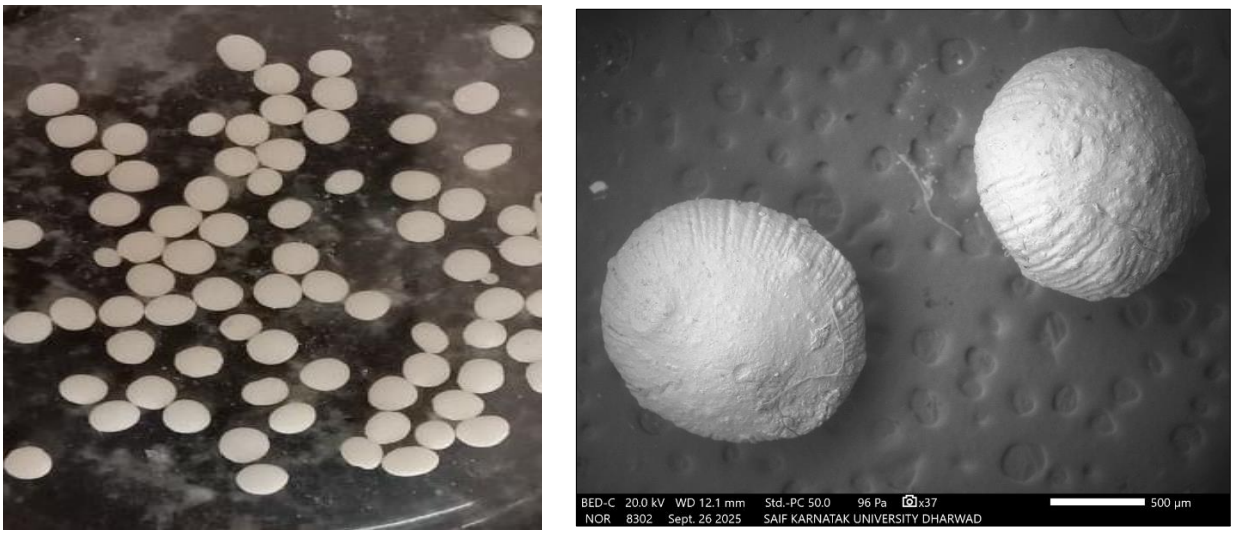

Figure 9 Digital photograph and SEM image of OP-LIN-MS

The *in vitro* mucoadhesion was done through wash-off test for 6 hr by standard procedure, the results shows good *in vitro* mucoadhesion on sheep intestine, where 55% of microspheres were intact on tissue after 6 hr. 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 in drug release studies.

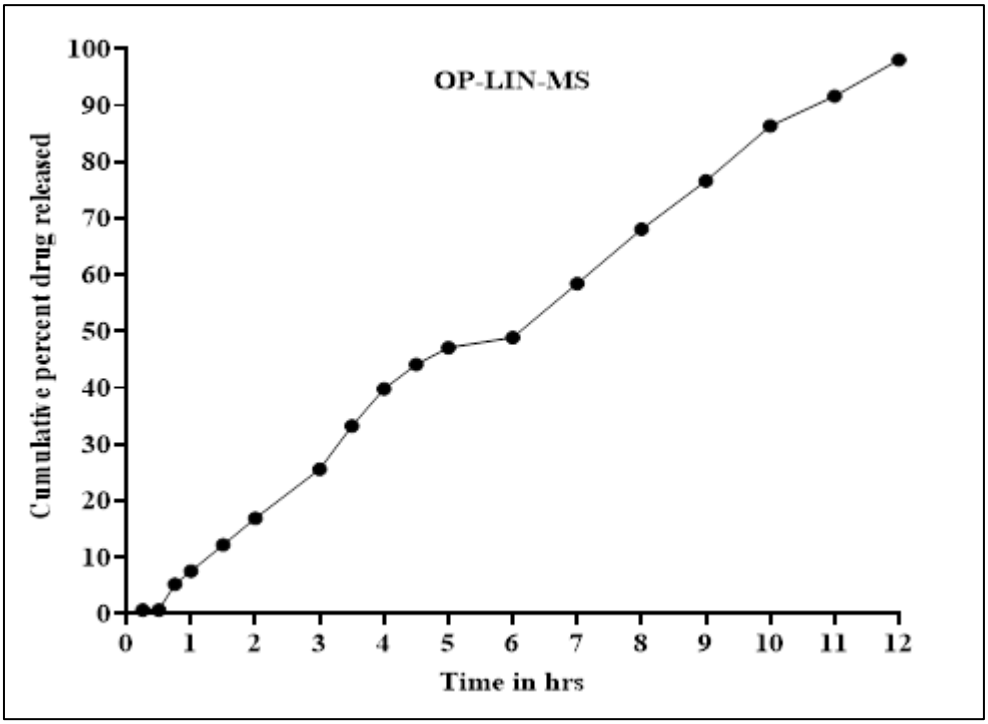

Figure 10 *In vitro* dissolution profile of OP-LIN-MS

Simulate the *in vitro* drug release of OP-LIN-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 10) was 16.914 ± 1.35 after 2h; 54.614± 1.11 after 6 hr and 98.086 ± 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 0.9953 (Zero order), 0.8895 (1st order), 0.9333 (Matrix), 0.9271 (Peppas) and 0.9639 (Hix.Crow) suggest best fit model was zero order and exponential 'n' value was greater than 1 indicate the drug release mechanism follows super case II transport i.e. swelling followed by erosion diffusion controlled. The Keltone concentration in the formulation greatly influenced the steady state release of LIN from the microspheres. The principle of gelation or cross-linking of Keltone with calcium chloride 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 LIN release becomes slow.

3.2. Validation

The formulated OP-LIN-MS was evaluated for CQAs within the design space, studies such as MVD, % EE and t_{50} (collected from dissolution data) were compared with the predicted data as given table 6. The results were validated and ratified with predicted data, it clearly indicates the DoE studies can be used to study the influence of CMA on CQAs. The study indicate high degree closeness between the predicted and experimental values of the responses and confirmed excellent prognostic ability of the employed mathematical model.

4. Conclusion

The studies concludes that ionotropic gelation method was appropriate for fabricate Linagliptin mucoadhesive microspheres using novel polymers such as Keltone, Carbopol and Pectin. The results shows good *in vitro* mucoadhesion, drug content and controlled drug release for 12 hr. Further the response surface design CCD studies suggest optimized formulation with significant relationship between CMA on CQAs. Overall study concludes that QbD approach can by successfully applied using DoE to optimize and characterize Linagliptin mucoadhesive microspheres.

Compliance with ethical standards

Acknowledgments

We wish to thanks to the principal and management of V. L. College of pharmacy for providing the facilities to carry out the work.

Disclosure of conflict of interest

There are no conflicts of interest.

Author's contribution

The collaborative efforts of all authors have successfully led to study of design, optimization and evaluation of linagliptin mucoadhesive microspheres. Ragini and Bhomika under the mentorship of Y. Anand Kumar conducted the research work provided guidance in crafting the manuscript and structuring the research paper in accordance with the journal's guidelines.

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