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Establishment and Escalation of Amino Acid Stacked Repressible Release Embedded System Using QbD

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Abstract

Objectives: Traditional approach of developing a new delivery system is an exhaustive task and requires a number of resources like man, money, material and time. To overcome this problem Quality by Design (QbD) can be utilized to get the pharmaceutical product of desired (best) quality with minimum use of above resources as well as determination of impact of one factor over the desired associated process. The present research is focused on establishing a design for formulating optimized gelatin microspheres using QbD.

Materials and Methods: Characterization of formulated microspheres was done by infra red spectroscopy, scanning electron microscopy, percentage yield, microsphere size, drug entrapment efficiency and drug release. Impact of concentration of gelatin and ethyl cellulose was determine over dependent response like percentage yield, microsphere size and drug entrapment efficiency.

Results: Response surface curve was obtained by using 3^2 central composite design and optimized batch was obtained with percentage yield, microsphere size and drug entrapment efficiency as 89.98, 333.32 mm and 82.61% respectively. Validation of optimized batch was done by formulating four different batches with optimized values of independent response and a comparison of the observed responses with the predicted ones setting up and all these batches were found close to the predicted values and show validity of optimized data.

Conclusion: Hence QbD approach is quite efficient to get optimized drug delivery systems of L-Arginine without doing exhaustive study.

Key words: L-Arginine, Gelatin, Central Composite Design, microspheres, Characterization of microspheres.

Introduction

Development of repressed released systems primarily involve a number of complexity in selection of suitable polymers used in rate controlling, their suitable concentrations, formulation technique etc[1] to achieve maximum therapeutic benefits. Optimization of the formulation and the process involved in manufacturing systems using traditional one-factor-at-a-time (OFAT) is a difficult task with great expenditure of time, money and material[2,3]

Hence to overcome these problems Quality by design (QbD) approach was developed for systematic development of dosage form that starts with predefined objectives. QbD approach emphasised on development of quality product, better understanding of process as well as process control which are based on sound science and quality risk management (QRM) [4]. Design of Experiment is a statistical tool performs systematic scientific studies in order to determine the effect independent variable on a their observed dependent response. It makes controlled changes to input variables in order to gain maximum amounts of information on cause and effect relationships with a minimum sample size for optimizing the formulation[5-7]. Response surface methodology is carried out by employing experimental design; also considered as a decisive part of QbD. The experimental design helps in scaling the responses on the basis of the defined objectives.[1-8]

Oral repressible release formulations are also known as oral controlled released formulations. These are the dosage form in which the drug is released in a planned, predictable and slower than conventional dosage form. These formulations are developed to improve the problems associated with oral conventional dosage forms like they can reduce side effects, improve the therapeutic efficacy by delayed/prolonged drug release so that frequency of drug administration can be reduce. Thus assuring better patient compliance. [9,10] Various technique have been developed for controlled release formulations; which utilizes the cross-linking ability of polyelectrolyte in the presence of counter ions to form multiparticulte system. These delivery systems are spherical cross linked hydrophilic polymeric system which upon gelation and swelling in simulated biological fluids releases drug in controlled manner. These developed microspheres are can be loaded with high amount of drug as compared to the conventional delivery system.[11,12] Arginine an ergogenic (i.e., performance enhancing) supplement, most notably in the “nitric oxide” (NO) class of supplements is a semi-essential amino acid involved in multiple areas of human physiology and metabolism. NO produced from it improves outcomes in various diseases.[13] L-arginine is readily available over the counter and is popular as a nutritional supplement to increase muscle mass. More recently, L-arginine has been tested as a potential therapeutic in numerous acute and chronic disease states, including sickle cell chest crisis, pulmonary artery hypertension, coronary heart disease, pre-eclampsia and myocardial infarction, because of its bronchodilator and vasodilator actions.[14-15]

Materials

L-Arginine was obtained from CDH Laboratory Chemicals, Sodium Alginate (low viscosity grade, 250 cp of 2% solution at 25°C) from Loba cheime pvt ltd (Mumbai). Gelatin, Ethyl Cellulose and Span 80 were purchased from Thermo fisher scientific India Pvt. Ltd. (Mumbai). Glutaraldehyde and Light liquid paraffin were procured from Loba chemical, Mumbai. All other chemicals used in the study are of analytical grade. HPLC grade water, methanol and potassium dihydrogen orthophosphate purchased from Qualigens fine chemicals (Gujrat).

Methods

Defining the QTPP and CQAs

For developing drug product using QbD, the quality target product profile (QTPP) was defined setting up the summary of quality attributes of the drug product for obtaining the desired oral repressible release formulations for attaining maximum therapeutic benefits. In order to meet the QTPP, various patient-centric critical quality attributes (CQAs) referring to the quality of end product were defined. The key elements of QTPP for preparing the oral repressible release microdpheres and CQAs are enlisted in table 1 along with felicious justifications for them.[8]

Screening of Polymers

Preliminary studies were carried out to select the amounts of variables, with focus on the formation of microspheres. Further, these prepared formulations were evaluated for percent yields, microspheres size and drug entrapment efficiency for identifying the suitable polymer combination for the purpose.

Preparation of microspheres

Controlled released microspheres of L-arginine were prepared by performing cross linking of gelatin using glutaraldehyde. The required amount of gelatin was taken in a beaker; to this 8ml of distilled water was added and this mixture was heated at 40°C temperature for 3-4 min to get uniform polymer mixture. Different concentration of ethyl cellulose (EC) was added as shown in table-1. Then the specified amount of drug was dispersed thoroughly to the polymer solution. A mixture of Light liquid paraffin (200mL) and span 80 (0.1mL) was prepared. The mixture was maintained at 4°C with ice bath and stirred at 200 rpm and to this mixture previously prepared polymeric drug solution was added through a syringe with 22 gauge

niddle. After some time glutaraldehyde (2mL) was added drop wise to it with continuous stirring for 2 h. Microspheres were filtered, washed by iso-propyl-alcohol to remove liquid paraffin and dry at room temperature. Then dried microspheres were collected, weighed and stored.[16-17]

3^2 Central Composite Design

A 3^2 CCD was adopted for optimization study. Two independent variables investigated were functional excipients such as concentration of gelatin (X) and EC (Y). The impact responses of these independent variables were investigated on the dependent responses such as percentage Yield, Microspheres Size (MS) and DEE. The experimental points used according to the design shown in Table 1.

Polynomial equations were generated and used to express the function of independent variables. Common polynomial equation to observe the effect of independent variable can be expressed as

$$Y_1 = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1X_2 + b_4 X_{12} + b_5 X_{22} + b_6 X_1X_{22} + b_7X_{12}X_2 \quad \text{eqn.(1)}$$

Where Y_1 is the dependent variable, b_0 is the arithmetic mean response of the thirteen runs.

The main independent variables, that is, effects X_1 and X_2 represent the average result of changing one factor at a time from its lower values to its higher values. 3^2 CCD is most efficient tool in estimating the influence of individual variables (main effects) and their interactions using minimum experimentation. In the present research, 3^2 CCD was considered to be best as the values of the response surfaces were not known from the previous findings. Thus, this design was selected for optimization of formulated microspheres.

Evaluation of prepared microspheres

Characterization of microspheres

FTIR spectra was obtained by Jasco FTIR 6100 type A, Japan spectrometer, sample was prepared in KBr disks, and spectra was recorded over the wave number 4000- 400 cm-1. All three spectra were completely analyzed.[18]

Percentage yield

Microspheres dried at room temperature were weighed and the Percentage yield of microspheres was calculated using formula.[14]

$$\% \text{ yield} = \frac{\text{Amount of sphere prepared experimentally}}{\text{Theoretical amount of microspheres (mg)}} \text{x100 eqn. (2)}$$

Morphological analysis

Scanning electron microscope (Zeiss, Supra 40, India) was used to characterize surface topography of the microspheres. The microspheres were fixed on a brass support with a thin

adhesive tape and the samples were coated with thin layer gold under vacuum to render them electrically conductive (approximately 3000 Å. The surface picture was taken screened taken at 15kV and 20kV for the drug-loaded microsphere.

Particle Size determination of microspheres

Particle size analysis was done by sieving method. Microspheres were separated out in different size fractions by passing them through a set of sieves for 5 minutes. This set of sieves included standard sieves having nominal mesh apertures of 1.0 mm, 0.71 mm and 0. 5 mm (sieve no.16, 22 and 30 respectively). The particle size distributions of the beads were determined and mean particle sizes of beads were calculated using following formula.

$$\text{Mean particle size} = \frac{\sum(\text{Mean particle size of the fraction} \times \text{weight})}{\sum \text{Weight fraction}} \qquad \text{eqn. (3)}$$

Swelling index

Gelatin microspheres were kept in double distilled water for swelling for 1h to reach maximum swelling. Volumetric measurements were done by determining the increase in volume in the swelling medium at specific time intervals. The swelling index was calculated as[19]:

$$\text{Swelling Index} = \frac{\text{Volume of swollen particles}}{\text{Volume of dry particles}} \qquad \text{eqn. (4)}$$

Drug Entrapment Efficiency

Accurately weighed drug-loaded microspheres equivalent to 100 mg of L- Arginine, were added to Phosphate Buffer (pH 7.4) and kept for shaking on mechanical shaker for 24 h. Then the solution was filtered and the drug content was estimated spectrophotometrically using HPLC (Younglin, ACME-9000, China), where samples withdrawn were subjected for separation in HPLC column C-18 which was also guarded by guard column. Mobile phase (Phosphate Buffer of pH 7.4 and methanol of HPLC grade) was filtered through 0.45µ membrane filter before use, degassed and was pumped from the solvent reservoir in a ratio of 90:10 v/v was pumped into the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 30°C. The detection of effluents was performed at 210 nm and the run time was finalized as 6 min. The volume of injection was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

The drug entrapment efficiency was determined using following formula[20]:

$$\text{Drug Entrapment Efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \qquad \text{eqn. (5)}$$

***In-vitro* Drug Release**

In vitro release studies were carried out on L-Arginine loaded microspheres using USP XXIV dissolution test apparatus-I (Electrolab, TDT-06T, Maharashtra, India). Weighed quantity of microspheres equivalent to 100 mg of L-Arginine were introduced into a dissolution basket and the basket was placed in 900 mL of phosphate buffer solution (pH = 7.4 for 8 h) at 37 ± 0.5°C (Ph. US 24th edn) and 50 rpm.[21] Aliquots of 5 mL solution were withdrawn at specific time intervals and replaced with fresh dissolution medium. The withdrawn samples were analysed for drug content at HPLC (Younglin, ACME-9000, China) having UV detector. The samples were studied at 210 nm to obtain the retention time 2.2-2.4 m and AUC.[22] The results of in vitro release data were fitted into various release equations and kinetic models.[23-25] The amount of drug dissolved, percent release, rate of drug release, and log fraction released at different time interval were evaluated. [26]

Optimization and Data validation

The QbD optimization data analysis was done after evaluation of developed microspheres for various CQAs like % yield, MS, DEE and n. Statistical modelling was performed by multiple linear regression analysis followed by polynomial analysis. This modelling uses second-order quadratic model to explore the soundness of significant interaction(s) among the CMAs. Concentrations of gelatin were selected as 1000, 900 and 800 mg; whereas for EC were 50, 100 and 150 mg. Thirteen formulation was developed by selecting nine possible combination among which centre point was repeated four times and mean value was taken for further study. The dependent responses were analyzed using Design Expert® 8.0.7.1 (trial version). The models were tested for significance and optimized batch was selected with desired values of dependent responses. The criterion adopted for getting optimized batches was 80-90 %, 300-320 mm and 75-85 % for percent yield, MS and DEE respectively.

Validation of QbD technique was done by formulating three formulations (VCB1 to VCB4) as the confirmatory check points. The observed and predicted responses were critically compared by constructing the linear correlation plots. Linear correlation plots were constructed for the chosen check-point formulations. The residual graphs between predicted and observed responses were also constructed separately and the percent prediction error (% bias) was calculated with respect to the observed responses. Optimized batch was validated taking total three formulations selected as check-points.

Result

Preliminary studies to select the amounts of polymer were done using different concentrations of polymers and CQAs (percent yield, MS, and DEE) were determined. Amount of polymer with required CQAs like percent yield, MS, and DEE were selected for further study.

FTIR profile of formulated microspheres was done to identify the drug polymer interaction. Hence formulated microspheres were subjected to IR analysis to evaluate possible interaction between drug and polymer. Infra red curve of pure drug and optimized batch of microspheres shows similar peak at 3158.57, 2935.78, 1681.04, 1576.87, 1517.08, 1458.25, 1407.13, 1356.02, 1320.33, 1176.56, 899.46, 521.77, 447.50 for NH Str, CH3 Str, NH_2 Bend, CO Str, OH Bend, CH_3 Asy Bend, CH_3 Sym Bend, CH_3 Sym Bend, OH Bend SYM Str, CCC Bond, NH_2 Bend, CO Bend and NH_2 Rock respectively (Figure-2) which confirms that there is no interaction between drug and polymer.

Surface topography

Scanning electron microscopy was used to investigate the surface topography of prepared microspheres and is shown in Figure. 1.

The percentage yield and Mean particle size of the formulation were depicted in table-3.

Drug Entrapment Efficiency

Drug Entrapment efficiency is an important variable used to assess the drug loading capacity of

Microspheres and their drug release profile. DEE depends upon various parameters such as process used for preparation, physicochemical properties of the drug and various formulation variables (Table-3).

In vitro release behaviour of drug:

In vitro drug release behaviour of formulated glutaraldehyde cross-linked gelatin microspheres is shown in Figure-3. All batches were studied for their drug release profile for up to 8 hit was observed that in all formulated system the rate of release was varies due to use if different concentrations of dependent variables. It is clear that as amount of EC increases from 50-150 mg rate of drug release decreased which indicate hydrophobic nature of EC in the formulation hence increasing amount of Ethyl cellulose lead to retardation in drug release. Cross linking property of glutaraldehyde leads to formation of a rigid hydro gel to restrict the leaching thereby decrease the drug dissolution (Table 4 & Figure 3).

Data analysis and optimization

Drug release mechanism was investigated by fitting to models representing zero-order, first order, Higuchi's square root of time model and Korsmeyer-Peppas model. Results of ANOVA for Response Surface Quadratic Model for various dependent parameters are:

$$\% \text{ yield} = +80.47+5.45A+2.73B+0.97AB+0.51A^2+0.32B^2-0.48A^2B-0.037AB^2 \quad \text{eqn. (7)}$$

$$MS = +317.64+11.15A+2.77B-0.62AB+0.29A^2+0.82B^2+1.10A^2B+0.31AB^2 \quad \text{eqn. (8)}$$

$$DEE= +76.38-0.075A+2.39B-0.67AB+1.79A^2+0.082B^2+0.70A^2B-0.48AB^2 \quad \text{eqn. (9)}$$

Where A indicates concentration of gelatin while B represents to concentration of EC.

Validation of the statistical model

Validation of optimized batch was done by formulating four different batches using overlay plot (figure 5) by utilizing the optimum value as founds by statistical tool i.e. by considering the optimum value as found (Table 5) and a comparative study was done between predicted value and observed values to determine the prediction error (Figure 6).

Discussion

FT-IR spectra (figure 2) of pure L-arginine and formulated microspheres of L-arginine shows the identical peaks as that of standard L-arginine which proves that excipients incorporated in formulated microspheres do not interact with L-arginine and all ingredients of beads are compatible with each other.

Scanning electron microscopy of microspheres of L- Arginine shows well-rounded spheres with rough surface because of sudden cross linking of gelatin with glutaradehyde. The particle size of the formulations was found to be between 320- 351.11µm. It was observed that the mean particle size of formulated microspheres were decreased with respect to the increased the amount of ethyl cellulose in the formulation.

Results for drug entrapment efficiency indicates that as the concentration of EC increases the DEE increase which is due practically insoluble nature of hydrophobic polymer i.e. EC. DEE was increased as the amount of EC was increased in the formulation because of practically insoluble nature of EC in water.

In vitro drug release study of microspheres of L- Arginine was carried out in 900 mL of phosphate buffer solution (pH = 7.4 for 8 h) at 37 ± 0.5°C. In the fasted state gel microcarriers exhibited a biphasic release profile as an initial rapid drug release phase due to

burst which are loosely into or just beneath the surface of microspheres followed by a slower, gradually decreasing drug release phase after 1 hour extending up to 8 hours (Table 4 and Figure 3).

Drug release mechanism was investigated for number of models i.e. zero-order, first order, Higuchi's square root of time model and Korsmeyer-Peppas model. zero-order, first order, Higuchi's square root of time and Korsmeyer-Peppas model gave R^2 value 0.9101 to 0.9473, 0.9832- 0.9889, 0.9901- 0.9992 and 0.963-0.991 respectively**,** showing Fickian diffusion involving a combination of swelling, diffusion and/or erosion of matrices. Various response surface plots were also drawn to analyse impact of independent variables on dependent variables as discussed earlier**.**

Optimizations of formulated microspheres were done by using 3^2 CCD. The outcomes for response parameters, that is, %Yield, MS and DEE were subjected to regression analysis and statistical models were found to be significant. Observed dependent responses i.e. %Yield, MS and DEE shows fair relation between the dependent and independent variables. Percentage yield for formulated batches were found in the range of 74.65– 86.36% while particle size was found in range of 302.34-.333.32 mm. Drug entrapment efficiency of all the formulation was found to be between 73.59 ± 1.744 to 82.61 ±0.700

Figure 4(a) shows that values of % Yield, increases with increase in concentration of gelatin and also increases with increasing EC concentration. Maximum % Yield is observed at the highest levels of Gelatin and EC.

Figure 4(b) shows a nearly linear ascending pattern for MS, as the content of gelatin increased, this MS increase slowly with increasing gelatin. Value of MS achieves to its maximum value at the highest levels of gelatin and EC. Nonlinear pattern of contour lines indicates significant impact on gelatin and EC.

Figure 4(c) shows that the DEE increases almost linearly with increae in concentration of gelatin whereas it decreases very slowly and then increases with increase in EC concentration. Maximum value of DEE was observed at the highest gelatin and EC concentration.

Validation of optimized batch was done by setting up a comparison of the observed responses with the predicted ones (Table 5), the prediction error varied between -0.024 to 0.048, -0.990 to 0.090 and -0.013 to 0.159 for %yield, MS and DEE respectively. The linear correlation plots drawn between the predicted and observed responses, forcing the line through the origin, demonstrated high values of R (0.996 to 0.999, Figure 4), indicating excellent

goodness of fit ($p < 0.005$). The corresponding residual plots show nearly uniform and random scatter around the mean values of response variables.

Conclusion:

Spherical amino acid loaded gelatin microspheres were prepared to achieve sustained release by cross-linking technique. Successful establishment of L-Arginine stacked repressible release matrix delivery system was done and escalation was achieved by using QbD applying 3^2 central composite design. Hence it can be concluded that QbD is a powerful tool for present research that helps in developing desired formulation without wastage of time as well as man, money and material.

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Table 1: Formulation Design and their coded value

Batch code	Coded value		Actual Value	
	Factor A Gelatin (mg) (X)	Factor B EC (mg)(Y)	Factor A Gelatin (mg) (X)	Factor B EC (mg)(Y)
B1	+1	-1	1000	50
B2	+1	0	1000	100
B3	+1	+1	1000	150
B4	0	-1	900	50
B5	0	0	900	100
B6	0	+1	900	150
B7	-1	-1	800	50
B8	-1	0	800	100
B9	-1	+1	800	150
B10	0	0	900	100
B11	0	0	900	100
B12	0	0	900	100
B13	0	0	900	100

Table 2: IR interpretation of optimized L-Arginine microspheres

IR frequencies (cm^{-1})	Assignments
3158.57	NH Str
2935.78	CH3 Str
1681.04	NH2 Bend
1576.87	CO Str
1517.08	OH Bend
1458.25	CH3 Asy Bend
1407.13	CH3 Sym Bend
1356.02	CH3 Sym Bend
1320.33	OH Bend
1176.56	Sym Str CCC Bond
899.46	NH2 Bend
521.77	CO Bend
447.50	NH2 Rock

Table 3: Evaluation of Formulated batches of Microspheres

Batch code	%Yield	Mean particle size (MS) (µm)	DEE (%)
B1	83.55	326.81	75.32±2.261
B2	86.36	329.38	78.0±2.458
B3	89.98	333.32	80.15±0.794
B4	77.98	315.98	73.59 ± 1.744
B5	80.07	318.59	75.43 ± 1.877
B6	83.44	321.53	78.76 ± 1.572
B7	74.65	302.34	75.09 ± 0.872
B8	75.45	307.08	78.15 ± 0.519
B9	77.21	311.65	82.61 ±0.700
B10	81.07	317.73	75.87± 0.519
B11	80.32	317.89	76.67± 0.519
B12	80.54	316.52	77.05± 0.519
B13	79.96	315.65	75.14± 0.519

Table 4: Drug Release Kinetics Study

Batch Code	DR_8	Korsmeyer R^2	Higuchi R^2	First Order R^2	Zero Order R^2	n
B1	85.754	0.9781	0.9953	0.9881	0.9122	0.4590
B2	83.963	0.9773	0.9935	0.9835	0.9325	0.4955
B3	79.508	0.9796	0.9917	0.9824	0.9474	0.4875
B4	86.693	0.9774	0.9955	0.9837	0.9263	0.4848
B5	84.323	0.9757	0.9920	0.983	0.9346	0.4826
B6	80.454	0.9778	0.9923	0.9869	0.9396	0.4905
B7	85.255	0.9633	0.9901	0.9830	0.9193	0.4861
B8	85.617	0.978	0.9892	0.9833	0.9268	0.4566
B9	81.194	0.972	0.9918	0.978	0.9351	0.4664

Table 5: Validation checkpoint formulations and their results

Validation check batch	Response	Predicted Value	Experimental Value	Percent Error
VCB1	*%yield*	79.91	79.97	-0.075
	MS	315.56%	315.50	0.019
	DEE	96.71%	96.67%	0.041
VCB2	*%yield*	82.15	82.11	0.048
	MS	319.94%	319.94	-0.990
	DEE	77.26%	77.19%	0.090
VCB3	*%yield*	81.45	81.47	-0.024
	MS	320.07%	320.27	-0.064
	DEE	76.14%	76.15%	-0.013
VCB4	*%yield*	80.69	80.67	0.024
	MS	319.96%	319.93	0.009
	DEE	75.29%	75.17%	0.159
Optimized batch	*%yield*	89.98	89.95	0.033
	MS	333.32%	333.30	0.006
	DEE	82.61%	82.98%	-0.447

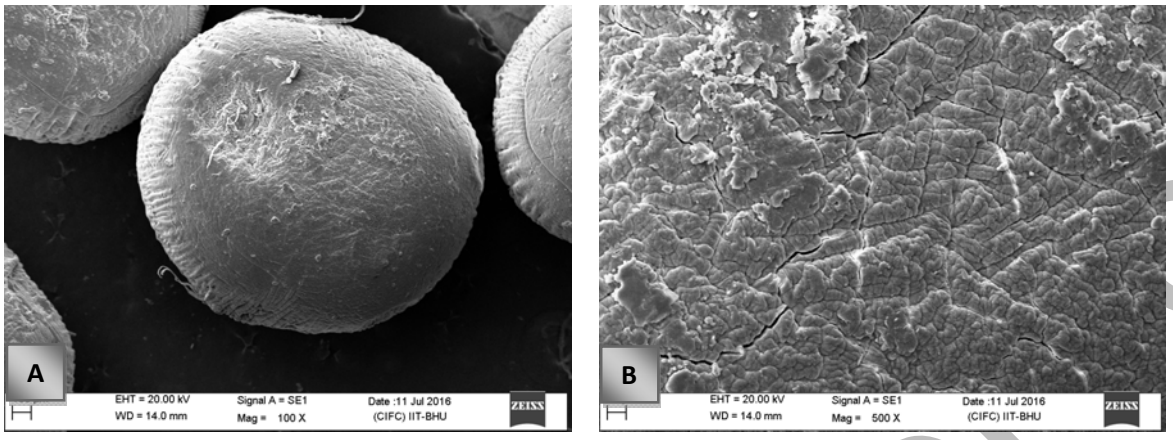

Figure 1: Scanning Electron Micrograph of microspheres (**A** = single bead, **B** = enlarged surface view)

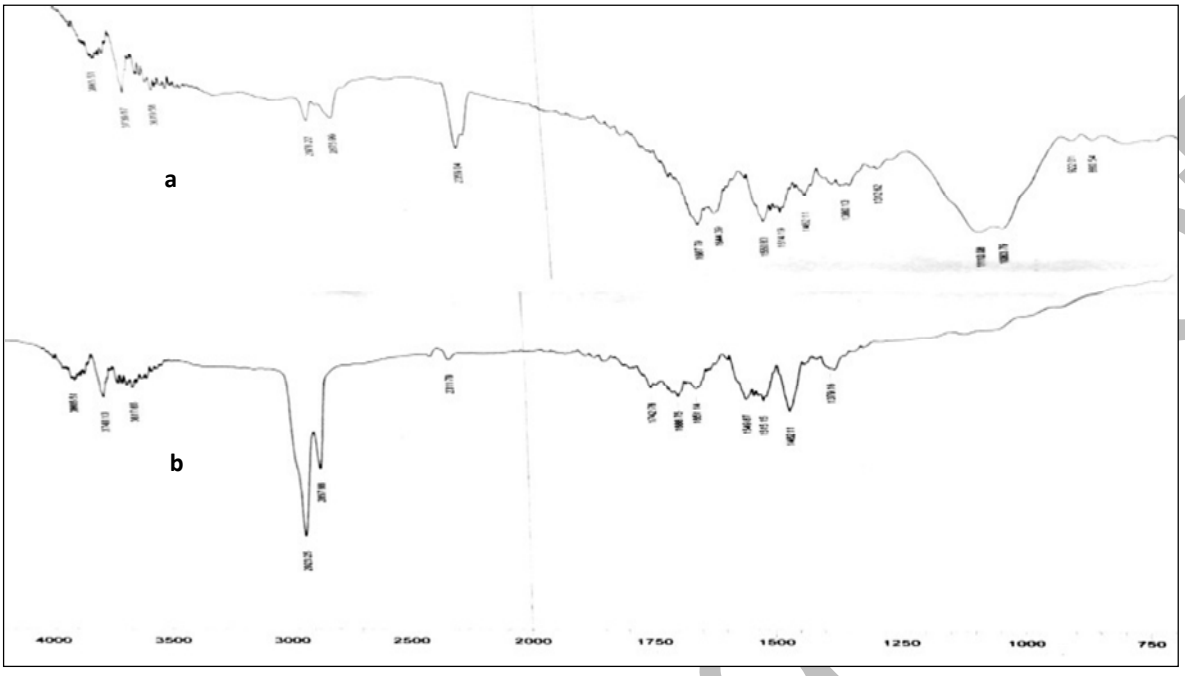

Figure 2: FTIR spectra of (a) L-arginine and (b)formulated Microspheres

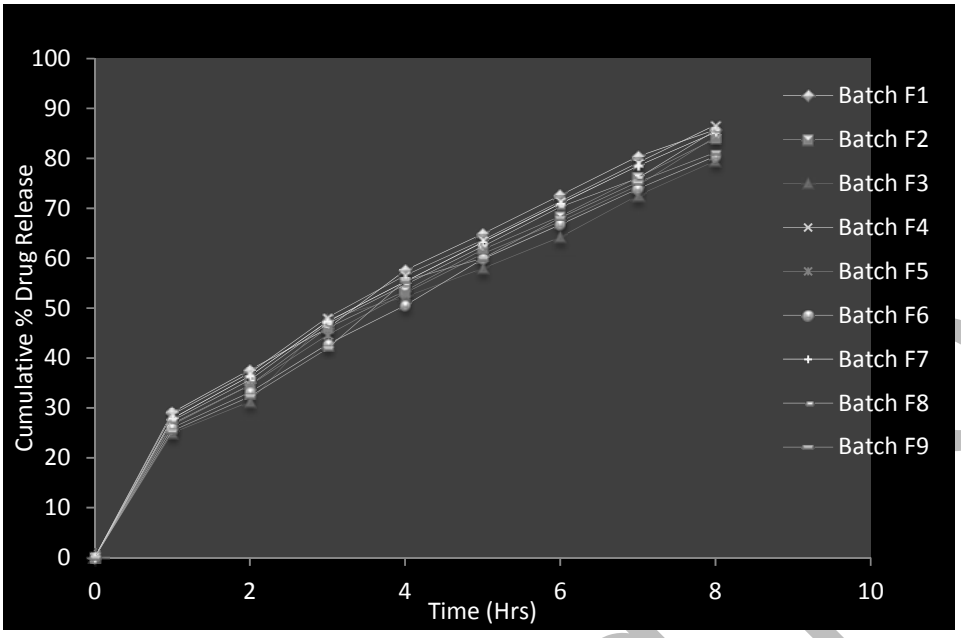

Figure 3: In vitro evaluation of L-arginine loaded gelatin microspheres

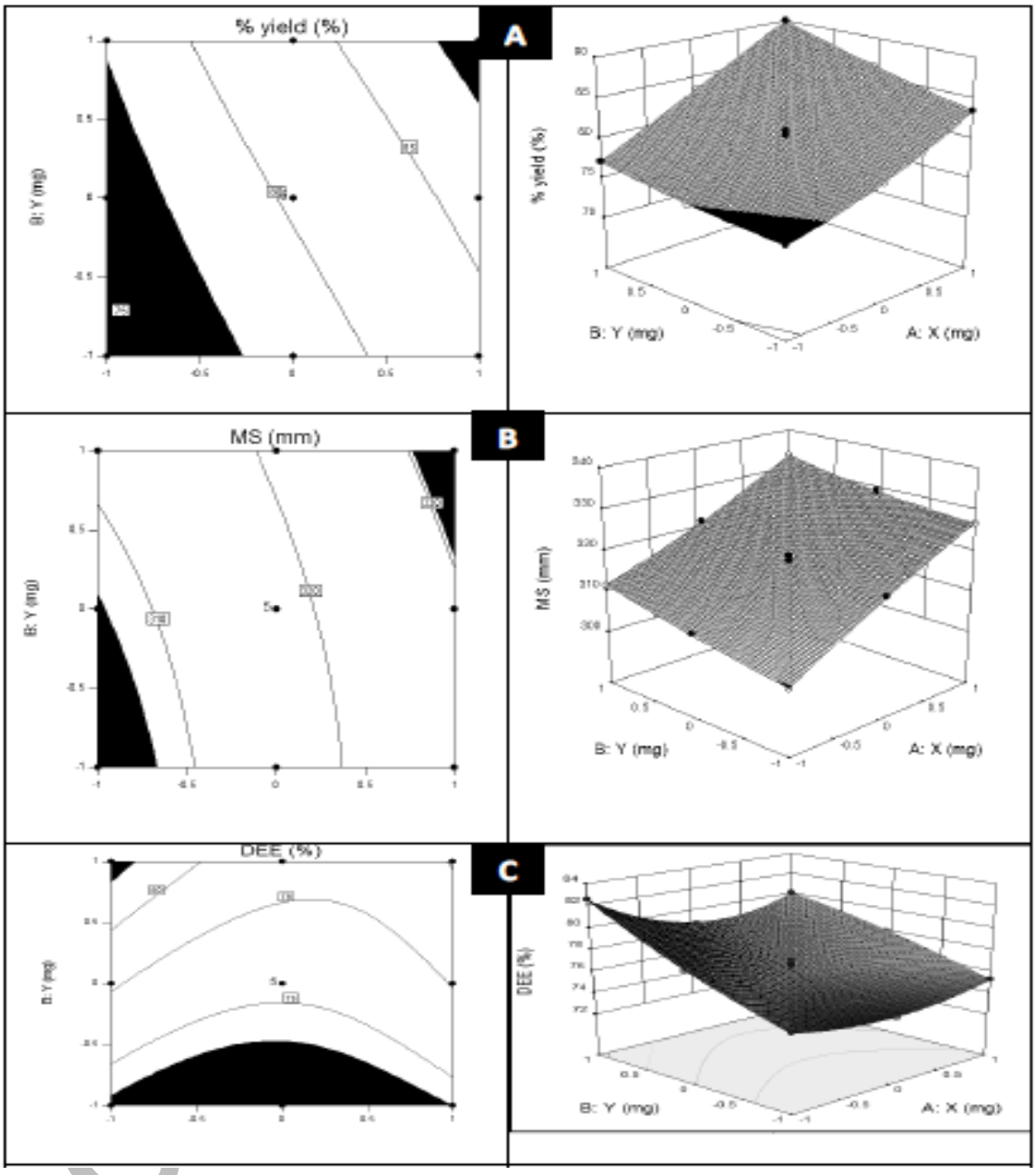

Figure 4: RSM and contour plots for a) Percentage yield b) Microsphere size c) Drug Entrapment Efficiency

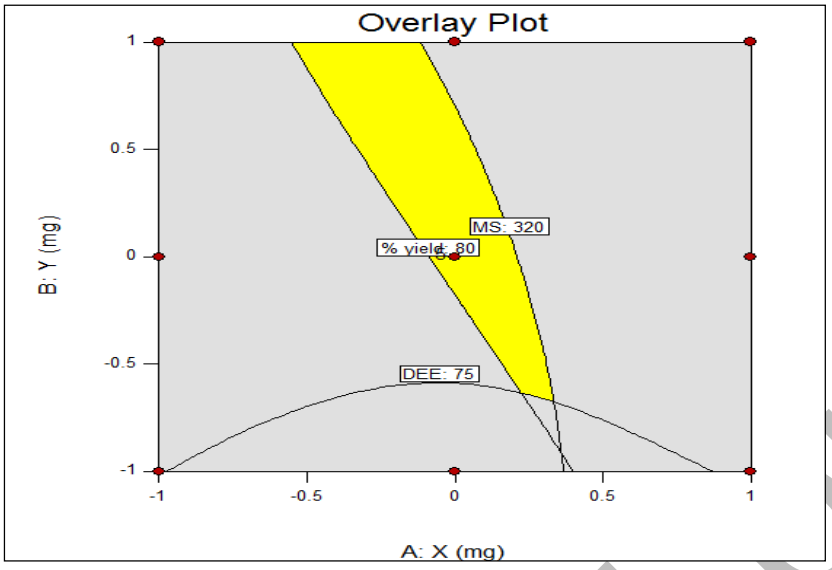

Figure 5: Overlay plot showing area for optimized product.