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Article

# QbD enabled Process Variable Study to Develop Sustained Release Chitosan-Alginate Embedded Delivery System for Improved Patient Compliance

**Vijay Sharma[1], Lalit Singh[2], Navneet Verma[1]**

*[1]Pharmacy Academy, IFTM University, Moradabad, India, [2]Future Group of Institutions, Bareilly, india*

The current investigation entail systematic Quality by Design (QbD)-enabled approach for the development of Sustained released embedded drug delivery systems of L-Arginine employing ionic gelation technique to attain improved patient compliance. Hence, in this QbD enabled systematic approach; quality target product profile (QTTP) was defined and critical quality attributes (CQAs) were identified. Further the risk assessment studies were undertaken through Ishikawa fish bone diagram to locate the critical material attributes (CMAs) and/or critical process parameters (CPPs) for the formulation of beads that may affect CQAs of drug product. A face centered central composite design (CCD) for two factors at three levels each with α =1 was employed for the optimization process to checkout the impact of concentration of sodium alginate and concentration of chitosan as CMAs which wereprior identified from risk assessment study and further evaluated for CQAs viz. bead size, swelling index and percent drug entrapment. The optimum formulation was embarked upon by using mathematical model being developed yielding desired CQAs. Thereby chitosan coated calcium-alginate delivery system was successfully developed by strategically employing QbD approach.In a nutshell, the presentinvestigation reports the successful development of optimized chitosan coated alginate beads employing QbD approach which can serve as a platform for other drugs too.

**Keywords:** Quality target product profile. Critical quality attributes. Critical material attributes.

## INTRODUCTION

A controlled drug delivery system is typically intended to deliver the drug at predetermined rate which helps in maintaining safe and effective drug concentration in blood., results in substantially constant plasma drug concentration when compared to the uncontrolled fluctuations which are being observed when multiple doses of immediate release conventional oral dosage forms are administered to the patient. Hence, in this drug delivery system, the release of the drug continues at a rate profile that is only predictable kinetically. (Majetin, Kumar,2000; Chien, 1992).

The objectiveof designing any controlled or sustained or delayed-delivery system is to improve patient compliance by reducing the frequency of dosing and thereby enhancing the therapeutic efficacy of the drug. This objective can be achieved by localizing the dosage form at the desired site of action orby reducing the dose of drug required, or bygiving the uniform drug conveyance or by prolonging the drug release; the present work mainly concentrates on prolonging of the drug releasefrom the dosage form to improve the patient compliance(Jantzen, Robinson, Lee, 1996).

L-Arginine (ARG) is a basic, semi-essential amino acid. Hedin in 1895 discovered its occurrence in mammalian protein. In 1886 it was first isolated from lupin seedlings (Schulze, Steiger, Das, 1886)**.** Synthesis of L-arginine, its subsequent conversion into L-ornithine and urea is catalyzed by the activity of arginase.This is an important pathway for the elimination of nonessential nitrogen-containing substances from the body. In 1932, Krebs and Henseleit reported their finding that ARG is an essential component in urea cycle metabolic pathway.

***Correspondence:** V. Sharma. Pharmacy Academy. IFTM University, Moradabad, India. Phone- +91 9045537375. Email- vijaysrampur@gmail.com. Orcid: https://orcid.org/0000-0003-1898-0476

ARG is metabolise through various enzymeslike: nitric oxide synthases, arginases, arginine glycine amidinotransferase, and L-arginine decarboxylase (Rodríguez, Ochoa,2008). Nitric oxide, along with L-citrulline, is generated from ARG by the enzyme nitric oxide synthase (Rodríguez, Ochoa,2008; Murrell *et al.,*1997) Arginases metabolize ARG to L-ornithine and urea. Following oral administration, ARG undergoes first pass metabolism (almost 38% in human) at the same time pre-systemic (i.e.by bacteria in GI flora) and systemic elimination (i.e.by arginases in gut and liver) (Schwedhelm *et al.,* 2008). Alginate and chitosan which are natural,biocompatible and biodegradable polymers,and are considered non toxic after oral administration. Alginate is a polysaccharide, composed of α-L-guluronic acid and β-D-mannuronic acid residues, arranged in homopolymeric blocks & hetro block of each kind. Alginates form strongcomplexes with polycations that are more resistant in the presence of calcium chelators, these can be utilized for both stabilize the gel and reduce its porosity (George, Abraham, 2006; Gombotz, Wee,1998; Tonnesen, Karlsen,2002). Among those polycations, Chitosan (CH) has received considerable attention for its safety and its mucoadhesive properties (Aral,Akbuğa,1998; Şenel, Hincal,2001).

## MATERIAL AND METHODS

### 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), calcium chloride and sodium hydroxide from Thermo Fisher Scientific India Pvt. Ltd. (Mumbai). Chitosan was purchased from Indian Sea Foods, Kerala. HPLC grade water, methanol and potassium dihydrogen orthophosphate from Qualigens Fine Chemicals Gujrat.

### Methods

#### *Defining the QTPP and identification of CQAs*

As per the Quality byDesign (QbD)-based approach for drug product development, Quality Target Product Profile (QTPP) was set-up for development of ARG beads with improved patient's compliance and toachieve desired therapeutic response. To attain QTPP (Table I), critical quality attributes (CQA) like bead size (BS), swelling index (SI), percent drug entrapment (PDE) and amount of drug release in 10 h ($Q_{10h}$) (Table II); were identified. BS was considered as indicative ofquality of dosage form and its stability; SI and PDE for encapsulation and drug retention efficiency of developed dosage form while $Q_{10h}$ for significant absorption of the drug throughout the GI tract.

**TABLE I -** QTTP for developing SR beads of L-Arginine

| QTTP components | Target | Justification(s) |
|---|---|---|
| Dosage form type | Beads | Selection of encapsulated SR matrix beads helps in improving the oral bioavailability of drug. |
| Drug delivery type | Encapsulated Sustained Release | To achieve desired therapeutic effect by continuous and sustained release of drug over an extended period of time. |
| Route of administration | Oral | Helps in improving patient compliance as well as most preferred route for delivery of proteins. |
| Packaging | Hard Gelatin capsule/ HPMC capsule | Beads can be easily filled in these capsules to get improved patient compliance as well as ease of manufacturing. |

*(continues on the next page...)*

**TABLE I -** QTTP for developing SR beads of L-Arginine

| QTTP components | Target | Justification(s) |
|---|---|---|
| Stability | 2 years minimum | To maintain the therapeutic/pharmacological potency of drug for 24 months during storage as per ICH guidelines. |
| Dosage strength | 800mg | Recommended dose for the management of disease(s). |

**TABLE II -** CQAs for the development of beads with their rational justifications

| CQAs | Target | CQA Yes/No | Justification |
|---|---|---|---|
| Beads Size | <2mm | Yes | Smaller beads size allows easy penetration throughout GI epithelial lining and paracellular pathways; hence was regarded as highly critical. |
| Swelling index | >200% | Yes | Although higher value of swelling index is desired because swelling of beads are followed by either dissolution or diffusion from the drug delivery system for attaining desired therapeutic response of drug. Thus SI is considered as a critical for the purpose. |
| Percent drug Entrapment | >70% | Yes | Higher values of entrapment efficiency are desirable for achieving maximum drug release characteristic from the beads to get the pharmacological action of the drug. Therefore, it was considered as a critical attribute. |
| Drug release in 10 hours ($Q_{10}$) | >80% | Yes | Sustained release characteristic is desired to attain prolonged drug release and absorption in GI tract, hence considered as critical for the purpose. |

### *Preparation of beads*

Sodium alginate (SA) solution was prepared (table III) by dissolving SA in a small amount of distilled water, in a mortar pestle. When a clear solution was formed, the volume was made up to 100 mL. ARG was added into each SA solution with 15 minutes stirring on magnetic stirrer to form a clear solution. Different amount of CH in calciumchloride (CC)solution was dissolved in 1% acetic acid solution. The pH of solution was adjusted to 5.0using 0.1 N NaOH solution to attain maximum crosslinking and was stirred for further 30 minutes. Drug containing SA solution was then dropped through a 21 gauge needle into 100 mL solution of CC containing chitosan and thus beads were formed. The gelation time of 30 minutes was allowed to complete the curing reaction in the CC solution and then beads were collected, filtered through whatman filter paper and washed thoroughly with water. The beads were then dried at 55-60$^0$C in a tray drier. The time of drying was optimised by weighing the beads repeatedly, until a constant weight was obtained (George, Abraham,2006).

**TABLE III -** Formulation composition of SR beads of ARG employing CCD

| Batch Code | Conc. of SA ($X_1$) | Level of factor | Conc. of CH ($X_2$) | Level of factor |
|---|---|---|---|---|
| F1 | 3.5 | +1 | 0.25 | -1 |
| F2 | 3.5 | +1 | 0.50 | 0 |
| F3 | 3.5 | +1 | 0.75 | +1 |
| F4 | 2.5 | 0 | 0.25 | -1 |
| F5 | 2.5 | 0 | 0.50 | 0 |
| F6 | 2.5 | 0 | 0.75 | +1 |
| F7 | 1.5 | -1 | 0.25 | -1 |
| F8 | 1.5 | -1 | 0.50 | 0 |
| F9 | 1.5 | -1 | 0.75 | +1 |
| F10 | 2.5 | 0 | 0.50 | 0 |
| F11 | 2.5 | 0 | 0.50 | 0 |
| F12 | 2.5 | 0 | 0.50 | 0 |
| F13 | 2.5 | 0 | 0.50 | 0 |

*Center point was taken as quintuplicate.

***Risk assessment studies***

The risk assessment studieswere carried out to identify the critical material attributes (CMAs) and/or critical process parameters (CPPs) for the formulation of beads that may affect CQAs of drug product. An Ishikawa fish-bone diagram (figure 1) was constructed using Minitab 18 software, trial version (M/s Minitab Inc., Philadelphia, USA). Risk Estimation Matrix (REM) was constructed for the qualitative evaluation of risk by assigning three different levels as: high, medium and low risk levelto establish a potential cause and effect relationship among CMAs and CPPs, and likely impact on the CQAs of beads (Table IV). Further, the Failure mode and effect analysis (FMEA)were performed for estimation of risk factors to identify failure modes associated with the dosage form. Rank order scores, ranging between 1 and 10 each, were assigned to the CMAs for their severity, occurrence and detectability to calculate risk priority number (RPN). Assignment of Rank was based on the prior knowledge and experience as well as extensive brain storming exercise(Fahmy, Kona, Dandu,2012, Table V).

RPN=Severity(S) x Occurrence (O) xDetectability (D) eq.(1)

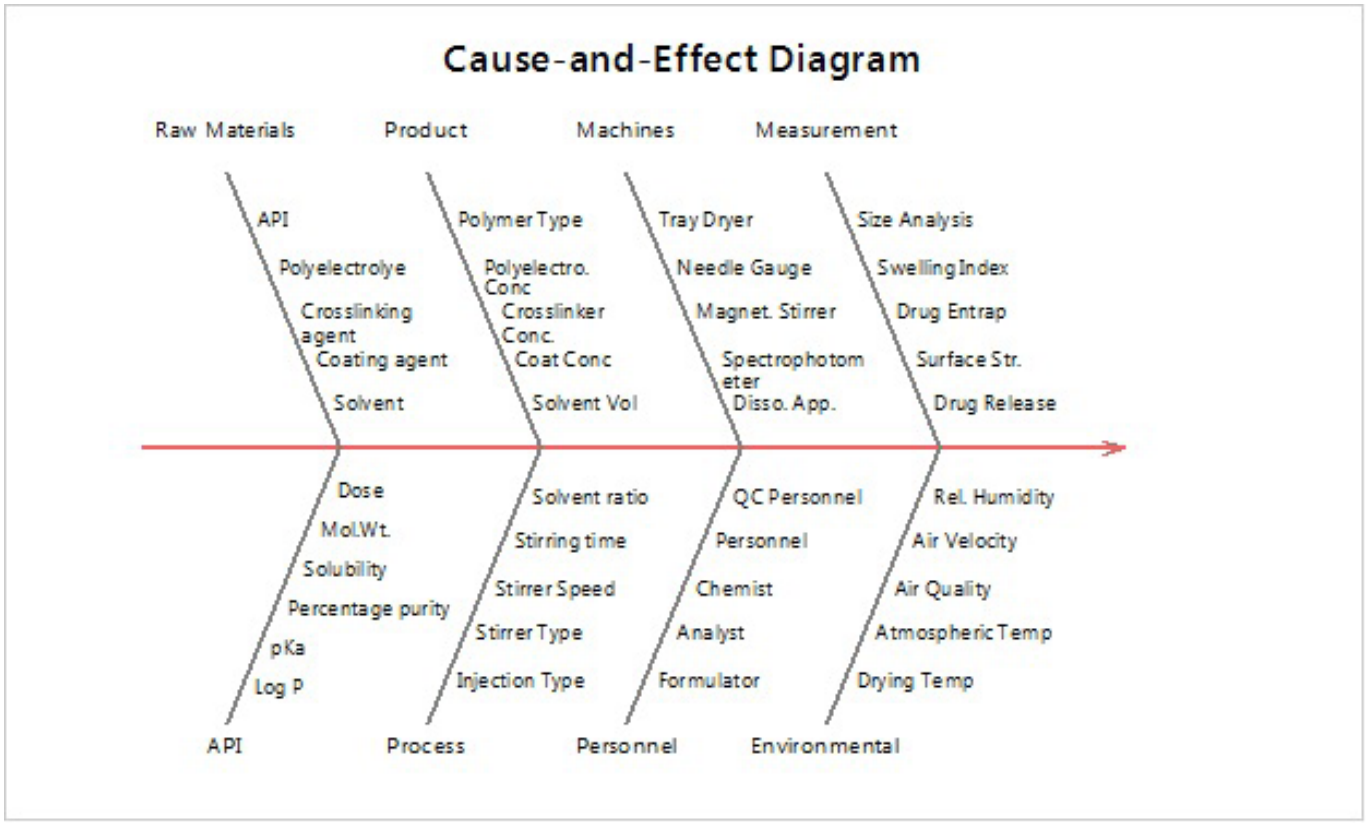


**FIGURE 1 -** Ishikawa fish bone diagram depicting Critical Quality Attributes for Alginate beads formulation.

**TABLE IV -** REM for initial risk assessment Beads of ARG

| CQAs | Amount of Poly electrolyte | Amount of cross linking agent | pH | Amount of Coating agent | Injection technique | Stirring speed | Curing time | Drying temp. |
|---|---|---|---|---|---|---|---|---|
| **BS** | High | High | Low | Med | Med | High | High | High |
| **SI** | High | High | Med | Med | Low | Low | Med | High |
| **PDE** | High | High | Med | Med | Low | High | Med | High |
| **$Q_{12h}$** | High | High | Med | Med | Low | Low | Med | Low |

*(continues on the next page...)*

**TABLE V -** FMEA analysis summary indicating RPN scores for process variables affecting the CQAs

| S. No. | CMAs/ CPPs | Name of factor | Severity | Occurrence | Detection | RPN | Likely risk on CQAs |
|---|---|---|---|---|---|---|---|
| 1 | CMAs | Amount of Polyelectrolyte | 8 | 8 | 7 | 448 | BS, SI, PDE, $Q_{12h}$ |
| 2 | | Amount of cross linking agent | 7 | 7 | 6 | 294 | BS, SI, $Q_{12h}$ |
| 3 | | pH | 7 | 6 | 5 | 210 | BS, PDE |
| 4 | | Amount of Coating agent | 8 | 7 | 7 | 392 | BS, SI, PDE, $Q_{12h}$ |
| 5 | CPPs | Injection technique | 7 | 4 | 5 | 140 | BS, PDE, $Q_{12h}$ |
| 6 | | Stirring speed | 7 | 6 | 5 | 210 | BS, PDE |
| 7 | | Curing time | 7 | 6 | 5 | 210 | BS, PDE |
| 8 | | Drying temperature | 6 | 4 | 3 | 72 | BS, $Q_{12h}$ |

| Risk Ranking | 0 to 150 | Green | 151 to 300 | Yellow | 301 to 512 | Red |
|---|---|---|---|---|---|---|

### *Optimization of beads using experimental design*

A face centered central composite design (CCD) for two factors at three levels each with α =1 was employed for optimization study to determine the impact of independent variables which were identified from risk assessment study. Design-Expert version -8 was employed for preparing optimization design. Independent variables *viz.* concentration of sodium alginate and chitosan were selected as the CMAs, and their responses *viz.* BS, SI and PDE were observed as CQAs. A total ofthirteen batches were formulated as per the trial runs suggested by the experiment where center point was taken as quintuplicate (Table VI).

**TABLE VI -** Systematic optimization of SR beads of ARG employing $3^2$ CCD

| S. No. | CMAs | | CQAs | | |
|---|---|---|---|---|---|
| | SA ( %) | CH (%) | BS (mm) | SI (%) | PDE (%) |
| **F1** | 3.5 | 0.25 | 1.634±0.0123 | 300±1.93 | 83.76±1.22 |
| **F2** | 3.5 | 0.50 | 1.643±0.0201 | 311±.035 | 96.14±0.21 |
| **F3** | 3.5 | 0.75 | 1.654±0.0097 | 323±1.66 | 99.00±1.76 |
| **F4** | 2.5 | 0.25 | 1.621±0.0161 | 287±0.77 | 80.43±0.93 |
| **F5** | 2.5 | 0.50 | 1.632±1.48 | 299±1.49 | 94.78±1.39 |
| **F6** | 2.5 | 0.75 | 1.644±2.12 | 313±1.35 | 97.02±0.22 |
| **F7** | 1.5 | 0.25 | 1.613±1.99 | 272±2.01 | 76.32±1.32 |
| **F8** | 1.5 | 0.50 | 1.625±2.08 | 284±1.21 | 92.31±2.01 |
| **F9** | 1.5 | 0.75 | 1.634±1.39 | 298±1.93 | 94.32±2.54 |
| **F10** | 2.5 | 0.50 | 1.629±0.28 | 289±1.85 | 94.99±0.83 |
| **F11** | 2.5 | 0.50 | 1.631±.1.17 | 289±2.77 | 95.79±2.38 |
| **F12** | 2.5 | 0.50 | 1.630±2.58 | 292±0.32 | 95.07±1.92 |
| **F13** | 2.5 | 0.50 | 1.629±2.49 | 286±1.07 | 96.09±0.19 |

## Characterization of formulations

*Beads size analysis*

In the test twenty five numbers of beads were randomly picked from each batch and their size was measured with digital vernier(Mitutoyo Japan). Methodwas repeated thrice for more precision.The results were expressed as the mean diameter (mm) ± standard deviation(Dorozynski *et al.,* 2004; Sangeetha*et al.,* 2010).

*Swelling properties*

The swelling behavior of beads was studied in simulated gastric fluid (50 mM NaCl, 3.3 mg *$ml^{-1}$ Pespin, pH 1.2 with HCl)for 0-2 h, simulated intestinal fluid (50 mM $KH_2PO_4$, 10 mg*$ml^{-1}$ Pancreatin) pH 6.8 for 2-5 h and simulated colonic fluid (6 g/L pectinase solution in a phosphate buffer pH 7.4 for 5-8 h respectively. A known weight of beads (equivalent to 100mg drug) was put into a beaker containing 100 mL of simulated gastric fluid, followed by simulated intestinal fluid and simulated colonic fluid respectively and allowed to swell. The swollen beads were removed after said time interval from the swelling medium. The weight of the swollen beads was estimated after blotting them with filter paper to remove excess moisture, immediately(Xu *et al.,* 2007, George, Nikolaos, 2006). Then the swelling ratio was calculated as per the following equation:

Swelling ratio (%) = weight of swelled beads/weight of dried beadsX100 eq. (2)

*Percent Drug Entrapment*

Amount of the drug inside the beads was determined by accurately weighing an amount of beads equivalent to 100mg L-Arginine.These beads were suspended in in standard phosphate buffer of pH 6.8±0.1 24 h. After 24h

beads were stirred for 5 minutes and then filtered.The same filtrate solution (2mL) was used for determination of concentration of drug usingHPLC with UV detector (Sato, Kawashima, Tekuchi, 2003; Sherina, Santhi, Sajeeth, 2012*)*.Percent drug entrapment (PDE) was calculated as per the following formula:

PDE = (Practical drug loading/Theoretical drug loading) ×100 eq.(3)

*Morphological study*

Scanning electron microscopic study(model- LEO 350, Japan) was used to determine the shape and surface characteristics of formulated beads. The beadswere vacuum dried and coated to 200 Å thicknesses with gold palladium prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-5000 magnifications (Patel, Sher, Pawar,2006).

*Assessment of in-vitro drug release*

The *in-vitro* drug release study of optimized alginate beads was carried out using a USP Type I dissolution apparatus (Electrolab, TDT-06T, Maharashtra, India). Accurately weighed quantity of alginate beads were introduced into a dissolution basket and the basket was placed in 900 mL of simulated gastric fluid (pH 1.2 for 0-2 h) followed by simulated intestinal fluid (pH 6.8 for 2-10 h)within dissolution beaker. These were stirred at 120 rpm at temperature 37±0.1°C. Samples (5 mL) were taken at regular time intervals from both media, and amount of L-Arginine was analyzed using HPLC (with C18 column at $25^0$C, filtered mixture of methanol:water through 0.45 micrometer pore size membrane in 1:1 v/v ratio at flow rate of 1mL/m, analysed at 210nm and retention time was 2.2-2.4 minutes). At each time of withdrawal, equal volume of fresh dissolution medium was replaced to maintain the sink condition(Prabhakara *et al.,* 2008; Sharma, Singh, Verma, 2017).The release studies were conducted in triplicate. Drug release data obtained from *in-vit*ro drug release studies were analyzed using ZOREL software. Further, Drug release data were applied to release models, like Higuchi model which indicates that the drug release mechanism deviates from Fick's laws and shows anomalous behavior. This is demonstrated by the equation 4.

$Q=KHt^{1/2}$ eq. (4)

Where, Q is the amount of drug release at time t, and KH is the Higuchi rate constant.

The dissolution data was also fitted to the well-known Korsmeyer equation (eq. 4a and 4b), which is often used to describe the drug release behavior from polymer systems.

$Mt/M\alpha = k.t^n$ eq. (5 a)

$Log (Mt/M\alpha) = log K + n Logt$ eq. (5 b)

Where 'Mt' is the amount of the drug release at time 't', 'Mα' is the amount of drug release after infinite time and 'K' is the release rate constant incorporating structural and geometric characteristic of the tablet and 'n' is the diffusion exponent which indicates the mechanism of drug release.

Data obtained from the analysis, the type of release, i.e., whether Fickian, non-Fickian (anomalous) or zero-order, was concluded. Further, Drug release data obtained from dissolution study were applied to release models, *viz.* zero order, first order, Higuchi model and Korsemeyer model (Lalit *et al.,*2014).

*Stability studies*

Optimized formulation was also subjected to accelerated stability studies to determine the changes in BS, SI, PDE and release profile on storage. The stability studies were carried out at 40±2°C/75±5% relative humidity (RH) for 6 months (zone II conditions as per ICH Q1 guidelines) in an environment chamber (Jindal S.M. Scientific 216, New Delhi). The samples were withdrawn periodically and evaluated for BS, SI, PDE and release profile (Abdelbary*et al.,*2010).

## RESULTS AND DISCUSSION

As per the QbD based approach, the QTPP was defined and summarized in table I. The CQAs for beads formulation were identified to evaluate the desired objectives of QTPP (Table II). These CQAs *viz.* were identified as BS, SI and PDE. Table IIsummarizes these

CQAs, their target range and their justification.

*Risk assessment studies*

A cause and effect diagram, often called a "fishbone" diagram for development of sustained release beads of ARG is shown in figure1. Possible contributing causes were listed on the smaller "bones" under various cause categories. This fishbone diagram helped in identifying possible CMAs and CPPs for the development of sustained release beads of ARG.REM was constructed to carry out the qualitative evaluation of risk which were associated with CMAs as mentioned in table IV. According to REM, the amount of polyelectrolyte, crosslinking agent, stirring speed, curing time and drying temperature were found to be at high risk while pH, injection technique and curing time were found to be at low risk. FMEA was performed to identify risk factors along with their impact on CQAs of beads. CMAs and CPPs identified enlisted in table IV and their RPN scores were calculated to describe their effect and plausible consequences on dosage form. Parameters with RPN value below 150 were selected as low risk parameters like drying temperature and injection technique while above 150 were selected as high risk parameters like amount of polyelectrolyte, cross-linking agent& coating polymer, pH of CC solution, stirring speed and curing time.

*Formulation optimization using CCD*

The systematic optimization of SR beads of ARG was done using $3^2$ CCD (TableVI&VII). Total thirteen batches were prepared for the formulation of beads. The experimental data so obtained as per the selected CCD was fitted to second-order quadratic polynomial model by MLRA method. Various mathematical equations were generated using MLRA for the studied response variables. Coefficients obtained for each CQA, revealed thepresence of interaction between CMAs and CQAs. Higher values of "R" from 0.9497 (p-value< 0.001) and0.9985 (p-value< 0.0001), vouch high prognostic ability of the RSM polynomials.

**TABLE VII -** Various coefficients for CQAs of SR ARG beads

| Coefficient variable | Coefficient values for CQAs | | |
|---|---|---|---|
| | **BS** | **SI** | **PDE** |
| **Intercept** | 1303.51 | 2315.57 | 95.34 |
| **b1** | 162.00 | 364.50 | 3.34 |
| **b2** | 264.50 | 338.00 | -8.28 |
| **b3** | 0.25 | 2.25 | 0.61 |
| **b4** | 20.63 | 113.16 | 0.31 |
| **b5** | 7.56 | 185.66 | -6.63 |
| **b6** | 2.08 | 0.75 | 0.055 |
| **b7** | 2.08 | 0.083 | -0.23 |
| **b8** | 4.48 | 78.22 | -0.76 |
| **R** | 0.9948 | 0.9909 | 0.9980 |
| **P-value** | p<0.0001 | p<0.0001 | p<0.0001 |

Model polynomial equation was generated and used to express the function of each CMAs.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1X_2 + b_4 X_1^2 + b_5X_2^2 + b_6X_1^2X_2 + b_7X_1X_2^2 + b_8 X_1^2X_2^2 \quad \text{eq.(5)}$$

Where Y is the dependent variable i.e. CQA, $b_0$; $b_1$to $b_2$; $b_3$; $b_4$ to $b_5$; $b_6$ to $b_7$; $b_8$ are the intercept; coefficients of linear model terms; linear interaction term; quadratic terms and quadratic interaction terms respectively. The main independent variables, that is, CMAs $X_1$ and $X_2$ represent the average result of changing one factor at a time from its lower values to its higher values.

Mathematical equations were generated using MLRA for the studied variables are as follows:

$$BS = 1630.2 + 9.0X_1 + 11.5X_2 - 0.25\ X_1X_2 + 3.8X_1^2 + 2.3X_2^2 - 1.25X_1^2\ X_2 + 1.25X_1X_2^2 - 2.55\ X_1^2\ X_2^2 \quad \text{eq. (6)}$$

$$SI = 291.0 + 13.50X_1 + 13.0X_2 - 0.75\ X_1X_2 + 6.5X_1^2 + 9.0X_2^2 - 0.75X_1^2X_2 - 0.25X_1X_2^2 - 8.25X_1^2X_2^2 \quad \text{eq. (7)}$$

$$PDE = 95.34 + 1.91X_1 + 8.29X_2 - 0.69X_1X_2 - 1.12\ X_1^2 - 6.62X_2^2 + 0.015X_1^2X_2 + 1.12X_1X2^2 + 0.74X_1^2\ X_2^2 \quad \text{eq. (8)}$$

All the polynomial equations were found to be statistically significant ($P$ <0.01), as determined using ANOVA (Table VIII), as per the provision of Design Expert software.

**TABLE VIII** - Analysis of variance (ANOVA) for observed dependent responses

| | Bead Size | | Swelling Index | | Percent Drug Entrapment | |
|---|---|---|---|---|---|---|
| Source | F | P value | F | P value | F | P value |
| Model | 82.23 | < 0.0001 | 11.16 | 0.0169 | 235.63 | < 0.0001 |
| $X_1$ | 71.78 | 0.0004 | 14.88 | 0.0182 | 23.03 | 0.0087 |
| $X_2$ | 117.20 | 0.0001 | 13.80 | 0.0206 | 432.10 | < 0.0001 |
| $X_1X_2$ | 0.11 | 0.7528 | 0.092 | 0.7770 | 5.98 | 0.0708 |
| $X_1^2$ | 8.08 | 0.0362 | 2.46 | 0.1916 | 5.62 | 0.0768 |
| $X_2^2$ | 1.40 | 0.2902 | 4.72 | 0.0955 | 196.52 | 0.0002 |
| $X_1^2\ X_2$ | 0.92 | 0.3808 | 0.031 | 0.8696 | 9.420 | 0.977 |
| $X_1\ X_2^2$ | 0.92 | 0.3808 | 3.040 | 0.9563 | 5.20 | 0.0847 |
| $X_1^2\ X_2^2$ | 2.64 | 0.1797 | 1.92 | 0.2385 | 1.20 | 0.3351 |

*Response surface methodology*

The 3D-response surface plots and 2D-contour plots for each CQAs were constructed employing Design expert software (Dx-8 trial version). The response surface analysis helped in revealing the significant impact of both the CMAs, i.e., amount of SA and CS and on all the CQAs, *viz.* beads size, swelling index, percent entrapment efficiency and $Q_{10h}$.

Figure 2(A) indicates that as the concentration of SA and CS increases, the corresponding BS also increases. The beads sizes were significantly increased with increase in the concentration of SA that can be attributed to the viscosity of the prepared gel. According to the studies in this regard, an increase in the viscosity of the starter gel leads to the formation of bigger beads. Increase in BS as the concentration of CS increases may be due

to formation of polyelectrolyte complex between carboxyl groups of SA and the amino groups of CS (Takahashi *et al.,* 1990), Douglas,Tabrizian,2005*).*

Figure 2(B) indicates almost linear increment in SI at pH 6.8 as the concentration of both SA and CS increases. Initially swelling of CS coated alginate beads at pH 6.8 may be due to exchange of calcium ions present in the poly mannuronate units of alginate with Na+ ions present in the buffer solution, which causes chain relaxation and enhancement of swelling. Further swelling of beads is due to binding of calcium ions with the carboxyl group of the poly-glucuronate units to form a tight egg-box structure which also starts to exchange with $Na^+$ ions in the buffer medium.

In acidic environment, the carboxylate groups of the alginate localized on the surface of particles get protonated with formation of alginic acid layer. Formation of hydrogen bond and alginic acid results into limited swelling ability in simulated gastric fluid. Although, CS is highly soluble in acidic environment but interaction between amino groups and protonated carboxylic groups do not promote swelling. Hence, swelling behavior in CS coated alginate beads in intestinal fluid is dominated by swelling behavior in gastric fluid.

In Figure 2(C) as the concentration of SA increases; PDE also increases a bit but when concentration of CS increases it shows negative effect on PDE i.e. declination in PDE. The variation in concentration of polymers had a remarkable effect on the PDE in CS coated alginate beads. Higher PDE was obtained as the concentration of alginate increased that 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 SA increased. Increase in PDE with increase in CS concentration may also be attributed to greater availability of active calcium binding sites in the polymeric chains. Nearly linear contour lines corroborate the markedly significant influence of both the polymers (Marsh, Weiss, 1967; Yotsuyanagi*et al.,* 1987).

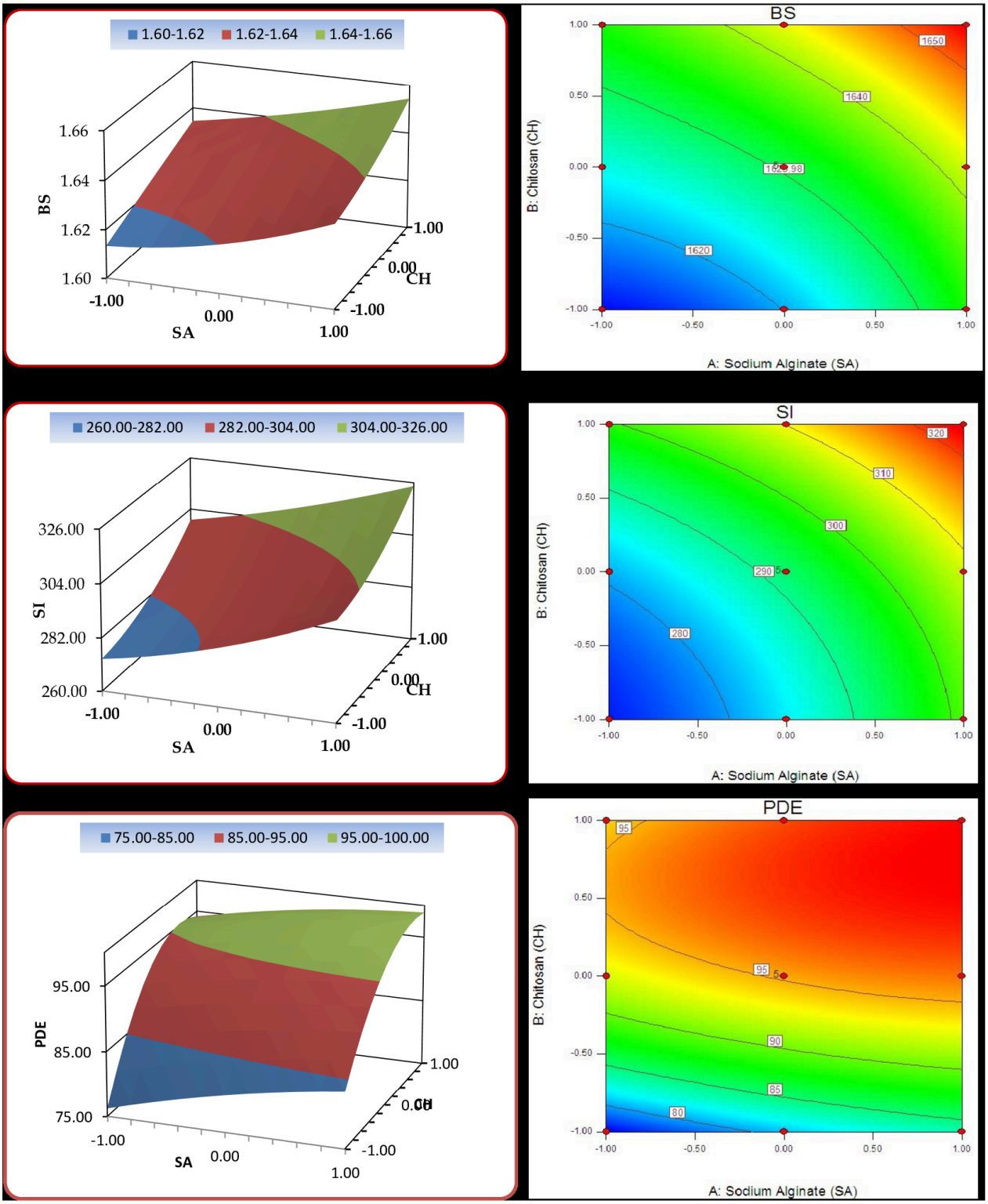


**FIGURE 2 -** RSM plot and contour plot for CQAs (A) BS, (B) SI, (C) PDE.

## Characterization

*Beads size*

The mean particle size of all beads was found in range of 1.613 to 1.654mm (table VI). It was observed that formulation F3 had largest while formulation F7 had lowest bead particle size. It may be concluded that polymeric drug solution which was added through a syringe with a 22 gauge needle resulted in the formation of particle with larger sizes of more than one millimetre. Particle size increases with increasing polymer concentration which may be due to increased viscosity of the dispersion.Bead size was found within the desired limit which is essential for improved patient compliance.

*Swelling index*

Swelling properties were estimated in phosphate buffer solution pH 6.8. Swelling of all formulated batches was found significant and was within the desired limit to attain prolonged release of drug which is desired for improving patient compliance. Batch F3 shows the maximum swelling within 3 hour and it was least for batch F6 (table VI). Swelling of CS coated alginate beads at pH 6.8 may be due to exchange of calcium ions present in the poly mannuronate units of alginate with Na+ ions present in the buffer solution, which causes chain relaxation and enhances swelling.

*Drug entrapment*

The drug entrapment efficiency was evaluated and results are shown in table VI. Batch B1 was having the highest drug entrapment whereas batch B9 had the lowest entrapment. Batch F3 is having highest polymeric content and batch F1 is having lowest entrapment. Hence it is clear that on increasing the polymer ratio the entrapment of the drug i.e. L-arginine increases. It may be due to an increment in CS concentration because, negative effect on the encapsulation efficiency, as high concentration of CS may lead to the formation of aggregates upon addition of SA.Higher PDE is desired for minimizing the dose of formulations required to be administration, therefore helps in improving patient-compliant formulations.

*Surface Topography*

Controlled release beads of L-arginine were found sphericalin shape with rough surface. The drug loaded beadswere brownish-yellow in color and did not change on increasing the concentration of SA or CS. Beadswere found to be discrete, spherical, free flowing and of monolithic matrix type (figure 3).

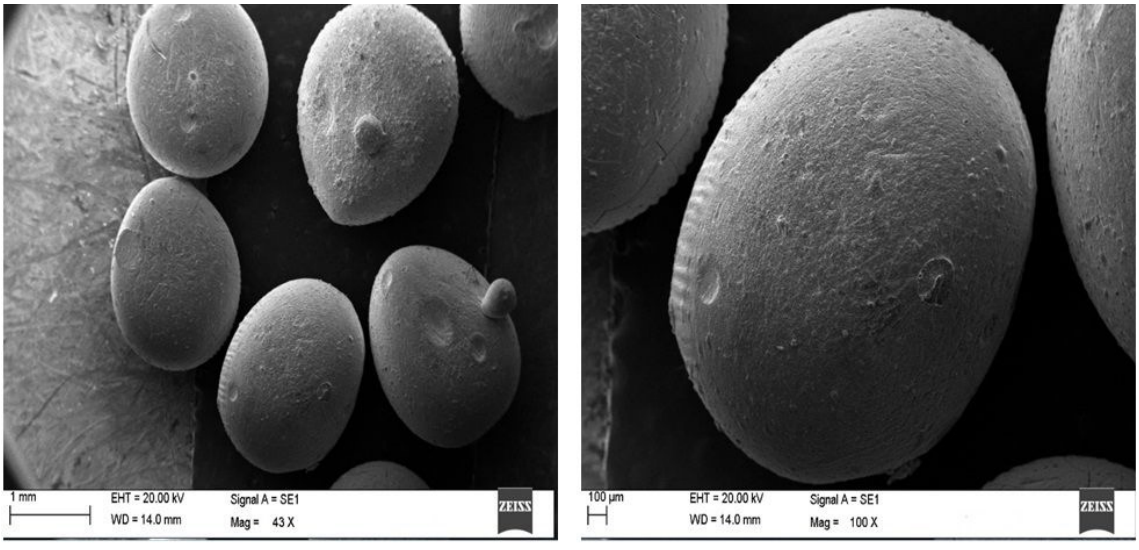


**FIGURE 3 -** Scanning Electron microscopy of optimized formulation.

*Optimization*

Optimized formulation was searched by "trading-off" the CQAs over the experimental domain using brute-force method, numerical optimization and graphical optimization techniques. According to brute-force method, feasibility search was done, which was followed by exhaustive grid search. Feasibility search was done by fixing the criterion for each CQAs i.e. beads size in range of 1.613-1.633 mm, swelling index in range of 280-323 % and entrapment efficiency > 95%. Then it was expanded for extensive grid search analysis. Grid search analysis suggested corresponding coded levels for concentration of SA (-0.45) and concentration of CS (0.55) (i.e., containing 2.05% of SA, and 0.3625% of CS) was selected as the optimized SR beads of ARG.

*Validation of Optimized Formulation*

The optimized formulations and various validation check batches were evaluated for their CQAs as listed in Table VII. A comparison study was done between the observed responses with those of the anticipated ones as observed by statistical analysis (Table IX &figure 4). Linear correlation plots were drawn between these predicted and experimental responses after forcing the line through the origin. High values of $R$ (0.964 to 0.999) indicated excellent goodness of fit ($p < 0.001$). Overlay plot was drawn from design expert software (Dx 8 trial version) also confirm the results of optimized formulation which was selected using brute force method (figure 5).

**TABLE IX -** Evaluation of validation check points

| Check point batch code | Amount of SA (mg) | Amount of CH (mg) | Response variables | Prediction values | Experimental values | Percentage error |
|---|---|---|---|---|---|---|
| Optimized Batch | 2.05 | 0.363 | BS (mm) | 1.634 | 1.631 | 0.183599 |
| | | | SI (%) | 294.36 | 294.3 | 0.020383 |
| | | | PDE (%) | 96.84 | 96.92 | -0.08261 |
| VC1 | 1.99 | 0.368 | BS (mm) | 1.632 | 1.63 | 0.122549 |
| | | | SI (%) | 293.48 | 293.42 | 0.020444 |
| | | | PDE (%) | 96.64 | 96.55 | 0.093129 |
| VC2 | 2.7 | 0.46 | BS (mm) | 1.633 | 1.631 | 0.122474 |
| | | | SI (%) | 291.58 | 291.52 | 0.020578 |
| | | | PDE (%) | 96.66 | 96.71 | -0.05173 |
| **VC3** | 2.83 | 0.418 | BS (mm) | 1.638 | 1.636 | 0.1221 |
| | | | SI (%) | 299.13 | 299.21 | -0.02674 |
| | | | PDE (%) | 97.84 | 97.79 | 0.051104 |
| **VC4** | 2.26 | 0.408 | BS (mm) | 1.633 | 1.631 | 0.122474 |
| | | | SI (%) | 292.21 | 292.22 | -0.00342 |
| | | | PDE (%) | 96.99 | 97.09 | -0.1031 |

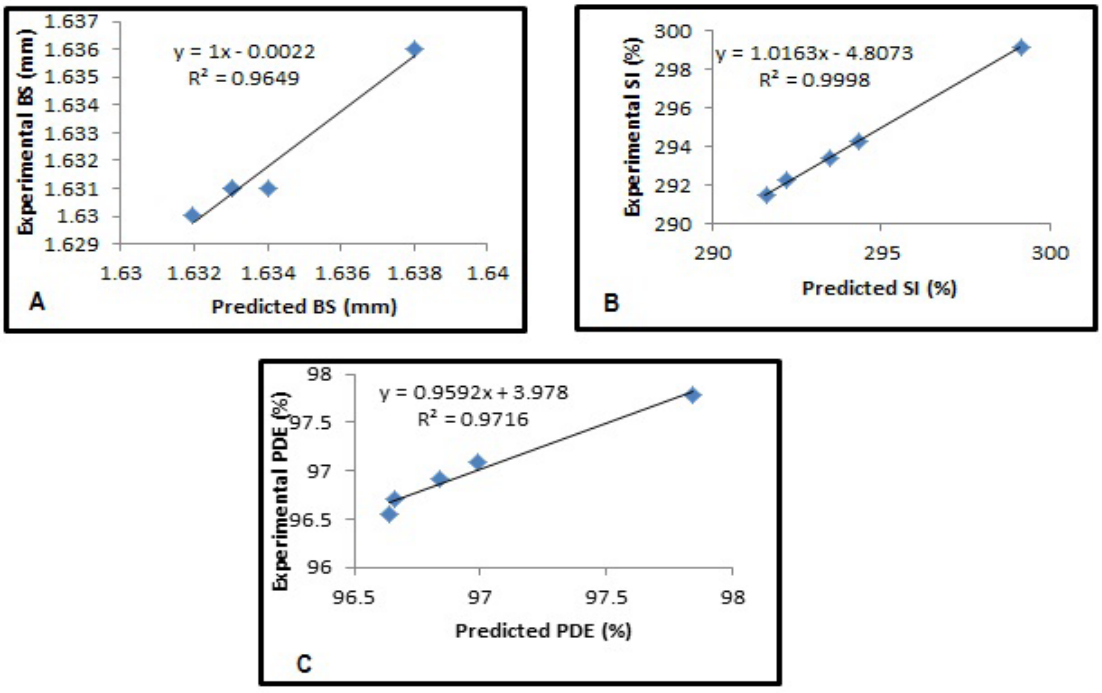


**FIGURE 4 -** Regression Coefficient between Anticipated and Experimental Response [A=BS mm, B=SI, C=PDE].

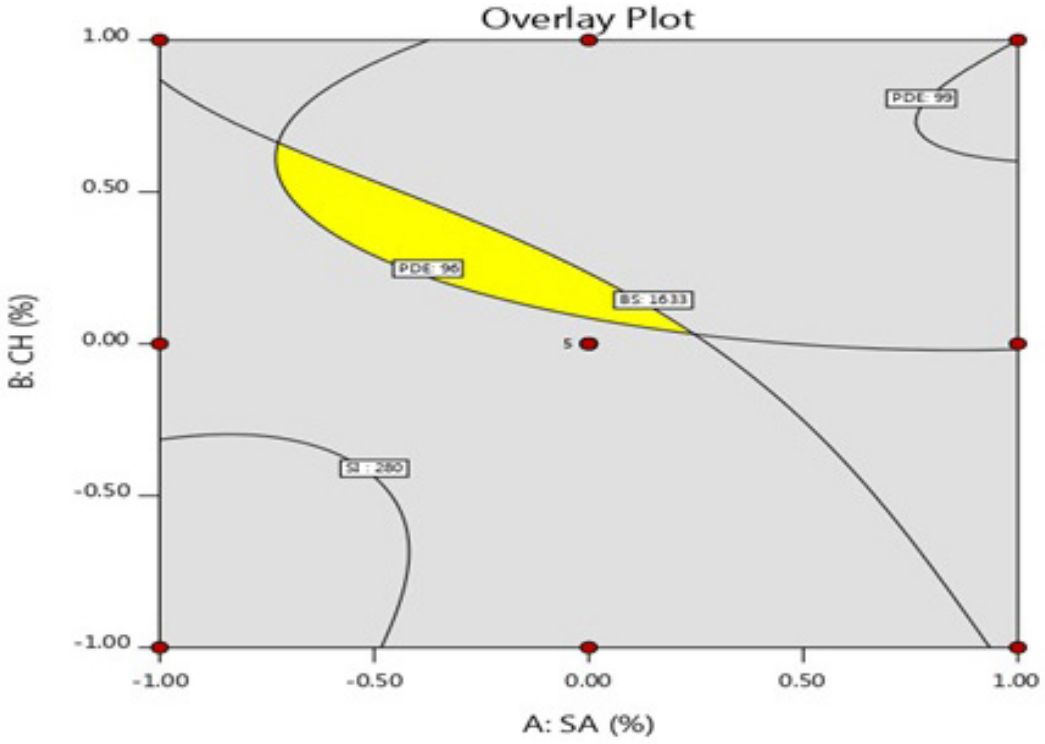


**FIGURE 5 -** Overlay plot showing the area for optimized formulation.

*In-vitro drug release of optimized formulation*

*In-vitro* drug release study of formulated beads was carried out in gastric media for 2 h and then in phosphate buffer pH 6.8 for 2-6 h.The *in-vitro* drug release behavior of formulated beads in SGF was found to be 13.75±1.02% while nearly 96.27±0.93 % drug release was observed in next 8 h using phosphate buffer pH 6.8 as dissolution media (figure 6). Drug release from the optimized formulation indicated delayed release of ARG, it may be because of poor swelling index of alginate beads. In acidic medium, calcium alginate matrices were depleted of calcium ions and converted to insoluble alginic acid within few minutes without changing morphology of beads. The formation of insoluble alginic acid delayed the penetration of media into the deeper layers of beads thus decreasesthe swelling rate, which ultimately affect the final release behaviour.*In vitro* drug release in phosphate buffer (pH 6.8) reveals that the beads may swell to a larger extent in medium because of the repulsion of fully negative charged –COO- groups of sodium alginate.Hence, *in-vitro*drug release study of optimized beads was observed within defined constrain limitsfor accomplishing improved patient-compliance.

The correlation coefficient ($R^2$) of the release profile was determined for the different mathematical models. The higher value of correlation coefficients (0.9912) was observed for the Higuchi model. The value of n was derived from the Korsmeyer–Peppas equation, it was found to be 0.41, and therefore the mechanism of ARG release may be Fickian diffusion through the chitosan coated alginate beads.

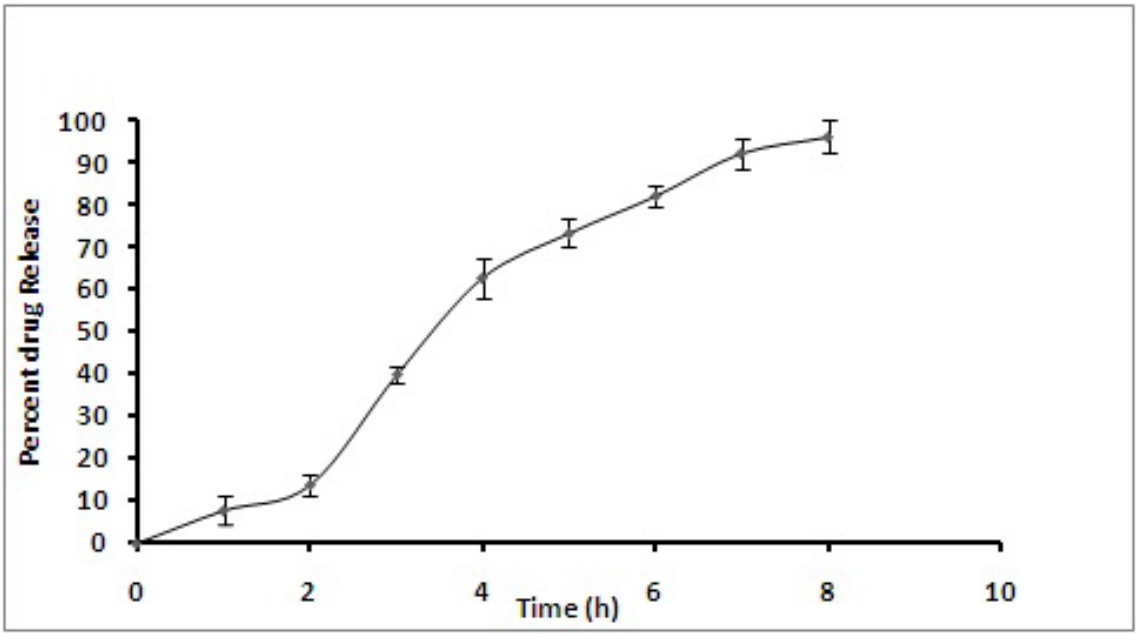


**FIGURE 6 -** *In-vitro* drug release of optimized formulation in simulated gastric fluid (pH 1.2 for 0-2 h), and simulated intestinal fluid (pH 6.8 for 2-10 h) respectively.

*Stability study of optimized formulation*

All the parameters viz. BS, SI, PDE and drug release were well within the desirable limits, showing negligible and random variation over six months of storage under accelerated conditions (table X). Drug release profile (8h) was determined during various time points of stability studies at temperature 40 ± 2 °C and 75 ± 5% RH which remained almost unaffected during the studies, suggesting the robustness of the optimized formulation with respect to dissolution characteristics.

**TABLE X -** Accelerated stability study of optimized formulation

| Conditions | Duration | BS (mm) | SI (%) | PDE (%) | $Q_{8h}$ (%) |
|---|---|---|---|---|---|
| **Zone II Accelerated (40 °C ± 2 °C/ 75% RH ± 5% RH)** | **1 month** | 1.634 | 296.33 | 96.84 | 96.69 |
| | **3 month** | 1.633 | 296.33 | 96.83 | 96.60 |
| | **6 month** | 1.633 | 296.32 | 96.83 | 96.60 |

## CONCLUSION

The present investigation undertaken successfully vouch the application of QbD-oriented approach for the development of optimized chitosan coated alginate beads of ARG employing central composite design for improved patient compliance along withsurpassing oral bioavailability. QTT was attained by identifying CQAs like bead size (BS), swelling index (SI), percent drug entrapment (PDE) and amount of drug release in 10 h ($Q_{10h}$) whereasthe risk assessment studies were carried out to identify the critical material attributes(CMAs) and/or critical process parameters (CPPs) for the formulation of beads that may affect CQAs of drug product. The optimized formulation fulfills the desired requisite of CQAs. Hence the present research was found useful in successful development of the optimized chitosan coated sustained release calcium-alginate delivery system by employing QbD approach for the improved patient compliance.

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