Optimization of chlorpheniramine maleate (CPM) delivery by response surface methodology – four component modeling using various response times and concentrations of chitosan-alanine, glutaraldehyde and CPM

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Abstract. The aim of the current study was to investigate the effects of formulation variables on the release of drug and to optimize the formulation of chitosan-alanine beads loaded with chlorpheniramine maleate (CPM) for controlled release using response surface methodology (RSM). Drug loaded beads of chitosan-alanine were prepared and crosslinked by using glutaraldehyde as crosslinker. The release behavior of drug was affected by preparation variables. A central composite design was used to evaluate and optimize the effect of preparation variables; chitosan concentration (X_1), percentage of crosslinker (X_2), concentration of drug (X_3) and release time (X_4) on the cumulative amount of drug release, Y in solutions of pH = 2.0 and pH = 7.4, respectively. The influence of each parameter was studied by factorial design analysis. Analysis of variance (ANOVA) was also used to evaluate the validity of the model. The statistical parameters reveal strong evidence that the constructed models for drug release in pH = 2 and pH = 7.4 are reliable.

Keywords: biodegradable polymers, chitosan, controlled drug release, crosslinking, response surface methodology (RSM)

1. Introduction

Chitosan is a deacetylated derivative of chitin which is a water insoluble polymer, (N-acetyl-d-glucosamine) found in nature, present in insect exoskeletons, outer shells of crabs, shrimps, lobsters etc. and fungal cell walls. It has been extensively studied as a carrier for drugs owing to its biocompatibility and biodegradability [1–5]. Chitosan based interpenetrating polymer networks (IPNs) currently received enormous interest for medical and pharmaceutical applications due to their good biocompatibility, low degradation and processing ease. The ability of these IPNs to swell and dehydrate depend on composition and environ-

ment which has been exploited to facilitate a range of applications such as drug release, its biodegradability and ability to form hydrogels [6].

Drug release from chitosan based IPNs can be controlled by the matrix density, which is affected by various factors, such as concentration of chitosan, percentage of crosslinking agent, amount of drug and response time [7–8]. However, quantitative aspects of the effects and relationships among these various factors have not been studied extensively. The authors [9–10] have prepared semi-IPNs consisting of chitosan-alanine and chitosan-glutamic acid and studied their cure kinetics [11]. The biocompatibility is the reason behind the use of L-ala-

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nine along with chitosan and it (alanine) acts as spacer in the formation of semi-IPN. In order to form semi-interpenetrating polymer network, two chitosan polymer chains are crosslinked by glutaraldehyde. Amino groups of chitosan and alanine can react with glutaraldehyde resulting in the attachment of alanine in pendent form. Hence, the resultant polymer system is characterized as semi-IPN (one phase consists of crosslinked chitosan and the other phase is made of alanine attached chitosan).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques, useful for developing, improving and optimizing processes [12–14]. It also has an important application in the design, development and formulation of new products as well as in the improvement of existing product designs. The basic components of response surface methodology include experimental design, regression analysis and optimization algorithms which are used to investigate the empirical relationship between one or more measured responses and a number of independent variables, with the ultimate goal of obtaining an optimal problem solution. RSM is a widely practiced approach in the development and optimization of drug delivery devices [15–17]. Based on the principle of design of experiments (DOE), the methodology encompasses the use of various types of experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain to determine the optimum formulation(s) [18–21]. The technique requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating dosage forms.

The objectives are to investigate the effects of formulation variables on the release of drug from crosslinked chitosan-alanine beads and to optimize the preparation of drug loaded chitosan-alanine crosslinked beads for drug release using RSM. Chlorpheniramine maleate (CPM), which is a sedating antihistamine drug used for the symptomatic relief of allergic conditions, is selected as model drug.

2. Materials and methods

2.1. Materials

Chitosan (percentage of deacetylation 80%; total nitrogen: 7% minimum, ignition residue (sulfate): <2% and loss on drying <15%) is procured from Tokyo Kasei Kogyo Co., Ltd. Japan and used as received. Chlorpheniramine maleate (CPM) [$C_{16}H_{19}ClN_2C_4H_4O_4$] (pK_a value = 9.4) is obtained as a gift sample from Japson Pharmaceuticals Ltd. Sangrur, India. Glutaraldehyde ($C_5H_8O_2$) (MW = 100.11) 25% aqueous, acetic acid and L-alanine [$CH_3CH(NH_2)COOH$] (MW = 89.09) are purchased from CDH (New Delhi, India) and are of analytical grade.

2.2. Experimental design

A central composite design (CCD) is employed to fit a second order model. The levels are calculated and experiments are performed using CCD described elsewhere [22]. The four independent formulation variables selected for this particular study are the amount of chitosan (X_1), the percentage of crosslinker i.e. glutaraldehyde (X_2), the drug loading level (X_3) and the time of drug release (X_4). The weight of alanine is taken proportional to weight of chitosan and all other parameters like 2% acetic acid solution (20 ml), amount of crosslinker (10 ml) and processing conditions such as temperature and drug release media (pH 2.0 and 7.4), are kept invariant throughout the study. The amount of drug release $Y(2)$ and $Y(7.4)$ in different pH solutions (pH 2 and 7.4) are studied as the dependent variables. The actual amounts and corresponding coded values of different variables taken for the design are reported in Table 1. The ranges of independent variables are chosen on the basis of the results obtained from the previous studies, conducted in our laboratory [9]. The range of concentration of chitosan is taken as 0.1–0.9 g/20 ml beyond which, it becomes very difficult to extrude

Table 1. Independent variables and their coded values for the central composite design

X_i	Independent variables	Coded values				
		–2	–1	0	1	2
X_1	chitosan [g]	0.1	0.3	0.5	0.7	0.9
X_2	glutaraldehyde (10 ml) [%]	0	6.25	12.5	18.8	25
X_3	drug [mg]	25	50	75	100	125
X_4	drug release time [hrs]	2	3	4	5	6

Table 2. Prepared formulations as per the experimental design

Trial No.	Formulation variables			
	Chitosan [g]	Glutaraldehyde (10 ml) [%]	Drug (CPM) [mg]	Response time [hrs]
	(X ₁)	(X ₂)	(X ₃)	(X ₄)
F1	0.3	6.25	50	3
F2	0.7	6.25	50	3
F3	0.3	18.8	50	3
F4	0.7	18.8	50	3
F5	0.3	6.25	100	3
F6	0.7	6.25	100	3
F7	0.3	18.8	100	3
F8	0.7	18.8	100	3
F9	0.3	6.25	50	5
F10	0.7	6.25	50	5
F11	0.3	18.8	50	5
F12	0.7	18.8	50	5
F13	0.3	6.25	100	5
F14	0.7	6.25	100	5
F15	0.3	18.8	100	5
F16	0.7	18.8	100	5
F17	0.1	12.5	75	4
F18	0.9	12.5	75	4
F19	0.5	0	75	4
F20	0.5	25.0	75	4
F21	0.5	12.5	25	4
F22	0.5	12.5	125	4
F23	0.5	12.5	75	2
F24	0.5	12.5	75	6
F25	0.5	12.5	75	4
F26	0.5	12.5	75	4
F27	0.5	12.5	75	4
F28	0.5	12.5	75	4
F29	0.5	12.5	75	4
F30	0.5	12.5	75	4

the solution through the syringe due to the high viscosity of the solution. At the same time, it is not possible to prepare the beads without substantial amount of chitosan. The drug release time is chosen as 2 to 6 hrs because a burst release is observed in the first hour and almost a plateau formation is there after sixth hour of drug release. The concentration of drug release $Y(2)$ and $Y(7.4)$ in pH solutions of 2.0 and 7.4, respectively are selected as response variables. Table 2 summarizes an account of thirty experimental runs conducted during study. The combinations of factors employed during the study and their responses are given in Table 3. The following second order model (Equation (1)) in X_1 , X_2 , X_3 and X_4 is fitted using the data in Table 3:

$$Y(\text{pH}) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 \quad (1)$$

where β_0 is the intercept representing the arithmetic average of all quantitative outcomes of 30 runs; and β_1 to β_{44} are the coefficients computed from the observed experimental values of Y ; and X_i 's are the coded independent variables. Statistical validity of the polynomials is established on the basis of ANOVA technique. Subsequently, the feasibility and grid searches are performed to locate the composition of optimum formulations. The 2-D contour plots for drug release in acidic and basic medium are constructed using program developed in MATLAB V.5. The formulations corresponding to the design are prepared and evaluated for various

Table 3. Translation of actual units into coded levels and response for central composite design

Trial No.	Chitosan	Glutaraldehyde	Drug (CPM)	Response time	Response ($\cdot 10^{-4}$) [g/ml]	
	(X ₁)	(X ₂)	(X ₃)	(X ₄)	Y(2)	Y(7.4)
F1	–1	–1	–1	–1	7.24	8.33
F2	1	–1	–1	–1	6.84	7.71
F3	–1	1	–1	–1	6.74	8.27
F4	1	1	–1	–1	6.47	7.63
F5	–1	–1	1	–1	7.62	9.02
F6	1	–1	1	–1	7.50	8.46
F7	–1	1	1	–1	6.92	8.60
F8	1	1	1	–1	6.69	8.18
F9	–1	–1	–1	1	7.75	8.99
F10	1	–1	–1	1	7.16	8.32
F11	–1	1	–1	1	7.66	8.51
F12	1	1	–1	1	6.83	8.29
F13	–1	–1	1	1	8.25	9.35
F14	1	–1	1	1	7.94	8.61
F15	–1	1	1	1	7.54	8.86
F16	1	1	1	1	7.10	8.52
F17	–2	0	0	0	6.83	8.43
F18	2	0	0	0	6.10	7.34
F19	0	–2	0	0	9.16	9.92
F20	0	2	0	0	6.97	9.02
F21	0	0	–2	0	6.33	7.81
F22	0	0	2	0	7.89	9.48
F23	0	0	0	–2	6.24	8.69
F24	0	0	0	2	7.97	9.67
F25	0	0	0	0	7.40	9.01
F26	0	0	0	0	7.40	9.02
F27	0	0	0	0	7.40	9.01
F28	0	0	0	0	7.40	9.01
F29	0	0	0	0	7.41	8.98
F30	0	0	0	0	7.38	9.02

response properties. The resultant experimental data of response properties are quantitatively compared with that of the predicted values. Also, linear regression plots between actual and predicted values of the response properties are drawn using Microcal Origin 6.0, forcing the line to pass through origin.

2.3. Preparation of chitosan-alanine beads

Table 1 enlists the central composition design of different chitosan-alanine formulations prepared using varying amounts of the chitosan, alanine, glutaraldehyde and drug. Drug loaded beads of chitosan and alanine crosslinked with glutaraldehyde are prepared, as reported earlier [9]. Briefly, a known amount of CPM, chitosan and alanine are dissolved in 2% acetic acid solution by stirring conditions for three hours at room temperature. The

composition of various formulations is given in Table 2. The homogeneous mixture is extruded in the form of droplets using a 0.56 mm diameter syringe into alkali-methanol solution (1:20 w/w) under stirring conditions. The beads are washed with hot and cold water, respectively. The resultant beads were allowed to react with 10 ml of glutaraldehyde solution (25, 18.8, 12.5 and 6.25%) at 50°C for about 10 min for crosslinking. The formation of crosslinked chitosan-alanine semi-IPN beads is shown in Figure 1. To form semi interpenetrating polymer network (semi-IPN), two chitosan chains are crosslinked by glutaraldehyde. Amino groups of chitosan and alanine can react with glutaraldehyde resulting in attachment of alanine in pendent form. Finally, the crosslinked beads were successively washed with hot and cold water and air dried at 37°C. After cross-linking, the strength of unreacted glutaraldehyde was determined by

Figure 1. Schematic representation of crosslinking of chitosan and L-alanine by glutaraldehyde for the formation of interpenetrating network

Brady's reagent. The strength of residual glutaraldehyde, obtained after crosslinking the beads was in the range of 2–3.5%. In other words, more than 96% of glutaraldehyde was consumed during the crosslinking process and hence, a highly crosslinked structure was obtained.

2.4. In vitro drug release studies

The drug release experiments are performed in acidic and basic medium (30 ml each) for all the formulation combinations as reported elsewhere [9]. Briefly, the drug release experiments are performed in a glass apparatus at 37°C under unstirred conditions. Out of the prepared beads only 0.2 g are taken for the drug release studies which contain approximately 5, 10, 15, 20 and 25 mg of CPM drug, respectively. Different pH solutions (pH = 2 and pH = 7.4) are used as the drug release medium,

and the samples of 3 ml are withdrawn periodically at predefined time intervals and analyzed under UV spectrophotometer at 193.5 nm. All the release experiments are carried out in triplicate and the average results are reported. In order to maintain nearly constant release environment and to get the cumulative drug release concentration, the samples withdrawn for the record of absorbance are immediately added back to the release medium after recording the absorbance. Drug release data are analyzed using ANOVA software.

3. Results and discussion

Semi-IPN beads of chitosan and L-alanine are formed by using glutaraldehyde as crosslinker. Alanine acts as a spacer between the chitosan polymer chains. The presence of alanine increases the void content of the semi-IPN and thus, lowers the tendency of 'blocking' effect of glutaraldehyde. It is further believed that due to the presence of a spacer, it is easier for the crosslinker to penetrate

Figure 2. a) and b) drug release profiles through acidic medium from 30 formulations of chitosan-alanine crosslinked beads prepared using experimental design

into the core of the beads resulting in fully crosslinked network.

In vitro drug release studies are performed in acidic and basic environments. The crosslinked beads are immersed in solutions of pH = 7.4 and pH = 2.0, respectively. The dependence of drug release on concentration of chitosan, degree of crosslinking and nature of release environment is displayed in Figures 2 and 3. It is observed during the drug release experiments that the size of beads increases as a result of diffusion of solvent. Due to increase in the surface area of beads, the resistance to drug transport increases which lowers the rate of drug release in the latter part of study. Thirty experiments are required for central composite design to study the effect of various independent variables on drug release. The designed formulations and the observed responses for thirty experiments are given in Table 3. The polynomial equations relating the drug release responses $Y(2)$ and $Y(7.4)$ and independent variables are (Equations (2) and (3)):

$$Y(2) = 7.4(\pm 0.099) - 0.19(\pm 0.049)X_1 - 0.36(\pm 0.049)X_2 + 0.25(\pm 0.049)X_3 + 0.32(\pm 0.049)X_4 - 0.02(\pm 0.06)X_1X_2 + 0.06(\pm 0.06)X_1X_3 - 0.07(\pm 0.06)X_1X_4 - 0.11(\pm 0.06)X_2X_3 + 0.03(\pm 0.06)X_2X_4 - 0.22(\pm 0.046)X_1^2 + 0.18(\pm 0.046)X_2^2 - 0.06(\pm 0.046)X_3^2 - 0.06(\pm 0.046)X_4^2 \quad (2)$$

$$Y(7.4) = 9.01(\pm 0.098) - 0.27(\pm 0.049)X_1 - 0.15(\pm 0.049)X_2 + 0.29(\pm 0.049)X_3 + 0.22(\pm 0.049)X_4 + 0.06(\pm 0.06)X_1X_2 + 0.01(\pm 0.06)X_1X_3 + 0.02(\pm 0.06)X_1X_4 - 0.04(\pm 0.06)X_2X_3 - 0.02(\pm 0.06)X_2X_4 - 0.07(\pm 0.06)X_3X_4 - 0.33(\pm 0.046)X_1^2 + 0.06(\pm 0.046)X_2^2 - 0.14(\pm 0.046)X_3^2 - 0.01(\pm 0.046)X_4^2 \quad (3)$$

The Equations (2) and (3) represent the quantitative effect of the independent variables (X_1 , X_2 , X_3 and X_4) and their interactions on the responses $Y(2)$ and $Y(7.4)$, respectively. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships, respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. The negative coefficients of X_1 and X_2 in both the models refer to the decreasing amount of drug release as the concentration of chitosan and concentration of glutaraldehyde increases. Similarly, the positive coefficients of X_3 and X_4 indicate the increase in drug release with increasing concentration of drug and response time.

In order to check the reliability of the models to predict drug release response four more experiments, consisting of different values of variables (X_1 , X_2 , X_3 and X_4) other than the design values, are conducted. The theoretical values of drug release are calculated using Equations (2) and (3). The theoretical and experimental values of drug release are compared and are shown in Table 4. Comparison of experimental and calculated values of drug release response shows that the predicted models are in reasonably good agreement with experimental data, outside the design values. Table 5 presents the ANOVA demonstrating that the model is significant. The probability values, $P < 0.0001$ in ANOVA, indicate significant effect of model on drug release response.

Figure 4 indicates the effect of concentration of chitosan, X_1 and percentage of glutaraldehyde, X_2

Figure 3. a) and b) drug release profiles through basic medium from 30 formulations of chitosan-alanine crosslinked beads prepared using experimental design

Table 4. Comparison of the calculated and experimental response values for the formulations other than the design values

Chitosan [g]	Glutaraldehyde (10 ml) [%]	Drug (CPM) [mg]	Response time [hrs]	Calculated response ($\cdot 10^{-4}$) [g/ml]		Experimental response ($\cdot 10^{-4}$) [g/ml]		Absolute error
(X ₁)	(X ₂)	(X ₃)	(X ₄)	Y(2)	Y(7.4)	Y(2)	Y(7.4)	
0.1	6.25	100	3	–	8.45	–	8.49	0.04
0.3	0	100	5	–	9.99	–	9.91	–0.08
0.3	6.25	25	5	7.35	–	7.34	–	–0.01
0.3	18.8	100	6	7.83	–	7.85	–	0.02

Table 5. Summary of results in analyzing lack of fit (LOF)

ANOVA for response surface quadratic model (drug release in pH = 2.0)					
Source	Sum of squares	DF	Mean square	F value	P
Model	11.12	14	0.794	13.584	< 0.0001
Residual	0.87684	15	0.05846		
Lack of fit	0.87634	10	0.08763	8.636	
Pure error	0.00051	5	0.0001		
Cor total	11.9936	29			
ANOVA for response surface quadratic model (drug release in pH = 7.4)					
Source	Sum of squares	DF	Mean square	F value	P
Model	9.34	14	0.667	11.485	< 0.0001
Residual	0.87092	15	0.05806		
Lack of fit	0.86989	10	0.08698	4.209	
Pure error	0.00103	5	0.00020		
Cor total	10.2067	29			

on drug release in acidic Y(2) and basic Y(7.4) media. In acidic medium, the drug release varies in a nonlinear manner, but in a descending pattern with an increase in concentration of chitosan and percentage of crosslinker as shown in Figure 4a. The contour plot shows that percentage of crosslinker has a comparatively greater influence on the drug release variable than concentration of chitosan. ‘Saddle’ point is observed at $X_1 = 0.42$ g and $X_2 = 18.8\%$. Figure 4b indicates the effect of simultaneous change in concentration of chitosan and crosslinker on the drug release in basic medium (pH 7.4). In our previous studies [9] on drug release from crosslinked chitosan-alanine beads, it is observed that the drug release in basic medium is higher than in acidic medium. It is also observed that at fixed concentration of crosslinker, the drug release increases with an increase in chitosan concentration, reaches a maxima, followed by its decrease. The drug release response increases to ‘peak’ maxima ($> 9.0 \cdot 10^{-4}$ g/ml) at the intermediate value of concentration of chitosan (0.42 g) and at somewhat higher concentration of crosslinker (15.5%). This may be due to the fact that a high concentration of chitosan results in a denser matrix, which causes reduced degree of swelling of beads. Consequently, the process of diffusion is slowed

Figure 4. Contour plots (2D) showing the influence of concentration of chitosan (X₁) and concentration of glutaraldehyde (X₂) on drug release a) Y(2), b) Y(7.4) for pH 2 and pH 7.4, respectively

down due to the decreased level of penetration of solvent, which leads to decreased release of drug. Bayomi *et al.* [1], Genta *et al.* [2] and Acikgöz *et al.* [23] also observed that there is a significant increase in drug release, when a more porous matrix having low concentration of chitosan was used.

The effects of concentration of chitosan, X_1 and concentration of drug, X_3 on drug release in acidic $Y(2)$ and basic $Y(7.4)$ media are plotted as contour and shown in Figure 5. The amount of drug release increases with the drug concentration in beads at fixed level of chitosan concentration. The drug release is maximum at nearly intermediate value of concentration of chitosan, $X_1 = 0.42$ g and a ‘rising ridge’ is observed corresponding to this point. According to Gupta and Kumar [7] and Kumar and Gupta [8], the total amount of drug released from the beads loaded with a higher concentration of drug is found to be higher in comparison to the beads loaded with a lower amount of CPM. At the

higher level of concentration of chitosan, the concentration of alanine is less. As a result of which, the intermediate space between the different layers of matrix is less causing lesser degree of swelling and hence, the amount of drug release is minimum. Figure 5b indicates that the drug release response is similar, but higher in basic medium as compared to drug release in acidic medium. Response to drug release increases to ‘peak’ maxima ($> 9.2 \cdot 10^{-4}$ g/ml) corresponding to concentration of chitosan, $X_1 = 0.42$ g and concentration of drug, $X_2 = 100$ mg.

Figure 6 illustrates the effect of concentration of chitosan, X_1 and response time, X_4 on drug release. The response time has the similar effect on drug release in acidic and basic media. In both the cases $Y(2)$ and $Y(7.4)$, the ‘rising ridge’ is observed corresponding to concentration of chitosan, $X_1 = 0.42$ g, similar to the studied effect of concentration of chitosan, X_1 and concentration of drug, X_3 on drug release in acidic medium $Y(2)$ in Figure 4a. At the

Figure 5. Contour plots (2D) showing the influence of concentration of chitosan (X_1) and concentration of drug (X_3) on drug release a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

Figure 6. Contour plots (2D) showing the influence of concentration of chitosan (X_1) and response time (X_4) on drug release a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

intermediate values of drug release time, the drug release in acidic medium $Y(2) = 7.45 \cdot 10^{-4}$ g/ml and in basic medium $Y(7.4) = 9.07 \cdot 10^{-4}$ g/ml. It is further observed that the effect of response time is more in acidic medium than in basic medium at fixed level of concentration of chitosan.

The effects of concentration of crosslinker, X_2 and concentration of drug, X_3 on drug release are depicted in Figure 7. Minimum drug release is found to be lying between the percentage of glutaraldehyde 9.8–20%, corresponding to lower range of concentration of drug i.e. 0–41 mg. At the lower values of percentage of crosslinker, the drug release $Y(2)$ increases linearly with the concentration of drug. The nature of response behavior changes to non-linear at higher concentration of crosslinker. Figure 7b observes the ‘region of maxima’ i.e. $9.4 \cdot 10^{-4}$ g/ml for the drug release lying between the high level of concentration of drug (100 mg) and low level of crosslinker (approximately 3%). At the lower values of drug load, the

release of drug decreases linearly with an increase in the percentage of crosslinker. The nature of drug release behavior changes from linear to nonlinear at the intermediate and higher values of drug load. The effect of concentration of drug seems to be more pronounced as compared with that of crosslinker.

Figure 8 describes the effect of percentage of crosslinker, X_2 and response time, X_4 on drug release. Contour plots in Figure 8a show the ‘region of minimum’ for concentration of the released drug. The amount of drug release is minimum ($6.6 \cdot 10^{-4}$ g/ml) at higher values of glutaraldehyde (18.8%) and at initial response time. At the fixed response time, as the concentration of glutaraldehyde decreases, the response behavior changes to linear. In Figure 8b, drug release decreases linearly with percentage of crosslinker. As the percentage of crosslinker increases, the penetration of solvent in to the matrix becomes difficult. Thus, the degree of swelling decreases, result-

Figure 7. Contour plots (2D) showing the influence of concentration glutaraldehyde (X_2) and concentration of drug (X_3) on drug release a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

Figure 8. Contour plots (2D) showing the influence of concentration glutaraldehyde (X_2) and response time (X_4) on drug release a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

ing in decreased rate of drug release. At the fixed value of crosslinker, as the response time increases, the drug release increases linearly because swelling is function of time.

The effects of concentration of drug, X_3 and response time, X_4 on drug release are shown in Figure 9. In Figure 9a, the drug release response surface is ‘hillside’ and varies in linear fashion with increasing concentration of drug as well as response time in acidic medium. However, the effect of response time seems to be more effective as compared to that of drug concentration. The maximum amount of drug release is $8.4 \cdot 10^{-4}$ g/ml at the higher values of concentration of drug and response time. In the basic medium, the drug release response is a linear function for the early values of concentration of drug and at all the values of response time as shown in Figure 9b. The response behavior becomes non linear at the higher values of concentration of drug.

Figure 9. Contour plots (2D) showing the influence of concentration of drug (X_3) and response time (X_4) on drug release a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

Figure 10 shows a linear correlation plots between the actual and predicted response variables. The linear correlation plots drawn between actual and predicted responses demonstrate reasonable values of R^2 (i.e. for $Y(2)$ $R^2 = 0.927$ and for $Y(7.4)$ $R^2 = 0.915$). The R -square (correlation coefficient) value provides a measure of how much of the variability in the observed response values can be explained by the experimental factors and their interactions. A good model explains most of the variations in the response. The closer the value of R -square to 1.0, the stronger the model and the better are the response predictions. Hence, high values of R -square indicate significant lack of fit ($P < 0.0001$) in both the cases.

The residual plots showing homogeneity of data for drug release are plotted in Figure 11. All the experimental data points are uniformly distributed around the mean of response variable showing the scatter of the residuals versus actual response values.

Figure 10. Linear correlation plots between actual and predicted response a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

Figure 11. Response surface plots for drug release response a) $Y(2)$, b) $Y(7.4)$ for pH 2 and pH 7.4, respectively

4. Conclusions

Optimization of drug release from chitosan-alanine crosslinked beads using RSM, central composite design, is performed. On the basis of analysis of variance (ANOVA), a second order model is established, describing the effect of the amount of chitosan (X_1), the percentage of crosslinker i.e. glutaraldehyde (X_2), the concentration of drug loading (X_3) and the time of drug release (X_4) on drug release response. The data obtained, based on designed formulations, is fitted in second order model for drug release in acidic and basic media. Both the polynomials are found to be statistically significant ($P < 0.0001$), as determined by ANOVA. It is observed from the probability data of both the models that the probability values of the coefficients of X_1 , X_2 , X_3 and X_4 are less than 0.1, which is a significant value. It implies that all the four independent variables have great influence on the drug release response. ANOVA is used to evaluate the adequacy of the fitted model. The prediction

from the model and the experimental results in this study conform to each other quite well, indicating the validity of the model. The obtained equations are plotted as contour. The increase in percentage of crosslinker and concentration of chitosan reduces the drug release response due to the formation of highly dense matrix. From the data obtained and contour plots, it may be concluded that the concentration of alanine has tremendous effect on the swelling and drug release properties of the matrix. As the concentration of alanine (which acts as spacer) increases, the density of the matrix decreases resulting in an increase in drug release. In all the cases, the drug release is maximum for chitosan concentration, $X_1 = 0.42$ g (Figures 4–6). The optimum drug level that can be loaded into the matrix corresponding to maximum drug release in basic medium is 100 mg (Figures 5b, 7b and 9b). It is observed that the concentration of drug has pronounced effect on drug release as compared to the concentration of crosslinker. For the predicted models, the range of percentage of glutaraldehyde for optimum drug release is 10–18% (Figures 4 and 8a). In general, the response time has shown a linear effect on drug release. As the response time increases, the percentage of swelling of the matrix increases and hence, the drug release increases linearly. It can be concluded that the response surface methodology (RSM) can be successfully used to optimize the drug release from chitosan–alanine crosslinked beads loaded with CPM.

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