OPTIMIZATION OF DRUG RELEASE FROM CHITOSAN-STARCH CROSSLINKED BEADS BY RESPONSE SURFACE METHODOLOGY

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The objectives of this study are to investigate and optimize the effect of relative concentrations of chitosan-starch, crosslinkers (sodium hexametaphosphate (SHMP) and glutaraldehyde) and the release time on release of chlorpheniramine maleate (CPM) at pH values of 2.2 and 7.4 by using response surface methodology. The process is optimized with the aim to achieve maximum drug release. The influence of each parameter is studied by factorial design analysis. Analysis of variance (ANOVA) is also used to evaluate the validity of the model. The optimum conditions obtained are 60% chitosan, 40% starch, 10% SHMP, 15% GA concentration and 6.25 h time for drug release at pH values of 2.2 and 7.4, corresponding to the optimum amounts of drug release of 117.7×10^{-4} g /ml and 121.2×10^{-4} g/ml, respectively. These optimized values agree with the predicted results, thus indicating the utility of the predictive models in determining the release of CPM in colon specific drug delivery systems.

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

INTRODUCTION

The study of drug release refers to the effect of orally consumed drugs after their interaction with fluid in the stomach. Drug release may be instant or slow depending upon the nature and reaction with stomach fluid.[1-2] Uncontrolled drug release does not usually provide specific target delivery, but results in a sharp increase in drug concentration to potentially toxic levels. Following a relatively short period at the therapeutic level, drug concentration eventually drops off until re-administration. Instant drug release has limitations such as undesired drug release above the therapeutic level, drug wastage and global costs. Such limitations may be reduced by improving prolonged gastric retention of drug and making it release in such a manner that the release rate is maintained within the permitted therapeutic range. Thus, controlled drug delivery signifies time-related release of a predictable amount of medication, minimizing the problems of patient compliance and undesirable side effects.[3-5] In order to control the drug release from a polymeric matrix, several important parameters should be considered, such as its hydrophilic character, molecular weight, length and structure of the spacer group linking the drug to the polymer, the liability of covalent linkages between the drug and the polymer, the amount of initial drug loading and the size and geometry of the particles *etc*.[6] Beads used as drug carrier present disadvantages such a slow strength, high dispersion and solubility. Chemical crosslinking improves the mechanical strength, thermal stability and swelling properties of the beads. The properties of chitosan can be improved by use of crosslinking agents.[7]

Chitosan, (1,4)-[2amino-2-deoxy-β-D-glucan] is a natural derivative of chitin, obtained by partial deacetylation of chitin.[8-9] Chitosan has an amine side group, which is responsible for its polycationic character and formation of well-known intermolecular complexes with carboxylic

acid and polycarboxylic acid. Chitosan is an inert, hydrophilic, biocompatible and biodegradable material.[10-12] The use of chitosan in the pharmaceutical industry is still very limited because of its high cost, poor mechanical strength, fast dissolution in the stomach for oral administration, and limited capacity for controlled drug release.[13] Hence, other biodegradable materials, such as pectin, guar gum, sodium alginate, starch *etc*., are used to enhance controlled drug release and to reduce the cost.[14] Starch can be used for making blends with other polymeric materials.[15-17] Corn starch is a low cost and easily available material with the extra advantage of its compatibility with chitosan. Starch is a water swellable excipient in nature, and it enhances the release of drug when added to controlled release formulations.[18]

Chitosan is used to prepare hydrogels, films, beads, fibers and sponges. The larger surface area of beads, as well as their ease of handling, makes them ideal agents of controlled drug release. Chitosan is the only pseudonatural cationic polymer and it also finds applications in wastewater treatment. Due to its unique molecular structure, chitosan has an extremely high affinity for many classes of dyes, including dispersed, direct, reactive, sulfur, acid and naphthol dyes. The rate of diffusion of dyes in chitosan is similar to that in cellulose. Few researches have modified chitosan for its potential application in the removal of dyes from wastewater.[19-22]

Response surface methodology (RSM) is a collection of statistical and mathematical techniques, useful for improving and optimizing processes. 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 and more measured responses and a number of independent variables, with the ultimate goal of obtaining an optimal problem solution.[23-26] This technique requires minimum experimentation and time, thus proving to be more effective and cost effective than conventional methods of formulating dosage forms.

Keeping in view the above aspects, the present work aims to optimize a drug delivery system synthesized by crosslinking chitosan and starch using two crosslinkers (*i.e.* sodium hexametaphosphate and glutaraldehyde). Sodium hexametaphosphate (SHMP) crosslinks starch and glutaraldehyde crosslinks chitosan to make the drug delivery matrix more compact and stable. The effects of process parameters, such as concentration of chitosan, starch, sodium hexametaphosphate, glutaraldehyde and release time of drug, have been studied as independent variables and optimized with the aim to achieve maximum drug release over an extended period.

EXPERIMENTAL

Materials

Chitosan of low viscosity (loss on drying <10% ash, insoluble matter >1%, viscosity <200 m Pa s) was supplied by FlukaBio Chemica (Germany) and corn starch was procured from Himedia (India). Acetic acid (99.5%) was purchased from Merck (Germany) and glutaraldehyde was procured from Central Drug House (P) Ltd, New Delhi. Sodium hexametaphosphate (SHMP) was purchased from the Pioneer Chemical Company, New Delhi. The drug chlorpheniramine maleate (CPM) [$C_{16}H_{19}ClN_2C_4H_4O_4$] was obtained as a gift sample from Japson Pharmaceutical Ltd. Sangrur, India. For the preparation of solutions, double distilled water was used.

Preparation of chitosan-starch beads

To prepare beads, a known quantity of chitosan was dissolved in 20 ml of 2% acetic acid solution at 25±1ºC with continuous stirring for three hours.The starch solution was prepared by dissolving a known weight of starch in 20 ml of distilled water at 85ºC, while stirring for 20 minutes, followed by natural cooling to room temperature. The solutions of chitosan and starch were mixed together and kept for 24 hours at room temperature (25 ºC) in order to get a bubble free clear solution.0.2 g of CPM drug was added to the resultant solution containing chitosan and starch and mixed thoroughly. This homogeneous mixture was 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 were washed with water and the resultant beads were allowed to react with 20 ml of SHMP (7.5%, 10%, 12.5%, 15%, and 17.5%) for 20 minutes at room temperature (25 ºC).The beads were washed with distilled water and the obtained beads were subjected to further crosslinking with 20 ml of glutaraldehyde (10%, 15%, 20%, 25% and 30%) at 60 ºC for 10 minutes. These beads were washed with distilled water to remove unreacted glutaraldehyde. The double crosslinked beads were dried at 40 ºC for 24 hours. Drug loading was done before extruding the polymeric mixture into the alkali-methanol solution. From previous studies conducted in our laboratory, it was found that the drug loading efficiency of the beads was approximately 55-60%. The lower encapsulation values may be due to the loss

of drug during the immersion of the beads into the alkali-methanol solution and subsequently during the crosslinking reactions.

Experimental design

Central composite design (CCD) and analysis of response surfaces were used to study the effect of multiple variables and to find an optimum formulation.[27]A central composite design (CCD) with four variables and five levels was used to study the response pattern and to determine the optimum combination of the variables. Four independent formulation variables were selected for this particular study: relative concentration of chitosan (X_1), percentage of crosslinkers *i.e.* SHMP (X_2) and GA (X_3) and drug release time (X_4).The weight of starch was taken proportional to the weight of chitosan and all other parameters like 2% acetic acid solution (20 ml), amount of crosslinkers (20 ml of each) and processing conditions, such as temperature and drug release media (pH = 2.2 and 7.4),were kept invariant throughout the study. The ranges selected for these variables are based on the preliminary study carried out in our laboratory and are given in Table 1.

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

X_i	Independent variable	Coded values				
		-2	-1	0	1	2
X1	Chitosan (%)	50	60	70	80	90
X2	SHMP(20 ml)[%]	7.5	10	12.5	15	17.5
X3	GA(20 ml)[%]	10	15	20	25	30
X4	Drug release time (hours)	1	2.75	4.50	6.25	8

Table 2
Prepared formulations as per the experimental design

Run	Factor 1 Chitosan (%)	Factor 2 SHMP (%)	Factor 3 GA (%)	Factor 4 Time(hours)
1	90	12.5	20	4.5
2	70	12.5	20	4.5
3	50	12.5	20	4.5
4	80	10	25	2.75
5	70	17.5	20	4.5
6	70	12.5	20	4.5
7	70	12.5	20	1
8	60	15	25	2.75
9	80	15	15	2.75
10	80	15	15	6.25
11	80	15	25	6.25
12	80	15	25	2.75
13	60	10	15	2.75
14	80	10	25	6.25
15	70	12.5	20	4.5
16	70	12.5	20	8
17	60	15	25	6.25
18	70	7.5	20	4.5
19	80	10	15	6.25
20	60	10	25	6.25
21	70	12.5	30	4.5
22	60	10	25	2.75
23	60	15	15	6.25
24	70	12.5	20	4.5
25	70	12.5	20	4.5
26	70	12.5	10	4.5
27	70	12.5	20	4.5
28	60	10	15	6.25
29	60	15	15	2.75
30	80	10	15	2.75

The amount of drug released Y (2.2) and Y (7.4) in different pH solutions (pH 2.2 and 7.4) were studied as dependent variables. The actual amounts and corresponding coded values of different variables taken for the design are reported in Table 1.

During the study, thirty experimental runs were conducted as per the design shown in Table 2. Polynomial models including the interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis approach. The polynomial equations represent the coefficients for intercept, first-order effects, interaction terms, and higher order effects. The sign and magnitude of the effects show the relative influence of each factor on the response. Combinations of factors were employed during the study and their responses are depicted in Table 2.The following second-order model in X_1, X_2, X_3 and $X_4$was fitted using the data:

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

where β_0 is the intercept representing the arithmetic average of all quantitative outcomes of thirty runs, 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.

Scanning electron microscopy (SEM)

The surface morphology of the chitosan-starch beads and crosslinked beads was studied with the help of SEM. SEM analysis was carried out on a JOEL scanning electron microscope. Before focusing the electron beam on the samples, the samples were gold-sputtered in order to make them conductive.

***In vitro* drug release studies**

The drug release experiments were performed in acidic and basic medium (100 ml each) for all formulations and combinations. The drug release experiments were performed in a glass apparatus at 37 ºC without stirring. Thirty experiments are required to study the effect of various independent variables on drug release by the central composite design. An amount of 0.2 g of prepared beads was taken for the drug release studies. Different pH solutions (pH = 2 and pH = 7.4) are used as the drug release medium. At predetermined intervals of time, samples of 3 ml were withdrawn, filtered and assessed by the absorbance at 193.5 nm through the UV spectrophotometer (HACH, DR/4000U). In order to maintain a nearly constant release environment, the samples were immediately added back to the release medium after recording the absorbance. All the release experiments were carried out in triplicate and the average results are reported. The amount of drug released through the crosslinked beads was calculated by using Beer Lambert's law:

$$A = \varepsilon \cdot c \cdot l = \log (I_0/I) \quad (2)$$

where *Io* is the intensity of incident radiations, *I* is the intensity of transmitted radiations, c is molar concentration of sample, l is the length in cm or the path of the light beam that passes through the sample cell, ε is molar extinction coefficient, and A is absorbance at a particular wavelength.

RESULTS AND DISCUSSION

SEM analysis

SEM analysis was used to determine the shape and surface morphology of the prepared beads. SEM images of uncrosslinked and crosslinked chitosan-starch beads are shown in Figure 1. The observed shape of the beads can be approximated as spherical, as depicted in Figure 1(a).The approximate size of the beads is in the range of 0.9-1.0 mm. Figure 1(b) presents the morphology of uncrosslinked chitosan-starch beads synthesized from 90% chitosan and 10% starch. One can observe that the rigidity and compactness of uncrosslinked beads improve after crosslinking. This is due to the fact that, with the addition of crosslinker, the polymer chains come closer to each other and give a regular and rigid structure. It is believed that glutaraldehyde mainly crosslinks chitosan, and to crosslink the starch present in the beads, another crosslinker, sodium hexametaphosphate (SHMP), was used. A highly compact and rigid matrix is exhibited by the morphology of doubly crosslinked beads (crosslinked by SHMP and GA), as shown in Figure 1(d).

Optimization of *in vitro* drug release studies

Thirty experiments are required to study the effect of various independent variables on drug release by the central composite design. To recognize the key process variables for the experimental design, which influence the synthesis of the chitosan-starch beads independently, the effect of parameters such as chitosan concentration, amount of different crosslinking agents, and drug release time, were studied by conducting the experimental runs at randomly selected different levels of the four parameters. A known amount of crosslinked drug loaded beads were immersed into solutions of pH = 7.4 and pH = 2.2.The dependence of drug release on the concentration of chitosan, degree of crosslinking and nature of the release environment is illustrated in Figures 2-3. The polynomial equations relating drug release response [Y (2.2) and Y (7.4)] and independent variables are:

$$Y(2.2) = 57.61(\pm583.984) - 7.26(\pm9.419)X_1 - 5.45(\pm16.274)\ X_2 - 9.56\ (\pm0.431)X_3 + 13.84(\pm22.635)X_4 + 2.73\ (\pm0.109)X_1X_2 - 0.38\ (\pm7.595E\text{-}3)X_1X_3 - 2.03\ (\pm0.116)\ X_1X_4 + 0.93\ (\pm0.074)X_2X_3 - 2.12\ (\pm0.484)X_2X_4 - 1.82(\pm0.208)X_3X_4 + 5.69\ (\pm0.057)X_1^2 + 1.79\ (\pm0.286)X_2^2 + 5.66(\pm0.226)X_3^2 + 1.23(\pm0.40014)X_4^2$$

$$Y(7.4) = 81.84(\pm521.298) - 5.42(\pm5.853)X_1 - 5.73(\pm12.660)X_2 - 5.90\ (\pm14.015)X_3 + 13.99(\pm5.231)X_4 - 0.068(\pm2.7275E\text{-}3)X_1X_2 + 1.04\ (\pm0.0208)X_1X_3 + 1.3(\pm0.0745)X_1X_4 + 2.7(\pm0.216)X_2X_3 - 1.6\ (\pm0.336)X_2X_4 + 3.71(\pm0.424)X_3X_4 + 3.28(\pm0.0328)X_1^2 + 1.97\ (\pm0.316)X_2^2 + 4.24(\pm0.169)X_3^2 - 2.16(\pm0.705)X_4^2$$

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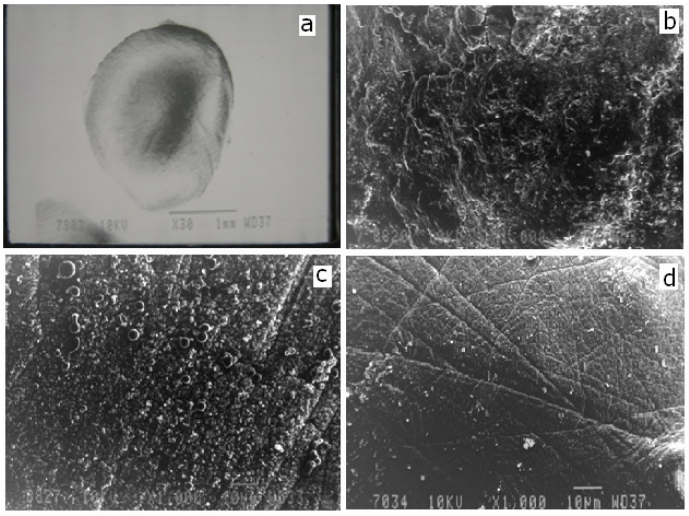

Figure 1: SEM micrographs of chitosan (90%)-starch (10%) uncrosslinked bead (a-b), bead crosslinked with GA (25%) (c) and beads crosslinked with SHMP (12.5%) and GA (25%) (d)

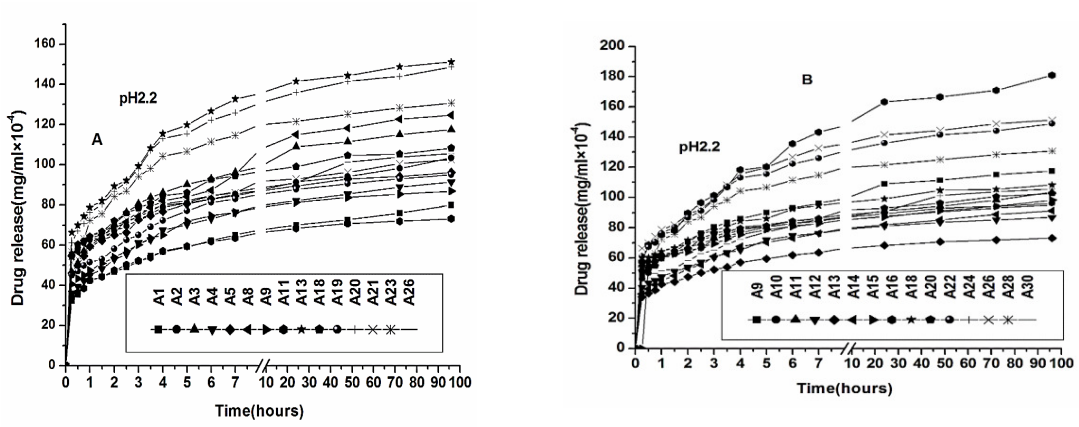

Figure 2: A) and B) Profiles of drug release through acidic medium from thirty formulations of chitosan-starch crosslinked beads prepared using the experimental design

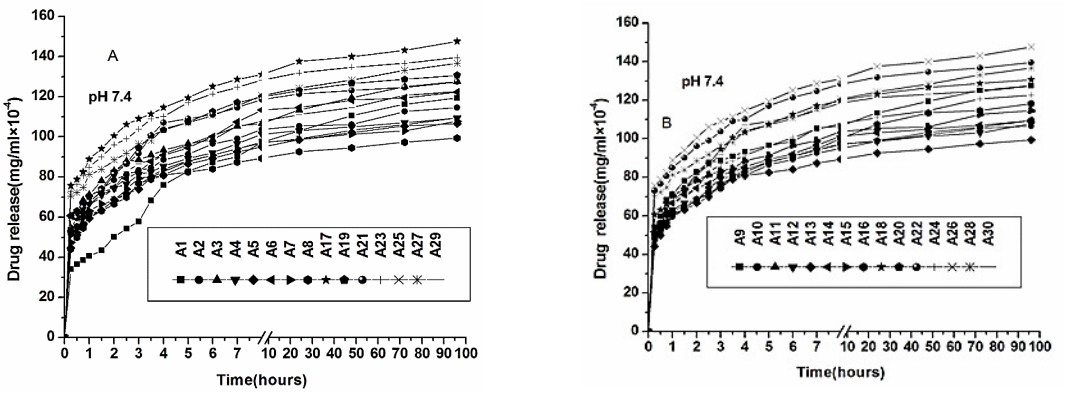

Figure 3: A) and B) Profiles of drug release through basic medium from thirty formulations of chitosan-starch crosslinked beads prepared using experimental design

Equations 3 and 4 represent the quantitative effect of independent variables (X_1, X_2, X_3 and X_4) and their interactions on the responses Y (2.2) and Y (7.4), respectively. The coefficient with more than one factor terms and those with higher order terms represent interaction terms and quadratic relationships, respectively. A positive sign represents a synergic effect, while a negative sign indicates an antagonistic effect. The negative coefficients of X_1, X_2 and X_3in the models refer to the decreasing amount of drug release as the concentration of chitosan, SHMP and GA increases. Similarly, the positive coefficient of X_4 indicates the increase in drug release with increasing response time.

Drug release profiles for acid and basic media for thirty experiments are given in Figures 2-3. For the release behaviors of CPM, all the plots show similar initial release behavior. It has been observed from the release profiles of CPM from the chitosan–starch crosslinked beads that the release of drugs from the beads in acidic medium is lower compared to that in basic medium. This can be explained by the fact that the release of drug depends mainly on the degree of swelling of the beads. At pH 2.2, there is less swelling; thus, the drug entrapped in the beads cannot be released easily. However, at pH 7.4, the beads are swollen to a greater extent, leading to a faster release of the drug as compared to the release in acidic environment. Further, the release of the drug is fast for the first hour in both media, followed by a moderate release over 7 hours and finally an almost constant release of the drug is observed for the studied period of 96 hours. Intermolecular hydrogen bonding is formed between NH^{3+} (ammonium ions) of the chitosan backbone and OH^- of starch. The amino groups (NH_2) of chitosan are protonated NH^{3+} in the acetic acid solution, whereas the ordered crystalline structures of starch molecules are destroyed with the gelatinization process, resulting in the OH^- groups being exposed to form hydrogen bonds with NH^{3+} of the chitosan.

Figure 4 illustrates the effect of the concentration of chitosan (X_1) and crosslinking agent (X_2) on drug release in acidic and basic environment. A 3D plot in Figure 4(a) shows that the amount of drug released increases with a decrease in the concentration of chitosan and SHMP. The low release of the drug from the beads at higher polymer concentration may be due to delayed swelling of the beads due to compact matrix formation. To understand the effect of the pH on the amount of drug released in basic medium, the effect of the concentration of chitosan (X_1) and crosslinking agent SHMP (X_2) on drug release was also analyzed. Figure 4(b) shows that the quantity of drug released increased with a decrease in chitosan concentration. Similarly, the quantity of drug released decreased continuously with an increasing concentration of SHMP. This is due to the formation of a dense matrix that reduces the degree of swelling of the beads, which results in reduced penetration of the solvent, and hence, in a reduced amount of drug released.[25,27-29] The drug release decreased with an increase in chitosan concentration.[24] It means that concentration of chitosan and crosslinker have significant effect on drug release. It means that the concentration of chitosan and crosslinker has a significant effect on drug release. Since, in alkaline medium (*i.e.* at pH 7.4), the swelling is mainly driven by solvent diffusion, while chain penetration due to protonation of amino groups is

absent, the amount of drug released is higher than at pH of 2.2.[30]

The response surface plot showing the influence of the concentration of chitosan (X_1) and crosslinking agent GA (X_3) on drug release in acidic and basic environment is presented in Figure 5. It can be noted that the quantity of drug released increases with a decrease in the concentration of chitosan and glutaraldehyde. The effect of the concentration of chitosan (X_1) and crosslinking agent (X_3) on the drug released at pH 7.4 is shown in Figure 5(b).The quantity of drug released increased with a decrease in the concentration of GA. This was due to the fact that a higher concentration of the crosslinking agent resulted in the formation of a dense matrix, which caused a reduction in the degree of swelling of the beads. The process of diffusion slowed down the penetration of the solvent, which led to a decreased quantity of drug released.[26-27] Hence, both the concentration of chitosan and crosslinker have a significant effect on the amount of drug released. This is due to the formation of ammonium salt in acidic medium, and in such a medium the process of drug release is fast.[28]

Figure 6 presents the effect of the concentration of chitosan (X_1) and drug release time in hours (X_4) on the amount of drug released in the solutions of pH 2.2 and 7.4.In both cases, it was observed that the quantity of drug released increased as the concentration of chitosan decreased. The quantity of drug released was maximum due to swelling of the beads at the highest limit of the specified range of time, which was also reported in earlier studies.[28-34] The amount of drug released increased as the drug release time increased due to the swelling of the beads over time.[24] Hence, the time factor is an important parameter for the amount of drug released.

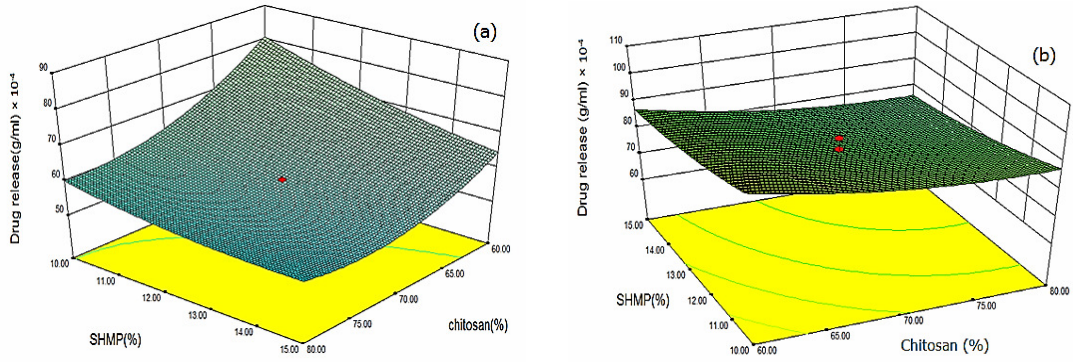

Figure 4: Effect of concentration of chitosan and crosslinking agent (SHMP) on drug release at pH (a) 2.2 and (b) 7.4

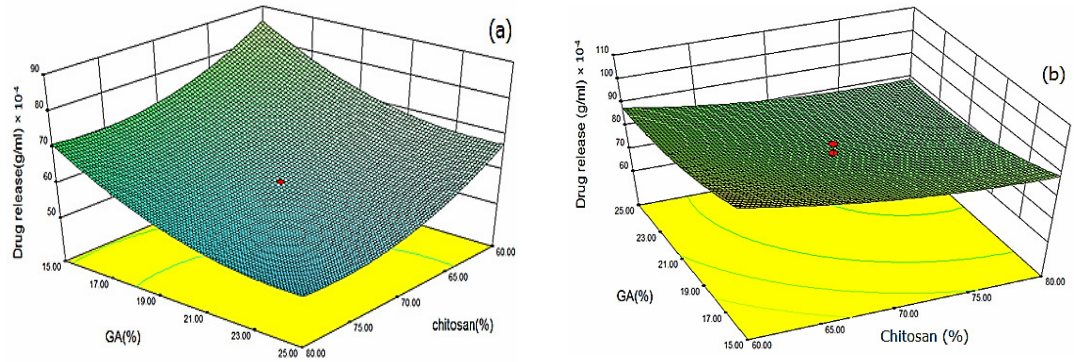

Figure 5: Effect of concentration of chitosan and crosslinking agent (GA) on drug release at pH (a) 2.2 and (b) 7.4

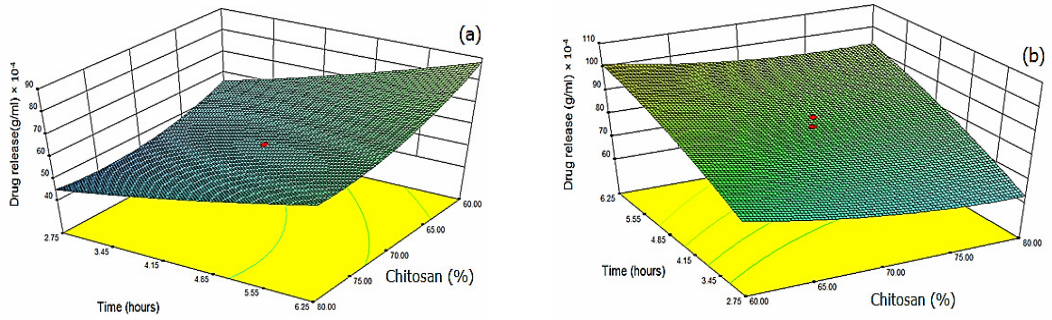

Figure 6: Effect of chitosan concentration and release time on drug release at pH (a) 2.2 and (b) 7.4

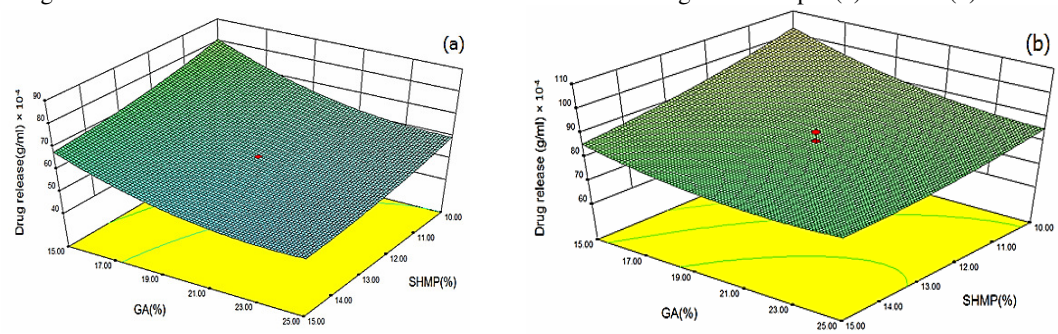

Figure7: Effect of crosslinking agents SHMP and GA on drug release at pH (a) 2.2 and (b) 7.4

The effect of the concentration of both crosslinking agents (X_2) and (X_3) on the drug release in solutions of pH 2.2 and 7.4 is illustrated in Figure 7. It can be observed from the plot that with a decrease in the concentrations of both crosslinkers, the rate of drug release reached the maximum. This is due to the fact that a higher concentration of crosslinking agent increases the crosslinking density of the bead matrix and reduces the degree of swelling. The process of diffusion slows down for further penetration of the solvent, which results in a decreased release of the drug.[31]

The response surface plot shows the effect of the concentration of crosslinking agent SHMP (X_2) and release time (X_4) on the drug released at pH 2.2 and 7.4 (Fig. 8).The drug release rate decreases with an increase in the concentration of crosslinking agent and the rate of drug release increases proportionally to the release time. An increase in drug release is observed with an increase in swelling time due to rapid hydration/high swelling, governed by the dissolution and diffusion of the drug in the polymer matrix formed by swelling.[35-36] The release of a water soluble drug from as well able matrix occurs only after the penetration of the release medium into the polymeric matrix, which allows swelling of the polymer and drug dissolution, followed by the diffusion along the same path to the surface of the beads. Thus, the drug release takes place from the compact matrix due to the increase in swelling of the beads and penetration of the solvent over a period of time.

Figure 9 illustrates the effect of the concentration of crosslinking agent GA (X_3) and time in hours (X_4) on drug release at pH 2.2 and 7.4. The quantity of drug released decreases with an increase in the concentration of glutaraldehyde. This is due to the fact that a higher concentration of crosslinking agent results in the formation of a dense matrix, which reduces the degree of swelling of the beads.

The release time also increases the quantity of drug released proportionally. This is due to the swelling of the beads over a period of time.

The results of the second-order response surface model in the form of analysis of variance (ANOVA) for pH 2.2 and 7.4 are given in Tables 4 and 5. Regression analyses for drug release indicate that the fitted quadratic models accounted for more than 94% of the variation in the experimental data, which is highly significant (R^2 > 0.94). Multiple regression equations were generated relating responses to both coded and un-coded forms (levels) of process variables. The values of regression coefficients and p-levels for the coded form of process variables are presented in Tables 4 and 5.

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

Run	Factor 1 Chitosan (%)	Factor 2 SHMP (%)	Factor 3 GA (%)	Factor 4 Time (hours)	Drug release at pH 2.2 ($g/ml \times 10^{-4}$)	Drug release at pH 7.4 ($g/ml \times 10^{-4}$)
1	-1	-1	-1	-1	62.355	75.893
2	+1	-1	-1	-1	57.293	77.429
3	-1	+1	-1	-1	97.306	104.822
4	+1	+1	-1	-1	48.622	65.651
5	-1	-1	+1	-1	55.45	68.739
6	+1	-1	+1	-1	58.293	87.429
7	-1	+1	+1	-1	32.971	42.776
8	+1	+1	+1	-1	54.841	70.779
9	-1	-1	-1	+1	57.355	73.254
10	+1	-1	-1	+1	82.852	92.11
11	-1	+1	-1	+1	62.16	102.11
12	+1	+1	-1	+1	45.35	62.037
13	-1	-1	+1	+1	76.799	95.191
14	+1	-1	+1	+1	71.17	102.708
15	-1	+1	+1	+1	58.293	82.429
16	+1	+1	+1	+1	90.962	94.425
17	-2	0	0	0	96.502	104.275
18	+2	0	0	0	54.316	101.517
19	0	-2	0	0	61.52	114.215
20	0	+2	0	0	91.12	110.302
21	0	0	-2	0	57.22	78.246
22	0	0	+2	0	57.273	74.995
23	0	0	0	-2	91.12	101.1
24	0	0	0	+2	57.22	80.429
25	0	0	0	0	57.273	80.429
26	0	0	0	0	105.1	110.11
27	0	0	0	0	64.01	83.29
28	0	0	0	0	117.81	121.205
29	0	0	0	0	61.964	90.048
30	0	0	0	0	63.53	83.499

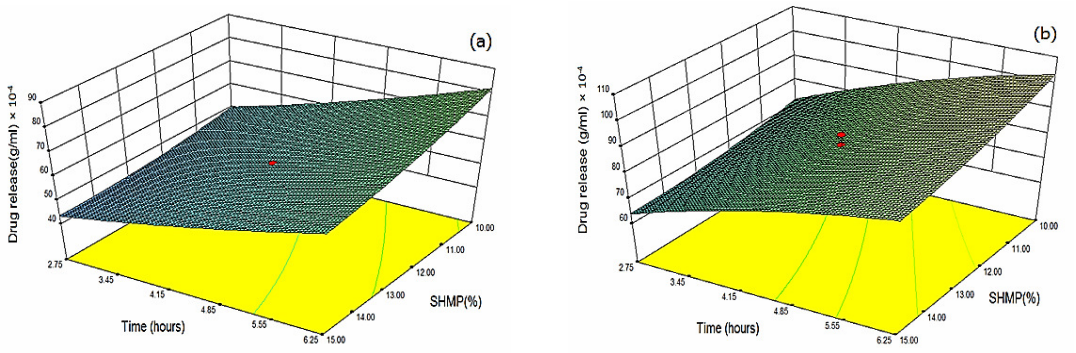

Figure 8: Effect of crosslinking agent SHMP and release time on drug release at pH (a) 2.2 and (b) 7

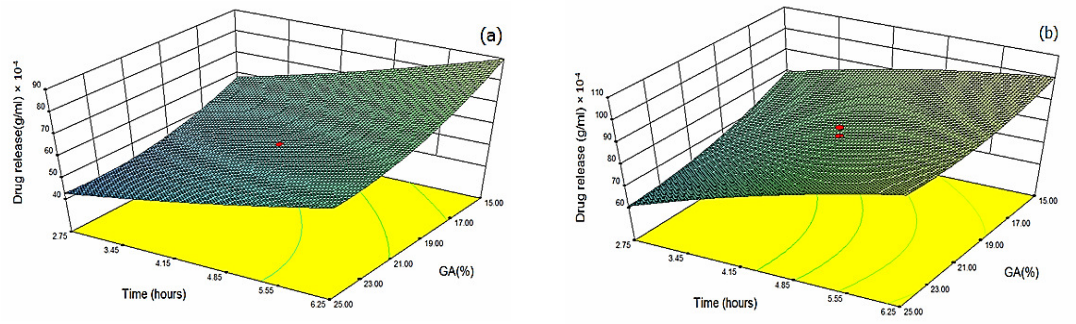

Figure 9: Effect of crosslinking agent GA and release time on drug release at pH (a) 2.2 and (b) 7.4

Table 4
Analysis of variance for response at pH 2.2

Source	DF	Coefficient, β	Sum of squares	F-value	p-value
Model	14	57.61	10771.40	59.59	<0.0001
Chitosan	1	-7.62	1394.28	107.99	<0.0001
SHMP	1	-5.45	713.27	55.25	<0.0001
GA	1	-9.56	2191.23	169.72	<0.0001
Time	1	+13.84	4595.05	355.91	<0.0001
Chitosan^2	1	+5.69	888.48	68.82	<0.0001
SHMP^2	1	+1.79	87.64	6.79	0.0199
GA^2	1	+5.66	878.94	68.08	<0.00001
Time^2	1	+1.23	41.19	3.19	0.0943
Chitosan×SHMP	1	+2.73	118.84	9.20	0.0084
Chitosan×GA	1	-0.38	2.31	0.18	0.6785
Chitosan×Time	1	-2.03	65.98	5.11	0.0391
SHMP×GA	1	+0.93	13.84	1.07	0.3169
SHMP×Time	1	-2.12	71.64	5.55	0.0325
GA×Time	1	-1.82	53.02	4.11	0.0609
Residual	15		193.66		

Table 5
Analysis of variance for response at pH 7.4

Source	DF	Coefficient, β	Sum of squares	F-value	p-value
Model	14	+82.76	8411.58	59.59	<0.0001
Chitosan	1	-5.42	706.22	107.99	0.0005
SHMP	1	-5.74	790.00	55.25	0.0003
GA	1	-5.90	834.41	169.72	0.0003
Time	1	+13.99	4699.77	355.91	<0.0001
Chitosan^2	1	+3.05	254.84	68.82	0.0127
SHMP^2	1	+1.75	83.75	6.79	0.1095
GA^2	1	+4.00	439.57	68.08	0.0024
Time^2	1	-2.39	156.82	3.19	0.0825
Chitosan×SHMP	1	-0.068	0.074	9.20	0.9648
Chitosan×GA	1	+1.04	17.31	0.18	0.4043
Chitosan×Time	1	+1.30	27.18	5.11	0.0962
SHMP×GA	1	+2.70	116.22	1.07	0.3081
SHMP×Time	1	-1.60	41.08	5.55	0.0275
GA×Time	1	+3.71	219.89	4.11	0.0609
Residual	15		530.06		

The p values (Table 4) indicate that all linear terms of the process variables have a significant effect ($p < 0.05$), whereas the quadratic term of time and the interactions of concentration of 'chitosan and GA' and 'SHMP and GA' have a non-significant effect at a 5% level of significance ($p > 0.05$) on drug release in the medium at pH 2.2. The relative magnitude of β values (Table 4) indicates the maximum positive effect of time (β = 13.84). This result indicates an increase in drug release with an increase in time. The concentration of GA has a maximum negative effect (β = -9.56), followed by the concentration of chitosan (β = -7.62) and the concentration of SHMP (β = -5.45) on drug release. Similarly, the p values for the response at pH = 7.4 (Table 5) indicate that all linear terms of the process variables have a significant effect ($p < 0.05$), whereas the quadratic term of time and the interactions of concentration of 'chitosan and GA', 'chitosan and SHMP', 'chitosan and time' and 'SHMP and GA' have a non-significant effect at a 5% level of significance ($p > 0.05$) on drug release in the basic medium (*i.e.* pH = 7.4). The relative magnitude of β values (Table 5) indicates the maximum positive effect of time (β = 13.99), resulting in an increase in drug release with an increase in time. The concentration of GA has a maximum negative effect (β = -5.90), followed by the concentration of SHMP (β = -5.74) and the concentration of chitosan (β = -5.42), on drug release.

Table 6
Results of optimization in different media

S.no.	pH	Chitosan (%)	SHMP (%)	GA (%)	Time(h)	Drug release (g/ml.10^{-4})
1	2.2	60.00	10.00	15.00	6.25	117.688
2	7.4	60.00	10.00	15.00	6.25	121.183

CONCLUSION

The optimization of drug release from chitosan-starch crosslinked beads using RSM, central composite design, was performed. The release characteristics of the prepared chitosan-starch beads depend on the solubility of the drug, the concentration of chitosan, starch and crosslinking agent, and on the release time. The crosslinking agents were used to control the swelling of the bead matrix, which is essential for controlled drug release. The synthesized beads can be used as a reliable drug delivery device for colon specific drug delivery, as the drug was found to be released to a greater extent in alkaline medium than in acidic medium.

On the basis of the analysis of variance (ANOVA), a second-order model was established, describing the effect of the amount of chitosan (X_1), the percentage of crosslinkers, *i.e.* sodium hexametaphosphate (X_2) and glutaraldehyde (X_3), and the time of drug release (X_4) on the drug release response. The data obtained, based on the designed formulations, were fitted to the second-order model for drug release in acidic and basic media. Both polynomials were found to be statistically significant ($p < 0.0001$), as determined by ANOVA. It was found that all four independent variables had a great influence on the drug release response. ANOVA was used to evaluate the adequacy of the fitted model. The prediction from the model and the experimental results in this study correspond to each other quite well, indicating the validity of the model. The obtained equations were represented as 3-D contour plots. The increase in the concentration of chitosan and percentage of crosslinkers reduced the drug release response because of the formation of a highly compact matrix. In general, the response time showed a linear effect on drug release. The percentage of matrix swelling increased and hence, the drug release increased linearly with the response time. It can be concluded that the central composite design can be successfully used to optimize CPM release from chitosan-starch crosslinked beads.

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