Optimizing Preparation of NaCS–Chitosan Complex to Form a Potential Material for the Colon-Specific Drug Delivery System

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ABSTRACT: A novel polyelectrolyte complex (PEC) formed by sodium cellulose sulfate (NaCS) and chitosan was prepared as a candidate material for colon-specific drug delivery system. It was found in experiments that the properties of two raw materials and the process parameters, such as the degree of substitution (DS) and concentration of NaCS, the viscosity and concentration of chitosan, were very important factors on the properties of the final product—NaCS–chitosan-PEC. The preparation of NaCS–chitosan complex was optimized by using response surface methodology to evaluate the effects of these parameters on the degradation properties of NaCS–chitosan in the simulated colonic fluid (SCF). The DS of NaCS was in the range from 0.2 to 0.6, the concentration of NaCS from 2 to 4% (w/v), the viscosity of chitosan from 50 to 550 mPa s, and the concentration of chitosan from 0.5 to 1.5% (w/v). A mathematical model was developed to describe the effect of these parameters and their interactions on the degradation of NaCS–chitosan complex. The optimum operation conditions for preparing NaCS–chitosan complex were determined to DS of NaCS of 0.2, the concentration of NaCS of 4.0% (w/v), chitosan viscosity of 327 mPa s, and the concentration of chitosan 0.5% (w/v), respectively. Validation of experiments with 5 confirmatory runs indicated the high degree of prognostic ability of response surface methodology. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 3001–3012, 2010

Key words: NaCS–chitosan complex; cellulose sulfate; colon-specific drug delivery system; biocompatible material; polyelectrolyte

INTRODUCTION

Delivery of orally administered drugs specifically to the colon has a lot of important implications in the field of pharmacotherapy. First, the diseases of the colon such as irritable bowel syndrome, Crohn's disease, and ulcerative colitis are effectively treated by using directly the anti-inflammatory agents to the affected area. Second, the colon is found to be a promising site for systemic absorption of peptides and proteins because of the less hydrolytic hostile environment in comparison with stomach and small intestine as well as the existence of specific transporters.[1,2]

To achieve a successful colon targeted drug delivery, a drug delivery system needs the efficient carriers and the relevant approaches to protect the drug loaded from degradation, release, and/or absorption in the upper portion of the gastrointestinal (GI) tract and then ensure abrupt or controlled release in the proximal colon.[3]

There are three kinds of carrier materials classified by the approaches used for colon targeting (i) pH-sensitive, (ii) time-dependent, and (iii) bacterially triggered. However, most of colon-specific drug delivery systems such as time- and/or pH-dependent release systems were found to be not very reliable in terms of "site-specific release", because many factors affect the drug transit time and pH in the GI tract such as age, sex, diet, intestinal motility, disease state, etc.[4–7] The microbially triggered systems seem to be more specific for colonic drug delivery due to the large population of microflora in colon.[8] Moreover, some colon-specific degradable materials have been investigated used in the bacterially triggered colon-specific drug delivery system,[7,9,10] such as chitosan.

Chitosan (poly-β-(1–4)-D-glucosamine) is a polycationic polysaccharide derived from naturally occurring chitin by alkaline deacetylation. It is nontoxic and biocompatible, can be digested by the colonic bacteria[11] and has ability to be conjugated with a

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variety of substrates via its amine. These properties make chitosan a good candidate for the development of the colon-specific drug delivery systems. However, chitosan can be dissolved in acidic solutions since it has a lot of amino groups. It is rapidly dissolved in the gastric cavity and is unable to protect its drug load during passage through the stomach and small intestine. To overcome the above problem, chitosan has to be modified by chemical or other methods.

In spite of limitation mentioned above, a number of articles have been published on the investigation of the application of chitosan in colon targeting.[11–16] Because two of the most important properties of chitosan are cationic nature and high charge density in acidic solution, chitosan can form insoluble polyelectrolyte complexes (PEC) with water-soluble polyanionic species in neutral conditions. A few reports on (PEC) applied in colon targeting chitosan–containing component have been published,[13,17–20] such as with pectin, alginate, etc.

Sodium cellulose sulfate (NaCS), is a polyanion, derived from cellulose by heterogeneous sulfating process.[21] It has the favorable biological properties such as nontoxicity, biocompatability, biodegradation, water-soluble, and a good film formation behavior.[22–24] In the recent years, a novel microcapsule formed by NaCS and poly-dimethyldiallyl-ammonium-chloride (PDMDAAC), another polycation, has been investigated for the immobilization of microorganisms, enzymes, animal, and plant cells etc.[25–28]

Chitosan and NaCS can be used to form PEC (NaCS–chitosan), which is insoluble at the acidic condition and can be degraded by the enzyme originated from the colon microflora. In our previous work,[29] a simple preparation process and the basic characteristics of NaCS–chitosan complex were measured. It was found that the degradation of NaCS–chitosan complex is one of the most important characteristic and can be varied with many factors including viscosity of chitosan, degree of substitution (DS) of NaCS, concentration of chitosan, concentration of chitosan, etc.

To obtain the optimal formulations with appropriate degradation and to investigate the effect of these factors on degradation of NaCS–chitosan complex, an optimization technique based on response surface methodology (RSM),[30–32] a statistical method based on the multivariate non-linear model, will be used in this PEC preparation process for its effectiveness in demonstrating the interactions between these factors on producing the optimum PEC form.

The objectives of the present work are to evaluate the effect of the factors on the degradation of the NaCS–chitosan complex in SCF by applying RSM, to develop a mathematical model between the degradation of NaCS–chitosan complex and the factors, to obtain the optimal formulations with appropriate degradation,[33] and finally to investigate the properties of NaCS–chitosan complex as a potential carrier material for the colon-specific drug delivery system by *in vitro* degradation experiment.

MATERIALS AND METHODS

Materials

Chitosan with a degree of deacetylation of 85% was supplied by Jinan Haidebei Co., (Jinan, China). The viscosity of 1% w/v in acetic acid solution (1% v/v) ranged from 50 to 550 mPa s. NaCSs with the different degree of substitution (DS = 0.2 $\sim$ 0.6) were prepared by the heterogeneous reaction as described previously in our lab.[21,22] Sprague-Dawley (SD) rats (male, 240 $\sim$ 260 g) were supplied by Zhejiang Academy of Medical Sciences (Hangzhou, China). All other chemicals and reagents used were of analytical grade and were used without further purification.

Preparation of NaCS–chitosan film

The formation reaction between NaCS and chitosan was showed schematically in Figure 1. NaCS–chitosan complex was produced from a pair of oppositely charged polysaccharides. An aqueous NaCS solution was prepared in deionized water. An aqueous chitosan solution was prepared by dissolving an appropriate quantity of chitosan powder in deionized water containing 1% (v/v) of acetic acid. Then two solutions were mixed together and stirred at 1500 rpm at room temperature. Thereafter the mixture was cast on a glass plate, thoroughly dried at 45°C in vacuum. A yellow-white film was formed on the glass plate and was carefully taken off.

Experimental design and data analysis

Response surface methodology is a collection of statistical and mathematical methods that are useful for modeling and analyzing multi-parameters optimization. It also can be applied to describe the relationship between several factors and the response surfaces. This optimization process involves three major steps: (i) performing statistically designed experiments, (ii) estimating the coefficients in a mathematical model, and (iii) predicting the response and checking the adequacy of the model.[34]

In this work, a Box-Behnken design was selected with the aim to calculate simultaneously the effects that produce changes on the variables and also their possible interactions.[35] Box-Behnken statistical design is one kind of RSM design that is an independent, rotatable or nearly rotatable, quadratic design having the treatment combinations at the midpoints of the edges of the process space and at

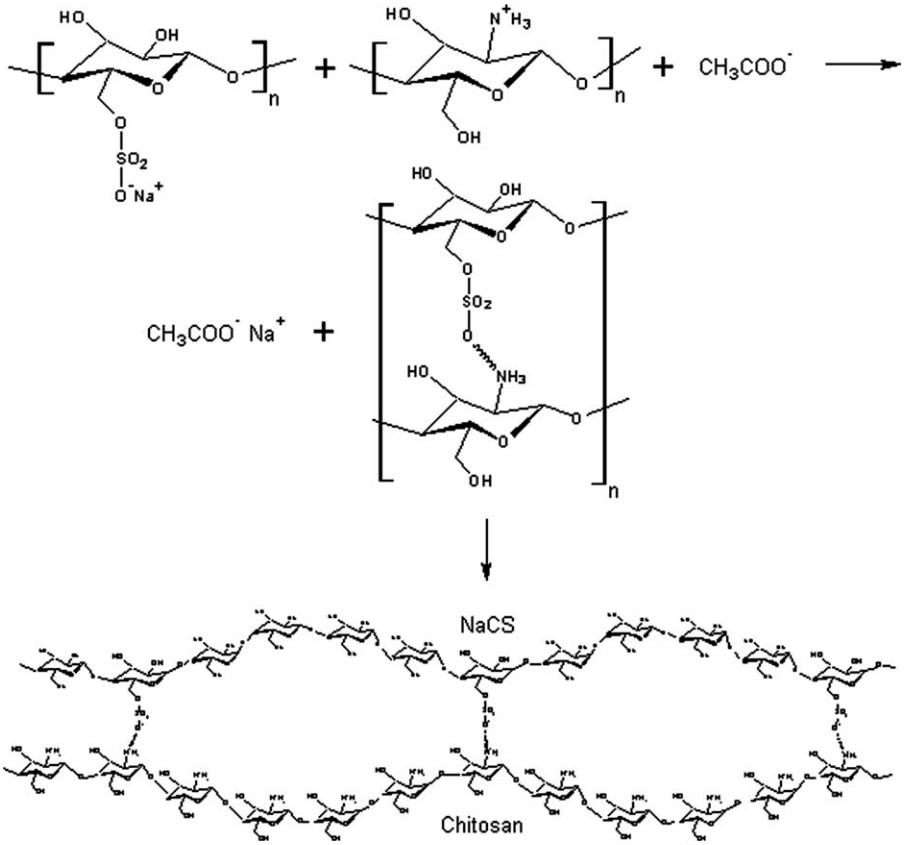

Figure 1 Polyelectrolytes complex reaction between NaCS and chitosan.

the center.[36] Additionally, it only has three levels (low, medium, and high, coded as −1, 0, and +1), and requires fewer experimental runs and less time. Therefore, it provides a far more effective and cost-effective technique than the conventional processes of formulating and optimization of dosage forms.

The design variables selected with actual and coded levels along with response variables are listed in Table I. These high, medium, and low levels were selected from the results of preliminary experimentation. Experiments were carried out according to the design points with independent variables such as DS of NaCS (X_1), concentration of NaCS (X_2), viscosity of chitosan (X_3), and concentration of chitosan (X_4). Table III showed the design matrix of a 27 trials experiment. The design consists of replicated center points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest.[33]

The nonlinear quadratic model generated by the design is of the form:

$$Y = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j \qquad (1)$$

where Y is the predicted response, the b_0 is the intercept term, the b_i values are linear coefficient, the b_{ii} values are quadratic coefficient and the b_{ij} values are interactive coefficients; X_1, X_2, X_3, and X_4 are the factors studied; X_iX_j are the interaction of two factors.

The software Design Expert (Version 7.0.0, Stat-Ease, Minneapolis) was used for the experimental design and regression analysis of the data obtained. The quality of correlation of the polynomial model equation was expressed by the coefficient of determination (R^2).

In vitro experimental conditions and media

Simulated gastric fluid

The simulated gastric fluid (SGF) was aqueous solution of 1% (w/v) pepsin that was adjusted pH to 1.5 using 0.1 *M* HCl.

Simulated intestinal fluid

The simulated intestinal fluid (SIF) was 0.05 *M* phosphate buffer, pH 7.5, with 1% (w/v) pancreatin present.

Simulated colonic fluid

Male Sprague-Dawley rats were anaesthetized and the cecum was exteriorized for collection of the

TABLE I
Box-Behnken Experimental Design for the Independent Variables (Actual and Coded Levels)

Factor	Variable	Unit	Range and level of actual and coded values		
			−1	0	+1
X_1	DS of NaCS	–	0.2	0.4	0.6
X_2	Concentration of NaCS	% (w/v)	2	3	4
X_3	Viscosity of chitosan	mPa s	50	300	550
X_4	Concentration of chitosan	% (w/v)	0.5	1	1.5

contents. The caecal contents were diluted with phosphate-buffered saline (PBS, pH 7) to 30% (w/v) dilution for release study. This step was conducted under N_2 to maintain an anaerobic environment.[37]

In vitro degradation behaviors

Degradation of the samples was monitored as the fractional weight loss. Initially at least three replicates (20 mm × 20 mm) of each dried NaCS–chitosan complex film were weighed (w_0). The NaCS–chitosan complex film was immersed in the simulated gastric fluid for 1 h, then removed and immersed in the simulated intestinal fluid for 4 h. The NaCS–chitosan complex film were finally immersed in the simulated colonic fluid for 12 h. The samples were maintained at 37°C and stirred at 100 rpm in shaker. Then the NaCS–chitosan complex samples were removed, washed with distilled water for 5 times and dried in vacuum at 45°C for 12 h. The masses of the samples were measured accurately and the weight loss expressed as a percentage of the original weight.

The degradation percentage (D_t) at t (h) was expressed by the eq. (2):

$$D_t = \frac{w_0 - w_t}{w_0} \times 100\% \quad (2)$$

where w_t was the weight of sample at time of t and w_0 was the initial sample weight.

Table II lists the composition of NaCS–chitosan complex sample to investigate the *in vitro* degradation behaviors.

Preparation of capsules with NaCS–chitosan complex

NaCS–chitosan complex was prepared as method described in Section 2.2. The NaCS–chitosan complex was placed in a water bath maintained at 80°C. Then 6 ∼ 8% w/v of gelatin and 1 ∼ 2% w/v of carrageenan, with respect to the volume of NaCS–chitosan complex, were added into the NaCS–chitosan complex, with stirring continuously. Then capsules were prepared by dipping a stainless steel rod into the mixture prepared above, followed by subsequent drying in vacuum at 45°C for 12 h. Then, the formed capsules were carefully denuded. Finally, the rim of each shell was clipped to form the capsule cap with a length of 13 mm, and diameter of 6 mm. The size of the capsule was 0# capsule whose length was 17 mm, inner diameter was 5.8 mm, and thickness was 0.2 mm. The capsules based on S1, S2, and S3 were named by C1, C2, and C3, respectively.

In vitro release profile of capsule based on NaCS–chitosan

The release profile of capsule based on NaCS–chitosan complex was investigated by using 5-aminosalicylic acid (5-ASA) as a model drug. First, 20 mg of 5-ASA were filled into a hard capsule formed by NaCS–chitosan complex manually. The joint of the capsule body and cap was carefully sealed by pressing them so that they fitted in the lock mechanism. The capsules were immersed in the simulated gastric fluid for 1 h, then removed and immersed in the simulated intestinal fluid for 4 h, and then removed and immersed in the simulated colonic fluid for 7 h. The rotation speed of the shaker was 100 rpm and temperature was maintained at 37 ± 0.5°C. The capsules were tied to a paddle with a cotton thread in each dissolution vessel to prevent floating. At predetermined time intervals, 2 mL of medium from vessel was sampled and replaced by

TABLE II
The Composition of NaCS–Chitosan Complex Sample

Sample code	DS of NaCS	Apparent viscosity of chitosan (mPa s)	Mixing ratio (NaCS : chitosan)	Concentration of chitosan (w/v)	Concentration of NaCS (w/v)	D_{12} (%)
S1	0.2	300	1 : 1	0.5%	4%	47.13
S2	0.4	300	1 : 1	1%	3%	35.18
S3	0.6	50	1 : 1	1%	3%	22.30

TABLE III
The Independent Variables and the Responses for All 27 Experimental Runs

Run No.	DS of NaCS X_1	Concentration of NaCS (%) X_2	Viscosity of chitosan (mPa s) X_3	Concentration of chitosan (%) X_4	Degradation of NaCS–chitosan (%) D_{12}
1	1	−1	0	0	23.49
2	1	1	0	0	30.93
3	−1	−1	0	0	32.05
4	−1	1	0	0	46.78
5	0	0	−1	−1	27.61
6	0	0	−1	1	27.61
7	0	0	1	−1	23.09
8	0	0	1	1	13.54
9	1	0	0	−1	31.83
10	1	0	0	1	25.92
11	−1	0	0	−1	50.55
12	−1	0	0	1	48.06
13	0	−1	−1	0	26.97
14	0	−1	1	0	5.98
15	0	1	−1	0	24.28
16	0	1	1	0	25.94
17	1	0	−1	0	22.30
18	1	0	1	0	8.30
19	−1	0	−1	0	31.05
20	−1	0	1	0	21.27
21	0	−1	0	−1	42.89
22	0	−1	0	1	35.46
23	0	1	0	−1	44.87
24	0	1	0	1	30.88
25	0	0	0	0	42.91
26	0	0	0	0	35.18
27	0	0	0	0	40.61

an equal volume of fresh medium. The drug content was assayed by HPLC. The drug release percent was determined using eq. (3)

$$\text{ratio of released } \% = \frac{R_t}{L} \times 100\% \quad (3)$$

where L and R_t represent the initial amount of drug loaded and the cumulative amount of drug released at time t.

HPLC analysis

The HPLC used in this work consisted of a modular chromatographic system (model 1100 series pump, variable wavelength detector (VWD), and manual injection valve; Agilent, Waldbroon, Germany). The detector was set at 300 nm. Chromatography was performed on a reverse-phase column (Hypersil BDS2 C-18 5U column, 250 × 4.6 mm I.D.; Yilite Co. Dalian, China) at room temperature. The mobile phase was methanol–phosphate buffer, pH 6.8 (5 : 95, v/v), and the flow rate was 1 mL/min.

RESULTS AND DISCUSSION

Principle of preparation of NaCS–chitosan complex

The electrostatic attraction between the cationic amino groups of chitosan (the macro p*K*a value is about 6.5)[38] and the anionic $-SO_4^-$ groups of the NaCS is the main interaction leading to the formation of the PEC. PEC reaction between NaCS and chitosan can be represented schematically in Figure 1.

Just as described in literatures,[39,40] the different characteristics of PEC can be obtained by changing the chemical characteristics of the component polymers. In preparation, four parameters, DS of NaCS, concentration of NaCS, viscosity of chitosan, and concentration of NaCS on the degradation of PEC, are main factors on the NaCS–chitosan formation and properties.

Response surface methodology

A four-factor, three-level Box-Behnken statistical experimental design as the RSM requires 27 experiments. The independent variables and the responses for all 27 experimental runs are given in Table III.

TABLE IV
The Analysis of Variance (ANOVA) for the Response Surface Quadratic Model

Source	Sum of squares	DF	Mean square	*F*-value	*P*-value (Prob > *F*)
Model	3174.38	14	226.73	12.60	<0.0001
Residual	216.00	12	18.00		
Lack of fit	184.49	10	18.45	1.17	0.5454
Pure error	31.51	2	15.75		
Cor total	3390.38	26			
$R = 0.9676$		*R*-squared = 0.9363		Adj *R*-squared = 0.862	
Adeq precision = 13.23		C.V.% = 13.96			

It can be seen from Table III that there was a considerable variation in the D_{12} depending upon the preparation parameters. The D_{12} ranged from 5.98% to 48.06%, and the run #14 and #12 had the minimum and maximum D_{12} values, respectively.

Fitting of data to the model

By applying multiple regression analysis methods, the experimental data were correlated based on eq. (1), the polynomial model for D_{12} was regressed by only considering the significant terms and was shown as follows:

$$D_{12} = 39.57 - 7.25X_1 + 3.07X_2 - 5.14X_3 - 3.28X_4 + 5.66X_2X_3 - 16.78X_3^2 \quad (4)$$

where D_{12} is the predicted response variable, viz., the degradation of NaCS–chitosan complex (%); X_1–X_4 are the coded values of the independent variables, viz., DS of NaCS, concentration of NaCS, viscosity of chitosan, concentration of chitosan, respectively.

Analysis of variance (ANOVA) was important in determining the adequacy and significance of the quadratic model. The analysis of variance (ANOVA) for response surface quadratic model is summarized in Table IV and Table V. The model *F*-value of 12.60 implies the model is significant.There is only a 0.01% chance that this large "Model *F*-Value" could occur due to noise. Values of "probability > *F*" is <0.0001, much less than 0.05, indicating that the model is highly significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here, the ratio of 13.23 obtained in this model indicated an adequate signal. A very low value of coefficient of the variation (C.V.) (13.96%) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values.

At the model level, the correlation measurements for the estimation of the regression equation are the multiple correlation coefficient *R* and the determination coefficient R^2. The closer the value of *R* is to 1, the better is the correlation between the experimental data and the calculated values.[41] In this experiment, the value of *R* was 0.9676, which indicated a

TABLE V
Results of Regression Analysis of the Quadratic Polynomial Model

Model term	Coefficient estimate	Sum of squares	Mean square	*F*-value	*P*-value prob > *F*
Intercept	0.3957				
X_1	−0.0725	630.61	630.61	35.03	<0.0001[a]
X_2	0.0307	113.10	113.10	6.28	0.0276[b]
X_3	−0.0514	317.24	317.24	17.62	0.0012[a]
X_4	−0.0328	129.17	129.17	7.18	0.0201[b]
X_1X_2	−0.0182	13.29	13.29	0.74	0.407
X_1X_3	−0.0105	4.45	4.45	0.25	0.628
X_1X_4	−0.0086	2.92	2.92	0.16	0.694
X_2X_3	0.0567	128.26	128.26	7.13	0.0204[b]
X_2X_4	−0.0164	10.76	10.76	0.60	0.454
X_3X_4	−0.0239	22.80	22.80	1.27	0.282
$X_1 \times X_1$	−0.0245	32.08	32.08	1.78	0.207
$X_2 \times X_2$	−0.0270	38.99	38.99	2.17	0.167
$X_3 \times X_3$	−0.1678	1501.03	1501.03	83.39	<0.0001[a]
$X_4 \times X_4$	0.0127	8.60	8.60	0.48	0.502

[a] Significant at 1% level.
[b] Significant at 5% level.

high consistency of correlation between the experimental and the predicted values. The value of R^2 (0.9363) for eq. (4) suggests that the total variation of 93.63% for D_{12} is attributed to the independent variables and only about 6.37% of the total variation can not be explained by the model. The adjusted R^2 value of 0.862 also suggested that model was significant.

The lack-of-fit measures the failure of the model to represent data in the experimental domain at points, which are not included in the regression.[42] The value of lack-of-fit for regression eq. (4) is not significant ($P = 0.5454$), indicating that the model equation was adequate for predicting the D_{12} under any combination of values of the variables.

The regression coefficients, along with the corresponding P-values, for the model of D_{12}, were presented in Table V. The P-values are used as a tool to check the significance of each coefficient, which also indicate the interaction strength between each independent variable. The smaller the P-values, the bigger the significance of the corresponding coefficient.[43] Table V showed that the regression coefficients of the linear term of X_1, X_3 and quadratic coefficients of X_3^2 were significant at 1% level, and the linear term of X_2, X_4 and one cross-product (X_2, X_3) was significant at 5% level.

A positive sign before a factor in polynomial equations represents that the response increases with the factor. On the other hand, a negative sign means the response and factors have reciprocal relation. It is evident that the linear term X_1, X_3, X_4 and quadratic coefficients (X_3^2) have negative effects on the response D_{12}. However, the linear term X_2 and one cross-product (X_2, X_3) has positive effect on the response D_{12}.

Coefficients with higher order terms or more than one factor term in the regression equation represented the quadratic relationships or interaction terms, respectively. It also showed that the relationship between responses and factors is not always linear. Used at different levels in a formulation or when more than one factors are changed simultaneously, a factor can produce different degree of response.[44] The interaction effect of X_2 and X_3 was favorable (positive) for response D_{12}. Higher and negative quadratic effects of X_3^2 were observed for the response.

From the equation, it is quite clear that the X_1 and X_3 play an more important role in the improvement of the degradation of NaCS–chitosan complex.

Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are very useful to illustrate the effects of the independent variables and interactive effects of each independent variable on the response variables. These types of plots showed effects of two factors on the response at a time. The shape of the corresponding contour plots indicated whether the mutual interactions between the independent variables are significant or not. An elliptical nature of the contour plots indicates that the interactions between the independent variables are significant. From the 3D response surface plots and the corresponding contour plots, the optimal values of the independent variables could be observed, and the interaction between each independent variables' pair could be easily understood.[43]

The fitted response surface for D_{12} by the above model was generated using the Design Expert software and is given in Figures 2–7. In all the presented figures, other factors were kept at a constant level. All the relationships among the four variables are non-linear, although Figure 3(a,b) exhibit a nearly linear relationship of factor X_1 with factors X_4, in the form of almost straight lines up to the medium level. The three-dimensional response surface based on independent variables concentration of NaCS (X_2) and viscosity of chitosan (X_3) was shown in Figure 2, while other independent variable, DS of NaCS (X_1) and concentration of chitosan was kept at a constant level. It can be seen that a maximal D_{12} could be determined under certain condition [$X_1 = 0.2$, $X_2 = 4.0\%$ (w/v), $X_3 = 250$–350 mPa s, $X_4 = 0.5\%$ (w/v)]. It means that further increases of X_3 would not increase the D_{12} any longer. Figures 2–7 show that the D_{12} increases with decreasing concentration of chitosan (X_4) and DS of NaCS (X_1) and increases with increasing concentration of NaCS (X_2).

From above analysis, the best value of D_{12} occurs at low DS of NaCS (X_1) and concentration of chitosan (X_4), high concentration of NaCS (X_2), and an appropriate viscosity of chitosan (X_3). By solving the inverse matrix from eq. (4), the optimum values of the test variables in actual units were DS of NaCS = 0.2, concentration of NaCS = 4.0% (w/v), viscosity of chitosan = 327 mPas, and concentration of chitosan = 0.5% (w/v). Under these conditions, the maximum predicted D_{12} was obtained, which is about 52.1%.

Validation of the models

To validate the adequacy of the model equations eq. (4), a total of five verification experiments were carried out under various conditions (within the experimental range). Table VI presents the design matrix of the independent variables in actual units along with the experimental results and values predicted by eq. (4).

The percentage of prediction error is helpful in establishing the validity of generated equations and

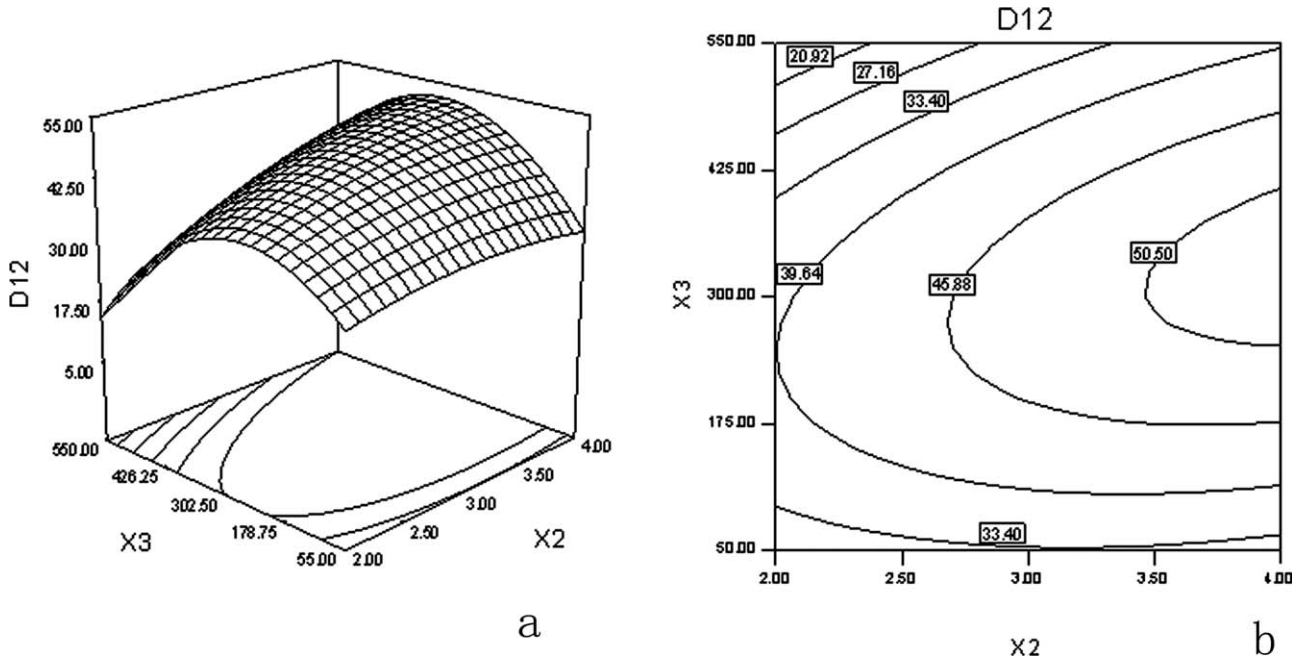

Figure 2 Surface plot and corresponding contour plot of D_{12} vs. X_2 and X_3.

to describe the domain of applicability of RSM model.[36] For validation of RSM results, the percentage prediction error was found to vary between −8.81% and +7.93%.

The results of analysis indicated that the experimental values were in good agreement with the predicted ones, and also suggested that the models developed were considered to be accurate and reliable for predicting D_{12} of NaCS–chitosan complex.

Degradation behavior of NaCS–chitosan complex film *in vitro*

The experiments of the degradation behavior of NaCS–chitosan complex film were carried out by immersing the NaCS–chitosan complex films (Samples S1, S2, and S3) in the SGF, SIF, SCF and phosphate buffered saline (PBS 7.0) in sequence. The degradation percentage of NaCS–chitosan complex films as a function of time was plotted in Figure 8.

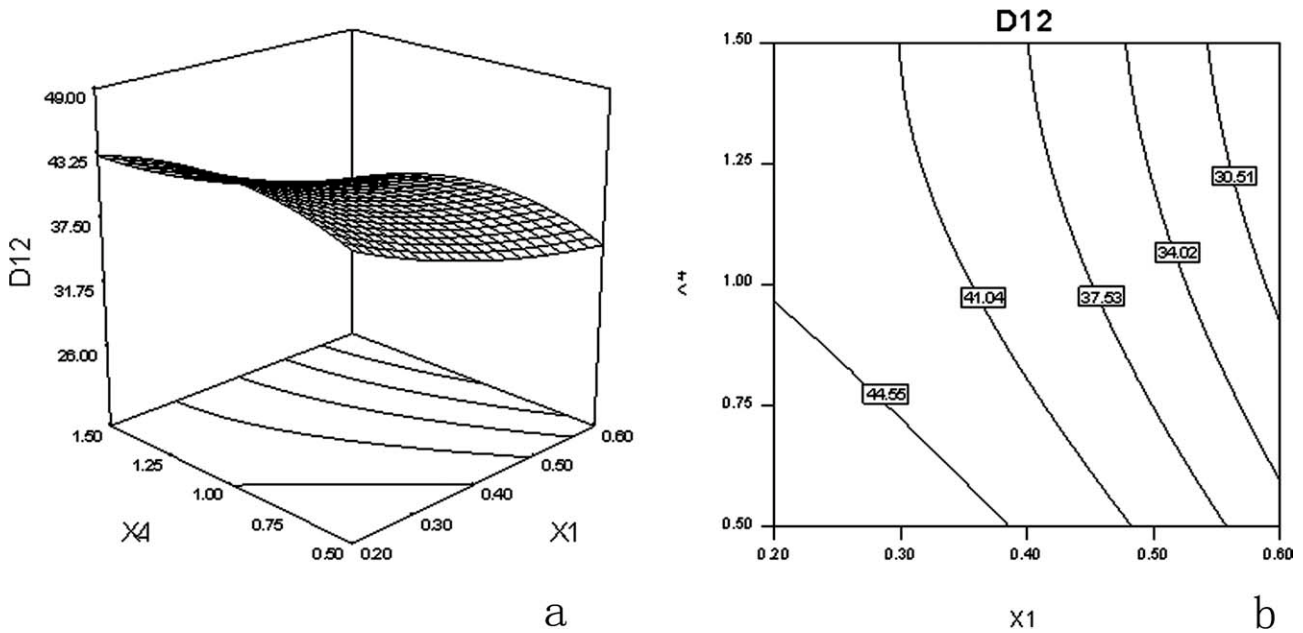

Figure 3 Surface plot and corresponding contour plot of D_{12} vs. X_1 and X_4.

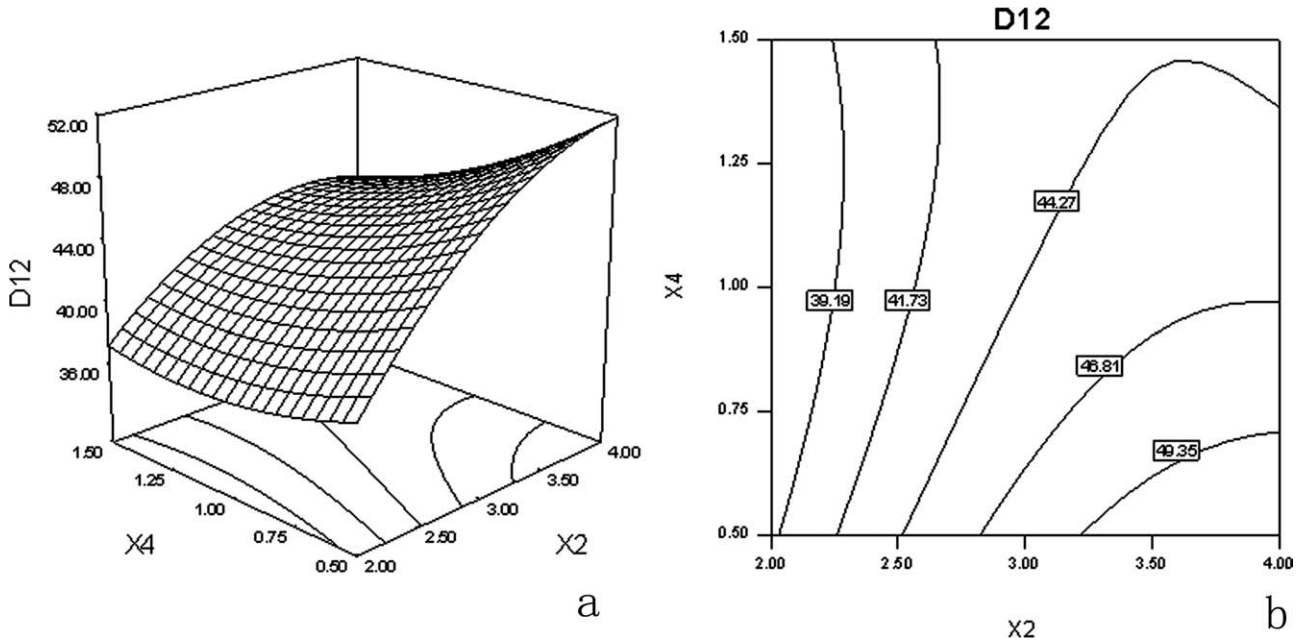

Figure 4 Surface plot and corresponding contour plot of D_{12} vs. X_2 and X_4.

As can be seen from Figure 8, when the NaCS–chitosan films were immerged in the SGF and SIF, no mass loss was observed that reveals that the NaCS–chitosan complex film is insoluble in the SGF and SIF and can not be degraded by pepsin and pancreatin. When the NaCS–chitosan complex samples were in the SCF, the degradation rate increased rapidly and more NaCS–chitosan film was degraded. However, little mass loss of the control samples in PBS was observed. These properties of the NaCS–chitosan complex make it a good candidate for the colon-specific drug delivery system.

In vitro drug release

5-ASA was chosen as a model drug to examine whether the capsule based on NaCS–chitosan complex may be degraded in the large intestine or not. The amount of 5-ASA released from the capsule based on NaCS–chitosan complex was determined

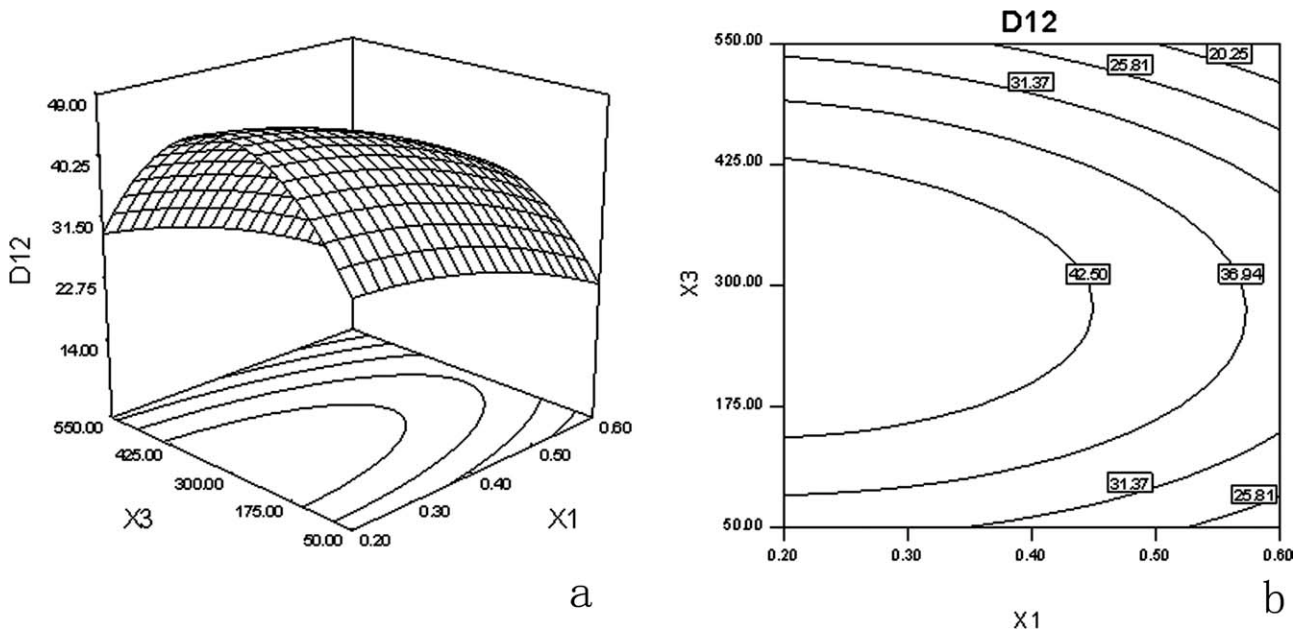

Figure 5 Surface plot and corresponding contour plot of D_{12} vs. X_1 and X_3.

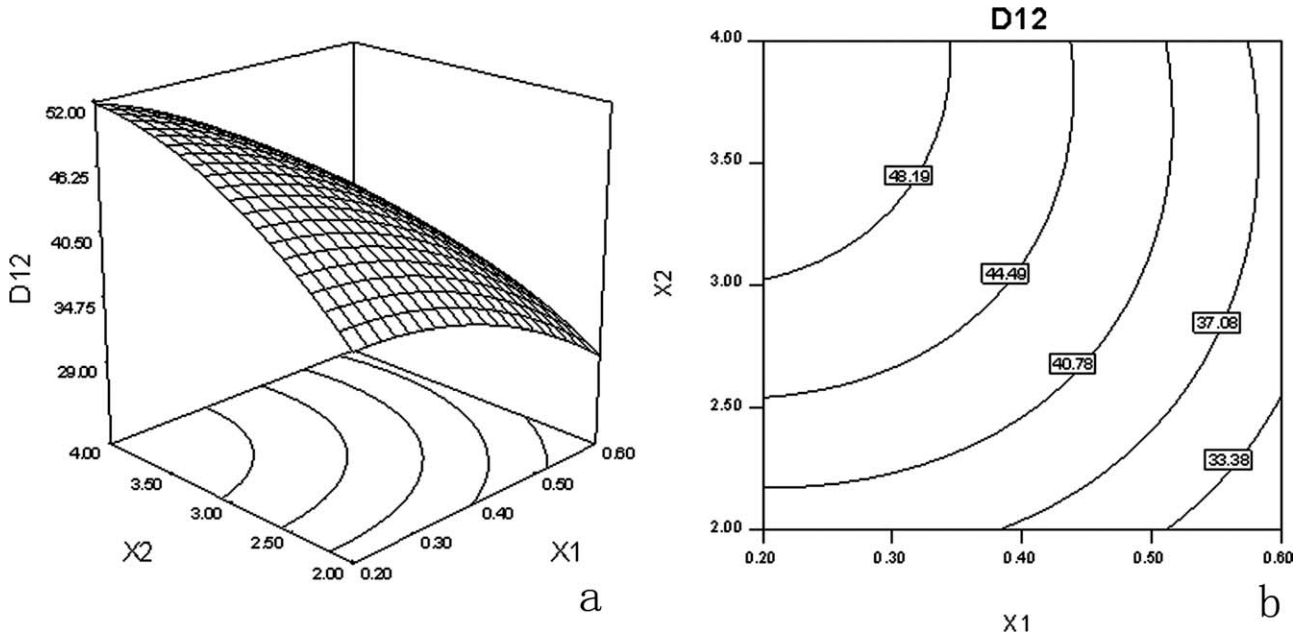

Figure 6 Surface plot and corresponding contour plot of D_{12} vs. X_2 and X_1.

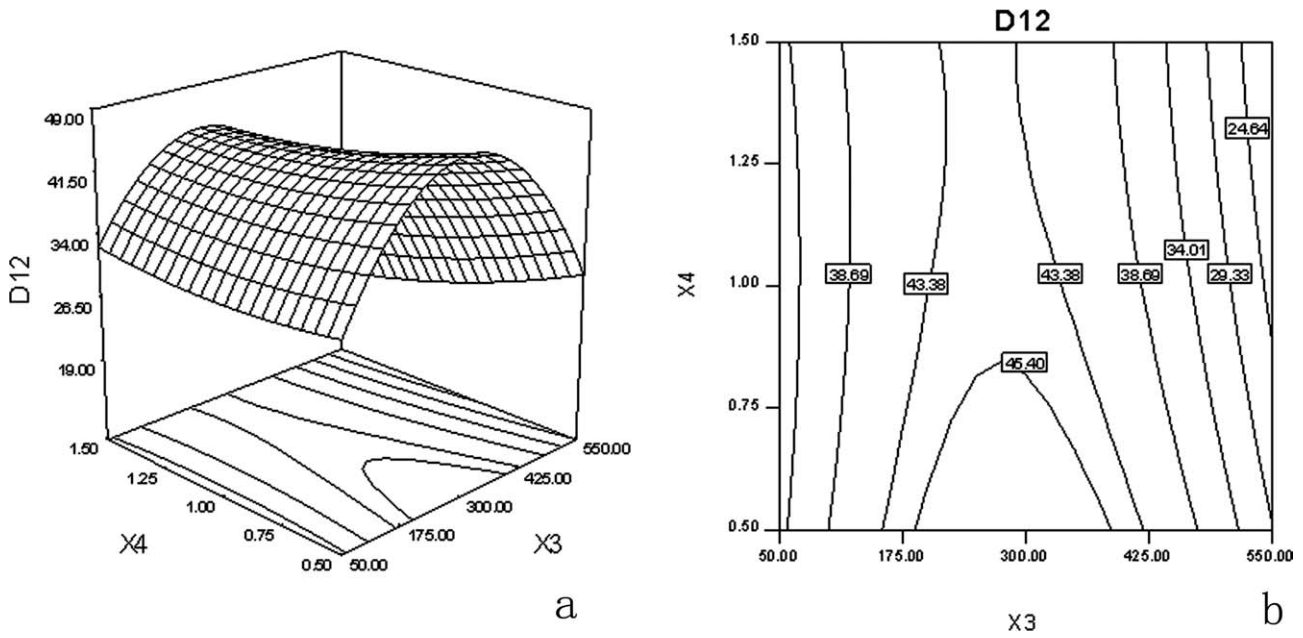

Figure 7 Surface plot and corresponding contour plot of D_{12} vs. X_4 and X_3.

TABLE VI
Comparison of Experimentally Obtained and Theoretically Predicted Values

	Actual level				D_{12}		
Tial No.	X_1	X_2	X_3	X_4	Experimental value	Predicted value	Percentage prediction error (%)
1	0.2	4	300	0.5	47.1	51.9	−8.81
2	0.6	3	300	0.5	31.3	35.3	7.93
3	0.4	3	200	0.5	44.9	42.5	5.65
4	0.4	2	400	1	28.1	26.8	4.85
5	0.4	3.5	50	1.5	24.3	25.5	−4.71

in the presence of rat cecal contents. The release-time profile of 5-ASA from capsules based on NaCS–chitosan complex was shown in Figure 9.

In the SGF, no release of 5-ASA was found, and in the SIF, 5-ASA began to be released from the capsules at a low rate. During 5 h, about 10% 5-ASA was found in SIF. The reason is that water permeated into the capsule, dissolved the 5-ASA and the drug began to be released from the capsule through the capsule shell. After the capsules immersed in the SCF, the release of 5-ASA was markedly increased in the presence of rat cecal contents. For capsules C1, a burst effect was observed between 6 and 7 h. After 7 h, almost 92% 5-ASA was released from the capsule based on NaCS–chitosan complex. For the capsules C2 and C3, the burst effect was observed between 7 and 9 h and the maximum drug release of 5-ASA were 82% and 75% for capsules C2 and C3, which appeared at 10 h and 11 h, respectively. In the control experiments, 5-ASA was released from the capsules based on NaCS–chitosan complex C1, C2, and C3 in phosphate buffered saline (PBS, pH 7.0) at almost constant rate and about 16 $\sim$ 18% 5-ASA was released from the capsules at time of 12 h. There was no significant difference in control experiment between capsules C1, C2, and C3.

From all the above observations, it was found that the D_{12} of NaCS–chitosan complex had little effect on drug release in PBS, but had significant effect on drug release in SCF. The lower D_{12} of NaCS–chitosan complex was, the later burst effect appeared and the lower the maximum drug release was. The reason was that increasing the D_{12} of NaCS–chitosan complex means the increased degradation in SCF, and then resulted in the capsules break and drug release. According to this result, different capsules could be made for different requirement. By applying Box-Behnken design to optimize and predict the selected factors may be an efficient method that design composition of pharmaceutical product. Besides, it converts the process factor correlations into a mathematical model that predicts where the optimum is likely to be located.

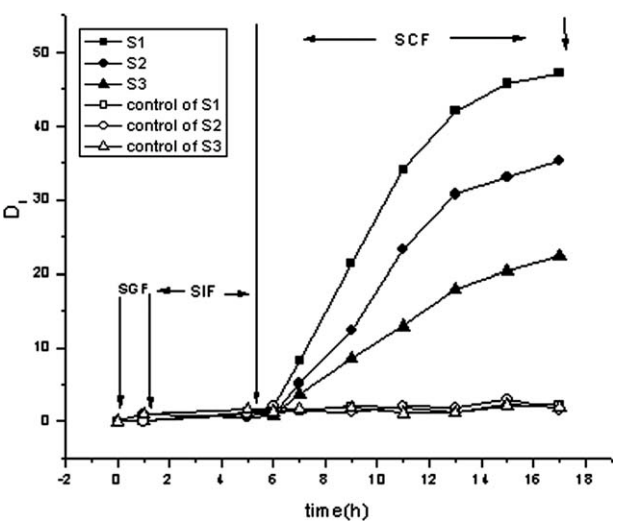

Figure 8 Degradation behavior of NaCS–chitosan film *in vitro.* The NaCS–chitosan films (Samples S1, S2, and S3) were immersed in the simulated gastric fluid (SGF), simulated intestinal fluid (SIF), simulated colonic fluid (SCF), and phosphate buffered saline (PBS 7.0) in sequence. The degradation percentage of Sample S1(■), Sample S2(●), Sample S3 (▲), Sample S1 in PBS after 5 h (□), Sample S2 in PBS after 5 h (○), Sample S3 in PBS after 5 h (Δ).

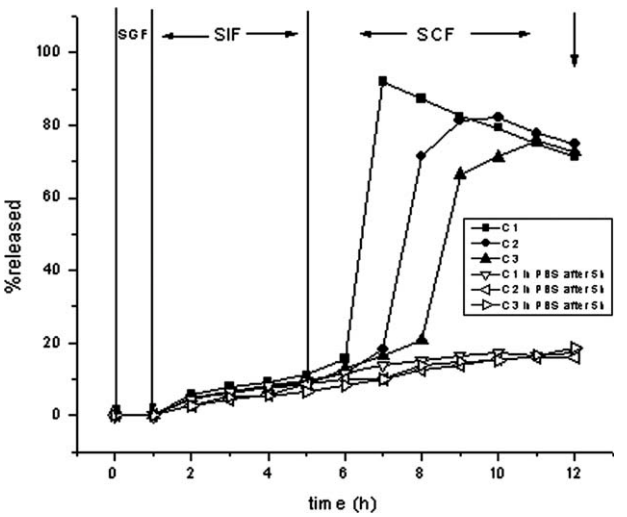

Figure 9 *In vitro* drug release of 5-ASA from capsules based on NaCS–chitosan. The capsules were immersed in the simulated gastric fluid for 1 h, then removed and immersed in the simulated intestinal fluid for 4 h, and then removed and immersed in the simulated colonic fluid for 7 h. The percent of released drug of Sample C1(■), Sample C2(●), Sample C3 (▲), Sample C1 in PBS after 5 h (□), Sample C2 in PBS after 5 h (○), Sample C3 in PBS after 5 h (Δ).

The results also revealed that NaCS–chitosan complex film has the good behavior at the colon-specific and could be a potential candidate for colon-specific drug delivery system.

CONCLUSIONS

In the present work, a four-factor-three-level Box-Behnken design combining with response surface methodology (RSM) was successfully employed to optimize and model the manufacturing process parameters of NaCS–chitosan complex. A mathematical model was then developed, and the quantitative effect of these factors at different levels could be predicted by using this polynomial equations. The optimum operation conditions for preparing NaCS–chitosan complex were determined as chitosan viscosity of 327 mPa s, DS of NaCS of 0.2, concentration of NaCS of 4.0% (w/v), concentration of chitosan 0.5% (w/v). In verification experiments, linearity observed

between the actual and predicted values of the response variables suggested the prognostic ability of the RSM design. Therefore, high degree of prediction obtained using RSM is quite efficient in optimizing drug delivery systems that exhibit non-linearity in responses.

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