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Original article

Chitosan–pectin polyelectrolyte complex as a carrier for colon targeted drug delivery

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ABSTRACT

Objective: The objective of present work was to prepare a polyelectrolyte complex (PEC) between chitosan (polycation) & pectin (polyanion) and to develop enteric coated tablets for colon delivery using the PEC.

Methodology: The PECs were prepared using different concentrations of chitosan and pectin. Drug loaded enteric coated tablets were prepared by wet granulation method using PEC to sustain the release at colon and coating was done with Eudragit S 100 to prevent the early release of the drug in stomach and intestine. Two independent variable, % PEC (chitosan/pectin) and % coating were optimized by 3^2 full factorial design. Statistical model were also used to supplement the optimization. DSC was performed to confirm the interaction between the polyions. Developed formulations were evaluated for physical appearance, weight variation, thickness, hardness, friability, % swelling, assay, *in-vitro* and *ex-vivo* drug release studies to investigate the PEC's ability to deliver the drug to colon. *Ex-vivo* release study using rat caecal content was also carried out on optimized formulation.

Results and discussion: DSC results confirmed chitosan/pectin interaction and subsequent formation of PEC. The optimized formulation containing 1.1% of PEC and 3% of coating showed highest swelling and release in alkaline pH mechanism of which was found to be microbial enzyme dependent degradation established by *ex-vivo* study using rat caecal content.

1. Introduction

In the development of colon targeted delivery interpolymer complex (IPC) hydrogels have generated considerable interest as vehicle for drug delivery. IPC provides free volume space for the easy encapsulation of drugs in its three-dimensional network structure commonly known as polyelectrolyte complex (PEC) obtained by cross-linking of two or more polymers.[1] The colon provides a site for drug delivery, with distinct advantages of a near neutral pH, a much longer transit time, relatively low photolytic enzyme activity, and greater responsiveness to absorption enhancers. Colon-specific delivery systems would prevent release of the drug in the upper-part of GIT but would require a triggering mechanism to affect an abrupt release on reaching the colon.[2,3] The successful targeted delivery of drugs to the colon via the gastrointestinal tract (GIT) requires the protection of a drug from degradation and release in the stomach and small intestine and then ensures abrupt or controlled release in the proximal colon.[4] Various properties of IPCs such as porosity, bio-adhesiveness, elasticity, swelling and stimuli-responsive behavior can be controlled by the appropriate choice of the network-forming polymers.[5] F. Bigucci et al (2008) have also earlier prepared chitosan/pectin polyelectrolyte complexes for colon-specific delivery of vancomycin but the present work focuses on economizing the development of drug loaded enteric coated tablets of PEC.

Several natural polysaccharides are extensively used to prepare the PEC. Chitosan is soluble at low pH of stomach hence there is a need to make enteric coated formulation that would protect it from stomach's environment. A coating with pH dependent polymer Eudragit S100 was done to prevent the early release of drug in stomach and intestine. As chitosan alone cannot provide sustained release at the site, pectin was used for cross-linking to formulate PEC. Pectin is highly water soluble, absorbed in the upper GIT, and completely degraded by the colonic bacterial enzymes. Accounting for all properties of PEC and coating polymer, tablets were prepared using various concentrations of PEC. The developed formulation showed pH dependent swelling and release in alkaline pH. *Ex-vivo* confirmed that polysaccharide degradation by colonic microbial enzyme was the prime source of drug release in colon.[6]

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2. Materials and methods

2.1. Materials

Theophylline was a gift from Sidmak Pharma, Vapi, India. Low molecular weight (LMW) chitosan (molecular weight = 50–190 kDa) was obtained from Balaji Drugs Ltd., Mumbai, India. Pectin, sodium hydroxide, glacial acetic acid, sodium acetate, sodium starch glycolate (SSG), potassium dihydrogen phosphate, magnesium stearate were obtained from S.D Fine Chemicals Ltd. Erythrosine was bought from Yarrow Chemicals Ltd, India. Talc was obtained from Chem Port Pte. Ltd., India. All other chemicals used were of analytical grade.

2.2. Drug – excipients compatibility study

2.2.1. Fourier transform infrared spectroscopy (FT-IR)

Compatibility of theophylline with the individual excipients was established by FT-IR. Any change in the chemical composition after combining with excipients was investigated with IR spectral analysis. The IR spectra of drug and drug + excipients were recorded in FT-IR (Bruker Optics Alpha) in the range of 4000–500 cm^{-1}.

2.3. Preparation of chitosan/pectin IPC

Chitosan solution (1% w/v) was prepared in acetic acid. Pectin solution (5% w/v) was prepared in distilled water. Both the solutions were sonicated separately till their clear solutions were obtained. After that both solutions were mixed, agitated for 30 min and then kept for drying for 5 h to yield a dry powder.

2.4. Characterization of IPC

2.4.1. By DSC

Chitosan, pectin and PEC were subjected to DSC study using Shimadzu DSC 60 (Kyoto, Japan). 10 mg sample was heated in aluminum pan at a rate of 20 °C/min in the temperature range of 50–300 °C under the nitrogen flow of 40 ml/min.

2.5. Tablet preparation

Drug and all excipients except PEC were passed through the 60 # sieve, and then PEC was mixed. Dry mass was granulated using isopropyl alcohol. The granules so obtained were dried at 50 °C for 1 h in the oven. Dried granules were passed through 22 # sieve and fines were separated using 44 # sieve. SSG was passed through 60 # sieve and mixed with dried granules. These granules were lubricated with magnesium stearate and talc. The lubricated granules were compressed into tablets using tablet compression machine (Rimek Mini Press-I, India). Weight variation, hardness, friability and disintegration test were performed for the core tablets. Formulation composition of the tablet is given in Table 1.

Table 1
Tablet composition.

Ingredient	Quantity
Theophylline	100 mg
Sodium starch glycolate	4% w/w
Chitosan:pectin IPC (1:5) (%w/w)	1%, 2%, 3%
Magnesium stearate	0.02 g
Talc	0.01 g
Lactose monohydrate q.s	400 mg

2.6. Preparation of coating solution

Coating solution was made by dissolving Eudragit S100 in isopropyl alcohol. A bath sonicator was used to homogenize the coating solution. After sonication PEG 400 (1.25%v/v) as plasticizer, was added to the solution. The coating solution so prepared was applied on tablets by intermittently spraying it through a pilot type spray gun fitted with 1 mm spray nozzle in pan coater (Hardik Manufacturers, India). The spray dispersion was maintained at a rate of 4–8 ml/min and bed temperature of 35–40 °C at 35 rpm. The 3^2 full factorial design was employed in which two factors were evaluated, each at 3 levels and experimental trials were performed at all 9 possible combinations. The amount of PEC and % coating were selected as independent variables while drug release at 4 h (Q_4) and at 12 h (Q_{12}) were selected as dependent variables (Table 2a and b).

As shown in Eq. (1), a statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1^2 X_2 + \beta_7 X_1 X_2^2 + \beta_8 X_1^2 X_2^2 \quad (1)$$

where, Y is dependent variable, b_0 is arithmetic mean response, β_1 is coefficient of factor X_1, The main effects X_1 and X_2 represent the average result of changing one factor at a time from its low to high value. The interaction terms, X_1X_2, $X_1^2X_2$, $X_1X_2^2$ and $X_1^2X_2^2$, show how the dependent variable changes when two or more factors are simultaneously changed. Statistical analysis was done using Microsoft Excel 2007. Contour plots and response surface plots were drawn using Design Expert software.[7–9]

2.7. Evaluation of core tablets

Core tablets were evaluated for appearance, diameter, thickness, weight variation, hardness, friability, hardness, disintegration, drug content and content uniformity test. Weight variation test and disintegration test were performed as per USP.[10,11] Hardness, thickness and friability were determined by Monsanto Hardness Tester, Digital Vernier Calipers and Roche Friabilator, respectively.

2.7.1. Swelling study

Three tablets from each batch were weighted accurately and immersed in dissolution medium (100 ml of 0.1 N HCl) for 2 h. For next 1 h tablets were kept in acetate buffer (pH 4.6), after that for next 4 h tablets were placed in phosphate buffer (pH 6.8) and then onwards tablets were placed in phosphate buffer (pH 7.4) for next 5 h. Tablets were taken out carefully after each time interval up to 12 h, blotted with filter paper to remove the fluid present on the surface and weighed accurately. Percentage swelling (swelling index) was calculated using the following formula.[12]

$$\%\ \text{Swelling index} = \frac{\text{Wet weight of tablet} - \text{Dry weight of tablet}}{\text{Dry weight of tablet}} \times 100 \quad (2)$$

2.7.2. Drug release study

2.7.2.1. In-vitro drug release study of Eudragit S 100 coated tablets. *In-vitro* dissolution studies of coated tablets were carried out using

Table 2a
Layout of factorial design.

Translation of coded values in actual units			
Independent variables	Levels used, actual (coded)		
	Low (−1)	Medium (0)	High (+1)
X_1 = amount of PEC	1	2	3
X_2 = % of coating	3%	5%	7%
Dependent variables			
1. Percentage cumulative release at 4 h (Q_4)			
2. Percentage cumulative release at 12 h (Q_{12})			

Table 2b
3^2 Full factorial design matrix with interaction terms.

Batch	X_1	X_2	X_{12}	X_{11}	X_{22}
F1	−1	−1	+1	+1	+1
F2	0	−1	0	0	+1
F3	+1	−1	−1	+1	+1
F4	−1	0	0	+1	0
F5	0	0	0	0	0
F6	+1	0	0	+1	0
F7	−1	+1	−1	+1	+1
F8	0	+1	0	0	+1
F9	+1	+1	+1	+1	+1

dissolution test apparatus USP I paddle type. The dissolution medium consisted of 900 ml of pH 1.2 for first 2 h followed by pH 4.6 and 6.8 phosphate buffers for 1 and 3 h respectively and finally pH 7.4 phosphate buffer for the remaining time period up to 12 h. The temperature of the medium was maintained at 37 ± 0.5 °C and the speed of rotation of the basket was 50 rpm. Aliquots of 10 ml were withdrawn after every hour and replaced with the fresh dissolution medium equilibrated at the same temperature. The absorbance of the samples was determined at wavelength according to the dissolution medium pH 1.2 (λ_{max} = 263 nm), 4.6 (λ_{max} = 271 nm), 6.8 (λ_{max} = 272 nm) and 7.4 (λ_{max} = 267 nm) using UV–Visible spectrophotometer (UV-1800, Shimadzu) against respective buffer solutions as a blank.

2.8. Release drug data modeling

2.8.1. Korsmeyer–Peppas model[13]

$$\frac{Mt}{M\infty} = kt^n \quad (3)$$

where, k is the rate constant and n is release exponent that characterizes the mechanism of drug release. For cylindrical matrix tablets, if $n = 0.45$ it indicates drug release mechanism by Fickian diffusion, and if $0.45 < n < 0.89$, it is non-Fickian or anomalous diffusion.

2.9. Ex-vivo release study

The drug release studies were carried out using USP dissolution rate test apparatus (apparatus 1, 100 rpm, 37 ± 0.5 °C). Each formulations after completing the dissolution study in 0.1 N HCl (2 h), acetate buffer pH 4.6 (1 h) and phosphate buffer pH 6.8 (3 h) was placed in the basket of the apparatus and immersed in 4% rat cecal content medium (phosphate buffer pH 7.4). At fixed time intervals, 10 ml of sample was withdrawn, replaced with 10 ml of fresh phosphate buffer pH 7.4 and the experiment was continued for another 6 h. Sample was filtered and analyzed for theophylline by UV Spectrophotometer at 263 nm, 271 nm, 272 nm, 267 nm for 0.1 N HCl, acetate buffer pH 4.6, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 respectively .[14]

3. Results and discussion

3.1. By FT-IR

The FT-IR spectra of theophylline, excipient mixture and theophylline and excipient mixture have been shown in Fig. 1. The spectra exhibited no change in the absorption peaks of theophylline vis-à-vis the theophylline + excipient mix so it can be concluded that there is no interaction between the components.

3.2. Characterization of prepared IPC

3.2.1. By DSC

The chitosan showed the first melting point peak at 56.7 °C and second at 312 °C. Pectin showed broad peak at 238 °C. DSC curve of PEC mixture (IPC) of chitosan and pectin showed peak at 215 °C. The results of DSC are in compliance with observations of Ghaffari et al[15] who had reported interaction (chitosan and pectin) temperature in the range of 210–220 °C. The shift to lower temperature in melting point peak of the complex indicates that the probable formation of ionic bonds between chitosan and pectin is correlated to the loss of organization (Fig. 2).[16]

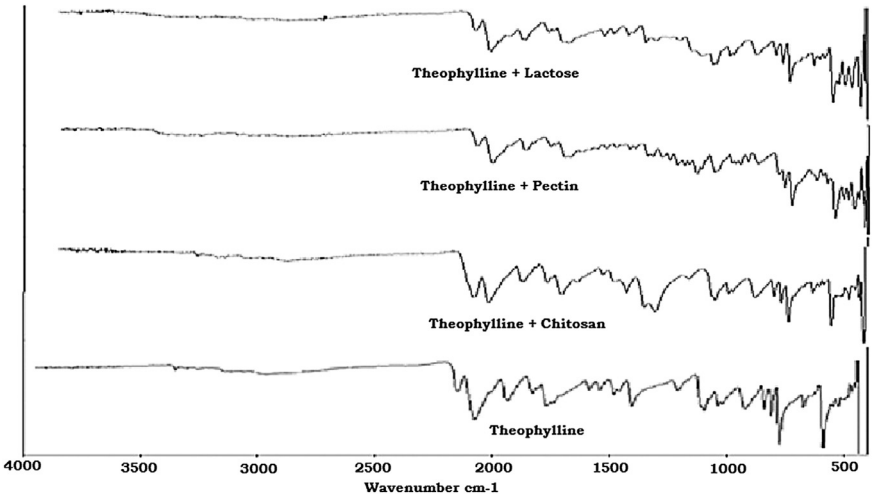

Fig. 1. FT-IR spectra of theophylline and excipient mixture.

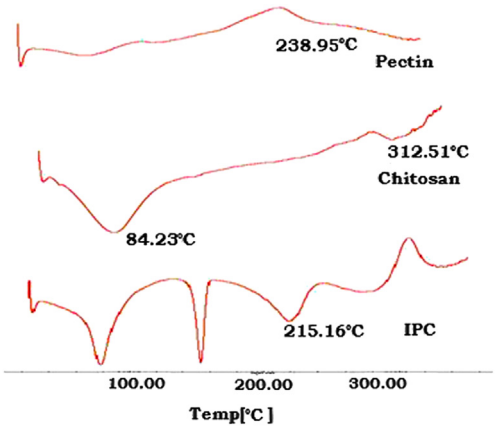

Fig. 2. DSC graph of pectin, chitosan, IPC.

3.2.2. Evaluation of physical properties of tablets

Table 3 lists the physical properties (thickness, diameter, weight, hardness, friability) and % assay of tablet. They produced with small weight variations (less than 6%); uniform thickness and hardness; acceptable friability (less than 1%) and % drug content. The average weight of tablet formulation was within the range of 387.6 ± 0.028 to 410.7 ± 0.091 mg. The hardness of the tablets ranged between 6.9 ± 0.14 to7.4 ± 0.14 kg/cm^2. In case of content uniformity test, drug content was found to be within 95.5 ± 0.03% to 98.23 ± 0.023% of labeled amount.

3.2.3. Swelling studies

The enteric coated tablets showed pH sensitive swelling behavior. The degree of swelling is mainly affected by pH of the medium, type of anionic polymer and amount of IPC (chitosan and pectin). As pH of the swelling medium changes (1.2, 4.6, 6.8 and 7.4) the degree of interaction between chitosan and pectin is modified and swelling occurs because of the dissociation of the complex. In acidic medium, pectin is neutralized and free positive charge ($NH3^+$) appears inside the gel; while in basic medium, chitosan is neutralized and free negative charge (COO^-) appears inside the gel. The mutual repulsion between positive or negative charges and the entry of water together with counterions to neutralize these charges cause swelling. All the tablets showed low swelling in pH 1.2 (0.1 N HCl), pH 4.6 (acetate buffer) and pH 6.8 (phosphate buffer). In pH 7.4 (phosphate buffer), the degree of swelling was increased due to deprotonation of chitosan. It was observed that as the concentration of IPC increased the swelling index decreased. Batch F4 (2% IPC, 5% coating) showed maximum swelling index of 79.51 ± 1.61%. Thus IPC tablet is hydrophilic and swells considerably in phosphate buffer solutions. This process shows a pH-dependent pattern. There also exists the possibility of intramolecular hydrogen bonding between the OH or $COOCH_3$ groups within the network of the IPC. However, at pH 7.4, the $-NH_2$ group of chitosan will be partially unionized. Therefore, at this pH, the IPC network will be loose as a result of suboptimal NH_3^+- $-OOC$ ionic interaction. In other words, stronger ionic interaction may result in a tightening of the IPC network leading to a reduced swelling capacity at pH 6.8 as compared with pH 7.4.

Table 4
Evaluation of enteric coated tablets.

Batch code	Weight of tablet[a] (mg)(n = 20)	% Coating	Disintegration time in pH 7.4 phosphate buffer
F1	412.25 ± 1.62	3	25 min 58 s
F2	420.32 ± 1.25	5	32 min 42 s
F3	428.86 ± 1.54	7	38 min 21 s
F4	412.14 ± 0.24	3	28 min 34 s
F5	420.28 ± 0.53	5	35 min 28 s
F6	428.58 ± 0.18	7	40 min 14 s
F7	412.21 ± 0.23	3	30 min 84 s
F8	420.38 ± 0.75	5	38 min 54 s
F9	428.67 ± 0.90	7	44 min 25 s

[a] Value expressed as mean ± SD.

3.2.4. Evaluation of enteric coated tablets

Tablets weight, % coating and disintegration time of coated second pulse tablets are reported in Table 4. Weight variation of enteric coated tablets for all batches (F1 to F9) was below 3%. The weight gain calculated from the average tablet weight for % Coating was found to be in the range of 3–7%. In the disintegration test, all the tablets remained intact in 0.1 N HCl for a 1 h and showed no cracks or fracture. Same tablets disintegrated within 45 min in pH 7.4 indicating that the disintegration time in pH 7.4 increased with increase in % weight. Eudragit S 100 was used in the preparation to target the colon. Isopropyl alcohol was used as a solvent for the coating dispersion due to its high flash point (15°), low boiling point (82.3°), and low heat of evaporation (667 J/g). Eudragit is a brittle polymer hence it was needed to lower the glass transition temperature of polymer and promote formation of a good elastic film. Polymer softening tendencies stimulate sticking at higher temperature, so the bed temperature was maintained at 35–40 °C using an infrared lamp. To get fine spray droplets the spray nozzle of 1 mm was used at an atomization air pressure of 4 kg/cm^2. By controlling the rotating pan at speed of 20 rpm and the rate of spray dispersion application the sticking tendency of the tablets was overcome.

3.2.5. Drug release study

3.2.5.1. In-vitro drug release of factorial batches. All the formulations were evaluated for *in-vitro* drug release. The cumulative % theophylline release, from all 9 formulations as the function of the time is shown in Fig. 3.

Cumulative drug release profile of factorial batches (F1 to F9) showed sigmoidal drug release pattern (Fig. 3). The *in-vitro* release of drug from formulations was mainly affected by concentration of

Table 3
Evaluation parameters of tablet.

Batch	Average weight (mg) (n = 20)	Hardness (kg/cm^2) (n = 3)	Friability (%) (n = 6)	Thickness (mm) (n = 6)	Diameter (mm) (n = 6)	Assay (%) (n = 3)	Disintegration time*(min) (n = 6)
F1	392.6 ± 0.018	7.08 ± 0.14	0.37 ± 0.21	4.26 ± 0.023	10.27 ± 0.009	99.3 ± 0.32	3.5 ± 0.124
F2	410.7 ± 0.091	7.17 ± 0.29	0.33 ± 0.34	4.26 ± 0.040	10.29 ± 0.015	99.1 ± 0.73	3.8 ± 0.125
F3	399.3 ± 0.012	6.92 ± 0.14	0.43 ± 0.23	4.25 ± 0.016	10.29 ± 0.010	99.54 ± 0.36	3.7 ± 0.124
F4	387.6 ± 0.028	7.17 ± 0.14	0.33 ± 0.25	4.25 ± 0.024	10.30 ± 0.010	99.52 ± 0.37	3.6 ± 0.123
F5	420.1 ± 0.1	7 ± 0.0	0.58 ± 0.31	4.24 ± 0.023	10.29 ± 0.005	99.57 ± 0.17	3.9 ± 0.127
F6	416.1 ± 0.01	7.42 ± 0.14	0.50 ± 0.26	4.25 ± 0.026	10.30 ± 0.007	99.59 ± 0.21	4.0 ± 0.127
F7	400.9 ± 0.085	6.92 ± 0.14	0.29 ± 0.36	4.27 ± 0.012	10.29 ± 0.014	98.72 ± 0.11	4.1 ± 0.128
F8	403.4 ± 0.018	7.08 ± 0.29	0.41 ± 0.38	4.25 ± 0.040	10.29 ± 0.007	99.64 ± 0.18	3.8 ± 0.126
F9	386.8 ± 0.056	7.50 ± 0.0	0.70 ± 0.35	4.24 ± 0.030	10.29 ± 0.009	98.68 ± 0.27	3.7 ± 0.127

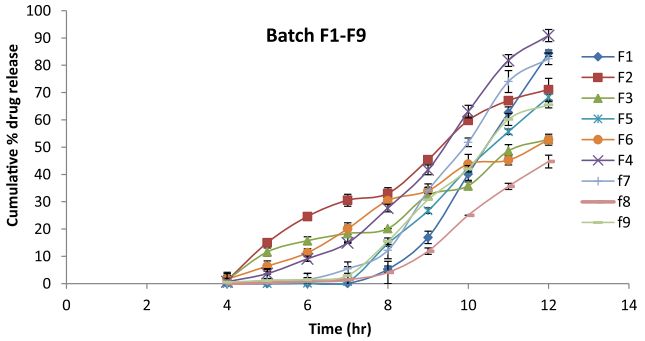

Fig. 3. *In-vitro* dissolution of all formulations.

chitosan, types of anionic polymers (pectin) used, % coating and swelling behavior of the polymer. Thus in turn it mainly depends upon the amount of IPC used. The *in-vitro* release study was performed in HCl buffer (pH 1.2) for initial first 2 h. Then the medium was replaced by acetate buffer (pH 4.6) for 1 h, followed by phosphate buffer (pH 6.8) for 3 h. Study was continued for next 6 h in phosphate buffer (pH 7.4). Eudragit S100 is soluble at pH near 7.0 hence it dissolves at pH 7.4 and leads to formation of porous surface at the coating layer which allows medium to diffuse into the core tablet to ruptures outer coat. Thus, the level of coating plays a very important role for in optimizing the formulation. At pH 1.2 and pH 4.6 none of the formulations showed any release of drug due to enteric coating, while some batches started releasing the drug at pH 6.8. The release of drug was a maximum of 90.90% from batch F4 (2% IPC, 5% coating) near to pH 7 at colon site. The ionic interaction between chitosan and negatively charged pectin was greatly reduced at colonic pH forming a loose network. This increased porous surface allowed entry of large amount of dissolution media leading to complete release of drug. Hence, it can be assumed that at pH 7.4 of phosphate buffer, rapid dissociation of IPC membrane occurs which leads to drug release.

3.2.6. Drug release data modeling

The mechanism of drug release from enteric coated tablets containing PEC is complicated and not completely understood. Some systems may be classified as either diffusion or erosion controlled, while the most systems exhibit a combination of these mechanisms.[17] The drug release data of all the 9 batches were mathematically analyzed using Korsmeyer–Peppas semi-empirical model equation. The linearity of the model was evaluated by calculating the linear correlation coefficient (r^2), while, the release mechanism was determined by evaluating the release exponent (n).

Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit. The r^2 and n values are given in Table 5. The r^2 values ranged between 0.9460 and 0.9759 for all the formulations. The n value were in the range of 0.555–0.686 (i.e. $0.5 < n < 1$), indicating that, the release mechanism of theophylline from these matrices is an anomalous (non-Fickian) transport, which suggests that, both diffusion of the drug from the hydrated matrix and its own erosion modulate drug release.[18,19]

Table 5
Results of kinetic model fitting for factorial batches (F1–F9).

Batch	Korsmeyer–Peppas	
	r^2	n
F1	0.9595	0.686
F2	0.9460	0.692
F3	0.9516	0.632
F4	0.9749	0.614
F5	0.9759	0.650
F6	0.9636	0.637
F7	0.9180	0.620
F8	0.9518	0.612
F9	0.9514	0.555

3.2.7. Data analysis

3.2.7.1. Data analysis for drug release at 4 h (Q_4). There was not much difference between actual and predicted values for all 9 batches. r^2 Value in plot of predicted v/s observed responses was 0.9899 which indicated excellent fit. The response (Y_1) obtained at three levels of the two independent variables (X_1 and X_2) were subjected to multiple regression to yield a polynomial Eq. (4). Equation clearly reflects the wide range of values for coefficients (b)

$$Q_4 = 0.11 + 0.47X_1 - 0.17X_2 - 0.22X_1X_2 + 0.80X_{12} - 0.30X_{22} \quad (4)$$

In the present study, coefficients b_1 and b_2 possessed positive and negative sign indicating synergistic and antagonistic effect of variables X_1 and X_2 on response Y_1 (Q_4). Among two independent variables, X_1 (amount of PEC) has prominent effect ($b_1 = 0.47$ and $p = 0.0022$) on Q_4, whereas to some extent X_2 (% coating) also affects the results ($b_2 = 0.17$ and $p = 0.1299$) in inverse manner. Significance (F value) is less than 0.05. The high values of the coefficient of determination indicate a good fit i.e. good agreement between the dependent and independent variables. The coefficients b_1 was found to be significant at $p < 0.05$ but b_2 was found to be insignificant at $p > 0.05$.

3.2.7.2. Data analysis for drug release at 12 h (Q_{12}). The r^2 value for Q_{12} in plot of predicted v/s observed responses was 0.9593 which indicated excellent fit. The Y_2 (Q_{12}) values observed for different batches showed wide variation i.e. values ranged from a minimum of 44.74 ± 2.34% to a maximum of 90.90 ± 2.25%. There was not much difference between actual and predicted values. Eq. (5) refers to polynomial equation (full model) for response Y_2.

$$Q_{12} = 0.105 + 0.474X_1 - 0.168X_2 - 0.217X_1X_2 + 0.797X_{12} - 0.297X_{22} \quad (5)$$

Coefficient b_1 and b_2 possessed positive and negative sign respectively which indicated positive effect of X_1 and antagonistic effect of X_2 variables on response Y_2 (Q_{12}). Independent variables, X_1 (amount of cross-linking) ($b_1 = 0.474$ and $p = 0.0039$) affects more prominently the Q_{12} then X_2 (% coating) ($b_2 = 0.168$ and $p = 0.4685$) which has inverse effect on Q_{12}. Significance (F value) is less than 0.05. The coefficient b_1 was found to be significant at $p < 0.05$ and b_2 was found to be insignificant at $p > 0.05$ Table 6.

Two-dimensional contour plots and three-dimensional response surface plots are presented in Fig. 4a–d which are useful tool to study interaction effects of the factors on responses. Fig. 4a and c exhibited non-linear pattern but with an increase in amount of PEC and % of coating it also showed that amount of PEC has a comparatively greater influence on response variable Q_4 then % of coating. In contrast to the result of drug release at 4 h contour plot of drug release in 12 h varies in non-linear manner with an increase in amount of PEC and % of coating (Fig. 4b and d). However, the effect of X_1 (amount of PEC) seems to be more pronounced as compared with that of % coating. The results was also confirmed through 3D response surface graph.

3.2.8. Selection of optimized formulation

From the polynomial equation and the contour plots, the optimized batch T1 was found. The optimum formulation was selected based on the criteria of attaining the constraints of variables

Table 6
Summary of results of multiple regression analysis of Q_4 and Q_{12}.

Dependent variable	$Q_4 = Y_1$		$Q_{12} = Y_2$	
	P value	Coefficient	*P* value	Coefficient
Intercept	0.0030	0.11	0.0390	67.57
X_1	0.0022	0.47	0.0039	−14.43
X_2	0.1399	−0.17	0.4685	−2.63
X_{12}	0.1299	−0.22	0.4081	3.68

response. Upon 'trading of' various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with amount of PEC (1.1%) and coating (3%) was found to fulfill the requisite of an optimum formula. *In-vitro release* data of optimized formulation are showed 92% theophylline release upon completion of 12 h but only 0.096% release in initial first 4 h. Composition of the optimized batch T1 was also very economical as compared to the next best batch, F4, obtained from factorization.

3.2.9. Validation of response surface methodology

Two check points were selected for validation of response surface methodology from contour and response surface plots. For all of the 2 checkpoint formulations, the results of the dependent variables (Q4 and Q12) were found to be within limits.

3.3. Ex-vivo release data

The drug release data in presence of rat caecal contents are shown in Fig. 5. Rat caecal microflora was used because of the inherent similarity with human intestinal microflora. The main aim of the drug delivery system targeted to the colon is not only to protect the drug from being released in the physiological environment of stomach and small intestine, but also to release the drug in the colon after enzymatic degradation of polysaccharide. Hence, the *in-vitro* drug release studies were carried out in phosphate buffer (pH 6.8) and phosphate buffer (pH 7.4) containing 4% w/v of rat caecal content. Optimized batch T1 shows 98.58% drug release in presence of rat caecal content. There was increase in drug release in the dissolution medium containing rat cecal content (4%) as compared to control group (without rat caecal content).

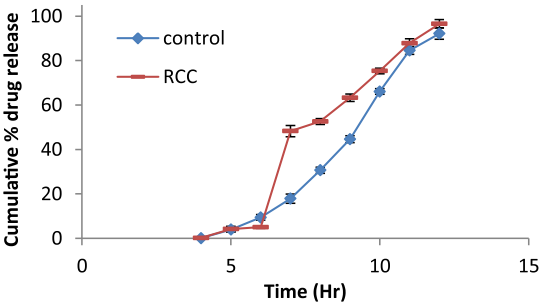

Fig. 5. Drug release profile of optimized formulation with and without rat cecal content. (No drug release in presence of rat caecal content was observed in 0.1 N HCl and phosphate buffer 4.6 after 3 h).

4. Conclusion

Chitosan–pectin polyelectrolyte complex was prepared by simple interaction of positively charged chitosan with negatively charged pectin. The interaction was confirmed by DSC. The optimized batch consisting of PEC (1.1%) and coating (3%) showed 92.16% drug release within 12 h. The *ex-vivo* drug release was found to be 98.58 ± 1.92% in presence of rat caecal content.

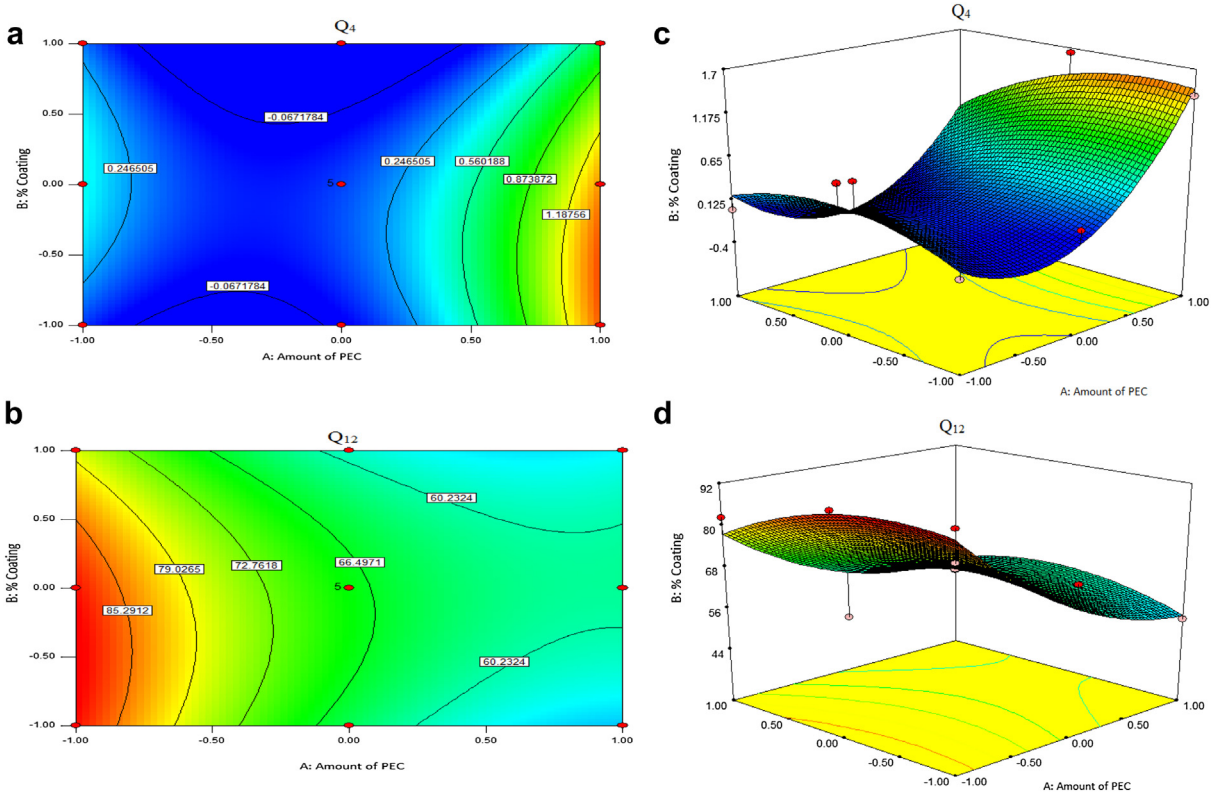

Fig. 4. (a): Contour plot Q_4, (b): contour plot Q_{12}, (c): response surface of Q_4, (d): response surface of Q_{12}.

Conflicts of interest

All authors have none to declare.

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