

# Studies on Applicability of Poly electrolyte Complex of Vancomycin Hydrochloride for Colon Targeting Beads Using Statistical Optimization

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## Abstract

The present study was initiated to develop colon targeted enteric coated hard capsule containing chitosan-based polyelectrolyte complex (PEC) beads of vancomycin HCl 500 mg. The chitosan-based PEC beads were developed and optimized using 3<sup>2</sup>-full factorial design based on two independent factors were evaluated, each at three levels for all nine possible combinations. The PEC beads were prepared by an ionic gelation method using positively charged chitosan and negatively charged hupu gum. The vancomycin HCl is an orally administered amphoteric glycol peptides, an antimicrobial substance used in the treatment of *enterocolitis* caused by *Staphylococcus aureus* and antibiotic-associated with pseudomembrane colitis caused by *Clostridium difficile*. Hence, localization of the drug at its site of action is more useful. The pharmacokinetic parameters of the drug also offer feasibility for colon-specific drug delivery and were developed with a view to have lag time 4–6 h, controlled release in the colon over a period of 16–20 h. The final optimized formulation is further subjected to enteric coating using Eudragit-S100 and subjected to *in vitro* studies. The optimized formulation showed <12% drug release during lag time, 91.34% at 20 h. The study was carried out to check the ability of PEC beads to release the vancomycin HCl in the presence of rat cecal content medium resembling the physiological environment of colon. The results for this *ex vivo* revealed that optimized formulations are susceptible to colonic enzymes released from rat cecal contents. The significance of differences was evaluated by analysis of variance.

**Key words:** Enteric coating, *ex vivo* studies, lag time, polyelectrolyte complex, vancomycin HCl

## INTRODUCTION

The interpolymer complex provides space for the easy encapsulation of drugs in its three-dimensional network structure commonly known as polyelectrolyte complex (PEC) which is obtained by cross-linking of two or more polymers; hence, the polyelectrolyte complex can be considered as a vehicle for colon targeted drug delivery.<sup>[1]</sup>

The colon provides the therapeutically acceptable site for drug delivery with advantages of neutral pH, longer transit time, low photolytic enzyme activity, and greater responsive to absorption enhancers. Colon-specific drug release systems would prevent the drug to release in the stomach, but it requires controlled the release of drug in the colon.<sup>[2]</sup>

The novel approach which can be considered for controlled drug delivery in the colon is polyelectrolyte complex, and also very few noted research, was done using colon-targeted delivery using PEC. Bigucci *et al.* (2008) have also earlier prepared chitosan/pectin PECs for colon-specific delivery of vancomycin HCl. However, the present work focuses on the development of drug loaded enteric coated hard capsule containing PEC beads using chitosan and Hupu

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gum with polyethylene glycol 400 and Eudragit S100 are extensively used to prepare the PEC. Chitosan is soluble at low pH of stomach; hence, there is a need to make an enteric coated formulation that would protect it from stomach's environment. A pH-dependent polymer Eudragit S100 was coated over the formulations to prevent drug release in the stomach region and starts erosion in the colon region. As chitosan along with hupu gum, polyethylene glycol 400 and Eudragit S100 will form PEC which can releases drug slowly from it, and considered in this colon-targeted controlled release formulation developments.

The developed formulation showed pH-dependent swelling and released the drug in alkaline pH. *Ex vivo* confirmed that polysaccharide degradation by colonic microbial enzyme was the prime source of drug release in the colon.<sup>[3]</sup> The development and optimization of enteric coated hard capsule containing PEC beads were carried out using 3<sup>2</sup> full factorial design based on evaluations of two independent factors each at three levels and subjected to all nine possible formulations.

Vancomycin HCl was considered as a model drug in PEC based colon-targeted drug delivery which is an orally administered amphoteric glycol peptides antimicrobial substance used in the treatment of *Enterococcus coliitis* caused by *Staphylococcus aureus* and antibiotic-associated to *Pseudo membrane coliitis* caused by *Clostridium difficile*. The mentioned diseases are the abnormal pathophysiological conditions of the colon. Hence, localization of the drug, vancomycin HCl targeting at its site of action is more useful. It is an orally not appreciably absorbed from the GI tract, as such the drug from a conventional dosage form does not reach the site of action in sufficient quantities and larger doses are needed for effective concentration levels in the colon. The present investigations of colon targeting drug delivery are done by a hard capsule containing PEC beads formulations. The pharmacokinetic parameters of vancomycin HCl also offer feasibility for colon-specific drug delivery and were developed with a view to have lag time 4-6 h, also to provide controlled release in the colon over a period of 16–20 h.

## MATERIALS AND METHODS

Vancomycin HCl was kindly supplied as gift sample by concord biotech limited Dholka (Gujarat) India. Low molecular weight chitosan was purchased from Kemphasol, Mumbai, India. Hupu gum was procured from Girijan Co-operative Corporation Limited Rajahmundry, India. Eudragit -S100 was obtained from Evonik industries. Polyethylene glycol 400, Sodium Tri polyphosphate, and glacial acetic acid were obtained from SD Fine Chem Ltd. (India). All other chemicals used were of analytical grade. In addition, a dissolution apparatus TDT-08L (Lab India Disso-8000), a ultraviolet-visible (UV-vis) spectrophotometer (Elico, Mumbai), a disintegration apparatus (Electrolab), an electronic balance (Shimadzu AX200), a pH meter (Systronics

model EQMK VI), a sonicator (Spectra Lab, model UCB 40), and a hot air oven (Labhosp) were used in this study.

### Drug and excipients compatibility study

Compatibility of vancomycin HCl with the individual excipients was established by Fourier-transform infrared (FTIR). The chemical composition changes after combining with excipients were investigated with IR spectra's. The IR spectra of drug and drug with excipients were recorded in FTIR (Bruker Optics Alpha) in the range of 4000–500 cm<sup>-1</sup>.

### Experimental design

A 3<sup>2</sup> full factorial design was considered for optimization of the best formulations among prepared formulations. Factors considered are the amount of PEC (X1) and %TWG of Eudragit-S100 coating (X2) acts as independent variables were evaluated at 3 levels, i. e., percentage drug release at 5<sup>th</sup> h (Y1) and percent drug release at 15<sup>th</sup> h (Y2) and percent drug entrapment (Y3) were selected as dependent variables which are shown in Table 1.

Nine formulations were prepared and evaluated for response. Statistical analysis was done using Microsoft Excel 2007. The obtained data were fitted into Sigma plot 12.0 software. Analysis of variance was used to validate the design.

As shown in Equation (1), the results obtained were analyzed for best curve fitting model using second order polynomial multiple regression analysis using Sigma plot 12.0 software and were fitted in the following equation.

$$Y = b_0 + b_{10} \times X_1 + b_{01} \times x_2 + b_{20} \times x^2 + b_{11} \times x_1x_2 \quad (1)$$

Where Y is the dependent variable, b<sub>0</sub> is arithmetic mean response of the nine trials, b<sub>i</sub> (b<sub>10</sub>, b<sub>01</sub>, b<sub>20</sub>, and b<sub>11</sub>) is the estimated coefficient for the corresponding factor X<sub>i</sub> (X<sub>10</sub>, X<sub>01</sub>, X<sub>20</sub>, and X<sub>11</sub>). The main effects (X<sub>1</sub> and X<sub>2</sub>) represent the average result of changing one factor at a time from its low to high values. The interaction terms x<sub>1</sub>x<sub>2</sub> show how the dependent variable changes when two or more factors are simultaneously changed.

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficients and mathematical sign it carries (i.e., positive or negative). Statistical analysis was done using Microsoft Excel 2007. Contour plots and response surface plots were drawn using software Sigma plot 12.0.

### Preparation of PEC beads

PEC beads were prepared by an ionic gelation method with chitosan and Hupu gum as primary colonic drug delivery

**Table 1:** Experimental design factors and responses

Factor (independent variables)	Level			Response (dependent variables)
X1=PEC ratio	-1	0	1	Y1=Percentage release at 5 h
X2=Coating level (%)	(1:3)	(1:3.5)	(1:4)	Y2=Percentage release at 15 h
	-1	0	1	Y3=Drug entrapment efficiency
	(6)	(8)	(10)	

polymers in the ratio of 1:1 (62.5: 62.5). The colonic drug delivery was further modified with polymers polyethylene glycol 400 and Eudragit S100 in the ratio of 1:3, 1:3.5, and 1:4 in the presence sodium tripolyphosphate as counter ions. The amount of vancomycin HCl was kept constant (1% w/v) which is shown in Tables 2 and 3.

The homogeneous aqueous solutions of chitosan and 10%w/v sodium tripolyphosphate used as coagulation media and the pH were adjusted to 6.0. The chitosan was dissolved in acetic acid solution (1% v/v) and the pH was adjusted using 1 M HCl and 1 M NaOH. The mixtures of hupu gum, polyethylene glycol 400, Eudragit-S100, and drug in distilled water were dropped through a 30-gauge needle into the coagulation media with appropriate mechanical stirring. Then, formed beads were filtered, washed, and dried at room temperature for 24 hours and were kept in desiccator till used further.

## EVALUATION PARAMETERS OF PEC BEADS

### Scanning electron micrograph of chitosan bead

The surface morphology of the beads was examined by scanning electron microscopy (SEM) using a JPG scanning electron microscope at 20 kV. Beads were coated with nickel under vacuum by SPI Sputter coating unit. The examinations were performed at three magnifications ( $\times 2000$ ,  $\times 1500$ , and  $\times 500$ ).

### Particle size

Particle size and mean beads diameter were measured with a calibrated optical microscope in which the calibration of eyepiece micrometer using stage micrometer, the calibration factor was calculated.

The fifty randomly chosen beads were considered to evaluate the particle size and were calculated no of divisions occupied by the particle is multiplying the by the calibration factor.

Moreover, an average of all these fifty beads was considered.

### Drug entrapment efficiency

This study was done by taking accurately weighed beads equivalent to 100 mg of the drug, was suspended in 100 ml

**Table 2:** Composition of experimental formulations

Batch code	X1 amount of PEC (%)	X2 coating level (%)
F1	1:3	6
F2	1:3	8
F3	1:3	10
F4	1:3.5	6
F5	1:3.5	8
F6	1:3.5	10
F7	1:4	6
F8	1:4	8
F9	1:4	10

PEC: Polyelectrolyte complex

of phosphate buffer pH 6.8. The resultant mixture was kept for shaking on the mechanical mixture for 24 h at 37°C, then the mixture was centrifuged at 5000 rpm for 10 min, and supernatant fluid is filtered.

1 ml of the filtrate was pipette out and diluted to 10 ml with the phosphate buffer pH 6.8 and was analyzed for drug content using spectrophotometer at 281 nm (Shimadzu-1700 UV-vis spectrophotometer) against a blank.

The drug entrapment efficiency was calculated using the following equation.

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

### Swelling studies

The core beads were subjected to 100 ml of HCl for 2 h, later the beads were replaced in phosphate buffer pH 7.4 for 3 h, and finally, the removed beads were kept in phosphate buffer pH 6.8 for 15 h.

The obtained tablets were blotted with filter paper and observed for the weight gain at different media exposures (0.1N HCl, Phosphate buffers pH 7.4, 6.8, respectively).

Percentage swelling (swelling index) was calculated using the following formula.

$$\% \text{ Swelling index} = \frac{\text{Wet weight of beads} - \text{Dry weight of beads}}{\text{Wet weight of beads}} \times 100 \quad (3)$$

### Preparation of coated beads containing hard capsules

#### Preparation of seal coating solution

The seal coating solution was prepared by dissolving PVP K30, Talc, and PEG-400 in isopropyl alcohol and was kept for a bath sonication to homogenize the coating solution. The obtained solution was left undisturbed for the escape of air bubbles from the solution.<sup>[4]</sup>

#### Preparation of enteric coating solution

The enteric coating solution was prepared by dissolving Eudragit-S100 in isopropyl alcohol and was kept for bath sonication to homogenize the coating solution. After sonication, 1.25% v/v of dibutyl phthalate was added to the solution as a plasticizer and was left undisturbed to allow for air bubbles to escape and then used for coating.<sup>[4]</sup>

#### Procedure for coating of beads containing hard capsules

The uncoated hard capsule containing PEC beads were coated for seal coating by the alcoholic spray containing 5% PEG-400 until 2% weight gain of the hard capsule containing PEC beads and were dried at room temperature; the obtained seal coated tablets were subjected to enteric coating with Eudragit-S100 coating dispersion by dip coating method until getting the weight gain 6 %, 8%, and 10% (w/w) of its initial beads containing hard capsules, i.e., seal coated hard capsule containing PEC beads and were dried at room temperature.

The tablets were analyzed for the weight gain of above-mentioned requirements and the coating process was continued until 6%, 8%, and 10% weight gain according to the formulation design.

Formula used for the analysis is

$$\text{Weight gain} = (W2 - W1) / W1 \times 100 \quad (2)$$

Where, W1: Weight (g) of uncoated tablet, W2: Weight (g) of coated tablet.

#### In vitro drug release studies

#### In vitro dissolution studies for enteric coated hard capsules containing beads

The prepared enteric coated hard capsules containing beads were placed for dissolution study by considering 900 ml of

0.1N HCl in the dissolution vessel for 2 h and were analyzed for drug release in the medium at each 1 h interval.

After the 2 h of dissolution study, the dissolution medium is completely replaced with phosphate buffer pH 7.4; dissolution was continued for 3 h and analyzed the drug release at each 1 h interval. Later the 5 h of study, the drug release study is done using phosphate buffer pH-6.8 for 15 h.

Specifications for dissolution study:

Apparatus type	USP type-I
Temperature of medium	37± 0.5 °C
Rpm	100
Sample size	5ml
Time points	at every 1 h interval
λ max	281 nm

#### Kinetic analysis of dissolution data

To study the mechanism of drug release, the release data were fitted to different equations such as zero-order ( $M = kt$ ), first-order equation ( $M = \ln M_0 + kt$ ), Higuchi model ( $M = k\sqrt{t}$ ) and Korsmeyer–Peppas equation ( $M = ktn$ ). A value of  $n = 0.5$  indicates case I (Fickian) diffusion,  $0.5 < n < 1$  is for anomalous (non-Fickian) diffusion,  $n = 1$  is for case II transport and  $n > 1$  indicates super case II transport. M is the amount of drug (%) released after time t;  $M_0$  is the amount of drug released at zero time; k is the release rate constant and n is the exponent. Drug release following a particular mechanism is adjudged by the linearity ( $R^2$ ) of plot.<sup>[5]</sup>

#### Ex vivo drug release studies

#### Preparation of rat cecal content for dissolution study

The healthy Wistar rats of either sex of weight 120–150 g were selected for the study. The approved protocol for the use of the animal followed was according to the protocol number (1269/PO/E/S/08/CPCSEA) as per the regulation of Institutional Animal Ethical Committee of Aditya College of Pharmacy, Kakinada, Andhra Pradesh, India.

The enzymes induction that is specific for the biodegradation of the Eudragit-S100 during its passage through the colon, the Wistar rats were intubated with Teflon tubing and 2 ml of 1%w/v solution of Eudragit-S100 in water was administered directly into the stomach for 7 days.

After 7 days of incubation and feeding Eudragit-S100, the rats were sacrificed, cecum was located in the abdomen, dissected and cecum content was removed before ½ h of dissolution study, and the content was suspended in phosphate buffer pH 7.4 to make 2% concentration of rat cecal content. Carbon dioxide (CO<sub>2</sub>) was continuously passed to pooled content to maintain an anaerobic environment.<sup>[6]</sup>



### Ex vivo drug release studies in the presence of rat cecal content

This investigation was carried out for the enteric coated hard capsule containing beads which removed after the 5<sup>th</sup> h of dissolution study from phosphate buffer pH 7.4. The *Ex vivo* drug release studies were carried out using USP dissolution rate test apparatus (basket type) with minor modifications with 100 ml of dissolution media maintained at  $37 \pm 0.18^\circ\text{C}$  and rotated at a speed of 100 rpm.

A 250 ml beaker containing 100 ml phosphate buffer pH 6.8 with 2% rat cecal content as dissolution medium, was immersed in the sufficient quantity water in the 900 ml vessel, and was kept in the water bath of the dissolution rate test apparatus and analyzed for drug release. The 2 ml of the dissolution media was withdrawn and replaced immediately with the same amount of fresh respective media bubbled with carbon dioxide. The studies were performed for 20 h.

The sample was filtered and analyzed for vancomycin HCl by UV spectrophotometer at 281 nm for 0.1 N HCl, phosphate buffer pH 7.4, and phosphate buffer pH 6.8.<sup>[7]</sup>

### Stability study

The stability study was conducted according to the International Conference on Harmonization (ICH) guidelines. The optimized formulation was stored in aluminum packaging laminated with polyethylene (cellophane packets) and kept in stability chamber at  $40^\circ\text{C} \pm 2^\circ\text{C}/75\% \pm 5\%$  RH (accelerated temperature studies) for 6 months. The hard capsules containing beads were analyzed after 0 day, 3 months, and 6 months. At the end of the study period, hard capsules containing beads were observed for the change in physical appearance, moisture content, and drug assay.<sup>[8]</sup>

## RESULTS AND DISCUSSION

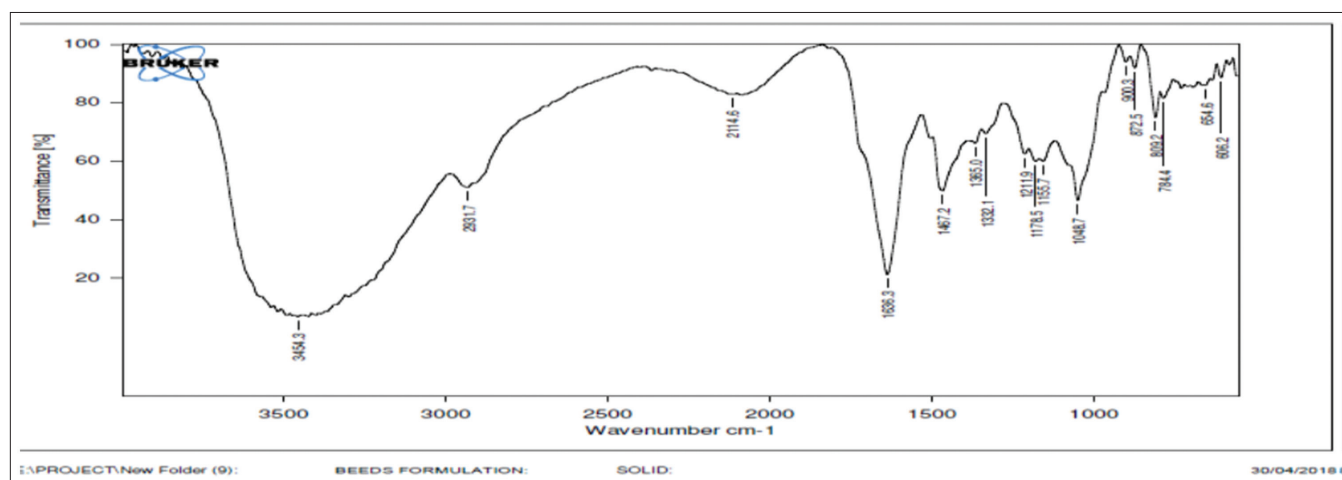
### Drug excipients compatibility study

The FTIR spectra's of vancomycin HCl, excipient mixture is shown in Figures 1 and 2. FTIR spectra of chitosan showed a sharp peak of strong intensity at  $1636.8\text{ cm}^{-1}$  which is the characteristic peak of the amino group in chitosan while in case of hupu gum peaks at  $1668.5\text{ cm}^{-1}$  indicate the presence of C-O stretching and amide (N-H) bending. The carboxylic acid of glucuronic acid in hupu gum is indicated by a peak at  $1400.9\text{ cm}^{-1}$  due to C=O stretching.

The FTIR spectra of PEG 600 showed a sharp peak of strong intensity at  $3439.4\text{ cm}^{-1}$  which is the characterized peak of N-H stretching, C-H stretching (alkane) observed at  $2933.5\text{ cm}^{-1}$ , C=O stretching (amide) was observed at  $1638.4\text{ cm}^{-1}$ . While in case of Eudragit-S100 peaks, at  $1636.8\text{ cm}^{-1}$  indicates the presence of N-H stretching.

The characteristic peaks for the optimized bead formulation showed N-H stretching at the wavelength of  $3452.6\text{ cm}^{-1}$ , N-H bending at  $1636.8\text{ cm}^{-1}$ , C=C stretching (aromatic group) at  $1474.7\text{ cm}^{-1}$ , C-N vibrations at  $1189.0\text{ cm}^{-1}$ , and C=S stretching observed at  $1049.3\text{ cm}^{-1}$ . This could be attributed to-NH<sub>3</sub><sup>+</sup> ion of chitosan and C=O ions of Hupu gum, PEG 600 and Eudragit-S100 forms an ionic complex to form a PEC. Eudragit-S600 contributed as release retardants and viscosity.

The spectra's revealed that there is no change in absorption peak of the vancomycin HCl in the FTIR spectra of excipient mixture, hence, it can be concluded that there is no interaction with the excipients.



**Figure 1:** Fourier-transform infrared spectrum for optimized formulation (F3). The spectra exhibited no change in the absorption peaks of vancomycin HCl and the vancomycin HCl with excipient mixture so it can be concluded that there is no interaction between the components

## Evaluation parameters of beads

### Particle size and surface morphology

Particle size and mean bead diameter measurements were carried out with an optical microscope. A stage micrometer was used to calculate the calibration factor. The 10-division of the stage micrometer was matched with the division of an ocular disc, and the calibration factor was calculated. Particle size was calculated by multiplying the number of divisions of the ocular disc that the particle occupied by the calibration factor. Fifty randomly chosen beads were taken to measure their individual sizes. The size of the beads is summarized in Table 4. Bead size was influenced by the concentration of chitosan and counterions and the pH of coagulation medium. The orifice of the needle and the rate of pouring the polymeric solution into the counterion solution were fixed. The average particle size of optimized formulation-3 (chitosan/hupu gum, polyethylene glycol 600 and Eudragit-S100) beads prepared with sodium tri poly phosphate was observed within the range of  $1.02 \pm 0.73$ – $1.07 \pm 0.82$  mm. The spherical shaped and smooth surface particles were observed in an electron microscope, which was noticeable on the  $\times 2000$  [Figure 9a], on the  $\times 500$  [Figure 9b], and on the  $\times 1500$  [Figure 9c].

## Scanning electron micrograph of chitosan hydrogel bead

### Drug entrapment efficiency

The results of entrapment efficiency are presented in Table 4. The prepared PEC beads showed high entrapment efficiency in the range of  $54.78 \pm 0.82$ – $95.39 \pm 0.78\%$ , suggesting that the ionic gelation method was effective for the entrapment of vancomycin HCl. The concentration of chitosan and the pH of the coagulation medium had an effect on the entrapment of vancomycin HCl. With an increase in polyethylene glycol 600 and Eudragit-S100 concentration and the pH of coagulation medium, there was an increase in the entrapment efficiency of vancomycin HCl. At higher concentrations of polymer and pH values corresponding to their ionized states, there will be an increase in both the charge density of the polymers, which leads to higher crosslinking and ionic interaction. As a result, less drug was lost from the PEC beads during gelation, and hence a higher percent entrapment occurred. The beads prepared with F3 showed less entrapment efficiency as compared with F7 because there was less cross-linking with low ionic interaction resulting in an increase in drug loss during the gelation process.

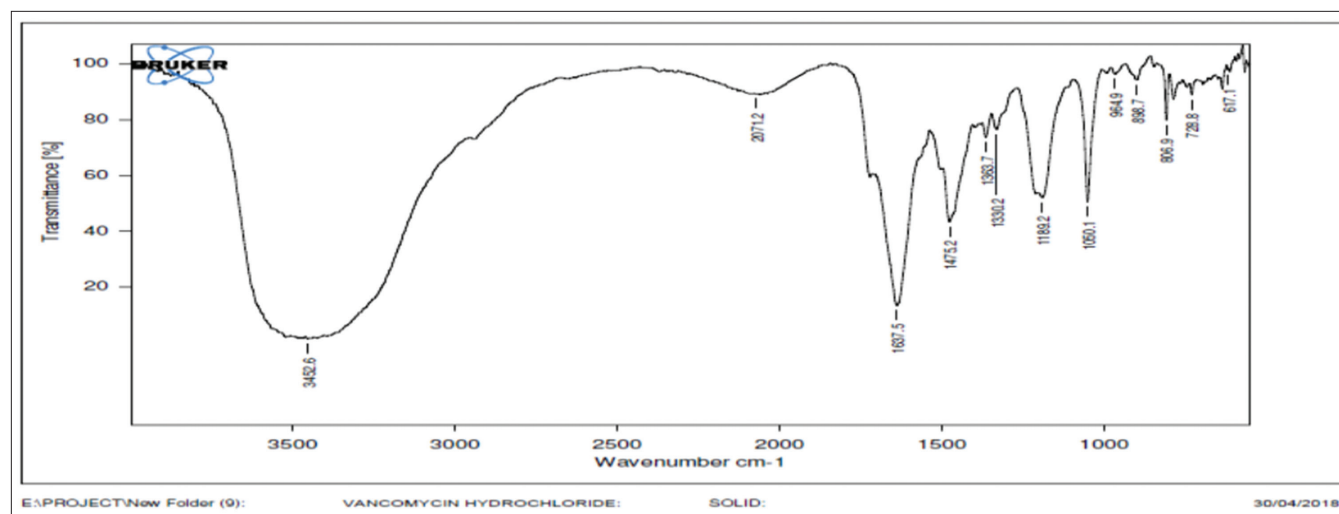


Figure 2: Fourier-transform infrared spectrum for pure vancomycin HCl

Table 3: Formulation details

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Vancomycin hydrochloride	125	125	125	125	125	125	125	125	125
Chitosan	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Hupu gum	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
PEG 400	125	125	125	187.5	187.5	187.5	250	250	250
Eudragit S100	125	125	125	187.5	187.5	187.5	250	250	250
Total weight gain (%)	6	8	10	6	8	10	6	8	10

**Table 4:** Evaluation parameters of beads

Formulation code	Particle size (mm) (mean±SD)	Percent drug entrapment (mean±SD)	Swelling index	Actual drug content (mg)	Percentage of yield
Formulation-1	1.02±0.73	64.30±1.05	90.82	49.46	92.32
Formulation-2	1.05±0.33	54.78±0.82	88.12	42.13	82.43
Formulation-3	1.07±0.82	60.85±1.33	86.32	46.80	85.33
Formulation-4	0.93±0.05	76.84±1.56	90.62	59.10	96.64
Formulation-5	1.02±0.73	74.43±0.60	90.23	57.25	90.98
Formulation-6	1.07±0.43	86.97±0.73	90.37	66.89	97.85
Formulation-7	1.04±0.29	95.39±0.78	74.83	73.43	97.92
Formulation-8	1.05±0.05	82.37±1.25	60.64	63.35	97.03
Formulation-9	1.08±0.33	91.15±1.33	50.49	70.11	99.47

SD: Standard deviation

**Table 5:** Kinetic analysis of dissolution data

Formulation code	Zero-order		First-order		Higuchi		Korsmeyer–Peppas	
	R <sup>2</sup>	K <sub>0</sub>	R <sup>2</sup>	K <sub>1</sub> (h <sup>-1</sup> )	R <sup>2</sup>	K <sub>H</sub>	R <sup>2</sup>	N
F1	0.8124	5.064	0.957	0.262	0.866	28.45	0.916	1.436
F2	0.831	4.814	0.952	0.227	0.881	27.64	0.919	1.441
F3	0.839	4.576	0.949	0.202	0.89	26.896	0.927	1.549
F4	0.818	4.766	0.955	0.230	0.875	27.488	0.918	1.463
F5	0.8123	4.454	0.961	0.204	0.877	26.42	0.903	1.396
F6	0.8287	4.443	0.947	0.181	0.883	26.182	0.904	1.472
F7	0.818	4.525	0.951	0.214	0.8795	26.776	0.909	1.442
F8	0.8217	4.423	0.951	0.188	0.8796	26.115	0.899	1.417
F9	0.8115	4.254	0.94	0.160	0.873	25.183	0.897	1.465

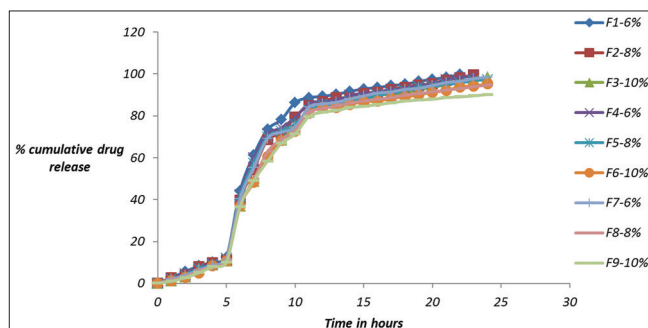
### Swelling studies

The results of the swelling index are presented in Table 4. All the prepared beads showed less solubility behavior in pH 1.2 (0.1 N HCl), little solubility behavior in phosphate buffer pH 7.4, but the swelling was increased in phosphate buffer pH 6.8, which may be due to protonation of chitosan. It was also observed that the swelling index in this buffer decreases by increasing the concentration of the PEC. F1 (1:3, 6% coating) showed a maximum swelling index of 90.82%. Thus, PEC beads are swelling considerably in phosphate buffer solutions. This process shows a pH-dependent pattern.

### In vitro drug release study

All the formulations were evaluated for *in vitro* drug release, and cumulative percentage vancomycin HCl release was considered in this study, which is shown in Figure 3.

This study was carried out using three different buffer as mentioned in the methodology, i.e., this study was initially done in the HCl buffer (pH 1.2) for initial first 2 h. Then, the medium was replaced by phosphate buffer (pH 7.4) for 3 h. The study was continued for the next 15 h in phosphate buffer (pH 6.8).



**Figure 3:** *In vitro* dissolution of all formulations (F1-F9). At simulating stomach pH of 1.2, none of the formulation showed any released drug due to enteric coating and only maximum of 12.5% of drug released at pH 7.4, but when pH of media was raised to pH 6.8, there was burst release. This was due to the fact that pH-sensitive Eudragits are ionized and solubilize at pH range. The release rate was slower at higher coating level due to the increased diffusion path length

During this study, none of the formulations showed drug release at pH 1.2 (0.1N HCl) due to enteric coating over the formulations. A maximum of 12.5% drug released at pH 7.4 buffer, but the drug started to release in pH 6.8 buffer, as enteric coat, i.e., Eudragit S100 will solubilizes in it

and buffer diffuses into the beads. The release rate of drug were observed slower for formulations which have higher coating levels and a higher level of polymer, i.e., PEC in the formulations.

The *in vitro* release of drug from all the formulations was analyzed and indicates that prepared formulations affected by coating level of enteric coated polymer, swelling behavior of the PEC.

### Kinetic analysis of dissolution data

All the nine batches of formulations were graphically, mathematically analyzed using zero-order, first-order, Higuchi, Korsmeyer–Peppas model equations for their release pattern and also to evaluate best suitable formulations with the ideal type of drug release for colon targeted beads.

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 ( $n$ ). The  $R^2$  and  $n$  values are given in Table 5 and observed all the formulations are best fitted with zero-order and Korsmeyer–Peppas plot than compared to the Higuchi plot.

The  $R^2$  values ranged between 0.8972 and 0.9271 for all the formulations. The “ $n$ ” value was in the range of 1.396–1.549, i.e., indicating that the release mechanism of vancomycin HCl from these beads follows super case II transport, which suggests that, drug release that is zero-order, where the release rate is constant and controlled by polymer relaxation. Finally, drug release that is erosion – controlled.<sup>[9]</sup>

### Data analysis

#### Data analysis for drug release at 5<sup>th</sup> h (Q5)

Data analysis for drug release at 5<sup>th</sup> h (Q5),  $R^2$  value in the plot was 0.9219, which indicated excellent fit. The response (Y1) obtained at three levels of the two independent variables (X1 and X2) were subjected to multiple regression to yield a polynomial Eq.(4). Equation clearly reflects the wide range of values for coefficients (b)

$$Y1 = 11.59 - 0.3567 \times x - 0.7317 \times y - 0.4033 \times x^2 + 0.0075 \times x \times y \quad (4)$$

In the present study, coefficients b1 and b2 possessed a negative sign which indicating increasing PEC concentration and coating level, the antagonistic effect of variables X1 and X2 on response Y1 (Q5). Among two independent variables, X1 (amount of PEC) has prominent effect ( $b1 = 0.3567$  and ( $P = 0.042$ ) on Q5, whereas to some extent X2 (% coating) also affects the results ( $b2 = 0.7317$  and  $P = 0.002$ ) in inverse manner.  $P < 0.05$  is considered statistically significant. The high values of the coefficient of the determination indicate

good fit, i.e., good agreement between the dependent and independent variables. The coefficients b1 and b2 were found to be significant at  $P < 0.05$ .

#### Data analysis for drug release at 15<sup>th</sup> h (Q15)

The  $R^2$  value for Q20 in plot was 0.9769, which indicated excellent fit. The Y2 (Q20) values observed for different batches showed wide variation, i.e., values ranged from a minimum of 84.49% to a maximum of 92.88%. Eq. (5) refers to polynomial equation for response Y2.

$$Y2 = 92.72 - 2.035 \times x - 2.34 \times y - 0.2417 \times x^2 - 0.3775 \times x \times y \quad (5)$$

Coefficient b1 and b2 possessed negative and negative sign respectively, which indicated that both the independent variables X1 and X2 having negative effects on Y2, i.e., release at 20<sup>th</sup> h. Independent variables, X1 (amount of PEC) ( $b1 = 2.035$  and  $P = < 0.001$ ) affects more prominently the Q20 then X2 (% coating) ( $b2 = 2.34$  and  $P = < 0.001$ ) which has inverse effect on Q20.  $P < 0.05$  is considered statistically significant. The coefficient b1 and b2 were found to be significant at  $P < 0.05$ .

#### Data analysis for drug entrapment efficiency

The  $R^2$  value for drug entrapment efficiency in the plot was 0.8599 which indicated excellent fit. The Y3 (Drug Entrapment Efficiency) values observed for different batches showed wide variation, i.e., values ranged from a minimum of 6<sup>th</sup> h to a maximum of 10<sup>th</sup> h. Eq. (6) refers to the polynomial equation (full model) for response Y3.

$$Y3 = 79.41 + 14.83 \times x + 0.4067 \times y - 4.607 \times x^2 - 0.1975 \times x \times y \quad (6)$$

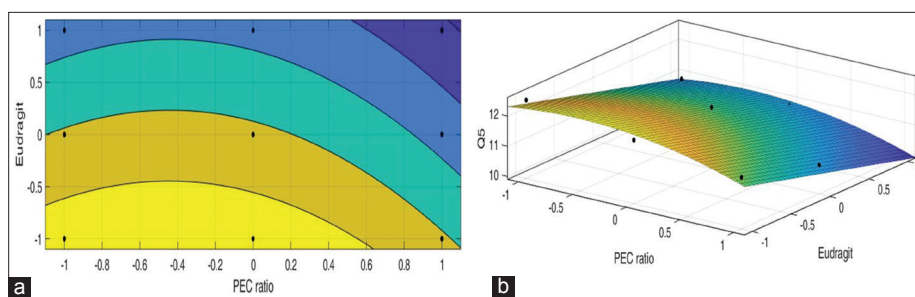
Coefficient b1 and b2 possessed positive and positive sign, respectively, which indicated that both the independent variables X1 and X2 having positive effects on Y3, i.e., drug entrapment efficiency. Independent variables, X1 (amount of cross-linking) ( $b1 = 14.83$  and  $P = 0.002$ ) affects more prominently the Y3 then X2 (% coating) ( $b2 = 0.4067$  and  $P = 0.886$ ).  $P < 0.05$  is considered statistically significant. The coefficient b1 was found to be significant and b2 was found to be non-significant at  $P < 0.05$ .

Tables 6 and 7 shows the results of multiple regression analysis of Q5, Q15, and drug entrapment efficiency.

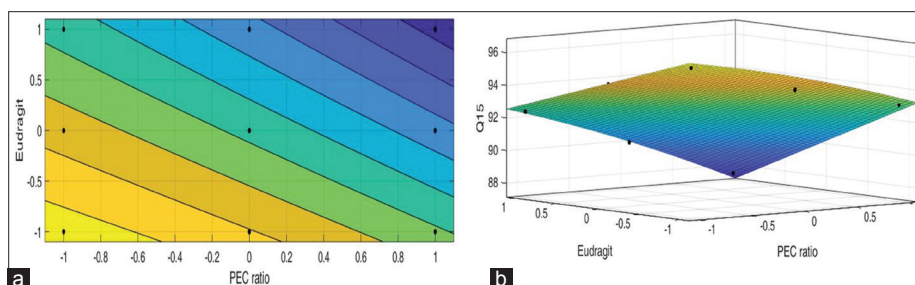
#### Response surface 3-d and contour plot analysis

The obtained results can be observed visually in two-dimensional contour plots and three-dimensional response surface plots are presented in Figure 4a,b and 5a,b, and 6a,b. Which are a useful tool to study interaction effects of the factors on responses. Figure 4a, b and 5a, b exhibited non-linear pattern, but with an increase in PEC concentration and

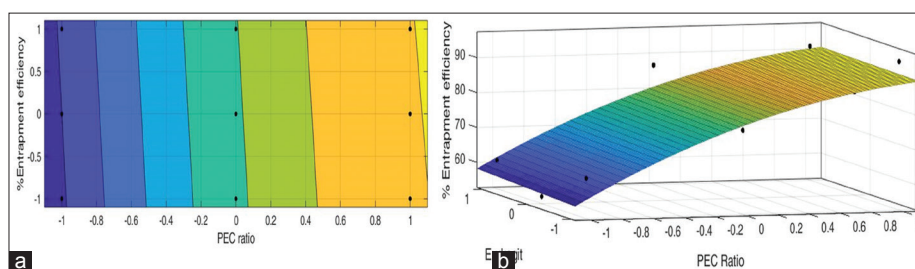




**Figure 4:** (a) Contour plot of 5<sup>th</sup> h drug release. The obtained results were observed visually in two-dimensional contour plots at 5<sup>th</sup> h and showed that with increasing polymer concentration and coating level, the drug release decreases, i.e. amount of polyelectrolyte complex (PEC) ratio code -1 (1:3) and coating level code -1 (6%) shows the highest rate of drug release whereas, PEC ratio code +1 (1:4) and coating level code +1 (10%) shows slow rate of drug release. (b) Surface plot of 5<sup>th</sup> h drug release



**Figure 5:** (a) Contour plot of 15<sup>th</sup> h drug release. The obtained results were observed visually in two-dimensional contour plots at 5<sup>th</sup> h and showed that with increasing polymer concentration and coating level, the drug release decreases, i.e., amount of polyelectrolyte complex (PEC) ratio code -1 (1:3) and coating level code -1 (6%) shows the highest rate of drug release whereas, PEC ratio code +1 (1:4) and coating level code +1 (10%) shows a slow rate of drug release. (b) Surface plot of 15<sup>th</sup> h drug release



**Figure 6:** (a) Contour plot of percentage entrapment efficiency. The results were observed visually in two-dimensional contour plots at percentage entrapment efficiency and showed that with increasing polymer concentration and coating level, the drug release decreases, i.e., amount of polyelectrolyte complex (PEC) ratio code -1 (1:3) and coating level code -1 (6%) shows a slow rate of drug release whereas, PEC ratio code +1 (1:4) and coating level code +1 (10%) shows the highest rate of drug release. (b) Surface plot of percentage entrapment efficiency

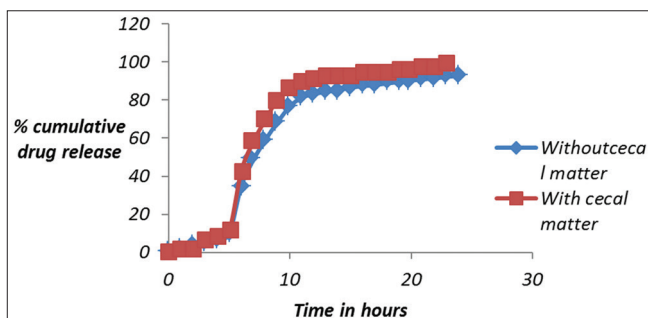
**Table 6:** Drug release data modeling

Formulation code	Independent variables (codes)		Dependent variables		
	PEC ratio	Eudragit S100 coating TWG	5 <sup>th</sup> h percentage of drug release	15 <sup>th</sup> h percentage of drug release	Percentage drug entrapment
F1	-1	-1	12.5	97.47	64.30±1.05
F2	-1	0	11.26	95.25	54.78±0.82
F3	-1	±1	9.88	93.43	60.85±1.33
F4	0	-1	12.01	95.28	76.84±1.56
F5	0	0	11.94	94.18	74.43±0.60
F6	0	±1	10.83	91.26	86.97±0.73
F7	±1	-1	11.62	94.46	95.39±0.78
F8	±1	0	10.85	91.37	82.37±1.25
F9	±1	±1	10.03	87.88	91.15±1.33

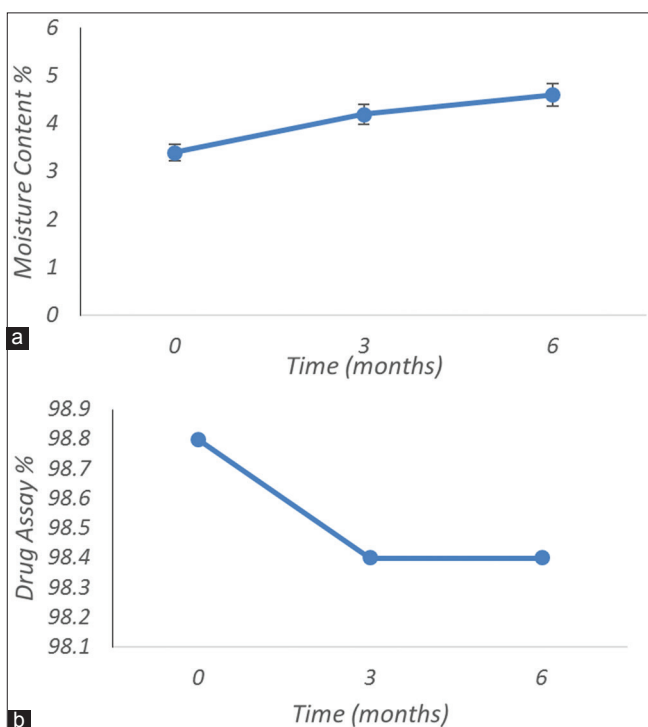
PEC: Polyelectrolyte complex, TWG: Total weight gain

**Table 7:** Results of multiple regression analysis of q5, q15, and drug entrapment efficiency

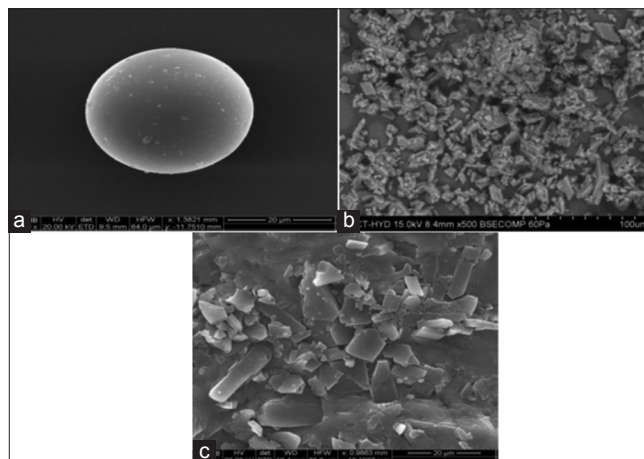
Dependent variable	Q5=Y1		Q15=Y2		Drug entrapment efficiency=Y3	
	P	Coefficient	P	Coefficient	P	Coefficient
Intercept	<0.001	11.324	<0.001	92.559	<0.001	76.342
X1	0.042	-0.357	<0.001	-2.035	0.002	14.830
X2	0.002	-0.732	<0.001	-2.340	0.886	0.407



**Figure 7:** Drug release profile of optimized formulation with and without rat cecal content. Optimized batch F3 shows 96.08% drug release in the presence of rat cecal content. There was an increase in drug release in the dissolution medium containing rat cecal content (2%) as compared to the control group (without rat cecal content)



**Figure 8:** (a) Accelerated stability testing of beads-moisture content was evaluated by conducting accelerated stability studies and was found 4.2%, 4.6 % at 3<sup>rd</sup>, 6<sup>th</sup> month of evaluation, respectively. This indicates accepted fluctuations in moisture contents than compared to the initial 3.4% at 0 month of study. (b) Accelerated stability testing of beads-drug assay was evaluated by conducting accelerated stability studies and was found 98.4%, 98.4% at 3<sup>rd</sup>, and 6<sup>th</sup> month of evaluation, respectively. These indicates accepted fluctuations in drug assay than compared to the initial 98.8% at 0 day of study



**Figure 9:** (a) SEM of chitosan hydrogel. Beads  $\times 2000$ . (b) SEM of chitosan hydrogel. Beads  $\times 500$ . (c) SEM of chitosan hydrogel. Beads  $\times 1500$

**Table 8:** Results of accelerated stability tests of beads at 0 day, 3, and 6 months

Time (months)	Moisture content (%) $\pm$ SD	Drug assay (%) $\pm$ SD
0	3.4 $\pm$ 0.75	98.8 $\pm$ 0.01
3	4.2 $\pm$ 0.56	98.4 $\pm$ 0.01
6	4.6 $\pm$ 0.23	98.4 $\pm$ 0.01

SD: Standard deviation

% coating level, it also showed that amount of PEC has a comparatively greater influence on response variable Q5 and Q15 then percentage of coating. In contrast to the result of drug release at 5<sup>th</sup> and 15<sup>th</sup> h contour plot of drug entrapment efficiency varies non-linear manner with an increase in the amount of PEC and percentage of coating [Figure 6a and b]. However, the effect of X1 (amount of PEC) seems to be more pronounced as compared with that of percentage of coating. The results were also confirmed through three-dimensional response surface graphs.

### Selection of optimized formulation

From the polynomial equation and the contour plots, the optimized batch F3 was found. The optimum formulation was selected based on the criteria of attaining the constraints of variables response. On “trading of” various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with

an amount of PEC (1:3) and coating (10%) was found to fulfill the requisite of an optimum formula. *In vitro* release data of optimized formulation are showed 93.43% vancomycin HCl release on completion of 20 h but showed a release of 9.88% at 5<sup>th</sup> h which meets the colon targeted delivery objective of the present work, i.e., a lag of 5 h and maximum release in between 16 and 20 h. Composition of the optimized batch F3 was also very economical when compared to the next best formulation, F7, as obtained from factorial design.

#### **Ex vivo drug release data in the presence of rat cecal content**

The *ex vivo* drug release study was performed for best formulations which optimized; using rat cecal microflora which is similar to human intestinal microflora, results of this study are shown in Figure 7.

The optimized batch F3 shows 93.43% drug release in the presence of rat cecal content. There was an increase in drug release in the dissolution medium containing rat cecal content (2%) as compared to the control group (without rat cecal content).

#### **Accelerated stability studies**

The stability tests for optimized formulation were performed according to the ICH guidelines. The temperature conditions followed was  $40 \pm 2^\circ\text{C}$  and 75% RH for 6 months. The results are shown in Table 8 and Figure 8a-c, which indicates that the optimized hard capsule containing beads, did not have any significant changes in the physical properties, assay and thus passed the stability testing.

## CONCLUSION

The factorial design used in the present study for optimization of chitosan-(Hupu gum with Polyethylene glycol 400 and Eudragit S100) PEC and Eudragit-S100 coating can be successfully used for colon delivery. Response surface and contour plots enabled for the designing formulation of desired release profile of vancomycin HCl beads consisting of PEC (1:3) and Eudragit-S100 coating (10%) level has shown (93.43% drug release within 20 hours) potential for colonic delivery. The *ex vivo* drug release was found to be 96.08% in the presence of rat cecal content.

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