ORIGINAL ARTICLE

Applicability of Natural Gums for the Development of Controlled Release Vancomycin Hydrochloride Tablets in Site Specific Colon region using Statistical Optimization

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Abstract

The present study was initiated to develop colon targeted enteric-coated matrix tablets of Vancomycin HCl 500 mg by incorporating chitosan-based polyelectrolyte complex (PEC). The matrix tablets were development and optimized using 3^2-full factorial design. The Vancomycin HCl is an antimicrobial substance used in the treatment of enterocolitis. Hence, localization of the drug at its site of action is more useful. The pharmacokinetic parameters of 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 optimized formulation showed 10% drug release during lag time, 98% at 20 h, and the study of antibacterial activity for the developed best formulation indicating effective killing of *Staphylococcus aureus*. The study was carried out to check the ability of PEC to release the Vancomycin HCl in the presence of rat cecal content medium resembling the physiological environment of colon.

Key words: Anti-bacterial studies, enteric coating, *ex vivo* studies, lag time, optimization, 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 PEC can be considered as vehicle for colon targeted drug delivery.[1]

The colon provides 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 stomach, but it requires controlled release of drug in the colon.[2]

The novel approach which can be considered for controlled drug delivery in colon is PEC and also very few noted research was done using colon targeted delivery using PEC. Bigucci *et al*. (2008) have also earlier prepared chitosan/pectin PEC for colon-specific delivery of Vancomycin HCl. However, the present work focuses on the development of drug-loaded enteric-coated tablets of PEC using natural polysaccharides, i.e., hupu gum, karaya gum, and chitosan

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are extensively used to prepare the PEC. As many gums are utilizing as release rate retardants in the drug delivery systems, but PEC developed from crosslinking behavior of cationic and anionic polymers offering great impact on rate of drug release from the drug delivery systems; hence, it is considered using chitosan containing cationic groups and is crosslinking with anionic groups of hupu gum and karaya gum will form PEC. Cationic groups of chitosan present in the PEC 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 pH-dependent polymer Eudragit S100 was coated over the formulations to prevent drug release in the stomach region and starts erosion in colon region. As chitosan along with hupu gum and karaya gum will form PEC which can releases drug slowly from it, and hence it is considered in this formulation developments.

The developed formulation showed pH-dependent swelling and released drug in alkaline pH. *Ex vivo* confirmed that polysaccharide degradation by colonic microbial enzyme was the prime source of drug release in colon.[3] The development and optimization of matrix tablets were carried out using 3^2 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 enterocolitis caused by *Staphylococcus aureus* and antibiotic associated to pseudomembrane colitis caused by clostridium dificile. 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 gastrointestinal 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 were done by tablet 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. Vancomycin HCl can directly act/kills *S. aureus* present particularly in colon region by providing the drug release and availability of drug in local region. Hence, the main objective of present work is to attain release of drugs in the local region, i.e., colon targeted drug delivery tablets.

MATERIALS AND METHODS

Vancomycin HCl was kindly supplied as a gift sample by Concord Biotech Limited Dholka (Gujarat) India. Chitosan was purchased from Kemphasol, Mumbai, India. Hupu gum and Karaya gums were procured from Girijana Co-Operative Corporation Limited Rajahmundry, India. Microcrystalline cellulose was obtained from Kemphasol, Mumbai, India. Eudragit –S100 was obtained from Evonik industries. Sodium hydroxide, glacial acetic acid, potassium dihydrogen phosphate, and magnesium stearate were obtained from S.D Fine Chemicals Ltd. All other chemicals used were of analytical grade. In addition, a dissolution apparatus TDT-08L (Lab India Disso-8000), a ultraviolet (UV)-visible spectrophotometer (Elico, Mumbai), a disintegration apparatus (Electro lab), a Friability apparatus (Electro lab), an electronic balance (Shimadzu AX200), a pH meter (Systronics model EQMK VI), a sonicator (Spectra Lab, model UCB 40), and a hot air oven (Lab hosp) were used in this study.

Drug excipients compatibility study

The compatibility of Vancomycin HCl with the individual excipients was established by Fourier-transform infrared spectroscopy (FT-IR). The chemical composition changes after combining with excipients were investigated with infrared (IR) spectra. The IR spectra of drug and drug with excipients were recorded in FT-IR (Bruker Optics Alpha) in the range of 4000–500 cm^{-1}.

Preparation of chitosan/hupu and karaya gum PEC

The present study was considered to prepare PECs using gums with chitosan and that PEC develops biocompatible matrix, which can effective entrapment drug. It can release the drug in controlled manner. The development of PEC using natural gums is economic and affective; hence, we considered development of PEC using different concentrations and evaluated the best concentrations based on the product yield, conductivity, and pH values which are shown in the below table.

Procedure for preparation PEC

Chitosan solution was prepared in acetic acid (1% w/v), and hupu, karaya gum solutions are prepared in distilled water and are sonicated separately until clear solutions. Later both Chitosan solution and hupu and karaya gum solution were mixed, agitated for 30 min and then kept for drying for 5 h to yield a dry powder.[4]

The conductivity test was done for prepared PEC from different concentrations of Chitosan – Hupu gum, Chitosan – Karaya gum, and Chitosan – Hupu and Karaya gum, proportion given in Table 1. The proportions with more chitosan concentration (acidic groups present) showed more conductivity indicating the presence of more moisture content, which may be the reason the yield obtained was less comparatively.

The proportion with less chitosan concentration showed less conductivity indicating the presence of less moisture content which capable to produce more yield.

The maximum PEC was found to be formed at 1:1 weight ratio of chitosan with hupu gum, chitosan with karaya gum, and chitosan with hupu gum and karaya gum. Hence, this weight ratio of chitosan with hupu gum, chitosan with karaya gum, and chitosan with hupu gum and karaya gum was selected for further studies.

Experimental design

A 3^2 full factorial designs were considered for the optimization of the best formulations among prepared formulations. Factors considered are the amount of PEC (X1), % total weight gain of Eudragit-S100 coating (X2) acts as independent variables were evaluated at 3 levels, i.e., percent drug release at 5^{th} (Y1), and percent drug release at 20^{th} h (Y2), and time required to release at 50% of the drug (Y3) were selected as dependent variables which are shown in Table 1.

The 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 design.

Table 1: Evaluation parameters of chitosan with hupu gum PEC, chitosan with karaya gum PEC and chitosan with hupu gum and karaya gum PEC

Ratio	Product yield	pH	Conductivity
Chitosan–hupu gum			
1:0.5	87.75	7.86	1.00
1:1	90.33	7.92	1.02
1:2	78.33	7.95	1.02
2:1	81.08	8.00	1.10
Chitosan–karaya gum			
1:0.5	82.75	7.63	1.08
1:1	88.16	7.61	0.98
1:2	84.5	7.51	0.91
2:1	82.1	7.55	1.14
Chitosan–hupu gum+karaya gum			
1:0.5	79.00	4.95	1.12
1:1	88.50	4.96	1.09
1:2	84.41	5.04	1.03
2:1	86.83	4.82	1.33

PEC: Polyelectrolyte complex

As shown in Eq. (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.

$$Y = b0 + b10*X1 + b01*X2 + b20*X2 + b11*X1X2 \quad (1)$$

Where Y is the dependent variable, bo is arithmetic mean response of the nine trials, b_i (b10, b01, b20, and b11) is the estimated coefficient for the corresponding factor Xi (X10, X01, X20, and X11). The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high values. The interaction terms X1X2 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 signs 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 vancomycin HCl core tablets

Vancomycin HCl and all the excipients except PEC are passed initially through 60# sieve, and resulting mixtures were mixed with PEC. A damp mass of this mixture is developed according to batch size (80 tablets), using polyvinyl pyrrolidone-K30 solution in water-ethanol mixture by passing it through sieve no 16# to obtain wet granules and were dried at 40°C for 24 h in a hot air oven.

The obtained granules were passed through sieve no. 20#, fines are separated to obtained uniform sized granules. These granules are lubricated with magnesium stearate and talc as per the formula. This is shown in Tables 2-4. The lubricated granules were compressed into tablets using multiple tablet compression machine (Shakthi Pharma tech, AN ISO 9001; 200 LAB PRESS India).

Limitations of the compression machine:

Maximum tablet size: 16.0 mm "B" tooling and 25 MM "D" tooling.

Table 2: Experimental design factors and responses

Factor (Independent variables)	Level			Response (Dependent variables)
XI=Amount of PEC	-1	0	1	Y1=percent release at 5 h Y2=percent release at 20 h
X2=Coating level (%)	-1	0	1	Y3=Time to release 50% of drug

PEC: Polyelectrolyte complex

Table 3: Composition of experimental formulations

Batch code	X1 amount of PEC (%)	X2 coating level (%)
F1	2	6
F2	2	8
F3	2	10
F4	4	6
F5	4	8
F6	4	10
F7	6	6
F8	6	8
F9	6	10

PEC: Polyelectrolyte complex

Maximum depth of fill: 17.0 mm "B" tooling and 20 MM "D" tooling.

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.[5]

Preparation of enteric coating solution

The enteric coating solution was prepared by dissolving Edragit-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.[5]

Procedure for coating of core tablets

The uncoated tablets were coated for seal coating by the alcoholic spray containing 5% PEG-400 until 2% weight gain of the tablets 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 tablet, i.e., seal coated tablet 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:

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

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

Evaluation of derived and flow properties of granules

The prepared granules of all formulations were analyzed for their derived and flow properties such as Bulk density, Tapped density, Car's index, Hausner's ratio, and Angle of repose.[6]

Evaluation of core tablets

All the core tablets were analyzed for their appearance, thickness by Vernier calipers, hardness by Monsanto Hardness Tester, weight variation, content uniformity and disintegration were done as per the USP, and friability by Roche friabilator.

Swelling study

The core tablets were subjected to 100 ml of HCl for 2 h, later the tablets were replaced in phosphate buffer pH 7.4 for 3 h, and finally, the removed tablets 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 (0.1 N HCl, Phosphate buffers pH 7.4, 6.8, respectively).

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

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

Drug release study

The prepared enteric-coated Vancomycin HCl tablets were placed for dissolution study by considered USP-I official methods to conduct dissolution method, and hence, we by considering 900 ml of 0.1 N HCl in the dissolution vessel for 2 h and was 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.

From the reference of reputed journals like Elsevier, International Journal of Pharmaceutics published research works on vancomycin, and by considering general working principal of UV Spectrophotometer, the dissolution samples were analyzed for their concentration of drug present. By placing buffer medium as reference sample, along with test samples absorbance is observed by running instrument with λ max 281 nm.

Specifications for dissolution study	
Apparatus type	**USP type-I**
Temperature of medium	37±0.5°C
Rpm	100
Sample size	5 ml
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 = l nM0 + kt), Higuchi model (M = k√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; M0 is the amount of drug released at zero time; k is the release rate constant; and n is the exponent. Drug release following particular mechanism is adjudged by the linearity (R^2) of plot.[7,8]

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 administrated directly into the stomach for 7 days.

After 7 days of incubation and feeding Eudragit- S100, the rats were sacrificed, the 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 6.8 to make 2% concentration of rat caecal content. Carbon dioxide (CO_2) was continuously passed to pooled content to maintain anaerobic environment.[9]

Ex vivo drug release studies in the presence of rat caecal content

This investigation was carried out for the tablet, which removed after the 5th 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 ± 0.18°C and rotated at a speed of 100 rpm.

The following are the minor modifications to carry out the *ex vivo* drug release studies. The 250 ml beaker containing 100 ml phosphate buffer pH 6.8 with 2% rat caecal 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.[10]

Antibacterial activity of vancomycin hydrochloride by cup plate method

Preparation of standard

Vancomycin hydrochloride contains 100 mg/ml from that 0.1 ml contains 10 mg of drug that is dissolved in 10 ml of 6.8 pH phosphate-buffered saline (PBS), which is regarded as 10 mg/10ml, from that, 1 mg/ml contains 1000 µg/ml. 0.1ml of solution was dissolved in 10 ml of same solvent which is regarded as 10 µg/ml.

Preparation of test

Equivalent to 10 mg drug was transferred into 10 ml volumetric flask containing 6.8 pH PBS, sonicated for 1 h, volume was made up to 10 ml with the same buffer, from that, 1 mg/ml contains 1000 µg/ml. From that 0.2 ml, 0.1 ml and 0.05 ml of solution were collected and then dissolved in 10 ml of the same solvent, which is regarded as 20 µg/ml (T-1), 10 µg/ml (T-2), and 5µg/ml (T-3).

Preparation of nutrient agar medium (composition)

Beef extract	10 g
Peptone	10 g
Sodium chloride	5 mg
Water	1000 ml
Agar-agar	15 g

Required quantities of beef extract, peptone, and sodium chloride were accurately weighed, taken in a beaker and dissolved in distilled water and then 15 g of agar-agar was added while in hot condition and to make up to 1000 ml with hot distilled water and the contents of the beaker were heated to boiling to dissolve the agar. The pH was adjusted to 7.3 ± 0.1 with 5 M sodium hydroxide and then again boiled for 10 min. Each 30 ml was transferred into test tubes while the

medium was still warm. The test tubes containing medium were closed with cotton plugs, and the medium was sterilized in Autoclave at 115°C for 30 min. A 400 ml of sterile nutrient medium was prepared separately for the preparation of inoculum.

Preparation of inoculum

The inoculum was prepared under aseptic conditions in the laminar airflow chamber. From a recently grown stock culture of above test organisms, each of the test organisms was sub cultured on the surface of a 50 ml volume of sterile nutrient agar medium by simple streaking method. The culture tubes were incubated at 37°C for 24 h. Using sterile water, bacterial strains were harvested and bacterial suspensions were prepared. These suspensions were used as inoculums.

Preparation of agar plates

Each of the test organisms was inoculated into the prepared sterile nutrient agar medium under aseptic conditions in the laminar airflow chamber. Then, 30 ml of inoculated medium was immediately poured into sterilized Petri dishes and allowed to solidify.

Test organism used: *S. aureus*.

Procedure for antibacterial activity

The antibacterial activity of optimized formulation-2 was investigated against *S. aureus* bacterial strains by the cup plate agar diffusion method. Into the solidified agar medium five wells (cups/holes) of uniform diameter (6 mm) were made using sterile aluminum borer. Then, using micropipette 50 µl of each test concentration, standard drug concentration, and vehicle control (sterile water for injection) were pipette and added directly into the respective wells in the laminar airflow chamber. The Petri dishes were carefully placed in refrigerator for 15 min to allow the diffusion of the solution in the wells into the medium. All the Petri dishes were then incubated at 37 ± 10°C for 24 h. Petri dishes were then examined for the presence of growth inhibition zones, and the diameters of the inhibition zone around each disc were measured in centimeters (including the diameter of the well [6 mm]). For each inhibition zone the diameter was measured 2 times from different directions, and the mean values were represented. The experiment was performed 3 times.

Stability study

The stability study was conducted according to International Conference on Harmonization guidelines. During this study, the only parameters which are highly effected by temperature and humidity were considered for the determination of stability, i.e., Physical Appearance, Drug content, and assay.

The optimized formulation (F_2) was stored in aluminum packaging laminated with polyethylene (cellophane packets) and kept in stability chamber at 40C ± 2°C/75 ± 5% RH (accelerated temperature studies) for 3 months. The tablets were analyzed after 0 day, 3 months, and 6 months. At the end of the study period, tablets were observed for the change in physical appearance, hardness, moisture content, friability, and drug content.[11]

RESULTS AND DISCUSSION

Drug excipients compatibility study

The FTIR spectra of Vancomycin HCl, excipient mixture is shown individually in Figures 1 and 2. The characteristic peaks of N-H stretching observed at the wavelength of 3452.6 cm^{-1}, N-H bending at 1637.5 cm^{-1}, C=C stretching (aromatic group) at 1475.2 cm^{-1}, C-N vibrations at 1189.2 cm^{-1}, and C=S stretching observed at 1050.1 cm^{-1}. The spectra 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.

Evaluation of derived and flow properties of granules

The results for the evaluations of derived and flow properties are given in Table 4. During the study, bulk density for the formulations was observed between 0.43 ± 0.012 and 0.51 ± 0.043 g/cm^3. The tapped densities were between

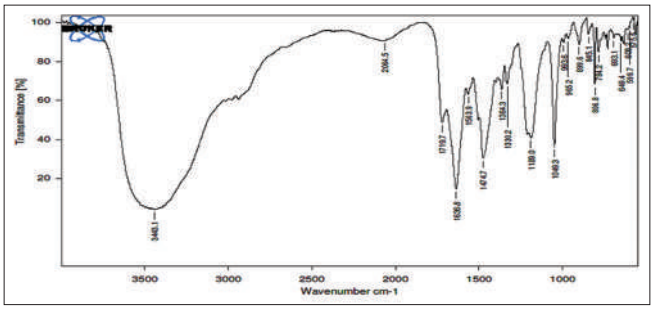

Figure 1: Fourier-transform infrared spectroscopy spectrum for optimized formulation (F2)

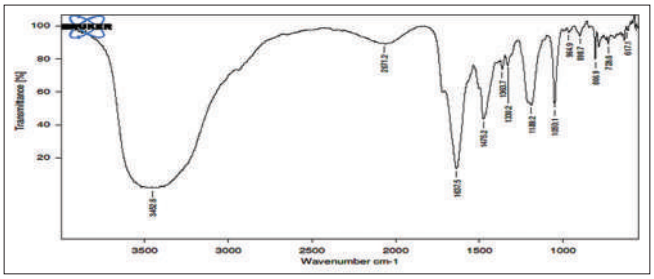

Figure 2: Fourier-transform infrared spectroscopy spectrum for pure vancomycin HCl

0.46 and 0.53 g/cm^3. Using the above two densities data, compressibility index and Hausner's ratio were calculated. The compressibility index ranges from 13.61 ± 1.43 to 17.69 ± 1.45. The Hausner's ratio ranges from 0.03 to 0.08. The angle of repose ranges from 21.80 ± 1.02 to 28.81 ± 1.03 indicates good to fair flow properties of the granules.

Evaluation parameters of tablets

The results for the evaluations of physical properties (thickness, weight variations, hardness, and friability) and % assay of tablet are given in Table 5. During the study, the formulations showed small weight variations (<6%); uniform thickness and hardness; acceptable friability (<1%); and % drug content. The average weight of tablet formulation was within the range of 647.3 ± 0.012–650.7 ± 0.018 mg. The hardness of the tablets ranged between 6.92 ± 0.15 and 7.36 ± 0.12 kg/cm^2. In case of content uniformity test, drug content was found to be within 99.35 ± 0.27%–98.14 ± 0.36% of labeled amount.

Swelling studies

All the tablets 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 (2% IPC, 6% coating) showed maximum swelling index of 79.83 ± 1.61%. Thus, IPC tablet is hydrophilic and swells considerably in phosphate buffer solutions. This process shows a pH-dependent pattern.

Evaluation of enteric-coated tablets

Tablets weight, % coating, and disintegration time of coated tablets are reported in Table 6. The Weight variation of enteric-coated tablets for all batches (F1–F9) observed was below 3.5%. The weight gain for as per the desired % coating was found to be within the range of 6–10%.

The disintegration test revealed that all the formulations remained intact in 0.1 N HCl for 1 h and observed no erosion of the coated solution over the tablet.

Table 4: Formulation details

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Vancomycin HCl	500	500	500	500	500	500	500	500	500
PEC (%w/w)	10	10	10	10	10	10	10	10	10
Microcrystalline cellulose	133	133	133	123	123	123	113	113	113
PVP-K30 (2%w/v)	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs
Magnesium stearate	2	2	2	2	2	2	2	2	2
Talc	4	4	4	4	4	4	4	4	4
Eudragit-S100 (%TWG)	6	8	10	6	8	10	6	8	10
Total weight (mg)	650	650	650	650	650	650	650	650	650

TWG: Total weight gain, PEC: Polyelectrolyte complex

Table 5: Evaluation of derived and flow properties of granules

Batch code	Bulk density (g/Ml) (*n*=3)	Tapped density (g/Ml) (*n*=3)	Carr's index (%)	Hausner's ratio	Angle of repose (°)
F1	0.43±0.01	0.46± 0.023	16.45±1.3	1.06±0.01	26.10±1.5
F2	0.50±0.06	0.52±0.016	14.01±0.9	1.04± 0.04	28.81±1.0
F3	0.41±0.05	0.43±0.034	14.65±1.2	1.04± 0.02	26.56±2.1
F4	0.43±0.04	0.46±0.09	16.45±2.0	1.06±0.03	25.64±1.0
F5	0.43±0.035	0.47±0.014	17.64±1.5	1.08±0.01	27.02±2.0
F6	0.48±0.02	0.52±0.019	17.69±1.4	1.08±0.03	21.80±1.0
F7	0.51±0.04	0.53±0.011	13.93±1.0	1.04±0.04	27.02±1.4
F8	0.50±0.03	0.52±0.023	13.61±1.4	1.03±0.02	28.07±1.4
F9	0.43±0.04	0.45±0.017	14.44±1.3	1.04±0.02	25.17±1.2

In vitro drug release study

All the formulations were evaluated for *in vitro* drug release and cumulative % 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 next 15 h in phosphate buffer (pH 6.8).

During this study, none of the formulations showed drug release at simulated pH 1.2 (0.1 N HCl) due to enteric coating over the formulations. A maximum of 12.85 ± 3.32% 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 tablets Table 7. The release rate of drug was observed slower for formulations which have higher coating levels and 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 ideal type of drug release for colon targeted tablets Table 8.

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 6 and observed all the formulations

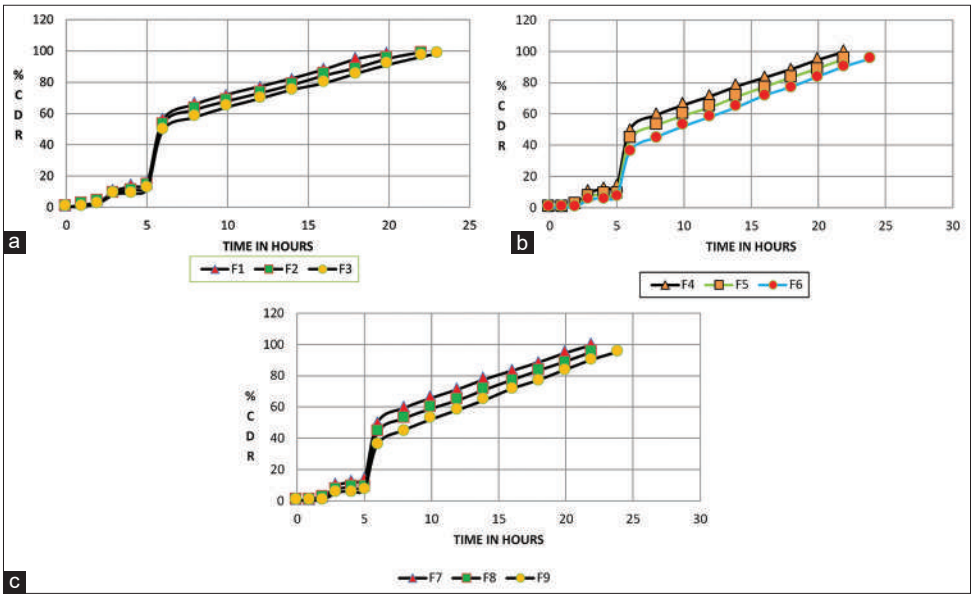

Figure 3: *In vitro* dissolution of all formulations

Table 6: Evaluation parameters of tablets

Batch code	Average weight (mg) (*n*=20)	Hardness (kg/cm²) (*n*=3)	Friability (%) (*n*=6)	Thickness (mm) (*n*=6)	Assay (%) (*n*=3)
F1	649.6±0.018	7.02±0.12	0.26±0.23	4.26±0.02	99.23±0.13
F2	650.4±0.091	7.14±0.12	0.36±0.34	4.26±0.040	99.23±0.03
F3	647.3±0.012	7.23±0.14	0.25±0.22	4.25±0.016	98.14±0.36
F4	648.6±0.028	6.94±0.14	0.34±0.21	4.25±0.024	99.35±0.27
F5	649.1±0.100	7. 15±0.11	0.28±0.31	4.24±0.023	98.57±0.17
F6	650.1±0.01	7.26±0.14	0.44±0.36	4.25±0.026	98.26±0.21
F7	648.9±0.085	6.92±0.15	0.36±0.26	4.27±0.012	98.36±0.11
F8	650.7±0.018	7.25±0.12	0.27±0.38	4.25±0.040	99.01±0.18
F9	649.8±0.056	7.36±0.12	0. 43± 0.35	4.24±0.030	98.62±0.27

Table 7: Evaluation of enteric-coated tablets

Batch code	Weight of tablet (mg) (*n*=20)	% coating	Disintegration time inpH 7.4 phosphate buffer
F1	689.25±1.62	6	43 min 50 s
F2	702.32±1.25	8	53 min 42 s
F3	715.86±1.54	10	55 min 31 s
F4	689.14±0.24	6	49 min 54 s
F5	702.28±0.53	8	53 min 28 s
F6	715.58±0.18	10	56 min 14 s
F7	689.21±0.23	6	51 min 10 s
F8	702.38±0.75	8	58 min 54 s
F9	715.67±0.90	10	60 min 15 s

Table 8: Kinetic analysis of dissolution data

Formulation code	Zero-order		First-order		Higuchi		Korsmeyer–Peppas	
	R^2	K_0	R^2	$K_1(hr^{-1})$	R^2	K_H	R^2	N
F1	0.9774	3.035	0.857	0.211	0.9047	27.97	0.9149	1.415
F2	0.9969	2.806	0.8189	0.174	0.9070	26.63	0.9112	1.407
F3	0.9962	2.794	0.8594	0.163	0.9207	25.53	0.9005	1.509
F4	0.9932	2.9295	0.8874	0.193	0.9134	27.26	0.9128	1.403
F5	0.9967	2.929	0.8504	0.172	0.9243	25.96	0.9079	1.396
F6	0.9929	3.046	0.8522	0.192	0.9347	25.24	0.9033	1.588
F7	0.9965	2.984	0.8871	0.163	0.9252	26.35	0.9213	1.491
F8	0.9988	3.105	0.8161	0.163	0.9286	25.18	0.9176	1.433
F9	0.9984	3.243	0.9022	0.130	0.9353	24.19	0.8960	1.814

are best fitted with zero-order and Korsmeyer-Peppas plot than compared to the Higuchi plot.

The R^2 values ranged between 0.9774 and 0.9988 for all the formulations. The *"n"* value was in the range of 1.3964-1.8142, i.e., indicating that, the release mechanism of vancomycin HCl from these matrices 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.

Data analysis

Data analysis for drug release at 5th h (Q5)

Data analysis for drug release at 5th h (Q5), R^2 Value in plot was 0.9625, 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).

$$Y1 = 11.87 - 2.66*x - 1.675*y + 0.7067*x^2 - 0.86*x*y \quad (4)$$

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 = 2.66 and $P \leq 0.001$) on Q5, whereas to some extent X2 (% coating) also affects the results (b2 = 1.675 and $P = 0.007$) an inverse manner, significance ($P < 0.05$). The high values of the coefficient of determination indicate a good fit, i.e., good agreement between the dependent and independent variables. The coefficients b1 and b2 were found to be significant at $P < 0.05$.

Data analysis for drug release at 20 h (Q20)

The R^2 value for Q20 in the plot of predicted versus observed responses was 0.9952 which indicated excellent fit. The Y2 (Q20) values observed for different batches showed wide variation, i.e., values ranged from a minimum of 84.12% to a maximum of 98.63%. Eq. (5) refers to polynomial equation for response Y2.

$$Y2 = 93.44 - 4.543*x - 3.08*y - 0.4925*x*y - 0.9967*y^2 \quad (5)$$

Coefficient b1 and b2 possessed negative and negative signs, respectively, which indicated that both the independent variables X1 and X2 having negative effects on Y2, i.e., release at 20th h. Independent variables, X1 (amount of

PEC) (b1 = 4.543 and $P \leq 0.001$) affects more prominently the Q20 then X2(% coating) (b2 =3.08 and $P \leq 0.001$) which has inverse effect on Q20. Significance ($P < 0.05$). The coefficient b1 and b2 were found to be significant at $P < 0.05$.

Data analysis for 50% of drug release (t50%)

The R^2 value for t50% in plot of predicted versus observed responses was 0.9832 which indicated excellent fit. The Y3 (t50%) values observed for different batches showed wide variation, i.e., values ranged from a minimum of 6th h to a maximum of 10th h. Eq. (6) refers to polynomial equation (full model) for response Y3.

$$Y3 = 7.0 + 1.167*x + 0.8333*y + 0.1667*x^2 + 0.75*x*y \quad (6)$$

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., 50% of drug release. Independent variables, X1 (amount of PEC) (b1 = 1.167 and $P = 0.005$) affects more prominently the Y3 then X (% coating) (b2 = 0.8333 and $P = 0.020$) significance

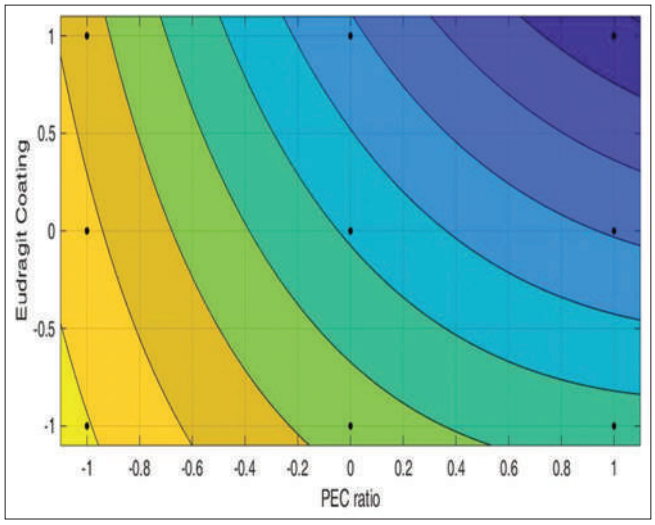

Figure 4a: Contour plot of 5th h drug release

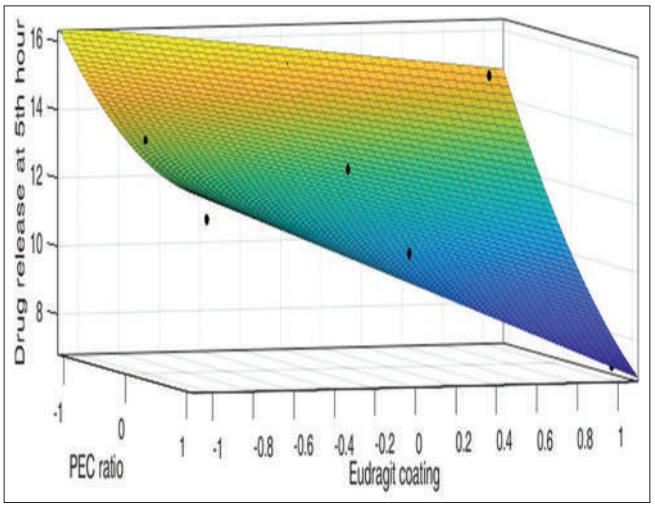

Figure 4b: Surface plot of 5th h drug release

Table 9: Drug release data modeling

batch code	Independent variables (codes)		Dependent variables		
	PEC ratios	Eudragit S100 coating TWG	5th h% of drug release	20th h% of drug release	50% of drug release
F1	−1	−1	15.89	99.93	6th h
F2	−1	0	15.10	97.37	6th h
F3	−1	+1	14.73	94.68	6th h
F4	0	−1	13.63	95.29	6th h
F5	0	0	12.65	93.68	7th h
F6	0	+1	9.34	89.28	8th h
F7	+1	−1	11.84	91.34	7th h
F8	+1	0	10.68	89.26	8th h
F9	+1	+1	7.24	84.12	10th h

TWG: Total weight gain, PEC: Polyelectrolyte complex

Table 10: Results of multiple regression analysis of Q5, Q20, and t50%

Dependent variable	Q5=Y1		Q20=Y2		t50% =Y3	
	P value	Coefficient	*P* value	Coefficient	*P* value	Coefficient
Intercept	<0.001	12.344	<0.001	92.772	<0.001	7.111
X1	<0.001	−2.660	<0.001	−4.543	0.005	1.167
X2	0.007	−1.675	<0.001	−3.080	0.020	0.833

($P < 0.05$). The coefficient b1 and b2 were found to be significant at $P < 0.05$.

Tables 9 and 10 shows the results of multiple regression analyses of Q5, Q20, and t50%.

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 Figures 4a and b-6 a and b, which are useful tools to study interaction effects of the factors on responses. Figure 4a and b, 5a and b exhibited non-linear pattern, but with an increase in PEC concentration and % coating level it also showed that amount of PEC has a comparatively greater influence on response variable Q5 and Q20 then % of coating. In contrast to the result of drug release at 5th and 20th h contour plot of time taken for 50% of drug release varies non-linear manner with an increase in amount of PEC and % of coating [Figure 6a and b]. However, the effect of X1 (amount of PEC) seems to be more pronounced as compared with that of % of coating. The results were also confirmed through 3D response surface graphs.

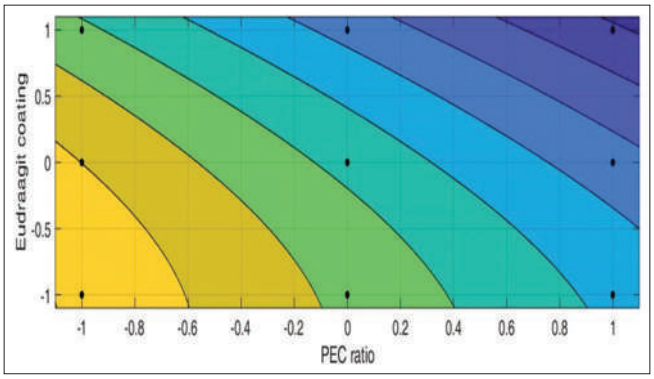

Figure 5a: Contour plot of 20th h drug release

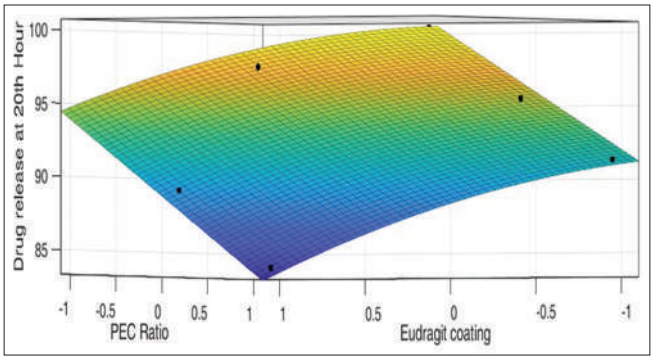

Figure 5b: Surface plot of 20th h drug release

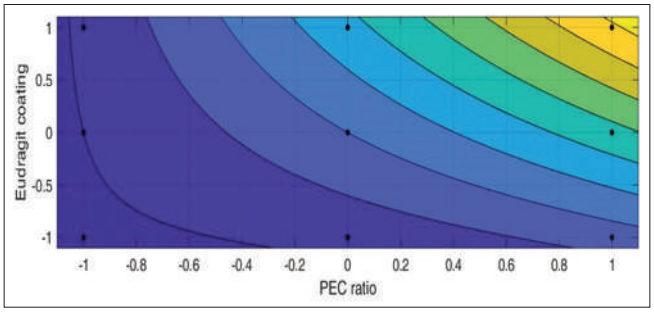

Figure 6a: Contour plot of 50% of drug release

Selection of optimized formulation

From the polynomial equation and the contour plots, the optimized batch F2 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 (2%) and coating (8%) were found to fulfill the requisite of an optimum formula. *In vitro* release data of optimized formulation are showed 98.63% Vancomycin HCl release on completion of 20 h but showed a release of 13.63% at 5th 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. The composition of the optimized batch F2 was also very economical when compared to the next best formulation, F7, as obtained from factorial design.

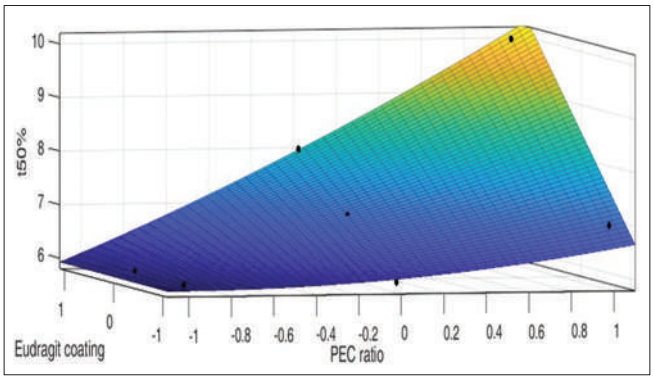

Figure 6b: Surface plot of 50% of drug release

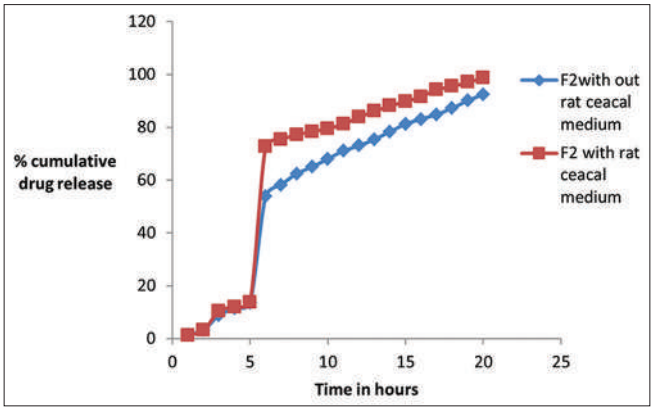

Figure 7: Drug release profile of optimized formulation with and without rat cecal content

Table 11: Percentage zone of inhibition for optimized formulation-2

S. No.	Bacterial strains used	% of inhibition zone (cm)				
		Drug concentration (µg/ml)			Control	Standard drug (10 µg/ml)
		20	10	5		
1	*Staphylococcus aureus*	3.87	2.91	1.12	-	2.85

Table 12: Results of accelerated stability tests of tablets at 0, 3, and 6 months

Time (Months)	Moisture content % (±SD)	Hardness (Kg/cm²)(±SD)	Friability %	Disintegration time (min) (in HCl)
0	5.2±0.24	5.8±0.1	0.12	No disintegration
3	5.8±0.32	5.4±0.2	0.01	No disintegration
6	6.9±0.45	5.2±0.1	0.01	No disintegration

Table 13: Drug content and assay values for the optimized formulation during the stability studies

Time (Months)	Drug content (mg) (±SD)	Assay (%) (±SD)
0	496.15±0.01	99.08±0.06
3	495.65±0.03	
6	494.45±0.08	

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 drug release studies were carried out at *in vitro* and *ex vivo* conditions, i.e., carried out using 100 ml phosphate buffer pH 6.8 with and without rat cecal content. Moreover, the difference in Vancomycin HCl release was observed in two conditions at 20th h and is significant. The amount of drug release from the optimized formulation was found to be 98.68% in dissolution medium with 2% w/v of cecal content and with no cecal content in dissolution medium, the drug release was found is 92.46% at 20th h [Figure 7].

The study reveals that the release of the drug in the physiological environment of the colon is due to the degradation of chitosan by colonic bacteria present in rat cecal content Table 11.

The optimized formulation (F2) shows 98.68% drug release in the presence of rat cecal content. There was increase in drug release in the dissolution medium containing rat cecal content (2%) as compared to control group (without rat cecal content).

Antibacterial activity of vancomycin hydrochloride by cup plate method

The results showed that the inhibition for a standard solution was increased on increasing the drug concentration. The

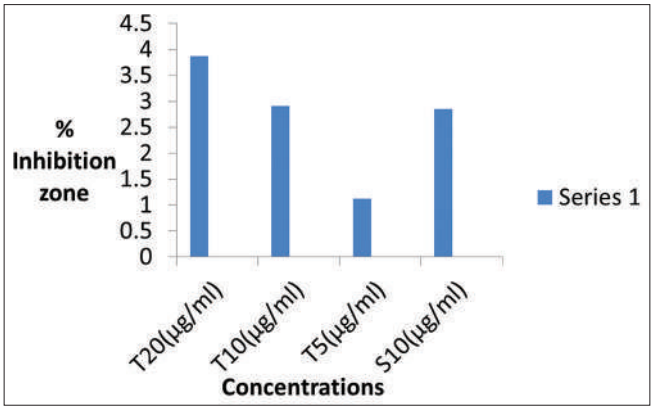

Figure 8: Microbiological studies and zone of inhibition for standard and formulation F2

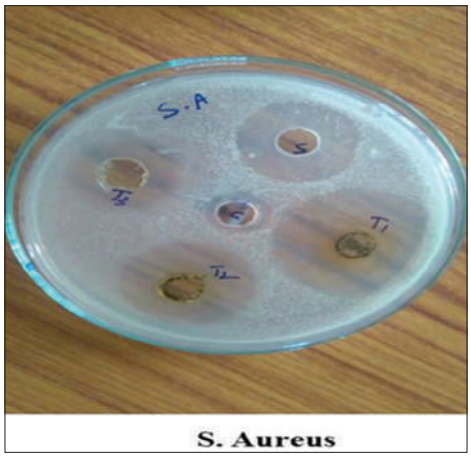

Figure 9: Pictures for antibacterial activity of vancomycin hydrochloride on *Staphylococcus aureus*

formulation F2 showed highest zone of inhibition after 24 h at 20 µg/ml concentration when compared with other concentrations. A graph was plot for the formulation F2 between log concentration and zone of inhibition and showed that on increasing drug concentration the percentage of inhibition also increased [Figure 8]. The results also showed that there was a steady release of the drug and is capable of inhibiting microorganism *S. aureus* for 24 h. This indicated

that the prepared vancomycin tablets are efficient and also can inhibit the growth of microorganism *S. aureus* due to high drug diffusion Figure 9.

Accelerated stability studies

The stability tests for optimized formulation were performed according to the ICH guidelines. The temperature conditions followed were 40 ± 2°C and 75% RH for 6 months. The results are shown in Tables 12 and 13 which indicates that the optimized tablets did not have any significant changes in the physical properties, drug content, assay, and thus passed the stability testing.

CONCLUSION

Vancomycin HCL colon targeted tablets were successfully prepared using PEC which coated with Eudragit-S100 and prepared formulations were optimized by full factorial design. The formulation F2 prepared with PEC (2%) and Eudragit-S100 coating (8%) level, which meeting objective of the present research work for the effective utilization and localized effect of prepared Vancomycin HCl Tablets in colon region. The antibacterial activity also showed highest zone of inhibition after 24 h at 20 µg/ml concentration when compared with other concentrations.

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REFERENCES

1. Gent AN, Hamed GR. 198 – adhesive. In: Encyclopedia of Polymer Science and Engineering. 1st ed. New York: Wiley Interscience; 1985. p. 476-517.
2. Jose S, Dhanya K, Cinu TA, Litty J, Chacko AJ. Colon targeted drug delivery: Different approaches. J Young Pharm 2009;1:13-9.
3. Geever LM, Cooney CC, Lyons JG, Kennedy JE, Nugent MJ, Devery S. Characterisation and controlled drug release from novel drug-loaded hydrogels. Eur J Pharm Biopharm 2008;69:1147-59.
4. Jana S, Banerjee A, Gandhi A. Preparation and characterization of chitosan based polyelectrolyte complex as a carrier of aceclofenac. J PharmaSciTech 2014;3:68-71.
5. Rao YM, Jithan AV. Advance in Drug Delivery. Vol. 3. USA: Pharmamed Press; 2014. p. 237-9.
6. Grey RO, Beddow JK. On the hausner ratio and its relationship to some properties of metal powders. Powder Technol 1969;2:323-6.
7. Korsmeyer RW, Gurney R, Dueler EM, Bury P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm 1983;15:25-35.
8. Peppas NA. Analysis of fickian and non-fickian drug release from polymers. Pharm Acta Helv 1985;60:110-1.
9. Mehta R, Chawla A, Sharma P, Pawar P. Formulation and *in vitro* evaluation of eudragit S-100 coated naproxen matrix tablets for colon-targeted drug delivery system. J Adv Pharm Technol Res 2013;4:31-41.
10. Salve P. Development and *in vitro* evaluation colon targeted delivery system using natural gums. Asian J Pharm Res 2011;1:91-101.
11. Nykänen P, Sten T, Jürjenson H, Veski P, Marvola M. Citric acid as a pH-regulating additive in granules and the tablet matrix in enteric-coated formulations for colon-specific drug delivery. Pharmazie 2004;59:268-73.

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