Research Article

ENHANCEMENT OF ANTIMICROBIAL ACTIVITY OF SUPPRESS RELEASE AMOXICILLIN CAMPHOR COMPLEXED MUCOADHESIVE TABLETS BY STATISTICAL EXPERIMENTAL DESIGN

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ABSTRACT

Objective: The purpose of the present work was to enhance antimicrobial activity of Suppress release Amoxicillin Camphor complexed Mucoadhesive tablets by Statistical Experimental Design using Sigma tech software version 3.1. Amoxicillin has short half-life (1 h) which requires frequent administration of the conventional amoxicillin tablets and is mainly used in the eradication of *Helicobacter pylori* that resides in the stomach. **Methods:** Amoxicillin-Sodium cholate (ASC) and camphor complexes (ACC) were prepared to enhance antimicrobial activity. **Results:** TLC and FT-IR studies confirmed the formation of drug complex. Zone of inhibition by agar well diffusion method of ACC showed greater inhibition than ASC, solubility of ACC was enhanced by re-crystallization technique. Hence ACC needle shaped re-crystallized was used to sustained release tablets using mucoadhesive polymers. Chitosan, HPMC K-15 and Ethyl cellulose (EC) were selected as independent variables and *ex vivo* mucoadhesion time, % drug release at 24 h and t₅₀ % (time to release 50 % drug) were selected as dependent variables. DSC studies indicated drug and excipients were compatible. Swelling studies and scanning electron microscopic analysis confirmed the drug release mechanism from sustained release tablets. *In-vitro* release studies and *ex vivo* studies of amoxicillin confirmed the sustained drug release profile with first order release kinetics better mucoadhesion and enhanced antimicrobial activity. The optimized formulation was found to be stable at accelerated storage conditions for 3 months. **Conclusion:** The results demonstrated the effectiveness of the proposed statistical design for optimization of ACC sustained release tablets.

Keywords: Statistical experimental design; Complexation; Chitosan; HPMC K-15; Ethyl cellulose; Mucoadhesion.

INTRODUCTION

Helicobacter pylori infection, a bacterial infection can be treated effectively using antibiotics with a broad spectrum activity. This infection is mainly responsible for the cause of chronic gastritis, gastric cancer and peptic ulcer disease¹. Half of the world's population affected by this bacterium, infection rates vary from country to country but developing countries have shown much higher rates of infection compared to developed countries, with prevalence rates (50 % - 69 %) in India¹. This bacterial infection can be exterminated using Amoxicillin (beta-Lactam family), where it exists in the epithelial surfaces of a cell or gastric mucosal layer. By increasing the concentration and residence time of amoxicillin in the stomach may eradicate this bacterium. The conventional amoxicillin dosage forms has short plasma half-life (1 hour) there by reducing its resident time in the stomach and degrades in gastric acid resulting in are duced concentration in gastric blood, hence it necessitates to extend residence time of the amoxicillin to provide better effective *Helicobacter pylori* eradication. Several attempts were made to extend the time of residence for eradication such as such as floating drug delivery tablets², mucoadhesive tablets³, mucoadhesive microsphere⁴, sustained release chitosan tablets⁵ extended-release tablets comprising HPC-H, HPMC K100 LV⁶. Antimicrobial activity can be further enhanced by increasing lipophilicity of the drug molecule.

Sustained delivery dosage forms provide the medication release for a prolonged period of time, a kind of controlled release system and controls the concentration of drug at required site. These systems show better patient compliance as there is the slower release of drug in the target site for prolonged period of time. Repeated administration of conventional dosage forms i.e. multiple administration may cause unwanted side effects which can be minimized by employing controlled release dosage forms. The residence time of Amoxicillin in the stomach can be enhanced by formulating with mucoadhesive polymers that maintain therapeutic concentration in a sustained manner. It has been reported so far that by employing many of hydrophilic polymers such as PMAA, starch, polyethylene glycol (PEG), polyacrylic acid (PAA), Hydroxy Propyl Methylcellulose (HPMC) and chitosan the therapeutic behaviour of amoxicillin formulations was improved to prevent its degradation and thereby increasing its plasma half-life^{2,5}. HPMC proved to be highly biodegradable, pH insensitive, mucoadhesive, biocompatible, swell able hydrophilic polymer. It forms a gelatinous layer, diffuses the water-soluble drugs upon contact with water. Chitosan is widely used as sustained release polymer in several dosage forms for sustaining the release of water-soluble drugs as a mucoadhesive polymer by swelling mechanism, which is non-toxic, biocompatible and biodegradable^{7,8}. Many amoxicillin formulations were developed by researchers such as mucoadhesive microspheres that adhere to the mucosal lining of the stomach, as well as to release the drug in a sustained manner⁹.

The sustained release of amoxicillin could be formulated in various forms, e.g. microspheres, beads and microcapsules, floating tablets. Ethyl cellulose (EC), a hydrophobic polymer which is non-toxic and inert, is used in most of the controlled release formulations as a mucoadhesive polymer for sustaining the drug release. Instead of formulating amoxicillin mucoadhesive sustained-release tablets by the normal trial method, a statistical experimental design i.e. Design of Experiments (DoE) was used which involves selection of independent and dependent variables. These concentrations of independent variables were selected by referring hand book of pharmaceutical excipients, literature search and experimentation done by other authors. Mucoadhesive polymers like Hydroxy Propyl Methyl Cellulose K15, Chitosan and Ethyl Cellulose were selected as independent variables to increase the residence time of amoxicillin in the stomach and *ex-vivo* mucoadhesion time and drug release at 24 h and t 50 % were selected as dependent variables/factors.¹⁰

The rationale of the present work was to formulate and optimize new sustained release mucoadhesive tablets of amoxicillin to minimize the frequency of administration and to enhance the time of residence of amoxicillin to eradicate *Helicobacter pylori* by Design of Experiment (2³ factorial design) including midpoint levels with fewer trials to obtain optimized formula. To the extent of our knowledge, the effect of mucoadhesive polymers (HPMC K15, chitosan, EC) on mucoadhesion and drug release for 24 h have not been reported for the drug Amoxicillin using experimental design (2³ factorial design).

MATERIAL AND METHODS

Amoxicillin obtained as a gift sample from Aurobindo Labs, Telangana, India. Chitosan, HPMC K15M, ethyl cellulose, Avicel pH 101, talc magnesium stearate and other chemicals were procured from SD fine Chemicals, Mumbai, India.

Pre formulation studies

API Characterization

Organoleptic properties

The pure Amoxicillin drug was studied for its organoleptic properties like description, color, odor and taste¹⁰.

Determination of melting point

One end of the Capillary tube was sealed, through the other end of the tube filled with the test substance and placed into the melting point apparatus. The melting point value of the drug was noted and compared with the standard literature value¹⁰.

Determination of Solubility

Supersaturated solutions of test substance in 0.1N HCl and water were prepared separately to study its solubility. It was then placed in an orbital shaker and was shaken for 24 hours, filtered the solution. The filtrate was collected and measured the absorbance using UV spectrophotometer (UV-1800, Shimadzu) to calculate solubility of Amoxicillin¹¹.

Preparation of Amoxicillin-Sodium cholate complex (ASC) and Amoxicillin-Camphor complex (ACC)

2 g of Amoxicillin was weighed and diluted to 57.14 ml with ethanol while 2.233 g of sodium cholate was reconstituted to 57.2 ml ethanol. Each was agitated properly. Sodium cholate solution

in a beaker was added to Amoxicillin solution drop wise while stirring and stirring continued till complete for evaporation of ethanol. Same procedure was followed for Amoxicillin- camphor complex¹².

Thin Layer Chromatographic detection of ASC and ACC

n-butanol-water-acetic acid selected as mobile phase ratio of 60:20:20. The chromatographic chambers were saturated with the mobile phase for 30 minutes. The plates were developed over a distance of 15 cm in filter-paper-lined chromatographic chambers, dried in a hot air oven. For the detection of spots in TLC plates, a few iodine crystals were placed on the base of fully closed chromatographic chamber which was then placed a fume cupboard. After 2 hours during which violet iodine vaporizes and distributes homogenously throughout the chromatographic chamber, the chromatographic plates were introduced in the chamber and kept for few minutes to get color spots¹³.

Fourier transformation-infrared spectroscopy (FT-IR)

The physical mixture of pure drug sample and other complexes were taken and subjected to IR spectral studies using FTIR spectrophotometer (FT-IR Spectrometer Bruker ALPHA)¹².

Determination of free Amoxicillin in complex

The absorbance of Amoxicillin-cholate complex and Amoxicillin-Camphor complex was determined at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/ml using UV-Visible spectrophotometer at 231 nm (λ_{max}) of Amoxicillin¹², the values of standard calibration curve were used to calculate the amount of Amoxicillin in the complex, given the formula as follows:

$$\text{Concentration (unknown)} = (\text{unknown-intercept})/\text{slope (standard)}$$

Antimicrobial activity

10, 20 and 30 µg/ml concentrations of Amoxicillin, ASC and ACC were prepared to evaluate the antibacterial property of prepared complexes by agar well diffusion method¹².

Determination of λ_{max} of ACC

100 mg of prepared complexes were dissolved in ethanol and then serial dilutions were made with water and these samples absorbance were analyzed under Shimadzu UV spectrophotometer from 200-400 nm¹².

Solubility enhancement of ACC

For the solvent evaporation method, 0.75 g of Amoxicillin-Camphor was dissolved in 25 mL of ethanol and 25 mL of methanol. This solvent mixture was heated for complete soluble of drug. The solution was cooled to room temperature and allowed to evaporate solvent completely. Formed Crystals were dried under vacuum at room temperature and stored in a desiccator for further use¹⁴.

Selection of excipients for formulation development of model drug

Mucoadhesive polymers as independent variables were selected to develop the formula of model drug based on literature search, by experimentation done by authors in the previous study and Pre formulation studies.

Drug-Excipients compatibility study**Differential scanning calorimetry (DSC)**

Accurately 5 mg of ACC was weighed and sieved through the #60 sieve, transferred to DSC aluminium pan and scanned at 25-250°C temperature at 10°C/min heating rate. The procedure was repeated by placing the blend of the optimized formulation to obtain thermo grams. The obtained thermo grams were compared to identify for any interaction between them¹⁰.

Mixing method

ACC weighed and mixed with HPMC K15, chitosan, EC, Avicel pH 101, talc, Magnesium stearate separately in different ratios and these mixtures were packed in transparent glass vials of 5 ml capacity, sealed with rubber closures. These vials were placed in stability chambers maintained at 50°C and 40°C/75 % RH. Observations for physical appearance were made at an initial loading of the sample, 2nd week and 4th week.

Formulation of amoxicillin controlled-release tablets by 2³ factorial design

In this study, 2³ factorial design with midpoint levels were selected for the experimental design of Amoxicillin camphor complexed mucoadhesive sustained-release tablets (Table 1). The amount of Chitosan, HPMC K 15M and Ethylcellulose were chosen as responses, mucoadhesion time (h), % drug release at 24th h, t50 % were selected as factors.

Wet granulation technique was employed for the preparation of Amoxicillin camphor complex mucoadhesive sustained-release tablets. All the other ingredients were weighed, sifted through sieve no. 60 separately. PVP K-30 was dissolved in ethyl alcohol and was added as a binder solution to the sieved ingredients (HPMC K15M, chitosan, EC, Avicel PH-101) for the formation of wet mass. Then sifted the mass through #100. The obtained wet granules were dried in a hot air oven at 60°C for 20 min. Percentage yield was calculated, the lubricants were added and mixed with the dried granules.

Table 1: Experimental design of tablets as per 2³ factorial level

S. No	Code	Name of variable	Unit	-1 (Low level)	0 (Mid-Point)	1 (High level)
1	X1	Chitosan (CS)	mg	11.875	13.75	15.625
2	X2	HPMC K 15M	mg	7.625	9.25	10.875
3	X3	Ethyl cellulose	mg	16.75	18.5	20.25

Evaluation of pre-compression parameters**Bulk Density**

100 ml graduated cylinder was held in inclined position and transferred accurately weighed blend of formulation into it. Initial weight and volume were noted.

$$\text{Bulk density} = \text{Weight of the sample} / \text{volume of the sample}^{15}$$

Tapped Density

To determine tapped density a measuring cylinder of 100 ml capacity was taken and an accurately weighed blend of sample was transferred (Electrolab Tapped Density Apparatus). Initial volume (V₀) was noted and tapped the cylinder for 10 times, noted the final volume; continued tapings for 500 times. Since the difference between the volume was more than 2 ml after 10 and 500 tapings so further continued for 1250 tapings¹⁵.

Hausner's Ratio

Hausner ratio (HR) was determined using the formula

$$\text{HR} = \text{Tapped density} / \text{Bulk density} \times 100$$

Carr's Index

Compressibility index (CI) was calculated using the formula

$$\text{CI \%} = \frac{\text{Tapped density of the powder} - \text{Bulk density of the powder}}{\text{Tapped density of the powder}} \times 100.$$

Angle of Repose

A funnel was taken and placed vertically to a cone height (h) of maximum level then poured the amoxicillin blend through it, heap radius (r) was measured. Calculated angle of repose using the formula:

$$\tan \theta = \text{Cone height} / \text{Heap radius}^{12}$$

Compression of Amoxicillin granules

Rimek tablet compression machine was used for compressing the formulated granules into tablets.

Evaluation of post-compression parameters**Physical appearance**

The tablets were inspected for smoothness, the absence of cracks, chips and other undesirable characteristics¹⁶.

Weight variation test

20 tablets were selected randomly; the weight of the individual tablet and the average weight of the tablets were noted¹⁷.

Thickness

Thickness was determined using digital vernier calipers for 20 tablets from each batch and expressed the average thickness in mm.

Hardness

Monsanto hardness tester was used to determine the hardness of tablets. 10 tablets hardness was noted and the average hardness was calculated, expressed in kg/cm²¹⁸.

Friability

10 tablets were taken its initial weight was noted and were placed in Roche friabilator rotated for 100 revolutions at 25 rpm and then de-dusted and reweighed¹⁹. The percentage friability was calculated using the formula:

$$\text{Percentage friability} = \frac{(A-B)}{B} \times 100$$

Where,

A = Initial weight of tablets, B = Final weight of tablets after 100 revolutions

Drug content

Randomly 20 tablets were selected and crushed. To 250 mg of ACC, equivalent powder was weighed and transferred to 100 ml volumetric flask. 80 ml of 0.1N HCl was added and shaken for 10 min using a mechanical shaker. Filtered the solution after making up the volume to 100 ml. 10 ml of the filtrate was collected and diluted to 100 ml with 0.1 N HCl. Further 10 ml of the resulting filtered solution was taken and diluted with 0.1N HCl to 100 ml. Then the final obtained solution was evaluated to determine drug content of tablets at 250 nm by UV-Visible spectrophotometer²⁰.

In vitro Dissolution study

Agilent HPLC (contained UV-VISIBLE detector Model) was used for analyzing *in vitro* dissolution samples. EZChrom Elite software was used for the collection of data and its integration. An Ecosil-C18 column (250 × 4.6 mm, 5 µm) was used for the ACC chromatographic separation at 25°C. A mixture of potassium dihydrogen phosphate (pH 5.0, 2 M) - acetonitrile (96.5: 3.5, v/v) used as mobile phase and was pumped at 1.0 mL/min with an injection volume of 20 µL at wavelength of 252 nm. The retention time was 3.6 min during a run of 10 min.

USP Type-II (Paddle) apparatus was used for dissolution study, 0.1N HCl buffer as dissolution medium at 75 rpm. The apparatus was maintained at 37°C ± 0.5°C temperature. At the regular time of intervals (2, 4, 8, 12, 16 and 24 h) samples were withdrawn and replaced with fresh 0.1N HCl media. Cumulative % drug release was analyzed by High Performance Liquid Chromatography (HPLC) method²¹.

Determination of swelling index

A tablet was selected from each batch which was formulated without drug, noted its initial weight and placed in a wire basket. For a period of 24 h, the basket was then dipped into 250 ml simulated gastric fluid (pH 1.2) maintained at 37°C. At the end of 24 h, the basket was removed from the fluid and cleaned the surface fluid with tissue paper. The basket including the tablet was weighed. Swelling index was calculated using the formula:

$$W_E = [(W_1 - W_0)/W_0] \times 100$$

Where, W_E is the swelling index (%), W_0 is the dry weight of tablet plus basket and W_1 is the weight of wet tablet plus basket after removal from the medium²².

Scanning Electron Microscopy (SEM) study

To study the mechanism of drug release from the optimized formulation, SEM study was performed. SEM photographs at 0 h, 2 h, 12 h and 24 h of dissolution were taken for the sustained release tablets²².

Ex-vivo mucoadhesion time

The *ex vivo* mucoadhesion time for the tablet was carried out using goat stomach mucosa. A fresh goat stomach mucosa within an hour of excision was collected from a local slaughter shop (Anantapuramu, Andhra Pradesh, India) and soon after its collection placed in cold normal saline then dripped in simulated gastric fluid (SGF, pH 1.2) for 2 min after washing with distilled water and normal saline. It was set to the inner-wall of a 500 ml beaker using cyanoacrylate glue. By the application of light force by fingertip for the 60s the tablet was pasted on to the mucosal surface, previously wetted with one drop of SGF. Simulated

gastric fluid (pH 1.2) of 450 ml quantity was added to the beaker, maintained at 37°C and to simulate the peristaltic movement it was stirred at 50 rpm for a period of 20 h. *ex vivo* mucoadhesion time was considered when the tablet got detached from the mucosal surface²³.

Ex-vivo mucoadhesion strength

Mucoadhesion strength of the tablet was measured on a modified physical balance employing the method. Right side of the arm contains a 100 ml beaker of weight M; freshly collected goat stomach mucosa is attached to another inverted beaker. Mucoadhesive tablet was wetted with pH 1.2 buffer and attached beneath the Left side arm. Weight of two arms of physical balance made equal. Water added drop wise in right arm beaker. At particular weight (N) tablet will get detached from mucous membrane²². Mucoadhesive strength calculated by using formula

$$[(N-M)/M] \times 100$$

Statistical analysis and Optimization

By using Sigma Tech software (version 3.1; Swaroop Tech. services Pvt. Limited – Hyderabad, India) the experimental design was generated to study and analyze the data obtained from all sustained release mucoadhesive tablet formulations. Based on several statistical parameters provided by experimental design software the best formulation was selected. Significant effects of factors on response regression coefficients were identified by Analysis of variance (ANOVA). A graphical optimization technique, Contour plots were used to generate the new formulations with desired responses. The generated formulation (predicted values) was evaluated for all the parameters. The predicted and experimental values were calculated for standard errors (%)²².

Stability

Stability chamber maintained at 40°C ± 2°C / 75 % ± 5% RH was used for stability studies of optimized formulation. The tablets were transferred into HDPE containers and loaded into the chambers. The tablets were evaluated for % drug release for 3 months respectively²³.

RESULTS

Melting point

Melting point of pure amoxicillin, amoxicillin-Camphor complex and re-crystallized amoxicillin-Camphor complex were found to be 193°C, 255°C, 230°C respectively.

Thin Layer Chromatography (TLC)

Retention factor (R_f) values for Amoxicillin, sodium cholate and camphor were found to be 0.41, 0.44 and 0.29 respectively whereas Amoxicillin-cholate complex and Amoxicillin-Camphor complex has shown 0.51 and 0.37 respectively.

Fourier transform infrared (FT-IR) Spectroscopy

Acid group (-COOH) present in Sod. Cholate reacted with alcohol (-OH) formed Ester (RCOOR), observed as a sharp characteristic peak of -C=O at 1737 cm⁻¹ and -C-O peak at 1377 cm⁻¹. Carbonyl (-C=O) group in camphor reacted with Amine (-NH₂) group in Amoxicillin that exhibited sharp peak at 1574 cm⁻¹ in FTIR Spectra.

Determination of free amoxicillin

Amount of free amoxicillin in ASC and ACC determined spectrophotometrically at 231 nm. The ingrained percentage purity of amoxicillin in ASC and ACC was about 97.2 % and 98.4 % respectively.

Determination of $\lambda_{\max}$

ASC and ACC showed $\lambda_{\max}$ at 324 and 280 nm respectively.

Antimicrobial activity

The agar well diffusion method was adopted to verify the antimicrobial activity. ACC showed greater zone of inhibition compared with ASC and pure Amoxicillin showed in Figure 1.

Figure 1A: Zone of inhibition of ACC B. ASC C. Amoxicillin

Solubility enhancement

Images of crystal habit of ACC before and after re-crystallization showed in Figure 2. ACC after re-crystallization formed needle shaped crystals which showed 10 folds increase in solubility than ACC without re-crystallization.

Figure 2A: Represents ACC before recrystallization B. ACC after recrystallization

Differential Scanning Calorimetry (DSC)

DSC thermographs revealed that the melting points of the ACC and that of the ACC in the Optimized formulation were found to be 230 and 235°C respectively. A slight shift of melting point in the Optimized formulation was observed as that of ACC, it indicated that the selected ACC was found to be compatible with excipients without any chemical and physical interaction.

Mixing method

ACC was mixed with water and other excipient(s) used in the formulation. The color of mixture was white. It remained same even after exposing to different temperature and humidity conditions continuously for four weeks and it can be concluded that the complex was compatible with the ingredients used in the formulation of tablets.

Pre and Post compression parameters

Results of pre-compression parameters for all formulations (F1-F10) were within the specifications. Bulk density and tapped density were in the range of 0.412-0.463 g/ml, 0.461-0.514 g/ml respectively. Hausners ratio was 1.092-1.128 whereas carr's index ranged from 7.11-11.35 % and angle of repose was 22.21-27.40°. Post compression parameters such as weight variation, Drug content, thickness, hardness, friability, swelling index and

mucoadhesive strength exhibited in the range of 495.70-509.80 mg, 99.2-101.1 %, 4.90-5.55 mm, 5.6-8.6 kg/cm², 0.08-0.31 %, 90.2-115.3 % (g/g) and 51.5-75.12 % respectively.

Scanning Electron Microscopy (SEM) study

The SEM image of the dry tablet was shown with the tough network between particles. A porous gelatinous film was formed upon polymer relaxation and hydration on the surfaces of the tablet.

Swelling index and Mucoadhesive strength

The highest water uptake and strength (121.3 %, 75.12 %) and lowest (90.2 %, 51.5 %) were shown by F4 and F8 batch, respectively. Among the batches containing higher quantities of chitosan and HPMC K15 showed higher swelling index and mucoadhesive strength which may be due to physical entrapment of more water that leads to the formation of bulky network branched matrix. Batch F4 showed higher swelling and strength due to the presence of hydrophilic polymers like chitosan and HPMC K15M.

Evaluation of critical formulation variables on Response variables

Results of dependent variables such as Mucoadhesive time (h), Drug release at 24 h and t50 % were performed and result were tabulated in Table 2.

Ex vivo Mucoadhesion time (Y1)

$$Y1 = 84.22375 + 0.8625 X1 + 2.0625 X2 - 2.0625 X3 + 2.3375 X1X2 - 2.1875 X1X3 - 1.6875 X2X3 - 3.0625 X1X2 X3$$

Mucoadhesion time (Figure 3) data was analyzed and found that interaction of X1 was highest with SS ratio (29.561 %) and a positive sign of the coefficient (2.3375). It showed that mucoadhesion time increased with increase quantity of Chitosan and HPMC K15, mucoadhesion time decreased with increase in quantity of EC. To identify significant effect ANOVA was used. The model was found to be significant at $p < 0.05$ since the obtained F value is larger than critical F-value. The critical value of F is 4.95, obtained F value (i.e. 6.87) is larger than critical value and so it can be determined that obtained F value is likely to occur

by chance with a $p < 0.05$ i.e. indicates significance at that level of probability. Since $R^2 = 0.997$, value was found to be greater than 0.70 suggesting that this model was reliable for all the selected factors. Hence used to establish predictions and contours/design space for developing Robust method.

Figure 3: Image showing *Ex vivo* Mucoadhesive time

% Drug release (Y2)

$$Y2 = 75.2375 - 0.6425 X1 - 4.0625 X2 - 2.0317 X3 - 4.2357 X1X2 - 1.2578 X1X3 - 2.5786 X2X3 - 4.0315 X1X2 X3$$

In-vitro drug release data was analyzed and found that interaction of $X1$ was highest with SS ratio (17.214 %) and a negative sign of the coefficient (- 4.2357). It showed that drug release decreased with increase in Chitosan, HPMC K15, EC due to greater mucoadhesion time and mucoadhesive strength. A representative chromatogram of drug in pH 1.2 buffer was used. Coefficient of determination $R^2 = 0.995$ and showed significance of model at $p < 0.05$ since the critical value of F is 4.95, obtained F value is 6.85.

t50% (Y3)

$$Y3 = 59.1 - 0.2625 X1 - 0.0625 X2 - 0.55 X3 - 0.8625 X1X2 + 0.2125 X1X3 - 0.025 X2X3 - 0.5125 X1X2 X3$$

t50% data was analysed and found that interaction of $X1$ was highest with SS ratio (38.3215 %) and a negative sign of the coefficient (- 0.8625). It was revealed that t50 % decreased with increase in Chitosan, HPMC K15 and EC due to greater mucoadhesion time and strength. Coefficient of determination $R^2 = 0.997$ and showed significance of model at $p < 0.05$ since the critical value of F is 4.95, obtained F value is 5.78.

Table 2 Evaluation of dependent variables

Formulation	Mucoadhesive time (h) *	Drug release at 24 h **	t50%	Swelling index (%) (g/g) ***	Mucoadhesive strength (%)****
F1	10±0.51	84.9±0.01	10.45	110.2±0.1	72.9±0.23
F2	9 ±0.62	80.2±0.03	11.4	96.3±0.3	55.3±0.31
F3	10.1±0.31	81.6±0.03	12.5	107.1±0.5	56.9±0.64
F4	>12	98.5±0.5	9.2	121.3±0.7	75.12±0.27
F5	10±0.89	82.4±0.06	11.3	108.9±0.6	61.3±0.72
F6	9.5±0.39	81.2±0.09	11.5	104.3±0.2	53.3±0.45
F7	10±0.61	84.6±0.09	11.2	109.3±0.3	69.1±0.62
F8	9.6±0.2	80.5±0.09	11.25	90.2±0.9	51.5±0.54
F9	10±0.4	85.9±0.03	10.3	110.9±0.9	65.9±0.81
				115.3±0.9	73.15±0.9

Values are expressed as the mean ± SD; $n = 3^*$, $n = 6^{**}$, $n = 6^{***}$, $n = 3^{****}$

Figure 4: Contour plots for ACC sustained release tablets A. *Ex vivo* Mucoadhesion time B. *In vitro* drug release C. t50 %

Establishing Design space

Total design space for *Ex-vivo* mucoadhesion 12-18 h, for drug release 84-96 % and t50 % 8-11 h. (Figure 4). Contour plots permitted the composition chitosan as 15.625 mg (+1), HPMC K15 10.875 mg (+1) and EC 18.5 (0).

Stability studies

Optimized formulation (F10) was subjected to stability studies as per ICH for 90 days at prescribed temperature and humidity conditions. It was observed that the F10 formulation showed 98.25 % drug release after 24 h.

DISCUSSION

Organoleptic properties showed that the Amoxicillin was a white, highly bitter, odourless and colourless powder. The melting point of the Amoxicillin was found to be 195°C. Amoxicillin was found to be more soluble in 0.1N HCl i.e., pH 1.2 (1.095). Anti microbial activity enhanced by choline and camphor complexes. Formation of ASC and ACC confirmed by difference in R_f value of TLC and FTIR spectra. Agar well diffusion method proved ACC has greater zone of inhibition than the other two.

Solubility enhancement of Amoxicillin-Camphor complex was carried out by re-crystallization which was then confirmed by compound microscopic images. DSC studies and hand mixing method revealed that ACC and selected excipients were found to be compatible. Controlled release tablets each containing 250 mg of ACC could be prepared by wet granulation method by using povidone K30 as a binder. Plastic and dilatency nature of bulk powder was the reason for selection of wet granulation technique. Granulation step is a key process in the production of tablets to increase the flow property. The granules were evaluated for Pre compression parameters. The results of Bulk Density (BD), Tapped Density (TD), Compressibility Index and Hausner's ratio indicated that granules possessed satisfactory flow properties. The tablets were examined after compression, showed smooth surface without chipping, cracking and other undesirable features. Formulated sustained release tablets were subjected to various post-compression evaluation tests based on the formulation as per experimental design (2^3 factorial design). The entire tablet formulations passed the weight variation test as per I.P. and showed un-deviated thickness ranging from 4.90 to 5.55 mm. Hardness of the tablets was in the range of 5.8 to 8.6 Kg/cm². Friability of the

tablet formulations was less than 0.4 %. The drug content (Assay) ranged from 99.2 to 101.3 %. The drug content values were within limits as per I.P. The drug release kinetics for the optimized formulation followed first-order kinetics. Based on Higuchi's equation, the value of regression coefficient for formulation trial F10 is greater than 0.9. Hence, the mechanism of drug release was found to be diffusion. As 'n' value of peppas for Optimized formulation was in between 0.45 and 0.89, Hence mechanism of drug release according to korsmeyer's peppa's equation was found to be non-fickian which means polymer relaxation followed by diffusion of the drug. Greater mucoadhesion time and mucoadhesive strength may be due to presence of HPMC which contains hydrogen bonds building groups (-OH) and its ability to diffuse drug by the formation of a gel layer, exhibits mucoadhesion and prolonged release, whereas mucoadhesion behaviour of chitosan is due to presence of (-OH) and (NH₂) groups which were responsible for hydrogen bonding. Order of mucoadhesion behavior and % drug release (Chitosan > HPMC K15 > EC). The decrease in drug release i.e., sustained release may be due to the presence of HPMC which increased the tortuosity or polymer gel strength. A viscous gelatinous layer (gel layer) was formed by rapid hydration, chain relaxation and presence of EC a hydrophobic polymer formed a strong matrix with reduced porosity sustained the drug release by reduced water penetration through the micro pores formation that increased diffusional path. The experimental values of optimized formulation exhibited closeness with predicted theoretical values which confirmed the validity of the model, the results were shown in (Table 3). Stability studies revealed that there was no significant change in the dissolution profiles of the optimized formulation F10 and were found within acceptable limits.

Table 3: Comparison of experimental results with predicted responses of ACC sustained release formulation

Ingredient	Composition (mg/tab)	Response	Predicted value	Experimental value	Standard error (%)
Chitosan	15.625	Y1 (h)	18.4	17.5	0.45
HPMC K15	10.875	Y2 (%)	92.8	90.7	1.05
EC	18.5	Y3 (h)	9.25	9.18	0.035

CONCLUSION

In this study the antimicrobial activity of antimicrobial drug enhanced by the formation of Amoxicillin camphor complex which was confirmed by agar well diffusion method. 2^3 factorial design with mid points (statistical experimental design) showed that the concentration of ACC mucoadhesive sustained release polymers have a profound and interactive effect on the dependent variables and the experimental design was successfully applied to optimize the concentration of mucoadhesive polymers to formulate sustained release tablets with desirable properties of high mucoadhesion time and prolonged drug release thereby enhancing the residence time of Amoxicillin in the mucosal layer to eradicate *Helicobacter pylori* infection. *Ex vivo* studies of the formed ACC showed better mucoadhesion. Solubility of the complex enhanced by re-crystallization technique which might shower better bioavailability, can be confirmed by performing *In vivo* experiments. It can be concluded that experimental design could be successfully applied for the development of Amoxicillin Camphor complexed mucoadhesive sustained-release tablets with minimal trials and better quality attributes there by reducing the cost of the final product.

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