International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 8, 2018

Original Article

QBD-BASED DEVELOPMENT AND EVALUATION OF ENTERIC COATED MUCOADHESIVE MICROCAPSULES OF AMOXICILLIN TRIHYDRATE AS A NOVEL CHRONOTHERAPEUTIC APPROACH FOR TREATMENT OF BACTERIAL INFECTIONS

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Received: 02 Jun 2018 Revised and Accepted: 12 Jul 2018

ABSTRACT

Objective: The present work entails design and characterization of enteric coated mucoadhesive microcapsules loaded with amoxicillin trihydrate as a novel chronotherapeutic approach for the treatment and management of bacterial infection.

Methods: The microcapsules were prepared by solvent evaporation technique using ethyl cellulose (EC) and hydroxypropyl methylcellulose (HPMC) as rate-controlling and mucoadhesive polymers, followed by a triple coating with Eudragit L100 as enteric coating polymer. Box-Behnken statistical design (BBD) was applied for optimization of formulations containing EC, HPMCK100M and Eudragit L100 as factors for selected responses like entrapment efficiency, mucoadhesive property and drug release in 24 h. The optimized microcapsules were also characterized for particle size, drug content, swelling index, mucoadhesive strength, and *in vivo* antiulcer activity.

Results: The optimized microcapsules exhibited good entrapment efficiency, particle size and mucoadhesive property. FT-IR studies revealed that there was no drug-polymer interaction. SEM studies revealed that microcapsules were non-aggregated, spherical in shape and smooth appearance. *In vitro,* drug release data from microcapsules was fitted to different kinetic models to explain release profiles. The correlation coefficient (r^2) value indicated that drug release followed Higuchi model. Analysis of variance (ANOVA) showed a significant difference in the release of drug from all the prepared formulations at $P<0.05$ level. Accelerated stability study of optimized formulation (F4) up to 6 mo showed there was no change in drug content and release characteristics during storage.

Conclusion: Overall, the present study indicated the successful development of mucoadhesive microcapsule.

Keywords: Gastric resistance, Mucoadhesion, Swelling index, *In vitro* drug release, Antibacterial activity

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i8.27699

INTRODUCTION

Amoxicillin trihydrate (AMT) is a semi-synthetic broad-spectrum β-lactam antibiotic used for the treatment of bacterial infections. It is primarily active against gram positive bacteria by inhibiting their cell wall synthesis [1, 2]. It exhibits lower stability in gastric acid due to cleavage of C≡N bond of the β-lactam ring which leads to loss of potency with reduced oral bioavailability [3]. Further, low half-life (<1h) with relatively high oral dosage regimen (250-300 mg b. i. d/t. i. d) requires the development of a novel once-a-day oral chrono-modulated drug delivery systems of AMT for the management of bacterial infections [4, 5].

Myriad formulation approaches have been tried viz. enteric coated tablets, sustained release mucoadhesive tablets, drug-coated beads and gastroretentive systems to protect the gastric degradation along with controlled drug absorption have limited fruition. Of late, polymeric microparticles appear to be the interesting device for their chronomodulated drug release mechanism satisfying the need of disease treatment [6]. Despite the more complex and onerous production of the multiple-unit systems, they have several advantages as compared to the single-unit products, including ready and uniform distribution in the gastrointestinal tract, minimizing the risk of local damage irritation caused by dose dumping. Furthermore, microparticles are less affected by pH change, gastric transit time, attain more constant plasma levels, give higher accuracy in reproducibility by dose and provide desired controlled release profile of drug delivery [7].

As these mucoadhesive drug delivery systems contains a diverse class of polymers and other inactive ingredients which may invariably affect the desired performance of dosage form. In such case, rational use of Design of Experiment (DoE) helps a lot in optimizing drug delivery systems to obtain robust formulations. Several DoE methodologies are used for optimization such as factorial design (FD), Box-Behnken design (BBD), Central-composite design (CCD), D-optimal design (DOD), Plackett-Burman design (PBD) and mixture design [8]. Of late, BBD is most widely accepted for optimization and formulation development of microspheres as the design execution and interpretation is easier over other designs. It allows the utilization of three or more components followed by optimization to obtain robust formulations with desired performance characteristics [9-11].

Therefore, the current research work entails design and characterization enteric coated controlled release mucoadhesive microcapsules of amoxicillin trihydrate as a novel chronotherapeutic system using optimized polymer blend containing ethyl-cellulose along with mucoadhesive polymers like HPMCK4M, HPMCK15M, and HPMCK100M, sodium CMC, HEC and HPC. The mucoadhesive microcapsules were prepared by a solvent evaporation method and enteric coated by dip coating technique. The microcapsules showed complete protection of amoxicillin trihydrate in the gastric acidic environment to enhance the systemic availability of the drug with desired sustained release action and to improve the patient compliance due to its chronotherapeutic action.

MATERIALS AND METHODS

Amoxicillin trihydrate was generously gifted by M/s Ranbaxy Labs. Ltd., Gurgaon, India. The polymers ethyl cellulose, HPMCK4M, K15M, K100M were obtained from M/s Colorcon Ltd., Goa, India. The sodium CMC, HEC, HPC and EudragitL-100 were obtained from M/s Evonik Ltd., Mumbai, India. Solvents like acetone, light liquid paraffin, Tween 80 were purchased from M/s Loba Chem Pvt. Ltd., Mumbai, India, while all other chemicals and reagents like sodium hydroxide, potassium dihydrogen phosphate used were of analytical grade. De-ionized double distilled water was prepared by Millipore

filtration unit (M/s Millipore, Mumbai, India), used throughout the experimental work.

Preparation of mucoadhesive microcapsules

The microcapsules were prepared using ethyl cellulose by a solvent evaporation technique. After dissolving the ethyl cellulose (2000 mg) and mucoadhesive polymer (1000 mg) in acetone (40 ml), amoxicillin trihydrate sodium (1000 mg) was added. The suspension was emulsified by light liquid paraffin (350 ml) containing Tween 80 (10-12 drops). The emulsion was mechanically stirred at 500 rpm for 2.5 h to remove acetone.

The microcapsules formed were collected by vacuum filtration, washed with n-hexane (250 ml) and air dried. The formulation composition of mucoadhesive microcapsules prepared is shown in table 1.

Table 1: Composition of preliminary mucoadhesive microspheres of amoxicillin trihydrate

Ingredients	Formulation code						
	F1	F2	F3	F4	F5	F6	F7
Amoxicillin trihydrate	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm
Ethyl cellulose	2 gm	2 gm	2 gm	2 gm	2 gm	2 gm	2 gm
HPMC K4M	_	1 gm	_	_	_	_	_
HPMC K15M	_	_	1 gm	_	_	_	_
HPMC K100M	_	_	_	1 gm	_	_	_
Sodium CMC	_	_	_	_	1 gm	_	_
Hydroxyethyl cellulose	_	_	_	_	_	1 gm	_
Hydroxypropyl cellulose	_	_	_	_	_	_	1 gm

Preparation of enteric coated mucoadhesive microcapsules

Enteric coating of mucoadhesive microcapsules was carried out by dip coating technique. The dried microcapsules were dipped into a coating solution containing Eudragit L100 (7.5%w/v) dissolved in 100 ml of acetone with the help of forcep. The microspheres were air dried, and the process was repeated twice with different concentration of coating solution containing Eudragit L100 (10 % w/v and 12.5% w/v).

Optimization using an experimental design

For the systematic optimization of mucoadhesive microcapsule formulations, the experimental design methodology was employed by BBD with the help of Design-Expert software 8.0.5 (Stat-Ease Inc., MN).

The BBD was specifically selected since it requires fewer treatment combinations than other experimental designs, which involves three to four factors to optimize the formulation performance using selected responses [12]. A 3-factor, 2-level (3^2) BBD was employed using concentration of EC(X_1), HPMCK100M(X_2)as selected factors, while percentage drug entrapment (Y_1), percentage drug release 24h(Y_2) and percentage mucoadhesion at 6h (Y_3) were selected as obtained responses. The levels at which factors were investigated along with the obtained responses are shown in table 2. Total thirteen different formulations obtained were characterized for selected responses and analyzed for the effect of factors by response surfaces. Table 3 depicts the formulations prepared as per the experimental design along with observed responses. The optimized formulation was selected by numerical optimization.

Table 2: Independent and dependent variables for experimental design optimization

Independent variables (Factors)	Range		
	Low	High	
X_1 = Concentration of EC (gm)	1.00	2.00	
X_2 = Concentration of HPMCK100 (gm)	0.50	1.00	
X_3 = Concentration of Eudragit L100 (% w/v)	7.50	12.50	
Dependent variables (Responses)	**Low**	**High**	**Goal**
Y_1 = Drug entrapment (%)	42	67	Maximize
Y_2 = Drug Release (%)	74	97	Maximize
Y_3 = Mucoadhesion (%)	40	73	Maximize

Table 3: Experimental runs for the formulation of mucoadhesive microcapsules as per the experimental design

Run	X1 Conc. of EC (gm)	X2 Conc. of HPMCK100 (gm)	X3 Conc. of Eudragit L100 (%w/v)	Y1 drug entrapment (%)	Y2 drug release (%)	Y3 mucoadhesion (%)
1	1.00	0.75	12.50	65	58	74
2	1.00	0.75	7.50	42	61	92
3	1.50	0.50	12.50	66	44	75
4	1.50	0.75	10.00	57	60	86
5	2.00	1.00	10.00	59	69	84
6	2.00	0.75	7.50	65	62	95
7	2.00	0.50	10.00	55	41	85
8	1.50	1.00	7.50	46	72	96
9	1.50	0.75	10.00	55	65	87
10	1.00	1.00	10.00	55	73	86
11	2.00	0.75	12.50	64	65	76
12	1.50	0.75	10.00	55	64	87
13	1.50	0.50	7.50	44	42	97
14	1.50	0.75	10.00	56	65	86
15	1.00	0.50	10.00	57	40	84
16	1.50	1.00	12.50	67	73	76
17	1.50	0.75	10.00	58	63	87

Characterization of uncoated microcapsules

Drug content

Accurately weighed microcapsules, equivalent to 10 mg of amoxicillin trihydrate sodium were crushed in a mortar-pestle, dissolved in 100 ml of phosphate buffer pH 7.4, mixed well and sonicated. The resultant dispersion was kept for 24 h for complete mixing and filtered through a Whatman filter paper. The drug content was determined spectrophotometrically using UV-Visible spectrophotometer (Shimadzu, Japan) at 273 nm.

Entrapment efficiency

The entrapment efficiency of prepared microcapsules was determined by dissolving accurately weighed microcapsules, equivalent to 10 mg of amoxicillin trihydrate sodium in 100 ml of phosphate buffer pH 7.4. The resultant dispersion was kept for 24 h for complete mixing and filtered through a Whatman filter paper. The drug content was determined spectrophotometrically using UV-Visible spectrophotometer (Shimadzu, Japan) at 273 nm. The entrapment efficiency was calculated by the formula (1):

$$Entrapment\ \ efficiency = \frac{Actual\ \ drug\ \ content}{Theoritical\ drug\ \ content} \times 100 \quad \text{..... (1)}$$

Loose surface crystals study

The drug encapsulated microcapsules were evaluated by loose surface crystal study to observe the excess drug present on the surface of microcapsules. Accurately weighed microcapsules, equivalent to 10 mg of amoxicillin trihydrate sodium were weighed, mixed with 10 ml of phosphate buffer pH 7.4 for 5 min, shaken and then filtered through Whatman filter paper. The amount of drug present on the surface was determined spectrophotometrically using UV-Visible spectro-photometer (Shimadzu, Japan) at 273 nm using regression equation (Y= 0.041X-0.006) derived from the standard plot, and calculated as a percentage of total drug content [13].

Swelling index

The dynamic swelling property of microcapsules was determined in phosphate buffer pH 7.4. Accurately weighed microcapsules (20 mg) from different formulation were placed in dissolution media (phosphate buffer pH 7.4) for 24 h. The swollen microcapsules were collected by centrifugation at 2000 rpm and weighed. Further, the swollen microcapsules were dried by keeping on a filter paper and the weight was noted down [14]. The percentage swelling was then calculated by the formula (2):

$$Sw = \frac{(Wt\ \ Wo)}{Wo} \times 100 \quad \text{..... (2)}$$

Where, Sw= Percentage of swelling of microcapsules, Wt = Weight of the microcapsules at time t in mg, Wo= Initial weight of the microcapsules in mg

Percentage moisture loss

The drug-loaded microcapsules were evaluated for percentage moisture loss which gives an idea about its hydrophilic nature. The microcapsules weighed 20 mg (W1) were initially kept in a desiccator containing calcium chloride at 37 ℃ for 24 h [15]. The final weight (W2) was noted when no further change in weight of the sample was observed. The percentage moisture loss was calculated by the formula (3):

$$Percentage moisture loss = \frac{(W_1\ \ W_2)}{W_2} \times 100 \quad \text{..... (3)}$$

Circulatory factors (Sphericity)

The particle shape was measured by computing circulatory factor (S). The tracing obtained from the triangular microscope (Olympus ch20i, Mumbai, India) were used to calculate area (A) and perimeter (P) of the particles [13]. Finally, the circulatory factor (S) was calculated by using formula (4):

$$S = \frac{P \times P}{12.56} A \quad \text{..... (4)}$$

Micromeritic properties of microcapsules

Micromeritic properties of the microcapsules were determined by using the angle of repose, Carr's index and Hausner's ratio. The angle of repose was determined by the fixed funnel method. Carr's Index and Hausner's ratio were determined by tapping method. The microcapsules were tapped using USP tapped density tester (Electrolab-1020, Mumbai, India) for 1000 taps in a cylinder and the change in volume was measured[2,16]. The angle of repose, Carr's index and Hausner's ratio were calculated by formulae (5-7):

$$Angle\ \ of\ \ repose\ \ (\theta) = tan^{-\frac{h}{r}} \quad \text{..... (5)}$$

$$Carr's\ Index = \frac{Bulk\ \ density\ \ \ Tapped\ \ density}{Bulk\ \ density} \times 100 \quad \text{..... (6)}$$

$$Hausner's\ ratio = \frac{Bulk\ \ density}{Tapped\ \ density} \times 100 \quad \text{..... (7)}$$

Where, h and r are the height and radius of the powder cone in cm, Bulk density and tapped density of the microcapsules are measured in gm/cm^3.

Fourier transform infrared (FTIR) spectroscopy

FT-IR spectroscopic studies were employed to characterize the possible interactions, if any, between the drug and excipients. The FTIR spectra of samples of pure drug, a physical mixture of the drug with polymers and prepared microcapsules were recorded using KBr disc using an FTIR spectrophotometer (Shimadzu, Japan) [17].

Scanning electron microscopy (SEM)

The shape and surface morphology of prepared microcapsules were observed by SEM (Joel Scanning Microscope JSM-5800, Japan). SEM analysis was carried out using an accelerating voltage of 20 kV after they were gold sputtered (Jeol 4B SVG-IN, Peabody, USA).

***In vitro* wash off test**

Modified disintegrating apparatus was used for determination of a mucoadhesive property of the prepared microcapsules by *in vitro* adhesion testing method, also called as a wash-off test. A freshly excised piece of goat intestinal mucosa (5.5 × 1.5 cm) was mounted on a glass slide (5.5 × 1.5 cm) with cotton thread. Glass slide was connected with a suitable support. About 25 microcapsules were spread on this wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating apparatus. When the disintegrating apparatus was operated, the tissue specimen was subjected a slow, regular up and down movement in the test fluid (phosphate buffer, pH 7.4) at 37 °C. At specific time intervals like 0.5, 1, 2, 3, 4, 5 and 6 h, the apparatus was stopped and a number of microcapsules adhering to mucosal tissue were counted [18, 19]. Percentage mucoadhesion was determined by formula (8):

$$\%\ Mucoadhesion = \frac{No.\ of\ \ microcapsules\ adhered}{Initial\ no.\ of\ \ microcapsules} \times 100 \quad \text{..... (8)}$$

***In vitro* drug release study**

The *in vitro* drug release from the mucoadhesive microcapsules were carried out using USP type II dissolution apparatus (Electrolab-TDT06L, Mumbai, India). The enteric coated microcapsules equivalent to 20 mg of amoxicillin trihydrate sodium were filled into the hard gelatin capsule, and subjected to dissolution in 900 ml of 0.1N HCl(pH 1.2) for initial 2 h followed by phosphate buffer (pH 7.4) up to 24 h, at 75rpm and 37 °±5 °C temperature. Sample aliquots of 5 ml were withdrawn periodically and replaced with 5 ml of the fresh media. The samples withdrawn were estimated for its drug content through UV spectroscopy at 273 nm and percentage drug release was calculated. The dissolution tests were performed in triplicate and the drug release data were fitted to various kinetic models like zero-order, first order, Higuchi, Korsmeyer-Peppas and Baker and Lansdale model [20, 21]. The

mean dissolution times (MDT) for 50% or 80% releases of the drug were calculated by the formula (9):

$$MDT = \frac{\sum_{j=1}^{n} t_j^{\wedge} \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \quad \text{..... (9)}$$

Where j is the sample number, n is the number of dissolution sample times, $t_{j^{\wedge}}$ is the time at the midpoint between t_j and t_{j-1} and it is easily calculated from $\frac{tj+tj-1}{2}$ and ΔM_j is the additional amount of drug dissolved between t_j and t_{j-1}

Antimicrobial assay

The antimicrobial assay of the optimized bilayer tablets was performed by using an agar plate diffusion method. The zone of inhibition (ZOI) and MIC was calculated to evaluate the efficacy of the prepared bilayer tablet formulation vis-à-vis conventional marketed preparation. The different dilutions of pure drug amoxicillin trihydrate (standard) were prepared in pH 7.4 phosphate buffer with concentrations ranging from 1-250 µg/ml. The prepared bilayer tablets (test) and conventional marketed immediate release tablet preparation (Amoxil®, GlaxoSmithKline, India) of amoxicillin trihydrate were subjected to dissolution in pH 7.4 phosphate buffer using the same method as mentioned earlier. The aliquots collected from dissolution study at different time intervals were filtered through 0.45 µm nylon filter and carefully transferred into the wells prepared on solidified agar plate in petridish inoculated with test organisms such as *Staphylococcus aureus*-ATCC29213 (gram positive cocci) and *E. coli*-ATCC25922 (gram negative bacilli) cultured at Department of Microbiology, Shri Venkateshwara University, Gajraula. The Petri dish was kept in an incubator at a controlled temperature (25 °C) condition. After 24h incubation, the ZOI for prepared bilayer tablets and marketed preparation were measured (in mm) and compared with a standard dilution of antibiotic in the concentrations of 0.5, 1, 2, 3, 4, 6, 8 and 12 µg/ml. On the basis of ZOIs, the MIC was calculated with respect to the amount of drug release at each specified time interval responsible to reduce the viable growth of microorganisms.

Statistical analysis

Analysis of variance (ANOVA) was performed to find out significance difference among various formulations using Prism Graph Pad software (CA, USA). Two-way ANOVA was applied on the amount of drug release at 3 h, 6 hand 10 h from all formulations.

Stability studies

Accelerated stability studies were carried out for the optimized formulation as per ICH guidelines. Optimized microcapsules were packed in vials and stored at 40 °C/75% RH up to 6 mo in a stability chamber. In the specified time intervals the drugs content and *in vitro* drug release rate was determined [23].

RESULTS AND DISCUSSION

Preparation of mucoadhesive microcapsules by experimental design

Initially, various mucoadhesive polymers were tried for preparation of microcapsules of amoxicillin trihydrate by a solvent evaporation technique. The preliminary screening showed that microcapsules prepared with mucoadhesive polymer ethyl cellulose-based HPMCK100M provides higher stability and protection to the drug and they were found to be non-aggregated (table 1). The combination of polymers in a ratio of 1:2:1 w/w of drug: ethylcellulose: mucoadhesive polymer (HPMCK100M) showed the formation of microcapsules satisfactorily.

Further, the microcapsules were prepared and optimized by employing BBD, at selected factors and levels. The response surface analysis was used to identify the effect of factors on the observed responses.

The optimized formulation was selected based upon the levels of factors which yielded maximum entrapment efficiency, higher sustained release profile and maximum mucoadhesion strength. The response variables considered for systematic optimization, i.e., %drug entrapment, % drug release in 24 h and % mucoadhesion were allowed to fit in the quadratic equations with added interaction terms to correlate the studied responses with the examined factors. Statistical analysis and validation of the design was carried out by establishing mathematical relationships the form of polynomial equations for the measured responses as listed below:

Y_1 (% Drug entrapment) =-48.05147+45.00000×X_1-11.50000×X_2+ 10.75000×X_3+12.00000×X_1×X_2-4.80000×X_1×X_3-0.40000×X_2×X_3

Y_2 (% Drug release in 24 h) =+95.02500+29.10000×X_1+2.70000×X_2- 2.49000×X_3-6.00000×X_1×X_2-0.20000×X_1×X_3+0.80000×X_2×X_3- 7.20000×X_1-0.80000×X_2-0.088000×X_3

Y_3 (% Mucoadhesion in 6 h) =-49.27500+ 20.15000× X_1+ 215.80000×X_2-1.51000×X_3-10.00000×X_1×X_2+1.20000×X_1×X_3- 0.40000×X_2×X_3-7.80000×X_1-91.20000×X_2+8.00000-003×X_3.

The quadratic polynomial equation represents the quantitative effect of variables (X_1, X_2, and X_3) and their interactions on the responses (Y_1, Y_2 and Y_3). The values of the coefficients X_1, X_2 and X_3 are related to the effect of these variables on the responses (Y_1, Y_2 and Y_3). The positive sign represents synergistic effect, while a negative sign indicates an antagonistic effect. Fig. 1 depicts the 3D response surfaces for the selected responses in the design *viz.* % drug entrapment, %drug release in 24 h and % mucoadhesion.

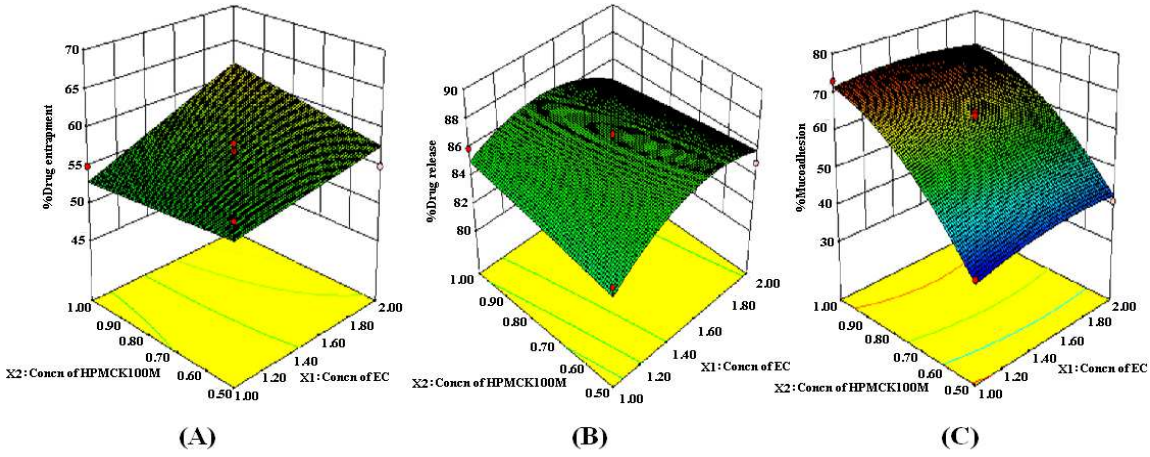

Fig. 1: 3D-Response surfaces for different dependent variables: (A) Y1 (% drug entrapment), (B) Y2 (% drug release) and (C) Y3 (% mucoadhesion) as per the Box-Behnken experimental design

Fig. 1(A) represents the effects of X_1 and X_2 and their interaction on Y_1 (%drug entrapment) at a fixed level of X_3. The response surface showed that entrapment efficiency was gradually increased with increase in the concentration of EC (X_1), while with an increase in the concentration of HPMCK100M the entrapment efficiency was decreased. Fig. 1(B) represents the effect of X_1 and X_2 on response Y_2 (%drug release). The response surface showed that initially drug release was increased with increase in (X_1) concentration of EC and at low level of HPMCK100M (X_2), however, increase the concentration of HPMCK100M (X2) at a low level of EC (X_1) revealed no significant increase in drug release. This indicated that HPMCK100M has no significant effect on drug release from microcapsules, while EC has a major role to sustain the release profile up to some extent. Fig. 1(C) indicated the effect of factor X_1 and X_2 on response Y3 (% mucoadhesion) and interaction between them. The response surface showed that % mucoadhesion was found to increase gradually with increase in the concentration of HPMCK100M (X_2), however increasing the concentration of EC (X_1) has no significant effect on mucoadhesion. From the response surface analysis, it has been concluded that HPMC has a major role on mucoadhesion and EC has a major role on drug release, however, the Eudragit L100 has no significant role on either of the responses Y1, Y2 and Y3. Hence, it was it taken as a null factor in all the experiments and during formulation development, the triple coating procedure was employed with a fixed concentration of enteric polymer. The model was evaluated by using two way ANOVA and the ANOVA results are enlisted in table 2. Finally, the optimized formulation for preparation of mucoadhesive microcapsules was selected by numerical optimization.

Table 2: ANOVA results for various dependent variables Y1 (% drug entrapment), Y2 (% drug release) and Y3 (% mucoadhesion) as per the Box-Behnken experimental design

ANOVA parameters	Y_1 (% drug entrapment)	Y_2 (% drug release)	Y_3 (% mucoadhesion)
SS	756.50	801.29	1978.52
df	6	9	9
MS	126.08	89.03	219.84
F-value	9.14	69.63	45.33
Prob>F	0.0014	<0.0001	<0.0001
Std. Deviation	3.71	1.13	2.20
R^2value	0.8458	0.9890	0.9831
Suggested model	Quadratic	Quadratic	Quadratic

Enteric coated microcapsules by dip coating technique

The enteric coated mucoadhesive microcapsules were prepared by dip coating mainly consists of three-layer coating of Eudragit L100. The coating composition for preparing enteric coated mucoadhesive microcapsules is shown in table 3. As a single layer and bilayer coat of enteric polymer on mucoadhesive microcapsules was unable to control the drug release in the gastric environment, therefore, trilayered coated microcapsules were prepared. The trilayered mucoadhesive microcapsules were found to protect the dosage form from the gastric acidic environment and allow the drug release in the intestinal fluid.

Table 3: Enteric coating composition for the preparation of enteric coated mucoadhesive microcapsules

S. No.	Concentration of coating solution (%W/V)	Enteric coating material	Solvent	Dissolution properties
1	7.5	Eudragit L-100	Acetone (100 ml)	>pH 6
2	10	Eudragit L-100	Acetone (100 ml)	>pH 6
3	12.5	Eudragit L-100	Acetone (100 ml)	>pH 6

Drug content and entrapment efficiency

The drug content and entrapment efficiency of uncoated microcapsules were determined and shown in table 4. Drug content and entrapment efficiency were found to be in the range of 43.06±0.09 to 70.22±0.01 % and 44.87±0.19 to 70.48±0.12 % respectively. A formulation containing ethyl-cellulose with HPMCK100M based mucoadhesive microcapsules showed maximum drug content and entrapment efficiency in comparison to other formulations. The amoxicillin trihydrate being highly soluble in water is having a tendency to diffuse out to the aqueous medium even though the sufficiently higher drug entrapment. This is due to hindered diffusion of medicament through the gel barrier formed by mucoadhesive polymer [9, 13].

Table 4: Characterization parameters for uncoated mucoadhesive microcapsules

Formulation code	Drug content (%) (mean±SD)	Entrapment efficiency (mean±SD)	Loose surface crystal study (%) (mean±SD)	Circulatory factors (mean±SD)	Moisture loss (%) (mean±SD)
F1	43.06±0.09	44.87±0.19	15.09±0.05	0.590±0.01	33.33±0.02
F2	65.75±0.05	66.09±0.07	14.14±0.04	0.451±0.02	11.11±0.05
F3	58.56±0.07	59.02±0.03	17.61±0.07	0.217±0.01	11.11±0.03
F4	70.22±0.01	70.48±0.12	4.71±0.07	1.068±0.03	12.25±0.19
F5	55.09±0.06	56.09±0.09	24.56±0.13	0.180±0.04	25.23±0.01
F6	48.09±0.12	49.98±0.06	29.35±0.04	0.939±0.04	5.26±0.03
F7	46.65±0.10	47.31±0.09	33.99±0.32	0.939±0.03	17.64±0.02
F8	42.23±0.15	41.79±0.09	17.17±0.54	1.287±0.09	32.73±0.08
F9	45.04±0.24	44.81±0.11	23.44±0.78	1.347±0.11	22.32±0.12

Data expressed as mean±SD (n=3)

Loose surface crystals study

The loose surface crystal studies lend a hand to estimate the excess amount of free drug present on the surface of microcapsules in adsorbed form. The study was executed with various prepared formulations and the results were obtained (table 4).

The loose surface crystal was found to be in the ranges of 4.71±0.07 to 33.99±0.32%. The formulation prepared with EC and HPMCK100M showed minimum drug particles on the surface of microcapsules because HPMCK4M formed a thick viscous gel layer over the EC matrices and prevent the escape of drug crystals outside the gel barrier [13].

Swelling index

The swelling indexes of microcapsules prepared as per the experimental design were found to be satisfactory (table 5). Formulation F4 showed maximum swelling up to 230±0.08%, whereas formulation F7 showed minimum swelling of 30±0.07%, as shown in fig. 2. The maximum swelling depends on the type of polymer used, concentration, viscosity, ionic strength as well as the presence of water. The microcapsules were undergone into swelling event due to the presence of HPMCK100M.

The swelling occurs when the polymer absorbs water and depends on the viscosity grade and ionic strength of the polymer [13, 27].

Table 5: Swelling index of uncoated mucoadhesive microcapsules

Formulation code	Swelling index (mean±SD)					
	1h	2 h	3 h	4 h	5 h	24 h
F1	50±0.04	25±0.13	30±0.04	45±0.05	20±0.10	50±0.13
F2	20±0.10	20±0.13	40±0.05	64±0.07	100±0.04	100±0.13
F3	10±0.13	45±0.05	60±0.08	60±0.10	80±0.08	170±0.03
F4	90±0.08	125±0.03	110±0.05	85±0.04	225±0.03	230±0.08
F5	30±0.08	70±0.10	45±0.04	25±0.08	55±0.07	60±0.07
F6	20±0.03	105±0.07	20±0.03	60±0.10	105±0.03	45±0.05
F7	15±0.07	65±0.05	25±0.13	55±0.10	30±0.04	30±0.07
F8	35±0.19	53±0.18	34±0.07	43±0.04	55±0.04	65±0.16
F9	47±0.19	33±0.04	25±0.11	36±0.04	67±0.04	41±0.21

Data expressed as mean±SD (n=3)

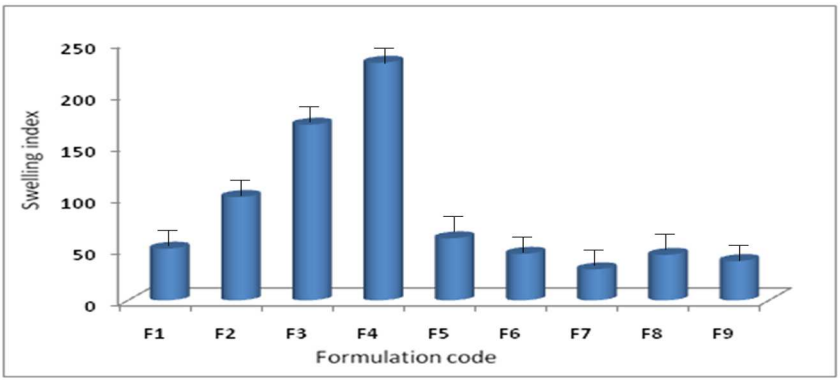

Fig. 2: Swelling index of the mucoadhesive microcapsules after 24h; Data expressed as mean±SD (n=3)

Percentage moisture loss

The percentage moisture loss by various mucoadhesive micro-capsule formulations prepared as per experimental design are shown in table 4. The minimum moisture loss was observed with formulation F4 and maximum moisture loss was observed with formulation F1, which ensures the presence of water content in the prepared microcapsule due to hygroscopic nature of the drug or mucoadhesive polymer. However, low proportion of water indicated proper drying and instant hardening of microcapsule upon storage [28].

Circulatory factors (Sphericity)

The circularity factor for mucoadhesive microcapsules was found to be very close to 1.00, confirm their spherical nature as observed from the table 4. Further, the SEM was used for better understanding of the morphology of the microcapsules.

Micromeritic properties

Table 6 enlists data on the micromeritic properties of the prepared microcapsules. The average particle size of microcapsules was found to be between 46.89±0.04 and 80.66±0.03 µm. The particle size depends on the amount, type or concentration of polymers used in the formulation which increases the viscosity of the solution. The tapped density was found to be between 0.312±0.02 to 0.5±0.07 gm/cm^3 and bulk density was found to be between 0.234±0.01 to 0.468±0.03 gm/cm^3. The Carr's Index was found in the range from 3.12±0.13 to 16.36±0.03%. The Carr's Index was found less than 17 %, showed good flow property. Hausner's ratio of mucoadhesive microcapsules was found to be less than 1.33±0.03 indicated good flow property of the prepared microcapsules. The angle of repose was found to be between 11.04±0.04 to 43.27±0.02 degree. From the values of angle of repose maximum data are less than 30° which indicate good flow property as compared to the drug [29].

Table 6: Micromeritic properties of prepared uncoated mucoadhesive microcapsules

Formulation code	Average particle size (µm) (mean±SD)	Tapped density (gm/cm^3) (mean±SD)	Bulk density (gm/cm^3) (mean±SD)	Angle of repose (θ) (mean±SD)	Hausner's ratio (mean±SD)	Carr's index (%) (mean±SD)
F1	50.22±0.01	0.312±0.02	0.234±0.01	43.27±0.02	1.33±0.01	15.00±0.01
F2	63.33±0.13	0.483±0.08	0.468±0.01	18.85±0.08	1.03±0.03	3.12±0.13
F3	50.12±0.13	0.483±0.06	0.405±0.08	17.52±0.07	1.19±0.08	16.21±0.13
F4	49.33±0.04	0.531±0.07	0.468±0.03	11.04±0.04	1.06±0.01	6.25±0.04
F5	46.89±0.05	0.539±0.04	0.375±0.03	24.94±0.08	1.33±0.03	16.36±0.03
F6	80.66±0.03	0.433±0.08	0.382±0.09	28.21±0.03	1.13±0.09	11.76±0.03
F7	52.66±0.08	0.365±0.03	0.388±0.05	29.43±0.13	1.21±0.05	12.99±0.08
F8	66.01±0.10	0.455±0.04	0.503±0.08	33.08±0.17	1.67±0.06	18.44±0.10
F9	43.67±0.12	0.539±0.07	0.375±0.10	21.35±0.09	1.25±0.08	10.33±0.15

Data expressed as mean±SD (n=3)

Fourier transform infrared (FTIR) spectroscopy

The FT-IR spectra of pure drug, a physical mixture of the drug with polymers and drug-loaded microcapsule are shown in fig. 3. The peak at 3583 cm^{-1} indicated-NH stretching, 1157 cm^{-1} indicated C-O-C symmetric stretching, 10298 cm^{-1} for–S=O stretching, 1384 cm^{-1} indicated–C-N vibrations and 1693 $cm^{-1}$for aromatic–C=N stretching. It was observed from the spectra's of pure drug and optimized formulations that there was neither remarkable shift in the wave number of the peaks nor in the intensity of peaks proved that there was no interaction between drug and selected polymers [17].

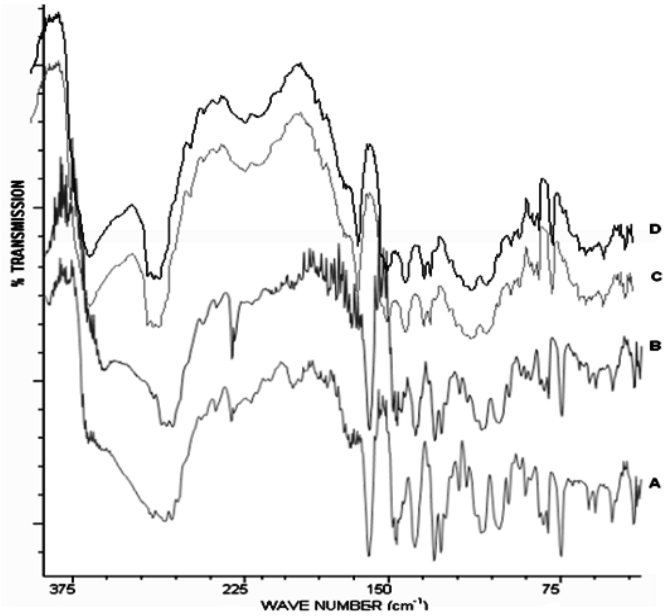

Fig. 3: FT-IR spectra of pure drug (amoxicillin trihydrate) (A); Physical mixture of pure drug+EC (B); Physical mixture of pure drug+EC+HPMCK100M (C); Optimized mucoadhesive microcapsule formulation (D)

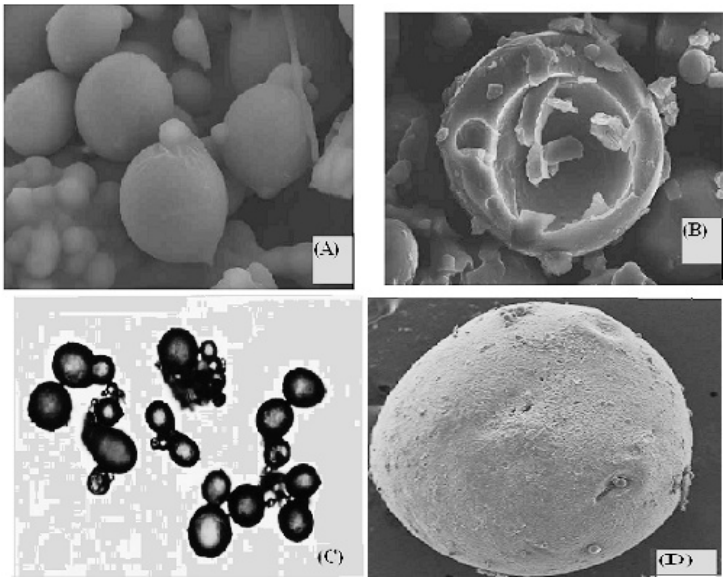

Fig. 4: SEM images of microcapsules of optimized mucoadhesive microcapsule formulation (A), broken microcapsules (B), optical microscopy images of microcapsules of optimized formulation (C) and optimized enteric coated microcapsules (D)

Scanning electron microscope (SEM)

The microcapsules were found to be discrete, non-aggregated, free-flowing and monolithic matrix type. Fig. 4 depicts the SEM photographs, which indicated that the microcapsules were spherical and completely covered with the coating polymer.

***In vitro* wash-off test**

Fig. 5 represents the percentage mucoadhesion exhibited by different batches of prepared mucoadhesive microcapsules. *In vitro* wash-off test showed that prepared microcapsules exhibited fair mucoadhesive property. The wash-off was faster at intestinal pH medium due to critical degree of hydration, molecular weight and mobility, ionic content, solubility and viscosity of the mucoadhesive polymers. The rapid wash-off observed at intestinal pH 7.4 is due to ionization of carboxyl and other functional groups in the polymers at this pH, which increases their solubility and reduces bioadhesive strength (table 7). The formulations containing a higher concentration of mucoadhesive polymer (HPMCK100M) showed higher mucoadhesion property and longer wash-off time attributed due to the electrostatic attraction

between HPMC and mucin. Also, the swelling index of the polymer affects the mucoadhesion property significantly. Higher swelling leads to the attachment of the microcapsules with mucosal surface for a longer period of time and showed slower wash-off [17, 18].

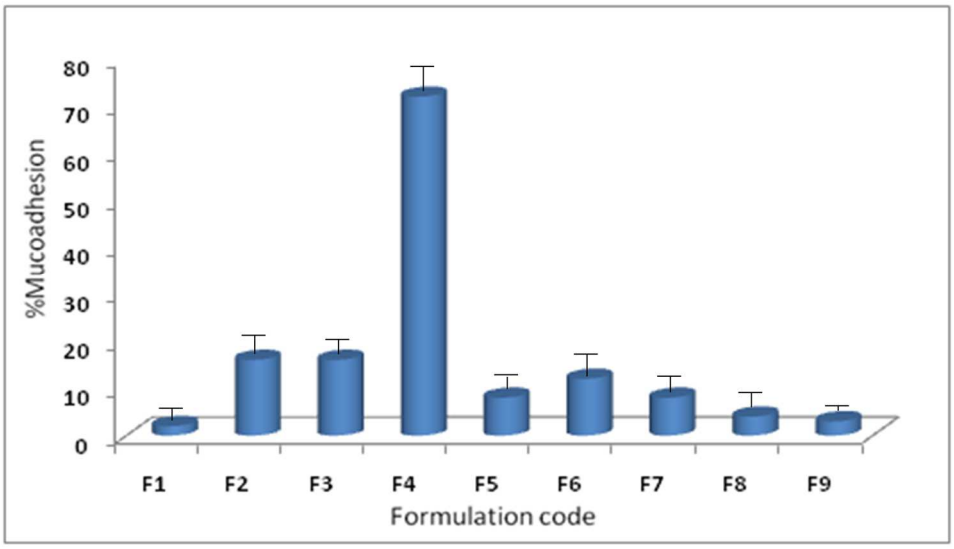

Fig. 5: % Mucoadhesion of microcapsules of different formulations after 6 h; data expressed as mean±SD (n=3)

Table 7: % Mucoadhesion of mucoadhesive microcapsules during *in vitro* wash off test

Formulation code	% Mucoadhesion (mean±SD)						
	0.5 h	1h	2h	3h	4h	5h	6h
F1	12±1	12±1	12±1	8±2	0	0	0
F2	80±3	64±3	44±3	28±1	20±3	20±3	16±2
F3	72±3	44±3	32±3	28±2	24±2	16±2	16±3
F4	100±1	100±1	100±1	72±1	72±3	72±3	72±2
F5	76±2	48±1	44±1	40±2	32±3	12±2	8±2
F6	60±3	48±2	16±2	12±1	12±1	12±1	12±1
F7	20±2	20±3	20±3	16±2	36±3	16±1	10±2
F8	18±1	23±2	33±1	12±3	33±1	25±2	13±1
F9	45±2	17±3	23±3	10±1	24±2	22±3	23±3

Data expressed as mean±SD (n=3)

Gastric-resistance of enteric coated microcapsules

The dissolution was carried out in the acidic medium (0.1N HCl) for first 2 h and it was found that there was no release of drug from the enteric coated microcapsules. The microcapsules remain intact during the acidic medium because the degree of ionization of carboxylic acid groups in the Eudragit L-100 increases with pH of the medium and remains intact in the acidic medium, and prevents drug release. In alkaline medium initially the enteric coating retard the drug release to some extent but as the enteric coating has no effect on drug release due to rapid dissolution of the coating layer in phosphate buffer pH 7.4, hence the drug release depends on the viscosity of the mucoadhesive polymer present in the mucoadhesive microcapsules. Thus, enteric coated microcapsules provide good barrier property against under low pH conditions to prevent drug diffusion [26].

***In vitro* drug release study**

The *in vitro* drug release showed that enteric-coated microcapsules provide a good barrier against drug diffusion under acidic pH conditions to protect the drug from degradation. The drug release was found to be sustained up to 24 h and depends on the concentration of HPMCK100, viscosity/molecular weight. The cumulative % drug release from mucoadhesive microcapsules was significantly decreased with an increase in the drug-polymer ratio (HPMCK100M) as compared to the EC. In the present study, HPMCK100M was used as a hydrophilic matrix agent because it forms a strong viscous gel on contact with aqueous media, which may be useful in the controlled delivery of highly water-soluble drugs. Faster release of the drug from the hydrophilic matrix was probably due to faster dissolution of the highly water-soluble drug from the core and its diffusion out of the matrix forming the pores for entry of solvent molecules. Incorporation of ethyl cellulose has little effect on controlling the release rate rather it helps in microencapsulation of the active pharmaceuticals [31].

Fig. 6 depicts the cumulative *in vitro* drug release from the enteric coated mucoadhesive microcapsule formulations prepared as per experimental design. From the *in vitro* drug release study the formulation F4 was considered as an optimized formulation with optimum mucoadhesion, swelling and sustaining drug release pattern. The mean dissolution data was calculated showed that formulation F4 with maximum MDT (10.17 h) and formulation F7 showed minimum MDT (3.68 h), indicated that the drug release was faster at low concentration of HPMCK100M and intermediate concentration of EC. $MDT_{50\%}$, the value of optimized formulation was found to be 7.05 h. Increase in MDT value indicated that the drug release is slower, which is attributed due to increase in the thickness of barrier layers HPMC on the matrix core.

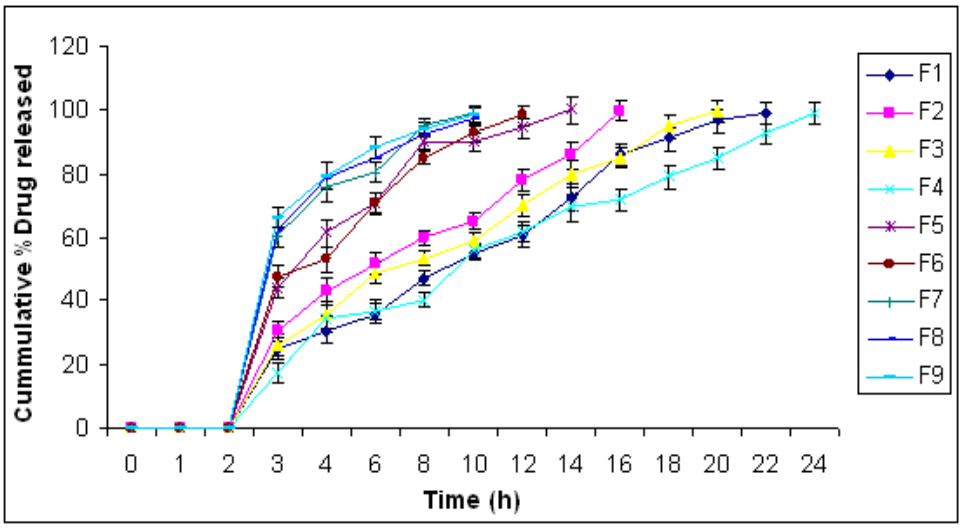

Fig. 6: Cumulative % drug release profiles of different formulations; Data expressed as mean±SD (n=3)

Mathematical modeling of kinetic data obtained revealed that drug release from trilayer microcapsules showed non-Fickian diffusion or super case type-II (n>1.0) mechanism. The higher (r^2) value signifies that the developed layered microcapsules follow Higuchi kinetics and represents a swellable system [13, 20, 31] (table 8).

Table 8: Various parameters of the model equations on the *in vitro* drug release kinetics

Formulation code	Zero-order		First order		Higuchi model		Korsmeyer-peppa's model		Baker-lansdale model	
	r^2	K_0	r^2	K_1	r^2	K_h	r^2	n	r^2	K
F1	0.966	-11.01	0.863	0.172	0.949	-56.90	0.869	1.541	0.966	0.011
F2	0.924	-14.48	0.629	0.274	0.935	-63.37	0.846	1.767	0.935	0.145
F3	0.947	-11.85	0.776	0.198	0.949	-58.81	0.855	1.606	0.944	0.128
F4	0.955	-9.58	0.788	0.138	0.957	-52.37	0.873	1.481	0.955	0.096
F5	0.836	-18.10	0.737	0.453	0.878	-75.99	0.818	1.945	0.836	0.179
F6	0.884	-21.12	0.944	0.324	0.878	-79.59	0.829	2.085	0.884	0.214
F7	0.812	-26.18	0.946	0.451	0.813	-89.05	0.810	2.310	0.812	0.264
F8	0.849	-20.04	0.962	0.433	0.820	-77.51	0.833	1.876	0.982	0.187
F9	0.883	-22.28	0.987	0.467	0.834	-82.04	0.846	1.662	0.957	0.192

r^2=Coefficient of correlation, K_0, K_1, K_h, K= Release rate constants for zero order, first order, Higuchi, Korsmeyer-Peppas and Baker-Lansdale model

Antimicrobial assay

Table 9 and 10 depicts the ZOI of standard dilutions of pure antibiotic, prepared bilayer tablets and marketed tablet formulation. It has been observed that as per the designed drug release profiles, there was a significant decrease in ZOI of the bilayer tablet formulation at 3 hr of dissolution with value 19.3 mm and 22.0 mm for G. positive cocci and G. negative bacilli, which matched with the ZOI of pure drug with dilution at 2 µg/ml. On the contrary, the marketed formulation showed ZOI value of 29.3 mm and 29.7 mm, which were matched with the ZOI of the pure drug with dilution at 5 µg/ml. This indicated that bilayer tablet formulation has lower value of MIC vis-à-vis the marketed formulation in both gram positive as well as gram negative microorganisms. Moreover, the prepared formulation indicated higher efficacy of chronomodulated release bilayer tablet formulation over the conventional marketed product.

Table 9: Antibiotic sensitivity of pure drug at standard dilution against

Conc. (µg/ml)	ZOI (in mm±SD) for gram positive cocci	ZOI (in mm±SD) for gram negative bacilli
0	0±0.00	0±0.00
1	0±0.00	0±0.00
2	19.1±2.5	18.0±2.6
5	23.2±1.6	22.9±2.2
10	28.2±2.0	30.1±1.4
15	33.2±1.7	34.8±2.0
20	40.5±1.4	38.4±1.3
50	46.4±2.6	43.6±2.6
100	51.2±1.4	45.8±1.6
200	53.4±1.7	50.2±1.7
250	56.3±1.9	55.3±2.2

Data expressed as mean±SD (n=3)

Table 10: Antibiotic sensitivity of optimized mucoadhesive microcapsules and marketed formulation of amoxicillin trihydrate

Dissolution time (h)	Optimized microcapsules		Marketed product (Amoxil)	
	ZOI (in mm±SD) for gram positive cocci	ZOI (in mm±SD) for gram negative bacilli	ZOI (in mm±SD) for gram positive cocci	ZOI (in mm±SD) for gram negative bacilli
0	0±0.00	0±0.00	0±0.00	0±0.00
0.5	7.6±2.07	10.2±3.10	12.8±2.07	16.2±2.10
1	10.4±1.53	13.3±2.33	19.0±3.51	21.3±1.09
2	12.9±3.06	16.3±3.53	21.7±3.06	25.2±1.80
3	18.3±3.91	21.0±1.00	20.3±3.93	28.7±2.90
4	31.2±2.02	30.7±1.38	30.4.±2.00	36.9±1.05
5	35.7±2.59	36.7±3.08	33.7±2.92	38.3±2.10
6	42.4±1.03	39.7±4.53	41.7±1.53	40.1±2.01
8	47.1±4.01	40.7±3.06	44.7±2.01	42.7±2.32
12	49.1±2.04	42.0±1.03	46.7±1.05	44.4±1.07

Data expressed as mean±SD (n=3)

Stability studies

The accelerated stability studies of the optimized formulation showed that prepared microcapsules were stable for 6 mo without any change in physiochemical parameters. The drug content and dissolution rate of the formulations showed no significant change upon storage [23].

CONCLUSION

The mucoadhesive microcapsules of amoxicillin trihydrate were prepared effectively using a polymeric blend of ethyl cellulose and HPMCK100M. The gastric protection of drug release from mucoadhesive microcapsules was achieved by trilayer enteric coating with Eudragit L100 using novel dip coating technique. The physiochemical characterization of microcapsules was found to be satisfactory. The microcapsules exhibited good mucoadhesive properties under *in vitro* test conditions. *In vitro* drug release studies showed that mucoadhesive microcapsules well control drug release over an extended period of time. *In vitro* microbiological studies showed the superior antimicrobial effect of enteric coated mucoadhesive microcapsules on *E. coli* and *S. aureus* strains vis-à-vis conventional marketed formulation. Stability studies revealed that optimized microcapsules remained stable for 6 mo period of time with no change in drug content and dissolution profile.

AUTHORS CONTRIBUTIONS

The author SNP and AVG majorly responsible for planning, execution and compilation of the work, while the author AKS and HKP provided support in conducting formulation and characterization studies.

CONFLICT OF INTERESTS

We have no conflict of interest to declare

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