Development & In Vitro Evaluation of Gastroretentive Mucoadhesive Microspheres of Amoxicillin Trihydrate

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Abstract
Amoxicillin trihydrate mucoadhesive microspheres containing polyox WSR N 303 as mucoadhesive polymer and ethyl cellulose and HPMC K4M as carrier polymer were prepared by emulsion-solvent evaporation technique and optimized by 32 factorial designs with drug-to-polymer-to-polymer ratio and stirring speed as processing parameters. Formulation containing different ratio of Polyox WSR N 303: EC: methocel K4M have been studied for the swelling index, in vitro mucoadhesion, drug content, and t50%, which could result in more available therapy. The mucoadhesive microspheres of the best batch with ratio of 1:1:0.5 and 1500 stirring speed exhibited a high % in vitro mucoadhesion, 88.56 % drug entrapment efficiency and mean particles size of 113.05 µm. The in vitro release study indicates that the mucoadhesive microspheres of amoxicillin trihydrate could sustain the release of the drug for more than 12 hrs. The results of stability studies for all the formulations reveal that the optimized formulations have satisfactory stability.

Keywords: amoxicillin trihydrate; in vitro mucoadhesion; in vitro release

Introduction
Amoxicillin is a semi-synthetic, orally absorbed, broad-spectrum antibiotic. It is widely used in the standard eradication treatment of gastric and duodenal ulcers, which are associated with H. pylori infection combined with a second antibiotic and an acid-suppressing agent (Suleymanlar I et al., 1999; Vakil N et al., 1999; Buzas GM et al.,1999). Increase in the residence time may reduce the treatment time of such diseases. Therefore, researchers had prepared and reported formulations such as float tablets, mucoadhesive tablets, pH-sensitive excipients composition microspheres, etc., which were able to reside in the GIT for an extended period of time for a more effective treatment (Hilton AK et al., 1992; Nagahara N et al., 1998). This would lead to improvement in the bioavailability of the drug.

In 1994, a patent assigned to Reckitt and Colman Products described a raft-forming formulation using triclosan. The drug was mixed with alginic acid, sodium bicarbonate, calcium carbonate and mannitol. The mixture was granulated, citric acid added, and then packed into

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sachets or compressed to tablets. In contact with the acid conditions of the stomach, carbonate or bicarbonate salts produced effervescence which aerated the raft structure formed by the alginates, causing it to float. However, the authors noticed that, in some patients with H. pylori infections, the pH of the stomach contents had possibly been elevated (possibly to as high as pH 6) reducing effervescence and, consequently, reducing the ability of the rafts to float. For this reason, they had added citric acid to their formulation (Dettmar PW et al., 1994).

In 1998, Nagahara et al. formulated mucoadhesive microspheres containing amoxicillin. They dispersed the drug and bioadhesive polymers (carboxyvinyl polymer and curdlan [a polysaccharide]) in melted hydrogenated castor oil. Microspheres of 250 to 335 µm in diameter were obtained by a spray-chilling method followed by sieving. They compared these microspheres with an amoxicillin suspension in infected Mongolian gerbils under feeding conditions. The amoxicillin microspheres provided 10 times greater anti-H. pylori activity than the amoxicillin suspension. Moreover, adhesion of microspheres on the stomach wall was observed (47% and 20% remained in the stomach after 2 and 4 h, respectively). The authors concluded that these mucoadhesive microspheres containing an appropriate antimicrobial agent should be useful for the eradication of H. pylori (Nagahara N et al., 1998).

One reason for the incomplete eradication of H. pylori is probably due to the short residence time of antimicrobial agents in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where H. pylori exists (Cooreman MP et al., 1993; Atherton J C et al., 1995). The other may be the degradation of amoxicillin in gastric acid (Axon AT, 1994; Giacomo F et al., 2001).

EC is a non-toxic, inert, hydrophobic polymer that has been widely used to prepare pharmaceutical dosage forms (Agrawal AM et al., 2003). It is used extensively as a coating material for tablets and granules, as a tablet binder, in microcapsules and microspheres and film or matrix-forming material for sustained release dosage forms (Desai J et al., 2006).

The purpose of this study was to design amoxicillin mucoadhesive microspheres for H. pylori eradication therapy, to study the in vitro and ex vivo evaluation of the microspheres, by formulating mucoadhesive microspheres using ethyl cellulose and polyox WSR N 303 polymer combinations.

Materials and Methods

Materials

Amoxicillin trihydrate was received as gift sample from Sehat Pharma Pvt. Ltd., India. Poly (ethylene oxide) WSR N 303 was received as a gift sample from Colorcon (India). All other ingredients and chemicals were of analytical grade. All materials used for study conformed compendial requirements.

Preparation of amoxicillin trihydrate mucoadhesive microspheres

The mucoadhesive microspheres were prepared by emulsification-solvent evaporation method. The preparation techniques were optimized 3² full factorial designs. 1.0 g of ethyl cellulose (EC)
dissolved in 20 ml ethyl alcohol and disperse 0.6 g of amoxicillin trihydrate (equivalent to 0.5 g amoxicillin); and 0.1 g Polyox WSR N 303 powder was dissolved in dichloromethane (20 ml), mix both mixtures and added to 500 mL of light liquid paraffin using syringe (gauge no.20) containing 2.5 % v/v Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at different rpm for 6 h. The system temperature was kept at 25°C all through the process. The microspheres were washed with petroleum ether and dried at room temperature. The microspheres were then dried at room temperature (25°C and 60 % RH) for 24 hrs.

**Experimental design**

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses. 

\[ Y=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_{12}+b_{22}X_2+… \] (1)

Where, \( Y \) is the dependent variable, \( b_0 \) is the arithmetic mean response of the nine runs, and \( b_1 \) is the estimated coefficient for the factor \( X_1 \). The main effects (\( X_1 \) and \( X_2 \)) represent the average result of changing one factor at a time from its low to high value. The interaction terms (\( X_1X_2 \)) show how the response changes when two factors are simultaneously changed. The polynomial terms (\( X_{12} \) and \( X_{22} \)) are included to investigate non-linearity. A \( 3^2 \) factorial design study was undertaken to assess the effect on formulation with regards to % entrapment, % in vitro mucoadhesion, size and \( t_{50\%} \).

The formulation variables that were studied were (A) EC: amoxicillin trihydrate: polyox WSR N 303 ratio, (B) stirring speed. Each factor was studied at three levels. The low, medium and high values were decided based on tests of polymers as studied in preliminary batches. The design matrix for the experiment is shown in Table 1.

**Evaluation of Amoxicillin trihydrate Mucoadhesive Microspheres**

**Yield of microspheres**

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres. 

\[ \% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}} \times 100 \ldots (2) \]

**Drug loading determination**

A certain amount of microspheres were ground to powder. About 250 mg of it was accurately weighed and transferred into a 100 ml volumetric flask with phosphate buffer (pH 7.8) to the volume of 90 ml. The suspension was sonicated and vibrated in a water bath for 3 hrs. Then, it was cooled to room temperature and distilled water was added to 100 ml. After the suspension was filtrated through a 0.45 µm cellulose acetate membrane filter, 20 µl aliquot was withdrawn. The drug loading was determined by a UV spectrophotometer at 272 nm.

**Drug entrapment efficiency**

The drug entrapment efficiency was calculated using the following formula:

\[ \text{Practical drug content/Theoretical drug content} \times 100. \]

The drug entrapment efficiency for batches AX1 to AX9 is reported in Table 1.

All samples were analyzed in triplicate and the drug loading (DL) and encapsulation efficiency (shown in Table 2) (EE) was
calculated according to the following equation:

\[ DL \% = \frac{W_D}{W_T} \times 100 \quad \ldots \quad (3) \]

DL: drug loading; \( W_D \): the weight of the drug loaded in the microspheres; \( W_T \): the total weight of the microspheres.

\[ EE \% = \frac{W_A}{W_T} \times 100 \quad \ldots \quad (4) \]

EE: encapsulation efficiency; \( W_A \): actual drug content; \( W_T \): theoretical drug content.

**Particle size of microspheres**
The particle size of the microspheres was determined by using optical microscopy method. Approximately 200 microspheres were counted for particle size using a calibrated optical microscope (Labomed CX RIII, Ambala, India).
The particle size of microspheres of batches AX1 to AX9 is reported in Table 1.

**Morphological characterization of microspheres**
The surface and inner part of the microspheres were observed via scanning electron microscopy (JSM 5610 LV; Tokyo, Japan)

**Mucoadhesion of microspheres**
The mucoadhesive properties of microspheres were evaluated by the method designed by Ranga Rao and Buri (1989) using stomach isolated from mice. Mice were fasted for 24 h the stomach was dissected immediately after the mice were sacrificed. The stomach mucosa were removed and rinsed with physiological saline. 100 particles were scattered uniformly on the surface of the stomach mucosa. Then, the stomach mucosa with microspheres was placed in a chamber maintained at 93% relative humidity at room temperature. After 30 min, the tissues were taken out and fixed on a plate/slide at an angle of 45\(^\circ\). The stomach mucosa was rinsed with simulated gastric fluid (pH 1.3, without enzymes) for 5 min at a rate of 22 mL/min. The microspheres remaining at the surface of stomach mucosa were counted, and the percentages of the remaining microspheres were calculated. The results of in-vitro wash-off test after 1, 5 and 8 hrs of batches AX1 to AX9 are shown in Table 1.

**In vitro drug release behaviour of mucoadhesive microspheres in 1.0 N HCl**
Amoxicillin trihydrate release behavior in the pH 1.0 HCl medium was also carried out using USP XXIV Dissolution test apparatus Veego (VDA-6D) (Electrolab, Mumbai, India). The conditions and procedures were as follows: 500 mg of microspheres, which contained about 156 mg amoxicillin trihydrate, was suspended in 900 ml of pH 1.0 HCl medium, with the temperature maintained at 37 \(\pm\) 0.5 \(^\circ\)C. The rotating rate of the basket was adjusted to 100 rpm. At different intervals, the microspheres were taken out from the basket and washed twice with 10 ml each distilled water. Then, the microspheres were ground thoroughly and transferred with distilled water to a 100 ml volumetric flask, sonicated and vibrated in water bath until amoxicillin released completely. Distilled water was added to 100 ml, and the suspension was filtrated through a 0.45 \(\mu\)m cellulose membrane filter. The absorbance of the filtrate was measured via UV spectrophotometry at the wavelength of 272 nm.
Kinetic analysis of the release data
The kinetics of famotidine release from the various matrices was analyzed using the exponential Peppas equation.

\[ K t^n = \frac{M_t}{M_\infty} \]  

Where: \( M_t = M_1 \) the fraction of drug released at time \( t \), \( k \) a constant incorporating the structural and geometric characteristics of the drug/polymer system, \( n \) the release exponent indicative of the drug release mechanism. The values of \( n \) and \( k \) were determined from the slope and intercept of the plot of \( \frac{M_t}{M_\infty} \) as a function of time, on a logarithmic scale, according to Eq. 2. \( n \) values=0.45 for Fickian (case I) release, 0.45>n<0.89 for non-Fickian (anomalous) release, \( n=0.89 \) for case II (zero order) coupling drug diffusion and polymer relaxation (Ritger PL et al., 1987), and \( n>0.89 \) for super case II which is generally related to the dissolution of the polymeric matrix due to the relaxation of the polymer chain.

Statistical analysis
Tests for significant differences between means were performed by one-way ANOVA. Differences were considered significant at \( P < 0.05 \).

Stability study
Mucoadhesive microspheres of amoxicillin trihydrate formulated in the present study were subjected to accelerated stability studies in aluminum/aluminum pouch pack. Dose dumping and failure of mucoadhesion are probable effects anticipated during the stability study of such dosage forms. The tablets of check point optimized formulation were charged for accelerated stability studies at 40°C and 75% RH for 3 months in a stability chamber. %drug content, mucoadhesive strength, and drug dissolution profile of exposed sample was carried out.

Result & Discussion
The size of the drug-loaded complex microsphere was slightly smaller than the microsphere without the drug, and the yield of the drug-loaded complex microsphere was better than the microsphere without the drug, as shown in Table 1.

Morphological characteristics of the microspheres: The mucoadhesive microspheres of amoxicillin prepared in this study were well rounded spheres with the size ranging approximately from 70.82 to 123.05 µm. Crystals of amoxicillin adsorbed on the surface of the microspheres might give a burst release and help enhance the amoxicillin concentration for the effective H. pylori clearance shortly after oral administration.

In vitro evaluation of mucoadhesiveness: The in vitro mucoadhesiveness test showed that the percentage of microspheres remaining on the gastric mucosa was found in the range of 47% to 80% after 8h, which indicated that the microspheres containing polyox WSR N 303 and ethyl cellulose adhered to the gastric mucus more strongly.

Optimization of the selected formulations (AX1 and AX9): Modifications of all above formulations were affected by preparing the microspheres using different ratio of polymer have investigated. The mucoadhesive microspheres were prepared according to the method describe earlier.
The detailed composition was given in the Table 1.

Factorial equation for % Entrapment efficiency: The Model F-value of 85.34 implies the model is significant. There is only a 0.20% chance that a "Model F-Value" this large could occur due to noise. In this case A, B, B++2- are significant model terms. The "Pred R-Squared" of 0.9245 is in reasonable agreement with the "Adj R-Squared" of 0.9814. The R² value was 0.9988 obtained for the following equation 6.

Entrapment efficiency (\%) =
82.35+7.05X₁-2.16X₂+0.49X₁X₂-0.93X₁²-0.80X₂² ....... (6)

The drug entrapment efficiency and T50% are important variables for evaluating the drug loading of microspheres and their drug release profile, thus suggesting the amount of drug availability at site. These parameters are dependent on the process of preparation, physicochemical properties of drug, and formulation variables. The model generated for drug entrapment efficiency was found to be significant.

The Figure 1 shows that the % drug entrapment efficiency of microspheres increased from 76.11% to 81.38% and 89.13% to 92.56% at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed increased. Results of equation indicate that the effect of X1 (drug-to-polymer-to-polymer) is more significant than X2 (stirring speed). Moreover, the stirring speed had a negative effect on the percentage drug entrapment efficiency (ie, as the stirring speed increased, the particle size decreased, and thus drug entrapment efficiency decreased).

Factorial equation for % in vitro mucoadhesion: The in-vitro mucoadhesiveness test showed that the percentage of mucoadhesive microspheres remaining on the stomach mucosa (Table 1). Figure 2, figure 3 and figure 4 showed that, microspheres were well adhered to gastric mucous layer. The mucoadhesive microspheres of all the batches of the factorial design were spherical and free flowing. The model generated for % in vitro mucoadhesion was found to be significant with a p<0.005.

Factorial equation for % in vitro mucoadhesion after 1h: The Model F-value of 166.20 implies the model is significant. There is only a 0.07% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Pred R-Squared" of 0.9604 is in reasonable agreement with the "Adj R-Squared" of 0.9904. "Adeq Precision" measures the signal to noise ratio. The ratio of 32.880 indicates an adequate signal. The R² value was 0.9994 obtained for the following equation 7.

\[ \text{In vitro Mucoadhesion after 1h (\%)} = 91.56+13.00X₁+1.67X₂-0.50X₁X₂-10.33X₁²-0.33X₂² \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ll..
was 0.9949 obtained for the following equation 8.

\[
\text{In vitro Mucoadhesion after 5h (\%)} = 84.00 + 13.17X_1 + 1.83X_2 - 1.25X_1X_2 - 9.50X_1^2 - 0.50X_2^2 \ldots (8)
\]

Factorial equation for \% in vitro mucoadhesion after 8h: The Model F-value of 120.27 implies the model is significant. There is only a 0.12% chance that a "Model F-Value" this large could occur due to noise. The "Pred R-Squared" of 0.9411 is in reasonable agreement with the "Adj R-Squared" of 0.9868. The ratio of 28.411 indicates an adequate signal. The R^2 value was 0.9988 obtained for the following equation 9.

\[
\text{In vitro Mucoadhesion after 8h (\%)} = 73.56 + 13.83X_1 + 2.00X_2 - 0.75X_1X_2 - 6.83X_1^2 + 0.67X_2^2 \ldots (9)
\]

Factorial equation for t_{50\%}: The Model F-value of 53.22 implies the model is significant. There is only a 0.40% chance that a "Model F-Value" this large could occur due to noise. The "Pred R-Squared" of 0.9095 is in reasonable agreement with the "Adj R-Squared" of 0.9703. The ratio of 19.662 indicates an adequate signal. The R^2 value was 0.9975 obtained for the following equation 10.

\[
t_{50\%}(\text{hours}) = 6.05 + 1.58X_1 - 0.32X_2 - 0.15X_1X_2 - 0.93X_1^2 \ldots (10)
\]

The plot (Figure 5) shows that the % drug released in-vitro from microspheres decreased at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results depicted in Table 1 indicate that the % in vitro drug released is highly dependent on drug-to-polymer-to-polymer and stirring speed. The drug-to-polymer-to-polymer ratio has a negative effect on t_{50\%}. Factorial equation for Size: The Model F-value of 14.51 implies the model is significant. There is only a 2.59% chance that a "Model F-Value" this large could occur due to noise. The "Pred R-Squared" of 0.5354 is not as close to the "Adj R-Squared" of 0.8941 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc. The R^2 value was 0.9909 obtained for the following equation 11.

\[
\text{Size (\(\mu\)m)} = 84.92 + 18.63X_1 - 6.05X_2 - 3.04X_1X_2 + 9.50X_1^2 - 0.47X_2^2 \ldots (11)
\]

The plot (Figure 6) shows that the particle size of microspheres increased at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as stirring speed decreased. Results indicate that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Means, as the stirring speed increased, the particle sizes decreased that directly affect the percentage mucoadhesion (increased). Thus, we can conclude that the amount of polymer and stirring speed directly affects the percentage mucoadhesion and particles size.

The optimized microsphere formulation (AX8) was developed using 1:1:0.5 drug-to-polymer-to-polymer ratio and 1500 rpm stirring speed. The optimized formulation was evaluated for %entrapment efficiency, % in vitro mucoadhesion, and drug release and particle size. The results of experimentally observed responses and those predicted by mathematical models along with the percentage prediction errors were compared. The low values of error
indicate the high prognostic ability of factorial equation and counter plot methodology. The drug release from the optimized formulation was found to low $t_{50\%}$ (6.63h) seems to be potential formulation for achieving drug release up to 12 hrs.

In vitro release studies of all formulations were carried out in 0.1N hydrochloric acid; the study was performed for 12 h and cumulative drug release was calculated at specific time intervals. The dissolution profile (figure 7 to 10) of the all batches were fitted to various models such as zero order, first order, and Higuchi, Korsmeyer and Peppas models to ascertain the kinetic modelling of drug release.

The mechanism of release of amoxicillin trihydrate from all the formulated batch AX1 to AX9 was by anomalous non fickian diffusion i.e. diffusion coupled with erosion (Table 5). The in vitro release from AX8 batch was found near to 88.48% which was adding to assuring that retarding the drug release for satisfactory time period from the formulation in the stomach. The $R^2$ value and n value were found 0.995 and 0.789 respectively for optimized batch.

The drug release profile of batch AX8 is shown in Figure 7 to 10. The study focus was the preparation of mucoadhesive microspheres, thus the microspheres of batch AX8 were evaluated in gastric fluid (pH 1.2). In vitro release test showed that amoxicillin trihydrate released faster in pH 1.2 hydrochloric acid than in pH 7.8 phosphate buffer (data are not shown) but the results indicated that no significant difference was observed between dissolution at pH 1.2 and pH 7.8 (data are not shown) as the $f_2$ value calculations.

Better retention and bioadhesion of compacts containing polyox WSR N 303 could be attributed to its higher molecular weight (Schmitt RL, 2003; Betageri GV et al., 2001). During the process of bioadhesion, bioadhesive polymers undergo wetting, swelling, and interdiffusion or interpenetration into the mucus or epithelial surface. In this process, polymers with optimum molecular weights are believed to make strong entanglements and reside in the application site for prolonged period of time (Huang Y et al., 2000).

At present, most studies of mucoadhesive formulations loading amoxicillin trihydrate focused on prolonging the gastric retarding time. The stability of amoxicillin in acidic medium was neglected. Amoxicillin was also reported to be unstable in mediums with pH below 2 (Mascher HJ et al., 1998; Erah PO et al., 1997; Chadha R et al., 2003). Amoxicillin trihydrate can be quickly absorbed after its conventional dosage forms are orally administered. Therefore, it s residence time in the stomach is expected to be short (Cooreman MP et al., 1993), which might cover up its shortcoming of being unstable in acidic surrounding. But for the mucoadhesive microspheres, which would stay in the stomach for a much longer time, the stability of amoxicillin trihydrate should be seriously considered. The longer it stayed in the acidic medium, the more it was degraded. On the contrary, amoxicillin trihydrate entrapped within the microspheres kept stable. The result suggested that mucoadhesive microspheres
can protect amoxicillin trihydrate from being degraded.

Morphological examinations of microspheres were determined by scanning electron microscopy which was used to obtain the photographs of the microspheres. The scanning electron microscopy images of loaded mucoadhesive microspheres of amoxicillin trihydrate are presented in Figures 11. The image show spherical shapes of microspheres. The surface of polyox WSR N 303 containing microspheres was found smooth with presence of drug crystallization on the surface.

The values of drug content, % entrapment efficiency, % in vitro mucoadhesion, t50% and size were found after one and three months that indicate a good similarity between both the dissolution profiles. Similarly, no significant difference was observed in the swelling index, in vitro mucoadhesion, drug content, and t50% after stability studies. Hence, the results of stability studies reveal that the developed formulation has good stability (Table 6).

**Conclusion**

Amoxicillin mucoadhesive microspheres containing polyox WSR N 303 as mucoadhesive polymer and ethyl cellulose as carrier polymer, were prepared by emulsion-solvent evaporation technique. The results of a $3^2$ full factorial design revealed that the drug-to-polymer-to-polymer (amoxicillin trihydrate-EC-polyox WSR N 303) and stirring speed significantly affected the dependent variables entrapment efficiency, % in vitro mucoadhesion, t50%, and size. The microspheres of the best batch (AX8) exhibited a high % in vitro mucoadhesion 80% after 8 hr, 89.30 % drug entrapment efficiency and mean particles size of 113.05 µm. The in vitro release study indicates that the mucoadhesive microspheres of amoxicillin trihydrate could sustain the release of the drug for more than 12 hrs.

**References**

6. Cooreman MP, Krausgrill P, Hengels KJ (1993). Local gastric and serum amoxycillin concentrations after different oral...
Table 1 Amoxicillin trihydrate mucoadhesive microspheres batches using $3^2$ full factorial design layout

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Levels in coded form $X_1$</th>
<th>$X_2$</th>
<th>% Entrapment efficiency</th>
<th>In vitro Mucoadhesion ($%$) after $t_{50}%$ (hours)</th>
<th>Size ($\mu$m)</th>
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<tr>
<td>AX1</td>
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<td>76.11</td>
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<td>0</td>
<td>82.37</td>
<td>92 83 73</td>
<td>6.01</td>
</tr>
<tr>
<td>AX6</td>
<td>0</td>
<td>1</td>
<td>79.04</td>
<td>93 87 77</td>
<td>5.68</td>
</tr>
<tr>
<td>AX7</td>
<td>1</td>
<td>-1</td>
<td>89.13</td>
<td>93 87 80</td>
<td>6.87</td>
</tr>
<tr>
<td>AX8</td>
<td>1</td>
<td>0</td>
<td>88.56</td>
<td>94 88 81</td>
<td>6.63</td>
</tr>
<tr>
<td>AX9</td>
<td>1</td>
<td>1</td>
<td>86.14</td>
<td>95 87 82</td>
<td>6.59</td>
</tr>
</tbody>
</table>

Ethyl cellulose: Amoxicillin trihydrate: Polyox WSR 303 ($X_1$)

Stirring speed ($X_2$)

<table>
<thead>
<tr>
<th></th>
<th>1:1:0.1</th>
<th>1:1:0.3</th>
<th>1:1:0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AX1</td>
<td>91.42 ± 2.61</td>
<td>87.38 ± 2.13</td>
<td>83.89 ± 1.05</td>
</tr>
<tr>
<td>AX2</td>
<td>93.16 ± 3.07</td>
<td>88.36 ± 1.26</td>
<td>86.24 ± 1.05</td>
</tr>
<tr>
<td>AX3</td>
<td>94.13 ± 2.55</td>
<td>91.22 ± 1.87</td>
<td>89.31 ± 1.66</td>
</tr>
</tbody>
</table>

*All values are mean of three determinations
### Table 3 Data of $3^2$ factorial run using Design expert 7.1.6 software

<table>
<thead>
<tr>
<th>Factor</th>
<th>Name</th>
<th>Units</th>
<th>Type</th>
<th>Low Actual</th>
<th>High Actual</th>
<th>Low Coded</th>
<th>High Coded</th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>EC:AMX:Polyox WSR 303 ratio</td>
<td>Numeric</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td>0.817</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Speed</td>
<td>rpm</td>
<td>Numeric</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td>0.817</td>
</tr>
</tbody>
</table>

### Table 4 Summary of results of regression analysis

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$b_{12}$</th>
<th>$b_{11}$</th>
<th>$b_{22}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entrapment efficiency (%)</td>
<td>82.35</td>
<td>7.05</td>
<td>-2.16</td>
<td>0.49</td>
<td>-0.93</td>
<td>-0.80</td>
<td>0.9988</td>
</tr>
<tr>
<td>In vitro Mucoadhesion after 1h (%)</td>
<td>91.56</td>
<td>13.00</td>
<td>1.67</td>
<td>-0.50</td>
<td>-10.33</td>
<td>-0.33</td>
<td>0.9994</td>
</tr>
<tr>
<td>In vitro Mucoadhesion after 5h (%)</td>
<td>84.00</td>
<td>13.17</td>
<td>1.83</td>
<td>-1.25</td>
<td>-9.50</td>
<td>-0.50</td>
<td>0.9949</td>
</tr>
<tr>
<td>In vitro Mucoadhesion after 8h (%)</td>
<td>73.56</td>
<td>13.83</td>
<td>2.00</td>
<td>-0.75</td>
<td>-6.83</td>
<td>0.67</td>
<td>0.9988</td>
</tr>
<tr>
<td>$T_{50%}$ (hours)</td>
<td>6.05</td>
<td>1.58</td>
<td>-0.32</td>
<td>0.15</td>
<td>-0.93</td>
<td>0.00</td>
<td>0.9975</td>
</tr>
<tr>
<td>Size ($\mu$m)</td>
<td>84.92</td>
<td>18.63</td>
<td>-6.05</td>
<td>-3.04</td>
<td>9.50</td>
<td>-0.47</td>
<td>0.9909</td>
</tr>
</tbody>
</table>

*All values are mean of three determinations
Table 5 Release kinetics of Amoxicillin trihydrate from prepared mucoadhesive microspheres

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero-order plots correlation coefficient $R^2$</th>
<th>First-order plots correlation coefficient $R^2$</th>
<th>Higuchi’s plot correlation coefficient $R^2$</th>
<th>Korsmeyer Peppas plots correlation coefficient $R^2$</th>
<th>Diffusional exponent $n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AX1</td>
<td>0.977</td>
<td>0.924</td>
<td>0.959</td>
<td>0.986</td>
<td>0.670</td>
</tr>
<tr>
<td>AX2</td>
<td>0.957</td>
<td>0.979</td>
<td>0.984</td>
<td>0.989</td>
<td>0.605</td>
</tr>
<tr>
<td>AX3</td>
<td>0.971</td>
<td>0.893</td>
<td>0.975</td>
<td>0.991</td>
<td>0.643</td>
</tr>
<tr>
<td>AX4</td>
<td>0.993</td>
<td>0.967</td>
<td>0.951</td>
<td>0.991</td>
<td>0.763</td>
</tr>
<tr>
<td>AX5</td>
<td>0.980</td>
<td>0.973</td>
<td>0.977</td>
<td>0.996</td>
<td>0.654</td>
</tr>
<tr>
<td>AX6</td>
<td>0.983</td>
<td>0.983</td>
<td>0.976</td>
<td>0.999</td>
<td>0.689</td>
</tr>
<tr>
<td>AX7</td>
<td>0.994</td>
<td>0.961</td>
<td>0.940</td>
<td>0.995</td>
<td>0.842</td>
</tr>
<tr>
<td>AX8</td>
<td>0.992</td>
<td>0.960</td>
<td>0.951</td>
<td>0.995</td>
<td>0.789</td>
</tr>
<tr>
<td>AX9</td>
<td>0.993</td>
<td>0.953</td>
<td>0.947</td>
<td>0.996</td>
<td>0.815</td>
</tr>
</tbody>
</table>

Table 6 Results of stability studies of optimized formulation AX8

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Duration of storage (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Off white coloured, free flowing microspheres</td>
<td>Off white coloured, free flowing microspheres</td>
</tr>
<tr>
<td>Drug content (%) as per drug loading in microspheres</td>
<td>100 ± 0.54</td>
<td>97.69 ± 0.17</td>
</tr>
<tr>
<td>% in vitro mucoadhesion after 8h</td>
<td>81.00 ± 5.18</td>
<td>77.00 ± 5.33</td>
</tr>
<tr>
<td>$t_{50%}$ (hours)</td>
<td>6.63± 0.039</td>
<td>6.12 ± 0.12</td>
</tr>
<tr>
<td>Size</td>
<td>113.05 ± 5.68</td>
<td>112.95 ± 8.45</td>
</tr>
</tbody>
</table>

All values are mean of three determinations
Figure 1 Response Surface plot with quadratic design model showing the entrapment efficiency using different combination of $X_1$ and $X_2$ with Design expert 7.1.6 software.

Figure 2 Response Surface plot with quadratic design model showing the mucoadhesion after 1h using different combination of $X_1$ and $X_2$ with Design expert 7.1.6 software.

Figure 3 Response Surface plot with quadratic design model showing the mucoadhesion after 5h using different combination of $X_1$ and $X_2$ with Design expert 7.1.6 software.
Figure 4 Response Surface plot with quadratic design model showing the mucoadhesion after 8h using different combination of $X_1$ and $X_2$ with Design expert 7.1.6 software.

Figure 5 Response Surface plot with quadratic design model showing $t_{50\%}$ using different combination of $X_1$ and $X_2$ with Design expert 7.1.6 software.
Figure 6 Response Surface plot with quadratic design model showing size in µm using different combination of $X_1$ and $X_2$ with Design expert 7.1.6 software

Figure 7 Zero order release profile (Cumulative % drug released vs time in h) of formulation AX1 to AX9 of amoxicillin trihydrate mucoadhesive microspheres.
Figure 8 First order release profile (Log Cumulative % drug retained vs time in h) of formulation AX1 to AX9 of amoxicillin trihydrate mucoadhesive microspheres.

Figure 9 Higuchi plot (Cumulative % drug released vs time in h) of formulation AX1 to AX9 of amoxicillin trihydrate mucoadhesive microspheres.
Figure 10 Korsemeyer peppas plot (Log M/M∞ vs Log time in h) of formulation AX1 to AX9 of amoxicillin trihydrate mucoadhesive microspheres.

Figure 11 SEM photographs of mucoadhesive microspheres of formulation AX8