



Development and Evaluation of Amoxicillin Loaded Carbopol 934P Mucoadhesive Microcapsules for Sustained Drug Release for *H. pylori* Treatment

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Abstract

Amoxicillin (α -amino-p-hydroxybenzyl-penicillin) is a semi-synthetic, orally absorbed and widely prescribed β -lactam antibiotic. It is now widely used for eradication of gastric *Helicobacter pylori* infection combined with a second antibiotic and an acid-suppressing agent despite its short elimination half-life of one hour. The purpose of this study was to develop and evaluate amoxicillin loaded carbopol 934P mucoadhesive microcapsules for sustained drug release at the gastric mucosa to prolong the residence time of dosage form in the stomach and to achieve controlled drug release for more effective *H. pylori* eradication. Amoxicillin mucoadhesive microcapsules were formulated by ion gelation technique using 3^2 factorial designs. A 3^2 full factorial design was used to derive the statistical equation, ANOVA analysis, contour plots and 3D response surface plots. FT-IR (4000 cm^{-1} - 450 cm^{-1}) analysis of amoxicillin and with polymers was performed by using potassium bromide pellet method. Different polymer ratios of carbopol 934P and sodium alginate were used to formulate by nine formulations (F1 to F9) amoxicillin mucoadhesive microcapsules. The prepared formulations were characterized by determining their percentage of yield, particle size, percentage of entrapment efficiency, swelling index, percentage of mucoadhesion and percentage of drug release. Amoxicillin and used polymers are found to be compatible with no interaction reported by FTIR analysis. The optimized formulation (F9) exhibited a high drug entrapment efficiency of $96.04\pm 0.03\%$, particle size of $847.2518\pm 0.06\ \mu\text{m}$, yield of $98.86\pm 0.01\%$, swelling index of $120.09\pm 0.06\%$, and mucoadhesion of 67.00 ± 0.02 after 8 h. A successful sustained drug release was achieved for more than 14 h. *In-vitro* dissolution test for optimized formulation (F9) demonstrated a slower release behavior in 0.1N hydrochloric acid followed by linear release profile in pH 7.4 phosphate buffer. The drug-to-polymer-to-polymer ratio had a more significant effect on the dependent variables. The release kinetic study of optimized formulation (F9) displayed a good fitting with zero order behavior and Korsmeyer-Peppas model has confirmed a non-Fickian release. The developed mucoadhesive amoxicillin microcapsules were observed to have adhered strongly with gastric mucosa with approximately 14 h of prolonged stay expecting improved bioavailability and reduced dosing frequency and subsequently improving patient's compliance. The concentration of carbopol 934P and sodium alginate had highly significant effects on depended variables. The present study concludes that amoxicillin mucoadhesive microcapsules can be effectively used for the more effective treatment of *H. pylori* infection.

Keywords: Amoxicillin, ANOVA analysis, *Helicobacter pylori*, Ion gelation technique, Microencapsulation, Mucoadhesive drug delivery

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1. Introduction

H. pylori is a gram-negative bacterium which is found in the mucus layer and in the mucosa of the stomach, and it is associated with several stomach diseases like chronic gastritis, gastric ulcers, duodenal ulcers and gastric cancer [1, 3]. This infection is affecting nearly 50%-75% of the worldwide demography, It is found to be more prevalent in (almost 70%) developing countries compared to 25%-50% in developed countries [4, 5]. The causes of treatment failure are poor drug penetration, low drug concentration; short gastric residence time and antibiotic resistance. Besides, the poor stability of antibiotics in gastric content requires frequent administration and leads to patient noncompliance [6].

Amoxicillin (α -amino-p-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed and widely prescribed β -lactam antibiotic. It is now widely used in a standard eradication of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent [7, 8]. It is a broad-spectrum antibiotic that reaches as to C_{max} (8 μ g/ml) approximately within two hours after administration with a short elimination half-

life of one hour [9]. Firstly the reason for the incomplete eradication of *H. pylori* is probably due to the short residence time of dosage form in the stomach which consequently results in ineffective antimicrobial concentration in a gastric mucous layer or epithelial cell surfaces where *H. pylori* exists and may be due to the degradation of amoxicillin in gastric acid [10, 11].

Microencapsulation is a method by which an active ingredient is entrapped inside miniature capsules. Very tiny droplets, or particles of liquid or solid material, are surrounded within a second material or coated with a thin film of polymeric material for the purpose of protecting the active ingredient from the surrounding environment [12, 13]. Microcapsules are capable drug carrier particles that control the release rate or target the active drugs to a specific body absorption site for particulate drug delivery system, thereby enhancing drug absorption, reduced toxicity, superior patient compliance and convenience [14]. Therefore, the development of new controlled or sustained release of the drug delivery system is one of the most excellent fields of research in pharmaceutical sciences that deliver the drug to the target tissue in the body. As a result, it has overcome difficult problems of conventional therapy such as drug toxicity and, stomach irritation, resulting in the enhanced therapeutic efficacy of the administered drug and reduced toxicity [15]. It also improves the intimate contact of the dosage form and prolongs the residence time of dosage form, enhancing the bioavailability of the drug and having a great

impact on the formulation and progress of novel drug delivery systems [16, 17].

Mucoadhesive polymer has low toxicity and non-absorbable, non-irritant to the mucous membrane, rapidly adheres to moist tissue and possess some site specificity, form the strong non-covalent bond with the mucous tissue, allow easy incorporation of the drug and without the hindrance of drug release, affordable, easily available and good shelf life. It is adhering to the tissue surface, absorbed into the mucous tissues, and releases slowly on site of action [18, 19]. Hence mucoadhesive drug delivery systems can enhance the efficiency of the drug for *H. pylori* infection treatment.

The aim of this study was to formulate amoxicillin mucoadhesive microcapsules by ion gelation technique using the different concentration of sodium alginate and carbopol 934P and calcium chloride as a cross-linker; which is achieved a gastric prolong the

residence time of the gastric mucosa and controlled drug release for more effective treatment of *H. pylori* eradication.

2. Materials and Methods

2.1.1. Materials

Amoxicillin was purchased from Sigma-Aldrich Company, Germany. Sodium alginate, carbopol 934P, and calcium chloride were obtained as a gift sample from MAHSA University, Malaysia.

2.1.2. Formulation of Amoxicillin Mucoadhesive Microcapsules

Formulation of amoxicillin mucoadhesive microcapsules was formulated by ion gelation technique [20]. Sodium alginate-carbopol 934P (mucoadhesive polymers) were dissolved in 10 ml of purified water to form a homogeneous polymer solution. 250 mg of amoxicillin was added to the polymeric solution and mixed thoroughly with a stirrer to

Table 1. Amoxicillin mucoadhesive microcapsules by carbopol 934P with their experimental coded level of variables for 3^2 factorial designs.

| Formulation Code | Variable Levels in Coded Form | |
|------------------|---|---|
| | X ₁ (concentration of sodium alginate) | X ₂ (concentration of carbopol 934P) |
| F1 | -1 | -1 |
| F2 | -1 | 0 |
| F3 | -1 | +1 |
| F4 | 0 | -1 |
| F5 | 0 | 0 |
| F6 | 0 | +1 |
| F7 | +1 | -1 |
| F8 | +1 | 0 |
| F9 | +1 | +1 |

Table 2. Translation of coded levels of amoxicillin mucoadhesive microcapsules by carbopol 934P in actual units.

| Amoxicillin (250 mg) | | |
|---|---------------------|------------------|
| X ₁ = Concentration of sodium alginate (% w/v) | | |
| Low 125 mg (-1) | Medium 187.5 mg (0) | High 250 mg (+1) |
| X ₂ = Concentration of carbopol 934P (% w/v) | | |
| Low 250 mg (-1) | Medium 375 mg (0) | High 500 mg (+1) |

form a viscous dispersion. The resulting dispersion was added manually drop-wise into 40 ml of 10 % w/v calcium chloride solution through a syringe (no. 21). The added droplets were retained in the calcium chloride solution for 1 h to complete the curing reaction and to produce spherical rigid mucoadhesive microcapsules. The mucoadhesive microcapsules were collected by decantation and the products were separately washed frequently and dried at 40 °C for the 3 h in a hot air oven.

2.1.3. 3² Factorial Designs

A response surface method 3² factorial designs was applied to evaluate the relationship between the independent variables and their responses. Two variables and six responses were involved in the experimental design. The dependent response factor variables studied were percentage yield, percentage entrapment efficiency, particle size, swelling index, mucoadhesion percentage and drug release. Various formulations of amoxicillin-carbopol 934P mucoadhesive microcapsules were prepared individually by using all combinations of different levels of experimental variables as shown in table 1.

The independent variables are the concentration of sodium alginate (X₁) and the concentration of carbopol 934P (X₂) independent variables are classified as low, medium and high and their value is shown in table 2 [20].

2.2. Evaluation of Amoxicillin Mucoadhesive Microcapsules

2.2.1. Fourier Transform Infrared Spectroscopy (FT- IR)

Fourier Transform Infrared Spectroscopy (FT- IR) is a rapid, easy and inexpensive analytical technique that used to predict the drug-excipient interactions. This analysis was performed by using potassium bromide pellet method. Amoxicillin and amoxicillin with individual polymers (1:1) were weighed and mixed homogeneously with 300 mg of potassium bromide and the mixture was compacted into a translucent film by mechanical die press. It was recorded on Shimadzu's fourier transform infrared spectrometer (Japan) with a frequency range of 4000-450 cm⁻¹. It was recorded on Shimadzu's Fourier transform infrared spectrometer (Japan) with a frequency range of 4000-450 cm⁻¹ [21, 22].

2.2.2. Particle Size Measurement

The particle size of amoxicillin mucoadhesive microcapsules was evaluated by optical microscopy method. The amount was done under 10 x 45 (10x eyepiece and 45x objective) and 100 mucoadhesive microcapsules counted for particle size analysis by using a calibrated optical microscope. First of all, 100 mucoadhesive microcapsules were taken and kept in a glass slide. It was mixed with glycerin and set in an optical microscope, then determined the particle size [23].

2.2.3. Percentage Yield

Percentage yield of amoxicillin mucoadhesive microcapsules was calculated to know the efficiency of the methods used during the preparation, which might be useful in the selection of an appropriate method for future production. Percentage yield was calculated as the weight of mucoadhesive microcapsules recovered from each formulation in relation to the sum of starting material. The percentage yield of prepared mucoadhesive microcapsules was determined by using the formula, respectively [24, 25].

$$\text{Percentage yield} = \frac{\text{Weight of mucoadhesive microcapsule} \times 100}{\text{Theoretical weight of polymer and drug}}$$

2.2.4. Drug Entrapment Efficiency

100 mg of amoxicillin mucoadhesive microcapsule was crushed in a glass mortar and pestle, and the powdered microcapsules were suspended in 10 ml of pH 7.4 phosphate

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

buffer solution (pH 7.4), respectively. After 24 h, the solution filtered and the filtrate was analyzed for the drug entrapment efficiency using the following formula [20].

2.2.5. Swelling Index

The amoxicillin mucoadhesive microcapsules (100 mg) were placed separately, in a glass vial containing 10 ml of 0.1N HCl and kept at $37 \pm 0.5^\circ\text{C}$ in an incubator with occasional shaking. The swelled amoxicillin mucoadhesive microcapsules were removed at the predetermined time interval, dried with tissue paper and weighed. The Weight of the swollen microcapsules was recorded after a period of 8 h and swelling ratio was calculated using the following formula.

$$\text{Percentage swelling Index (SI)} = \frac{W_t - W_o}{W_o} \times 100$$

Whereas, W_t = Equilibrium weight of microcapsules after swelling and W_o = Initial weight of microcapsules [26, 27].

2.2.6. Mucoadhesion In-vitro Wash-Off Test

The Mucoadhesive property of the amoxicillin mucoadhesive microcapsule was evaluated by an *in-vitro* wash-off test using goat stomach mucosa. A piece of goat stomach mucosa (2cm x 2cm) was collected and tied onto a glass slide (7.5cm x 2.5cm) using thread. 100 mg amoxicillin mucoadhesive

microcapsules were separately placed onto wet tissue specimen and the prepared slide was hung into the groove of disintegration tester. The tissue specimen was given a regularly up and down movement in a beaker containing 900 ml of 0.1N HCl (pH 1.2) set at $37 \pm 0.5^\circ\text{C}$. At the end of the time interval, the number of mucoadhesive microcapsules that remained attached to the tissue was recorded [28, 29].

The mucoadhesion adhesion number was determined by the following formula

$$N_n = (N/N_0) \times 100$$

Where, N_n = Adhesion number, N = Number of mucoadhesive microcapsules attached to the mucosa after washing, N_0 = Initial number of mucoadhesive microcapsules in the intestinal mucosa.

2.2.7. *In-vitro* Dissolution Studies

Dissolution studies of amoxicillin mucoadhesive microcapsule, (equivalent to 250 mg of amoxicillin) was carried out by USP29 type-II tablet dissolution test apparatus (Electrolab India) at 50 rpm and $37 \pm 0.5^\circ\text{C}$, using 900 ml of 0.1N HCl (pH 1.2) as the dissolution medium. An aliquot of sample (5 ml) was withdrawn periodically, replaced with an equivalent volume of blank dissolution medium [30]. Samples were filtered through Whatman filter paper (0.45 μm), and analyzed spectrophotometrically at 272 nm. Drug release data obtained during *in-vitro* dissolution studies were analyzed for release kinetics of zero order, first order, and Higuchi

model equations and fitted into Korsmeyer-Peppas model for evaluation of release mechanism from mucoadhesive microcapsules [31].

2.2.8. Drug Release Kinetic Profile

To study the drug release kinetics and mechanism of amoxicillin mucoadhesive microcapsules, the *in-vitro* data was evaluated to find a suitable mathematical model to fit the *in-vitro* release behavior. The following mathematical models evaluated to determine the drug release per unit time, namely zero order and first order whereas Higuchi and Korsmeyer-Peppas model was used to evaluate the mechanism of drug release [32].

Zero-order:

The zero-order equation describes in which the drug release rate is independent of its concentration of dissolved substances. The Equation of zero-order release is

$$Q_t = Q_0 + K_0 t$$

Where, Q_t = cumulative amount of drug release a time "t", Q_0 = initial amount of drug, K_0 = zero order release constant and t = time in hours

$$C = k_0 t$$

K_0 = rate constant and concentration release is directly proportional to time.

First order:

First order kinetic is described absorption and/or clearance of the drug. The release rate of the drug is dependent on concentration.

$$\text{Log}C_t = \text{Log}C_0 - kt / 2.303$$

Where, C = initial concentration of drugs and indicates first order reaction constant.

Higuchi's model:

Higuchi's model determines the kinetic profile of different geometric and porous drug delivery system. It obeys Fick's law and is square root time dependent.

$$Q = K_H t^{1/2}$$

Where K_H = Higuchi dissolution constant to identify the diffusion controlled process. Drug release that calculated in time per unit area is plotted against a square of time.

Korsmeyer - Peppas model:

Determine the drug release mechanism of particular dosage form either by fickian or non-fickian.

$$\text{Log} (M_t / M_\infty) = \text{Log} k + n \text{Log} t$$

Where M_t / M_∞ = drug release at time t , n = exponent indicative of release mechanism manipulated by polymer and K = kinetic constant with structural and geometric properties of a dosage.

2.2.9. 3^2 Full Factorial Design Studies

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

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

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 polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity.

On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables, X_1 - concentration of sodium alginate (%w/v) and X_2 - concentration of polymer (%w/v) on dependent variables particle size, % drug entrapment efficiency, swelling index, drug release and percentage mucoadhesion. Factorial designs can screen for important drugs and drug interactions, as well as determine potential optimal drug dosages. Enable to build statistical models with a small number of runs. A statistical model was incorporating by using Design-Expert® Software Version 11.0.0 [20, 33].

2.2.10. Statistical Analysis

Factorial equation and ANOVA analysis were used for statistical analysis. Responses observed for each of the formulations (F1–F9) were simultaneously fitted to quadratic model using Design-Expert® Software Version 11.0

3. Results and Discussion

3.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy studies were performed to ensure that the processing time has not led to any interaction between the drug and polymer in the formulation. The FT-IR spectrum of the pure amoxicillin, sodium alginate, and carbopol 934P are shown in figure 1 to 3. Furthermore, the spectrum of carbopol 934P-sodium alginate mucoadhesive microcapsules containing amoxicillin is shown in figure 4. Spectra were recorded on Shimadzu's Fourier transform infrared

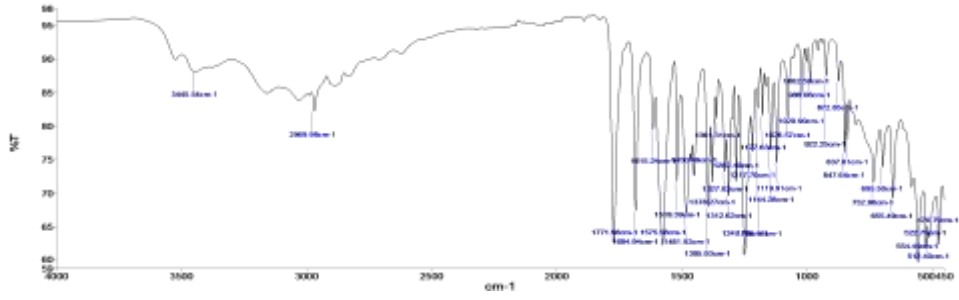


Figure 1. FT-IR spectrum of amoxicillin.

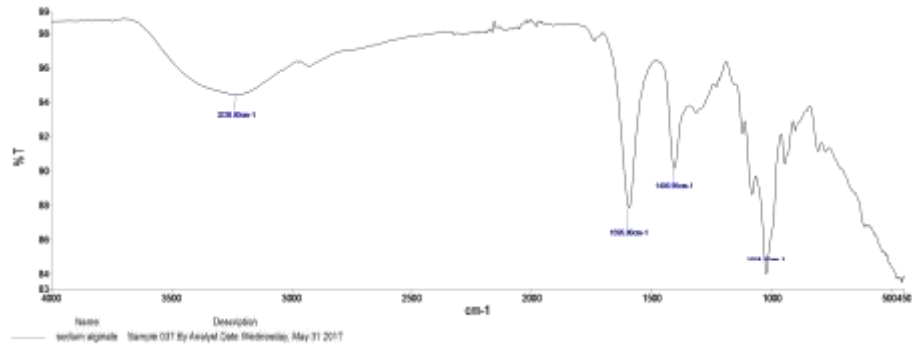


Figure 2. FT-IR spectrum of sodium alginate.

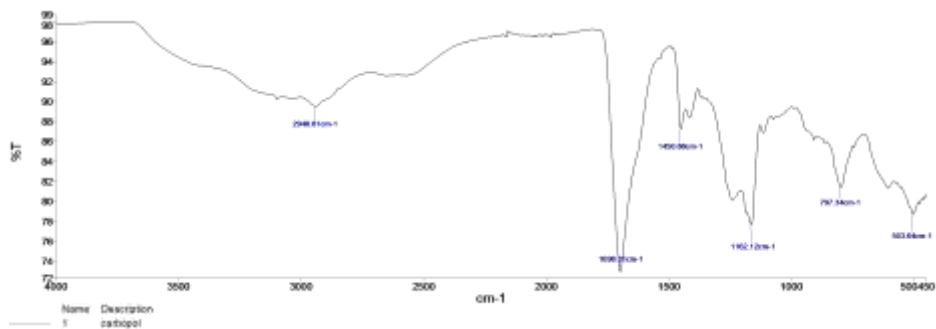


Figure 3. FT-IR spectrum of carbopol 934P.

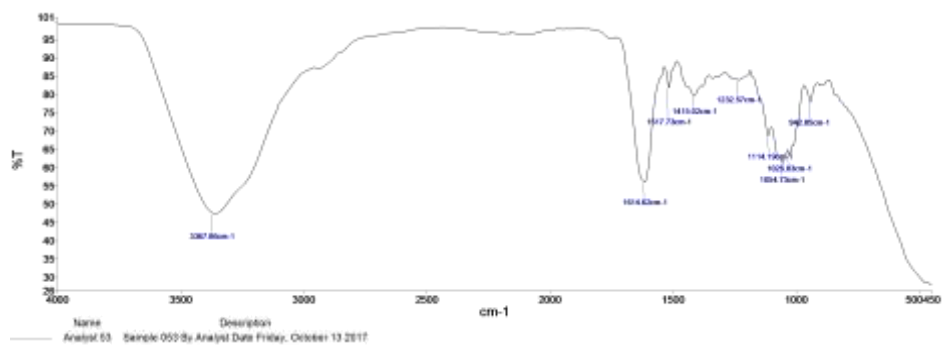


Figure 4. FT-IR spectrum of carbopol 934P-based mucoadhesive microcapsules containing amoxicillin.

spectrometer (Japan) with a frequency range of 4000-450 cm⁻¹.

The FT-IR spectrum of amoxicillin has shown the presence of absorption band at

approximately 3445.54 cm⁻¹ and 2969.98 cm⁻¹ corresponding to the O-H and C-H stretching vibrations, respectively. The β-lactam and amide C=O stretching frequency appeared at 1771.98 cm⁻¹ and 1684.94 cm⁻¹. The band peaks at 1519.39 cm⁻¹, 1481.92 cm⁻¹, 1395.93 cm⁻¹, and 1076.57 cm⁻¹ which are confirmed to the C=C bending, N-H bending, -CH₂ bending and C-N stretching vibrations spectrum. The FT-IR spectrum of sodium alginate has shown the peaks at about 3228.00 cm⁻¹, 1595.00 cm⁻¹, 1406.95 cm⁻¹, and

1024.32 cm⁻¹ indicating O-H stretching vibrations, COO⁻ stretching vibrations, -CH stretching vibrations and C-O-C stretching vibrations, respectively. The FT-IR of sodium alginate was almost similar to the finding reported by previous studies [34]. The FT-IR spectrum of carbopol 934P showed the band at around 2940.81 cm⁻¹, 1698.01 cm⁻¹, 1450.88 cm⁻¹, and 1162.12 cm⁻¹, corresponding to the stretching of O-H, stretching of C=O, stretching of -CH₂ and bending of C-H, respectively. Carbopol 934P FT-IR was found

Table 3. Particle size, percentage yield, drug entrapment efficiency, swelling index, percentage mucoadhesion, and drug release of amoxicillin mucoadhesive microcapsules.

| Formulation code | Particle size (μm) | Percentage yield (%) | Entrapment efficiency (%) |
|------------------|--------------------|----------------------|---------------------------|
| F1 | 805.00±0.05 | 97.06±0.01 | 77.23±0.3 |
| F2 | 814.08±0.03 | 97.78±0.05 | 85.25±0.02 |
| F3 | 828.02±0.02 | 89.52±0.01 | 93.07±0.04 |
| F4 | 825.09±0.08 | 97.57±0.7 | 81.19±0.01 |
| F5 | 832.10±0.05 | 97.25±0.04 | 93.03±0.05 |
| F6 | 842.04±0.02 | 98.86±0.01 | 92.06±0.02 |
| F7 | 837.15±0.04 | 97.78±0.06 | 83.28±0.09 |
| F8 | 838.50±0.05 | 97.60±0.08 | 95.18±0.02 |
| F9 | 847.25±0.06 | 93.66±0.01 | 96.04±0.03 |
| Formulation code | Swelling Index % | % Mucoadhesion | % Cumulative drug release |
| F1 | 90.24±0.02 | 48.00±0.05 | 80.50±0.03 |
| F2 | 95.08±0.01 | 56.00±0.03 | 66.40±0.04 |
| F3 | 98.14±0.05 | 60.00±0.02 | 57.14±0.04 |
| F4 | 100.50±0.06 | 52.00±0.07 | 74.30±0.08 |
| F5 | 99.05±0.02 | 58.00±0.05 | 64.90±0.07 |
| F6 | 115.20±0.06 | 61.00±0.04 | 55.40±0.02 |
| F7 | 110.50±0.08 | 57.00±0.03 | 67.80±0.06 |
| F8 | 117.78±0.02 | 62.00±0.04 | 58.30±0.06 |
| F9 | 120.09±0.06 | 67.00±0.02 | 53.50±0.01 |

to be similar to the result reported by previous studies [35]. Finally, the FT-IR spectrum for carbopol 934P-sodium alginate mucoadhesive microcapsules containing amoxicillin showed distinguished absorption characteristics of peaks of amoxicillin, sodium alginate, and carbopol 934P present in the drug-polymer combinations, indicating there is no interaction between polymer and drug used as shown in figure 4.

3.2. Particle Size Measurement of Amoxicillin Mucoadhesive Microcapsules

The particle size of amoxicillin mucoadhesive microcapsules was varied from $805.00 \pm 0.05 \mu\text{m}$ to $847.25 \pm 0.06 \mu\text{m}$, as shown in table 3. Researchers have reported that as polymer concentration increases, the particle size also improves, which could be due to increase in the viscosity of drug and polymer ratio, and coat thickness of polymer [36, 37]. Studies have reported that the particle size depends on rotational speed and temperature; an increase in rotational speed results in a decrease in particle size, while an increase in temperature leads to increase in particle size [38]. The present study indicated that the higher concentration of sodium alginate and carbopol 934P solution form large droplets with increased particle size compared to lower concentration of polymers which results in small droplet due to the difference of viscosity.

3.3. Percentage Yield of Amoxicillin Mucoadhesive Microcapsules

The percentage yield was found to be within the range of $89.52 \pm 0.01\%$ to $97.78 \pm 0.06\%$, as shown in table 3. The present study found that the percentage yield was increased with increase in polymer ratio. The previous studies have proved that the percentage yield of mucoadhesive microcapsules was improved within increasing concentration of sodium alginate too [39]. The percentage yield of amoxicillin-carbopol 934P mucoadhesive microcapsules formulation F1 and F6 were found to be $97.78 \pm 0.06\%$ and $98.86 \pm 0.01\%$, respectively, which sodium alginate was used within 125.00 mg and 187.50 mg. Their results indicate that the percentage yield was also affected by increased sodium alginate concentration.

3.4. Drug Entrapment Efficiency of Amoxicillin Mucoadhesive Microcapsules

The average efficiency ranges for amoxicillin-carbopol 934P mucoadhesive microcapsules were found to be 77.23 ± 0.3 to $96.04 \pm 0.03\%$, as shown in table 3. The formulated mucoadhesive microcapsules indicated that drug entrapment efficiency depends on polymer concentration (sodium alginate and carbopol 934P). The present research study has also shown that with the same sodium alginate concentration if carbopol 934P concentration was increased drug entrapment efficiency also increased; additionally drug entrapment also found to be increased with increased concentration of sodium alginate. Therefore, drug entrapment

efficiency depends on polymer concentration. Increased in drug entrapment efficiency with increase in polymer concentration is attributed to increase in viscosity of the formulation. Amoxicillin mucoadhesive microcapsules formulation F9 has shown highest drug entrapment efficiency (96.04 ± 0.03) due to high polymer concentration. Hence, the highest drug entrapment efficiency was observed in amoxicillin mucoadhesive microcapsules when sodium alginate concentration was increased and used in 10% CaCl_2 due to high cross-linking by sodium alginate and CaCl_2 . The present research work has showed that with the same sodium alginate concentration if carbopol 934P concentration were increased drug entrapment efficiency was also increased; additionally, drug entrapment also found to be increased with increased concentration of sodium alginate. Therefore, drug entrapment efficiency was lowest and highest depends on polymer concentration; the result was similar to study as reported by previous studies [40, 41].

3.5. Swelling Index of Amoxicillin Mucoadhesive Microcapsules

The swelling index of amoxicillin mucoadhesive microcapsules were found to be in the range of 90.24 ± 0.02 to $120.09 \pm 0.06\%$, as shown in table 3. Formulation F9 has shown highest swelling index due to maximum polymer concentration. The swelling index of all the formulations was reported to be improved with the increased concentration of polymers. The swelling index depends on drug and polymer concentration which influence the swelling behaviour of the mucoadhesive microcapsules. The result shows that maximum swelling index was achieved with highest polymer concentration. Similarly, the previous studies reported that increased polymer concentration leads to increased with swelling index [42].

3.6. Mucoadhesion Testing By In-Vitro Wash-Off Test

In-vitro bioadhesion study has demonstrated that amoxicillin-carbopol 934P mucoadhesive microcapsules had good

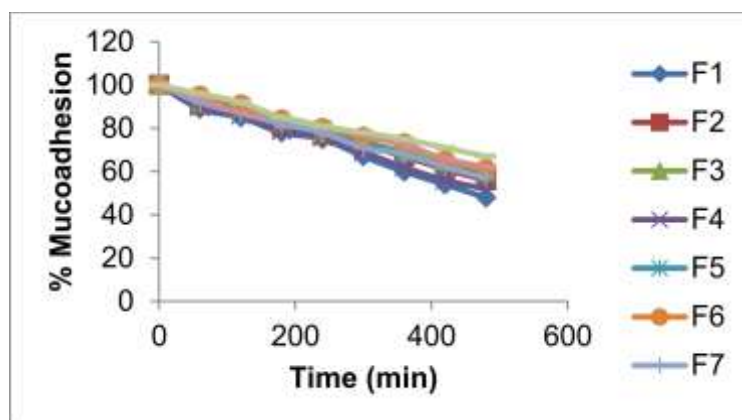


Figure 5. Mucoadhesion of amoxicillin-carbopol 934P mucoadhesive microcapsules. Values are presented as Mean \pm SD. n=9.

bioadhesive property ranging from 48.00 ± 0.05 to $67.00 \pm 0.02\%$, as shown in table 3. Formulation F9 for carbopol 934P had highest bioadhesive property due to the higher concentration of mucoadhesive polymer and expected to adhere for long time in the stomach. It was observed that mucoadhesion of the amoxicillin mucoadhesive microcapsules significantly ($P < 0.05$) increased with increasing polymer concentration due to increase in viscosity and produces stronger mucus gel network which helps to increase mucoadhesion as shown in Figure 5. Amoxicillin mucoadhesive microcapsules have shown good mucoadhesion properties to mucosal tissue and could adhere to intestinal mucosa for an expanded period of time.

These results were finding may be attributed to the change in particle size that affects mucoadhesion when the drug-polymer ratio increased the percentage of mucoadhesion also increased because higher amounts of polymer provides excessive free –COOH groups, which are responsible for

binding with sialic acid groups in mucous membrane and thus results in improved properties of mucoadhesive microcapsules [43].

3.6. In-Vitro Dissolution Studies of Amoxicillin Mucoadhesive Microcapsules

Amoxicillin microcapsules have shown negligible amounts of drug released in simulated gastric fluid (0.1N HCl, pH 1.2); whereas the microcapsules have shown as substantive drug release in phosphate buffer pH 7.4, as shown in table 3 and figure 6. The percentage of cumulative drug release (CDR %) were found to be within the range of $80.50 \pm 0.03\%$ to $53.50 \pm 0.01\%$ for amoxicillin mucoadhesive microcapsules over 8 h. The drug release from the sustained release of amoxicillin mucoadhesive microcapsules varied depending on the amount of sodium alginate and carbopol 934P. Formulation F1 and F4 had faster release rate than F2, F3, F5, F6, F7, F8 and F9. Formulation F6, F8, and F9 showed much slower release rates within the

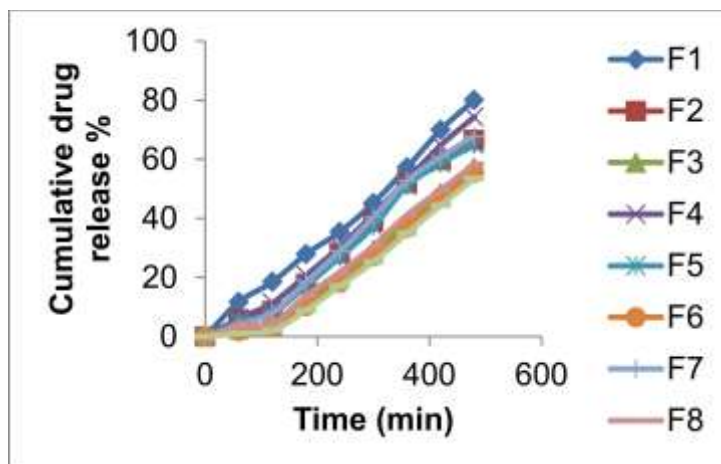


Figure 6. Amoxicillin-carbopol 934P mucoadhesive microcapsules drug release. Values are presented as mean \pm SD. n=9.

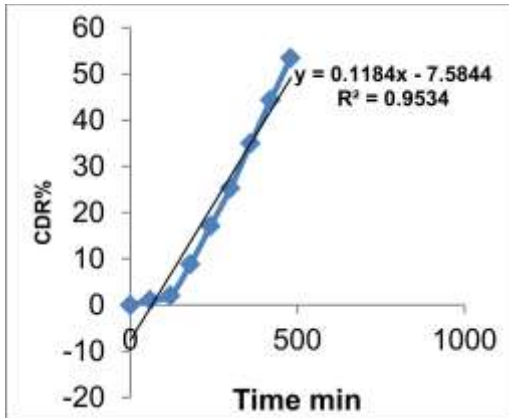


Figure 7. Zero order release of F9.

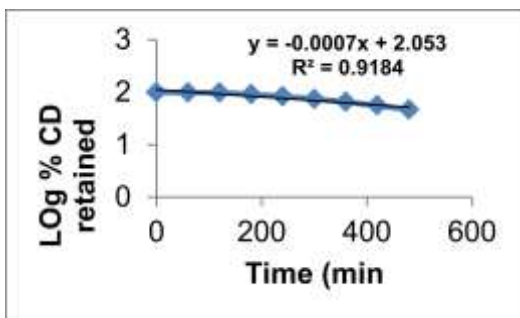


Figure 8. First order release of F9.

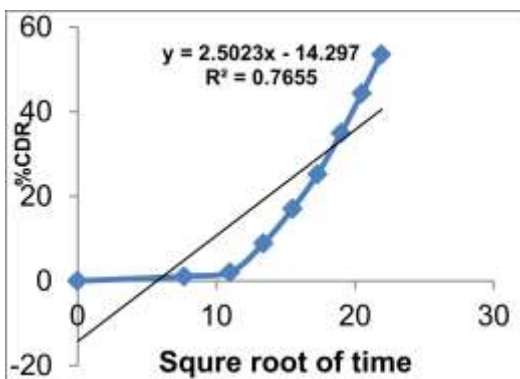


Figure 9. Higuchi model of F9.

range of $55.40 \pm 0.02\%$ to $53.50 \pm 0.01\%$ after 8 h; whereas formulation F1, F2, F3, F4, F5 and F7 showed faster release rates up to 60% by 8 h due to polymer concentration. Table 3 and Figure 6, indicates that the drug release rates were decreased with increased polymer concentration. Formulation F9 has shown

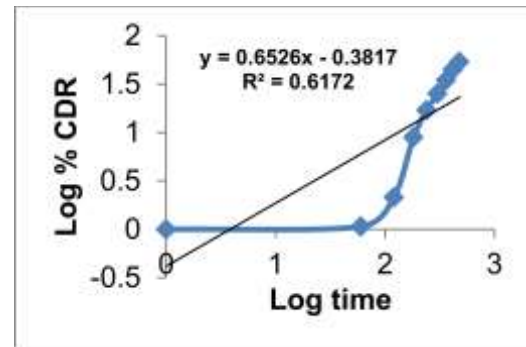


Figure 10. Korsmeyer-Peppas release of F9.

slower drug release rates due to higher polymer concentration.

All formulation F1 to F9 has shown a decreased rate of drug release with increased polymer concentration, the results are in accordance with previous studies [44]. It was also observed that highest drug to polymer ratio resulted in slowest drug release due to dense polymer matrix which creates layer diffusion path length for drug release [45].

3.7. Drug Release Kinetic Profile

Formulation F9 was selected as the most potential for its drug release kinetics model fitting like zero order, first order, Higuchi and Korsmeyer-Peppas models. The results of the curve fitting into various mathematical models are shown in table 4 and figure 7 to 10. The suitability of the model has been observed on the basis of best fit to the model using the correlation coefficient value (R^2). The zero order, first order, Higuchi and Korsmeyer-Peppas models are shown in figure 7, 8, 9, and 10. The Korsmeyer-Peppas model was used in the *in-vitro* release behaviour evaluation of the formulations to distinguish between release mechanisms based on n value: Fickian release

Table 4. *In-vitro* release kinetic models of amoxicillin-carbopol 934P mucoadhesive microcapsules.

| F9 | Zero- order | | First-order | | Higuchi | | Korsmeyer-Peppas | | Mechanism of drug release |
|----|----------------|----------------|----------------|----------------|----------------|-------------------------|------------------|-------|---------------------------|
| | R ² | K ₀ | R ² | K ₁ | R ² | K (min ^{1/2}) | R ² | n | |
| | 0.953 | 0.118 | 0.918 | 0.00 | 0.763 | 2.508 | 0.615 | 0.652 | Non-Fickian release |

Table 5. ANOVA analysis for the particle size, drug entrapment efficiency, swelling index, mucoadhesion and drug release of amoxicillin mucoadhesive microcapsules.

| Particle size of amoxicillin-carbopol 934P mucoadhesive microcapsules | | | | | | | |
|---|----------------|-------------------|-------------|---------|--------------------------|----------------------------|--|
| Source | Sum of squares | Degree of freedom | Mean square | F value | <i>p</i> -Value Prob > F | Significant/ Insignificant | |
| Model | 0.4094 | 2 | 0.2047 | 37.85 | 0.0004 | Significant | |
| A-Sodium alginate | 0.2835 | 1 | 0.2835 | 52.42 | 0.0004 | Significant | |
| B-Carbopol 934P | 0.1259 | 1 | 0.1259 | 23.28 | 0.0029 | Significant | |
| Entrapment efficiency of amoxicillin-carbopol 934P mucoadhesive microcapsules | | | | | | | |
| Source | Sum of squares | Degree of freedom | Mean square | F value | <i>p</i> -Value Prob > F | Significant/ Insignificant | |
| Model | 337.66 | 2 | 168.83 | 7.36 | 0.0243 | Significant | |
| A-Sodium alginate | 319.50 | 2 | 159.75 | 17.65 | 0.0031 | Significant | |
| B-Carbopol 934P | 259.65 | 1 | 259.65 | 28.68 | 0.0017 | Significant | |
| Swelling index of amoxicillin-carbopol 934P mucoadhesive microcapsules | | | | | | | |
| Source | Sum of squares | Degree of freedom | Mean square | F value | <i>p</i> -Value Prob > F | Significant/ Insignificant | |
| Model | 874.92 | 2 | 437.46 | 37.98 | 0.0004 | Significant | |
| A-Sodium alginate | 702.22 | 1 | 702.22 | 60.97 | 0.0002 | Significant | |
| B-Carbopol 934P | 172.70 | 1 | 172.70 | 14.99 | 0.0082 | Significant | |
| Model | 874.92 | 2 | 437.46 | 37.98 | 0.0004 | Significant | |
| Mucoadhesion of amoxicillin-carbopol 934P mucoadhesive microcapsules | | | | | | | |
| Source | Sum of squares | Degree of freedom | Mean square | F value | <i>p</i> -Value Prob > F | Significant/ Insignificant | |
| Model | 248.11 | 5 | 49.62 | 53.59 | 0.0039 | Significant | |
| A-Sodium alginate | 80.67 | 1 | 80.67 | 87.12 | 0.0026 | Significant | |
| B-Carbopol | 160.17 | 1 | 160.17 | 172.98 | 0.0009 | Significant | |
| Drug release of amoxicillin-carbopol 934P mucoadhesive microcapsules | | | | | | | |
| Source | Sum of squares | Degree of freedom | Mean square | F value | <i>p</i> -Value Prob > F | Significant/ Insignificant | |
| Model | 2.43 | 2 | 1.21 | 79.94 | < 0.0001 | Significant | |
| A-Sodium alginate | 0.3730 | 1 | 0.3730 | 24.58 | 0.0026 | Significant | |
| B-Carbopol | 2.05 | 1 | 2.05 | 135.29 | < 0.0001 | Significant | |

(diffusion-controlled release), non-Fickian release (anomalous transport) and case-II transport (relaxation-controlled release). When

n value is ≤ 0.43 , it is Fickian release. The n value between 0.43 and 0.85 is defined a non-

Fickian release. When, n value is ≥ 0.85 , it is case-II transport [46].

From the results shown in table 4, it can be observed that the release kinetics of amoxicillin mucoadhesive microcapsules formulation F9 showed good fitting with zero-order (R^2 values 0.953), and others first order and Higuchi model with R^2 values 0.918 and 0.763. The n value of Peppas model (0.652) indicates that the mechanism of drug release

follows non-Fickian diffusion.

3.9. 3^2 - Full Factorial Designs Studies in the Statistical Analysis

Quantitative results are expressed as mean \pm SD. The statistical differences were analyzed by ANOVA analysis, factorial analysis and P-values < 0.05 were considered significant. Responses observed for each of the formulations (F1–F9) were simultaneously

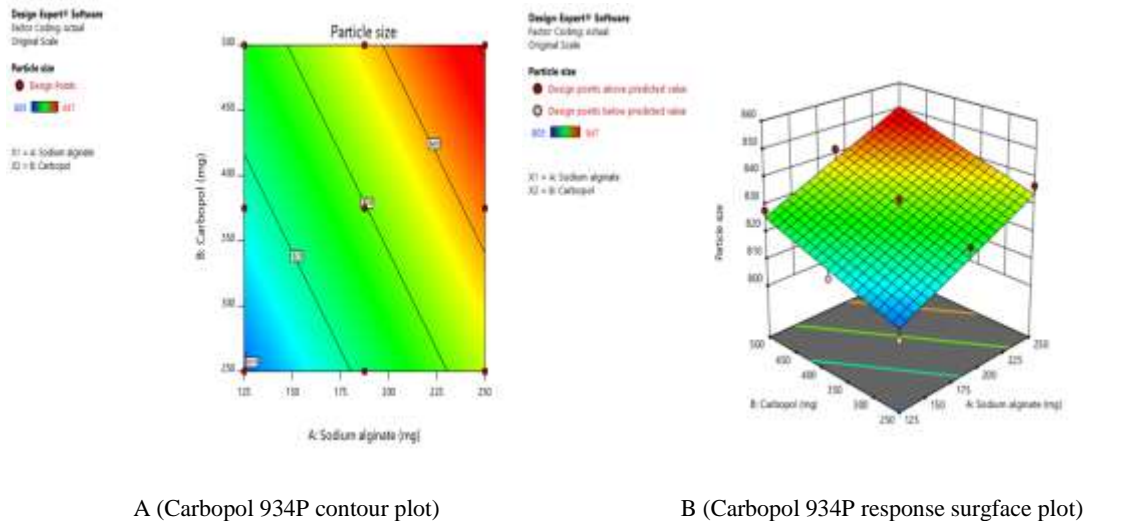


Figure 11. A (Contour plot) and B (response surface plot), showing the effect of independent variables on the particle size of mucoadhesive microcapsules.

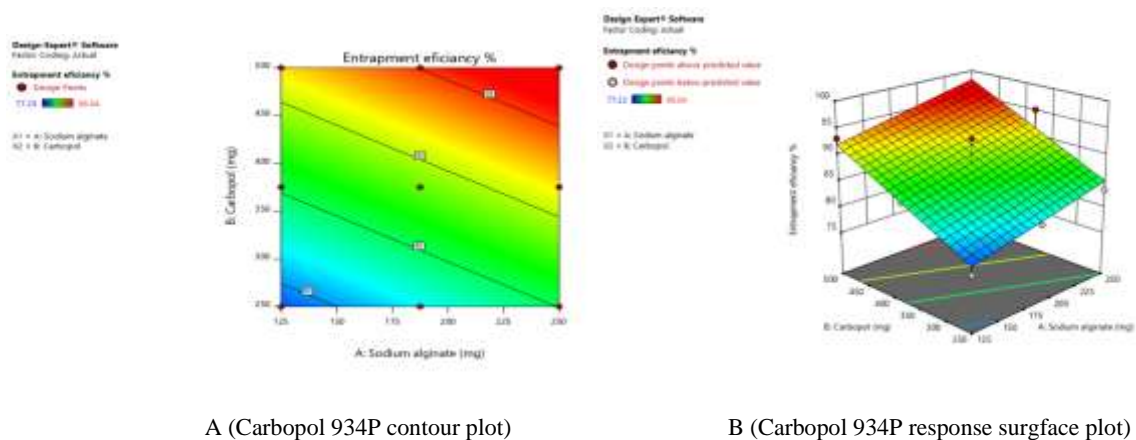


Figure 12. Amoxicillin A (Contour plot) and B (Response plot), showing the effect of independent variables on the entrapment efficiency of mucoadhesive microcapsules.

fitted to quadratic model using Design-Expert® Software Version 11.0.0. Statistical analysis was analyzed according to Table 3.

3.9.1. Factorial Equation

The result of equation Y that are indicated

for particle size, drug entrapment efficiency, swelling index, mucoadhesion and drug release for all batches (F1-F9) showed a wide variation of independent and dependent variables.

The factorial equation for particle size,

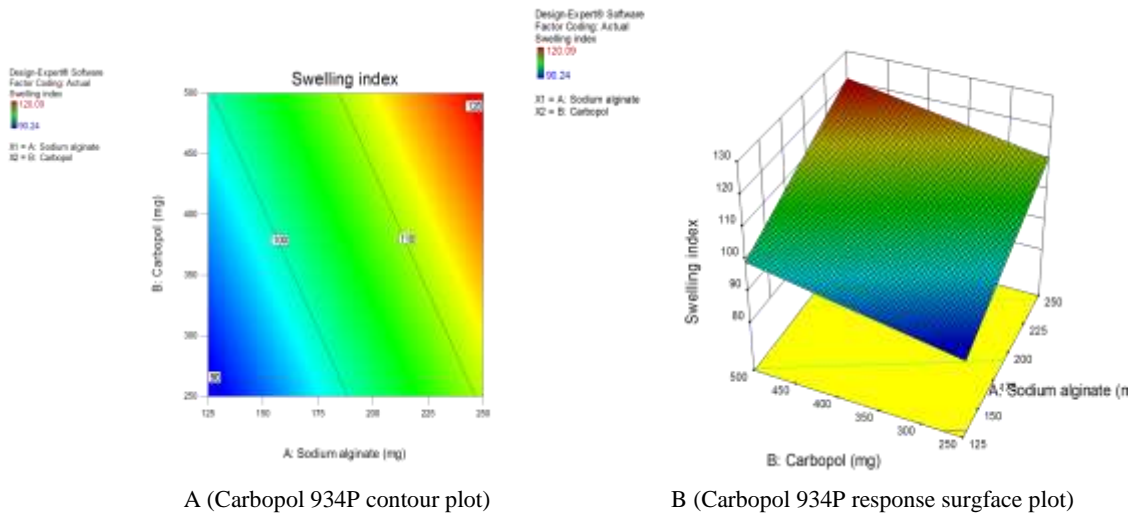


Figure 13. Amoxicillin A (Contour plot) and B (Response surface plot), showing the effect of independent variables on the swelling index of mucoadhesive microcapsules.

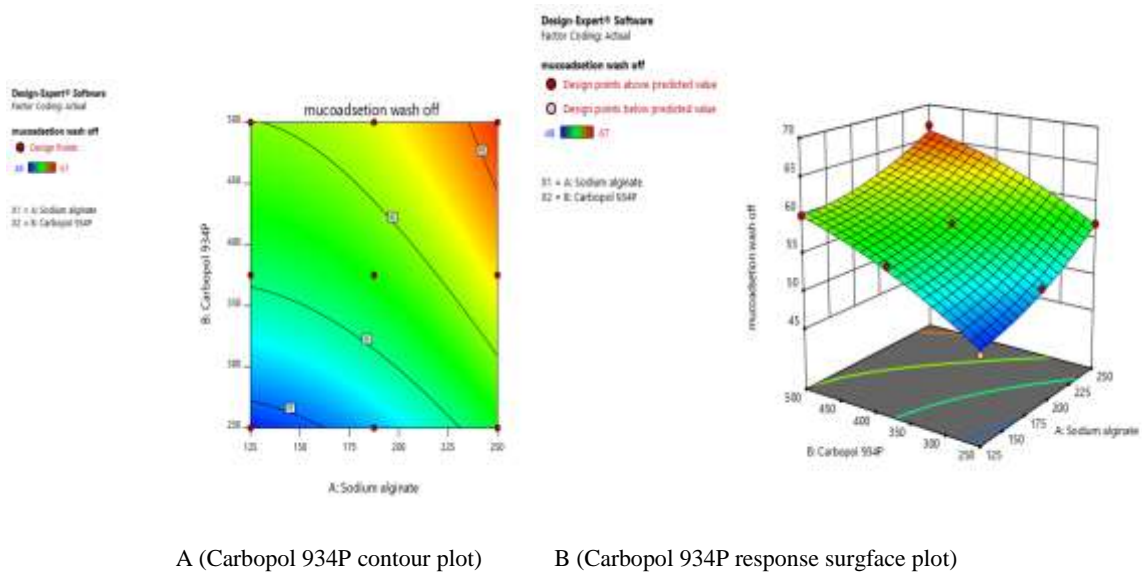


Figure 14. Amoxicillin A (Contour plot) and B (Response surface plot), showing the effect of independent variables on the mucoadhesion of mucoadhesive microcapsules.

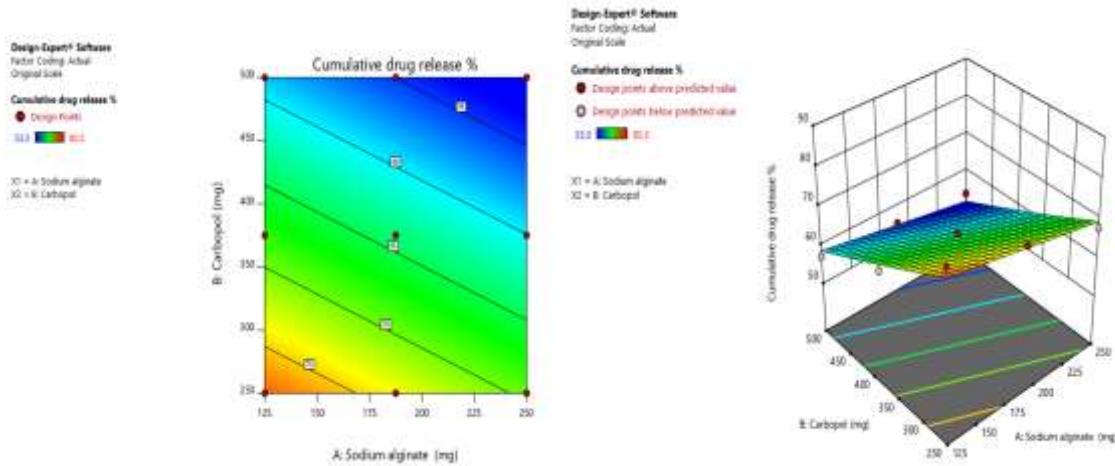


Figure 15. Amoxicillin A (Contour plot) and B (Response surface plot), showing the effect of independent variables on the drug release of mucoadhesive microcapsules.

drug entrapment efficiency, swelling index, mucoadhesion and drug release for amoxicillin mucoadhesive microcapsules carbopol 934P are shown in equation 1, 2,3,4 and 5. A positive co-efficient represents a synergistic effect, while a negative co-efficient indicates an antagonistic effect. Regression equations (1, 2, 3 and 4) with positive sign of X_1 (sodium alginate) and X_2 (carbopol 934P) illustrates the synergistic effect, and indicates that if polymer concentration increases; the value of particle size, entrapment efficiency, swelling index, and mucoadhesion are also increases. Negative effects of X_1^2 and X_2^2 suggest that as total amount of polymer increases, those values also increasing slowly. Amoxicillin mucoadhesive microcapsules equation showed that a X_2 had higher value of co- efficient than X_1 which indicates that X_2 has a great effect on Y.

$$Y = 831.39 + 12.63X_1 + 8.34X_2 - 4.74X_1^2 + 2.53X_2^2 - 3.23X_1 X_2 \dots\dots\dots (1)$$

$$Y = 91.44 + 3.15X_1 + 6.57X_2 - 0.4250X_1^2 - 4.01X_2^2 - 0.7800X_1 X_2 \dots\dots\dots (2)$$

$$Y = 103.71 + 10.82X_1 + 5.37X_2 + 0.3883X_1^2 + 1.81X_2^2 + 0.4225X_1 X_2 \dots\dots\dots (3)$$

$$Y = 57.78 + 3.67X_1 + 5.17X_2 + 1.33X_1^2 - 1.17X_2^2 - 0.5000X_1 X_2 \dots\dots\dots (4)$$

$$Y = 63.84 - 4.09X_1 - 9.43X_2 - 0.9100X_1^2 + 1.54X_2^2 + 2.27X_1 X_2 \dots\dots\dots (5)$$

3.9.2. ANOVA Analysis, 3D Response Surface, And Two- Dimensional Contour Plots For Amoxicillin Mucoadhesive Microcapsules

ANOVA analysis was used to determine the response of combination formulations and it is also used to identify that formulations is either significant or insignificant. On other hands, three-dimensional response surface plots were generated for every response to study the performance of the manner and also assisted the main and interaction effects of the independent variables (factors), as well as two-dimensional contour plot provides a visual representation of values of the response.

Table 5 shows that the P value for sodium alginate and carbopol 934P value is less than 0.05 which are statistically significant. Amoxicillin mucoadhesive microcapsules

were prepared using both polymers and model is observed significantly. The contour plots (Figure 11A to 14A) and response surface plots (Figure 11B to 14B) indicates that when the sodium alginate (X_1), carbopol 934P (X_2) concentration is gradually increased the particle size, entrapment efficiency, swelling index and mucoadhesion are also increased. The contour plots (Figure 15A to 15A) and response surface plots (Figure 15B to 15B) indicates that increased sodium alginate (X_1) and carbopol 934P (X_2) concentration result in decreased in drug release.

4. Conclusion

The present investigation has successfully demonstrated the feasibility of ion gelation technique for the preparation of amoxicillin mucoadhesive microencapsules. The prepared microcapsules were found to be a successful alternative for sustaining the drug delivery and prolonging the residence time in the stomach, which is an obligation for complete eradication of *H. pylori*. Carbopol 934P-sodium alginate mucoadhesive microcapsules encapsulated with amoxicillin drugs have shown a satisfactory *in-vitro* sustained drug release (53.50 ± 0.01 in 8 h) and mucoadhesive ability (67.00 ± 0.02) for prolonged residence time expected to effectively eradicate the *H. pylori* infection. Additionally, the microencapsulated forms of amoxicillin is also anticipated to have enhanced oral bioavailability, minimized harmful side effects and reduced dosing frequency which would be further helpful to improve patient compliance.

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