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FORMULATION AND *IN-VITRO* EVALUATION OF METRONIDAZOLE LOADED HPMC K15M MUCOADHESIVE MICROCAPSULES FOR *H. PYLORI* INFECTION USING 3^2- FULL FACTORIAL DESIGNS

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Keywords:

Metronidazole, Mucoadhesive drug delivery, Microencapsulation, Sodium alginate

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ABSTRACT: The purpose of the research was to develop and evaluate metronidazole loaded HPMC K15M mucoadhesive microcapsules for sustained drug release at the gastric mucosa. Metronidazole mucoadhesive microcapsules were formulated by ion gelation technique using 3^2 factorial designs. A 3^2 full factorial designs were used to derive a statistical equation, ANOVA analysis, contour plots, and 3D response surface plots. Different polymer ratios of HPMC K15M and sodium alginate were used to formulate nine formulations (F1 to F9) of HPMC K15M loaded mucoadhesive microcapsules of metronidazole. *In-vitro* drug release and mucoadhesion were carried out by USP29 type-II tablet dissolution test apparatus and disintegration tester using goat stomach mucosa. The formulation was characterized by determining possible drug-polymer interaction using FT- IR, the percentage of yield, particle size, the percentage of entrapment efficiency, swelling index, the percentage of mucoadhesion and percentage of drug release. FT-IR spectroscopy result shows the interaction between the drug and polymers combined. The optimized formulations F9 exhibited high drug entrapment efficiency of 92.07 ± 0.02%, particle size of 852.46 ± 0.04 (μm), percentage yield of 96.36 ± 0.04%, swelling index of 99.25 ± 0.02%, percentage of mucoadhesion after 8 h was 69.00 ± 0.04%, and the drug release (49.70 ± 0.01%) sustained more than 14 h. Metronidazole mucoadhesive microcapsules adhered more strongly to gastric mucous layer and could retain in the gastric mucosa for an extended period, followed by a non-Fickian type of release. The study shows that metronidazole mucoadhesive microcapsules can be effectively used for sustained drug release to the gastric mucosa in the treatment of *H. pylori* infection.

INTRODUCTION: Metronidazole [1-(2-hydroxyethyl)-2-methyl-5- nitroimidazole) is a broad spectrum antimicrobial agent. It is used in the eradication of *Helicobacter pylori* Infections which is responsible for developing gastritis, gastric ulcer and gastric carcinoma [1, 2]. Due to the short biological half-life (6-8 h), short gastric residence time and non-targeted drug release, and bitter taste which may lead to compliance issues [3-5].

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H. pylori are a motile pathogen which lives in the gastric mucus layer and penetrates deep in the mucous membrane close to the epithelial cells [6]. The eradication of *H. pylori* infection is the main troubles antibiotic resistance, patient's compliance and intolerance to therapeutic regimens [7, 8, 9]. The causes of resistance are poor drug penetration, low drug concentration; short gastric residence time and antibiotic resistance represent a significant health care burden on society. Besides, the poor stability of antibiotics in gastric content requires frequent administration and leads to patient noncompliance [10].

Mucoadhesive drug delivery systems (micro-capsules) have developed to raise the contact time or residence time with mucous layer and absorption tissue of the dosage forms thereby resulting

improving drug absorption, increase bioavailability and also work in sustained release of drugs which are influenced to reduce the gastric motility time and diminish peak plasma fluctuations [11, 12]. Dosage forms are designed by mucoadhesive polymers that drugs are achieved prolong retention time at the site of action, controlling and extending drug release over extended period time result in increasing bioavailability, improve patients compliance and better therapeutic effects [13, 14]. Hence, the mucoadhesive drug delivery systems can enhance the efficiency of the drug for *H. pylori* infection treatment.

Alginate is nature polymer which is obtained from marine brown algae. It exhibits mucoadhesion, biocompatibility, biodegradability, ability to form gels in the presence of ca^{2+} and more used in pharmaceutical preparations for controlled drug delivery system [15, 16, 17]. The medicinal use of sodium alginate which is achieved to sustained and controlled release drug delivery due to its hydrogel-forming properties [18]. Alginate mucoadhesive microcapsules are the re-swelling ability and drug release rate retardant a period of long time [19].

Microencapsulation is a method (ion gelation technique) by which an active ingredient is entrapped inside a miniature capsule. 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 to protect the active ingredient from the surrounding environment. These enclosed capsules, which range in size from a micrometer (diameter range of 1 to 1000 μm) to a millimeter, are referred to as microcapsules [20, 21].

Microencapsulation (microcapsules) are capable drug carrier particle, control the release rate or target the active drugs to a specific body absorption site for particulate drug delivery system, thereby it is enhanced drug absorption, reduced toxicity, superior patient compliance and convenience [22]. Therefore, the development of new controlled or sustained release of the drug delivery system is one of the most excellent fields of research in the pharmaceutical sciences which 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, stomach irritation, resulting in enhanced the therapeutic efficacy of an administered drug and reduced toxicity [23].

The ion gelation technique was used to prepare sustained release metronidazole loaded HPMC K15M mucoadhesive microcapsules. The influence of different formulation factors on the particle size, percentage yield, drug entrapment efficiency, swelling index, mucoadhesion, drug release mechanism, and *in-vitro* drug release was investigated. The present work was aimed at reducing the dosing frequency, improving oral bioavailability and sustained release an extended period of metronidazole mucoadhesive microcapsules for effective treatment of *H. pylori* infection.

MATERIALS AND METHODS:

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

Methods: Formulation of metronidazole mucoadhesive microcapsules was formulated by using ion gelation technique [24]. Sodium alginate-HPMC K15M as mucoadhesive polymers were dissolved in 10 ml purified water to form a homogeneous polymer solution. The metronidazole active ingredients were added to the polymeric solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion solution was added manually dropwise into 10% w/v calcium chloride solution (40 ml) 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.

3^2 Factorial Designs: A response surface method 3^2 factorial designs were 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 measured was percentage yield, % entrapment efficiency, particle size, swelling index, percentage mucoadhesion, and

drug release. The independent variables are the concentration of sodium alginate (X_1), and the concentration of polymer Carbopol 934P (X_2) was classified as low, medium and high, and their value was shown in **Table 2**. [24]

Various formulations of metronidazole-carbopol 934P mucoadhesive microcapsules were prepared individually by using all combinations of different levels of experimental variables as shown in **Table 1**.

TABLE 1: METRONIDAZOLE MUCOADHESIVE MICROCAPSULES BY CARBOPOL 934P WITH THEIR EXPERIMENTAL CODED LEVEL OF VARIABLES FOR 3^2 FACTORIAL DESIGNS

Formulation code	Variable Levels in Coded Form	
Metronidazole (250 mg)	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 METRONIDAZOLE MUCOADHESIVE MICROCAPSULES BY CARBOPOL 934P IN ACTUAL UNITS

Metronidazole (250 mg)		
X_1= Concentration of sodium alginate (% w/v)		
Low 125 mg (-1)	Medium187.5 mg (0)	High 250 mg (+1)
X_2= Concentration of HPMC K15M (%w/v)		
Low 250 mg (-1)	Medium 375 mg (0)	High 500 mg (+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 a potassium bromide pellet method. FT-IR of metronidazole and metronidazole with individual polymers was taken, weigh and mix homogenously with 300 mg of potassium bromide. After that, the mixture was compacted into a translucent film by using mechanical die press. It was recorded on Shimadzu's Fourier transform infrared spectrometer (Japan) with a frequency range of 4000-450 cm^{-1}. [25, 26]

Particle Size Measurement: Metronidazole mucoadhesive microcapsules of particle size were evaluated by using optical microscopy method. The amount was done under 10 × 45 (10x eyepiece and 45x objective) and 100 mucoadhesive micro-capsules 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 [27].

Percentage Yield: Percentage yield of metronidazole 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 about the sum of starting material. The percentage yield of prepared mucoadhesive microcapsules was determined by using the formula, respectively [28, 29].

Percentage yield = Weight of mucoadhesive microcapsule × 100 / Theoretical weight of polymer and drug

Drug Entrapment Efficiency: 100 mg of metronidazole mucoadhesive microcapsule was crushed individually in a glass mortar and pestle, and the powdered microcapsule was suspended in 10 ml of phosphate buffer solution (pH 7.4), respectively. After 24 h, the solution filtered and the filtrate was analyzed for the drug entrapment efficiency after it was calculated using the following formula [24].

Practical drug content / Theoretical drug content × 100

Swelling Index: The metronidazole mucoadhesive microcapsule (100 mg) was placed separately, in a glass vial containing 10 ml of 0.1N HCl at 37 ± 0.5 °C in an incubator with occasional shaking. The swelled metronidazole mucoadhesive micro-capsules were removed a predetermined time interval and weighed after drying the surface by using tissue paper. The weight of the swollen microcapsules was recorded after a period of 8 h, and swelling ratio was calculated using the following formula.

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

Whereas, W_t = Equilibrium weight of microcapsules after swelling and W_o = Initial weight of microcapsules [30, 31].

Mucoadhesion Testing by *in-vitro* Wash-Off Test: The Mucoadhesive property of the metronidazole mucoadhesive microcapsule was evaluated by an *in-vitro* wash-off test using goat stomach mucosa. A piece of goat stomach mucosa (2 cm × 2 cm) was collected and tied onto a glass slide (7.5 cm × 2.5 cm) using thread. 100 metronidazole 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) separately at 37 ± 0.5 °C. At the end of the time interval, the number of mucoadhesive micro-capsules that remained attached to the tissue was recorded [32, 33].

The following formula determined the mucoadhesion adhesion number

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

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.

***In-vitro* Dissolution Studies:** Dissolution studies of metronidazole mucoadhesive microcapsule, equivalent to 250 mg of metronidazole individually was carried out by USP dissolution test apparatus (Electrolab India) at 50 rpm and 37 ± 0.5°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 dissolution medium. Samples, filtered through Whatman filter paper (0.45 μm), was analyzed spectrophotometrically at 277 nm. Drug release data obtained during in vitro dissolution studies were analyzed for release kinetics using zero order, first order, and Higuchi model equations and fitted into Korsmeyer-Peppas model for evaluation of release mechanism from mucoadhesive microcapsules [34, 35].

Drug Release Kinetic Profile: To study the drug release kinetics and mechanism of metronidazole mucoadhesive microcapsule, 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 [36].

Zero-Order: 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 clearance of the drug. The release rate of the drug is dependent on concentration.

$$LogC_t = LogCo - kt / 2.303$$

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

$$Log (M_t / M\infty) = Log\ k + n\ Logt$$

Where M_t / $M\infty$ = 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.

3^2 Full Factorial Design Studies: A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0+b_1X_1+b_2X_{2+}b_{12}X_1X_{2+}b_{11}X_1^{2}+b_{22}X_2^{2}$$

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 [24, 36].

Statistical Analysis: Quantitative results were expressed as mean ± 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 fitted to quadratic model using Design-Expert® Software Version 11.0.0.

RESULT AND DISCUSSION:

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 metronidazole, sodium alginate, and carbopol 934P were shown in **Fig. 1 - 3**. Furthermore, the spectrum of carbopol 934P-sodium alginate muco-adhesive microcapsules containing metronidazole was shown in **Fig. 4**. It was recorded on Shimadzu's Fourier transform infrared spectrometer (Japan) with a frequency range of 4000-450 cm^{-1}.

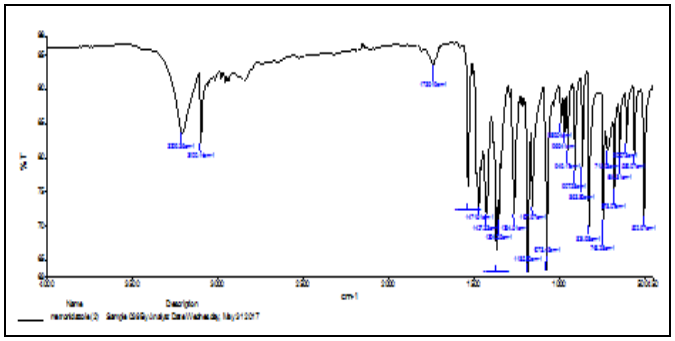


FIG. 1: FTIR SPECTRUM OF METRONIDAZOLE

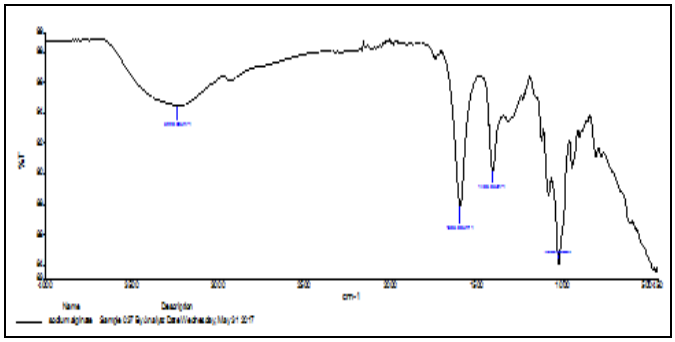


FIG. 2: FT-IR SPECTRUM OF SODIUM ALGINATE

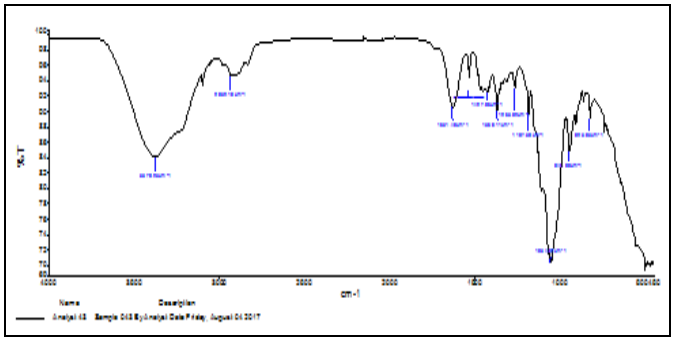


FIG. 3: FT-IR SPECTRUM OF HPMC K15M

The sample of pure metronidazole showed feature vibrations peaks for O-H, C-H and C=O stretching frequency at 3209.36 cm^{-1}, 3100.14 cm^{-1}, and 1739.19 cm^{-1}, respectively. The peaks at around 1471.91 cm^{-1} and 1354 cm^{-1} were attributed to symmetric and asymmetric stretching N=O, respectively. The band peaks at 1427.33 cm^{-1}, 1264.51-1185.80 cm^{-1}, and 1073.40 cm^{-1} were assigned to C-C stretching, C-O stretching, and C-N stretching, respectively. The FTIR spectrum of sodium alginate showed peaks at about 3228.00 cm^{-1}, 1595.00 cm^{-1}, 1406.95 cm^{-1}, and 1024.32 cm^{-}

[1] that were indicating of O-H stretching vibrations, COO- stretching vibrations, -CH stretching vibrations and C-O-C stretching vibrations, respectively. The vibration peaks of polymer HPMC K15M at 3370.25 cm^{-1} and 2932.10 cm^{-1}, which were due to O-H stretching and C-H stretching, respectively, as well as the presence of peaks at around 1427.96 cm^{-1}, 1369.11 cm^{-1}, 1187.38 cm^{-1}, 1054.91 cm^{-1} and 946.99 cm^{-1} were assured to –CH2 symmetric bending vibrations, -CH3 asymmetric bending vibrations, C-O stretching vibrations, C-O-C stretching vibrations and pyranose ring, respectively.

Finally, the FT-IR spectrum for HPMC K15M-sodium alginate mucoadhesive microcapsules containing metronidazole showed different absorption characteristics of peaks of metronidazole, sodium alginate and HPMC K15M were found that the almost same primary peaks were also present in the drug-polymer combinations, indicating there is no interaction between polymer and drug used as shown in **Fig. 4**.

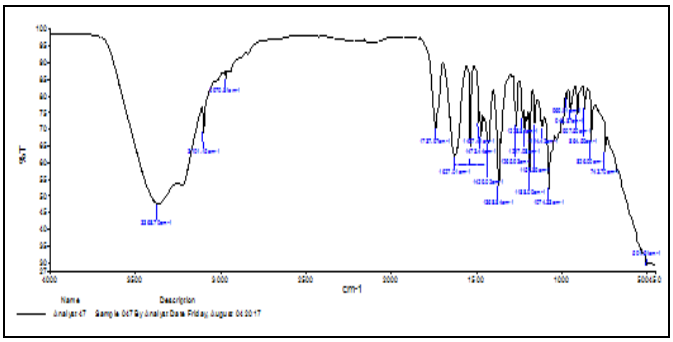


FIG. 4: FT-IR SPECTRUM OF HPMC K15M-BASED MUCOADHESIVE MICROCAPSULES CONTAINING METRONIDAZOLE

Particle Size Measurement of Metronidazole Mucoadhesive Microcapsules: The particle size within the range of mucoadhesive microcapsules of metronidazole was found to be 760.08 ± 0.04 (µm) to 852.46 ± 0.04 (µm), respectively. Researchers have suggested that as polymer concentration increased, the particle size also improved, which could be due to enhancing in the viscosity of drug and polymer ratio, and coat thickness of polymer [38, 39]. The present study indicated that the higher concentration of sodium alginate and HPMC K15M solution form large droplets with increased particle size than those of lower concentration polymers result in small droplets and diminish particle size due to the difference of viscosity, as shown in **Table 3**. Lower polymer concentration resulted in decrease in inner phase viscosity, which might efficiently promote the break-up of coacervate droplets and prevent coalescence. The smallest particle size was produced when sodium alginate, carbopol 934P was used at a low-level concentration. The highest particle size was achieved when polymer concentration was acquired higher level. Increased in sodium alginate concentration resulted in increased in particle size and this observation is found to be in the line of previous research reported [40].

Percentage Yield of Metronidazole Muco-adhesive Microcapsules: The Percentage yield of metronidazole mucoadhesive microcapsules were found to be within the range of 92.48 ± 0.04% to 93.16 ± 0.02% for metronidazole-HPMC K15M mucoadhesive microcapsules, respectively. The present study found that the percentage yield was increased when the polymer ratio was increased as well. The studies have proved that the percentage yield of mucoadhesive microcapsules was improved within increasing the concentration of sodium alginate also [41]. Other studies have reported that the percentage yield decreased, with an increase in sodium alginate due to the high viscosity of the drug-polymer solution, needle blockage wastage of the drug-polymer solution, loss transferring and washing [42].

Drug Entrapment Efficiency of Metronidazole Mucoadhesive Microcapsules: The entrapment efficiency is a vital parameter that assists in the identification of drug efficacy, and it depends on various concentrations of mucoadhesive polymers such as sodium alginate and HPMC K15M. The average efficiency ranges for metronidazole-HPMC K15M mucoadhesive microcapsules was 85.16 ± 0.01% to 92.07 ± 0.02% respectively, as shown in **Table 3**. Present work found that some formulation of metronidazole mucoadhesive microcapsules has lower entrapment efficiency due to decrease number of binding sites of alginate for Ca^{2+} ions consequently formulations are less compact gel membrane which, in turn, the superior influx of Ca^{2+} ions leading to decrease in drug entrapment efficiency and also lower polymer concentration. Metronidazole mucoadhesive microcapsules have highest entrapment efficiency due to increase polymer concentration especially with higher

sodium alginate concentration which provides increase number of binding sites of sodium alginate with calcium chloride. The result shows that the entrapment efficiency of mucoadhesive micro-capsules was increased with increased polymer concentration; the result was similar to previous studies [43, 44, 45].

Swelling Index of Metronidazole Mucoadhesive Microcapsules: The swelling index of metronidazole- HPMC K15M mucoadhesive microcapsules was found to be a range of 85.88 ± 0.03% to 99.25 ± 0.02%. The swelling index of all the formulations was reported to be improved with the increased concentration of polymers as shown in **Table 3**. The result shows that maximum swelling index was achieved in increasing polymer concentration; which was similar to the study as reported previous literature [46].

Mucoadhesion Testing by *in-vitro* Wash-off Test: The study of *in-vitro* bioadhesion demonstrated that metronidazole-HPMC K15M mucoadhesive microcapsules had good bioadhesive property ranging of 49 ± 0.01% to 69.00 ± 0.04% respectively, as shown in **Table 3**. The present study was carried out for a higher level of polymer concentration by factorial metronidazole muco-adhesive microcapsules formulations F9 was excellent mucoadhesion and strongly adhered to the gastric mucous layer. The results were observed that if the drug and polymer concentration was improved; the percentage of mucoadhesion was also increased, as shown in **Fig. 5**. It was observed that mucoadhesion of metronidazole mucoadhesive microcapsules significantly increased with increasing polymer concentration due to increase in viscosity and produced stronger mucus gel network which helps to enhance mucoadhesion.

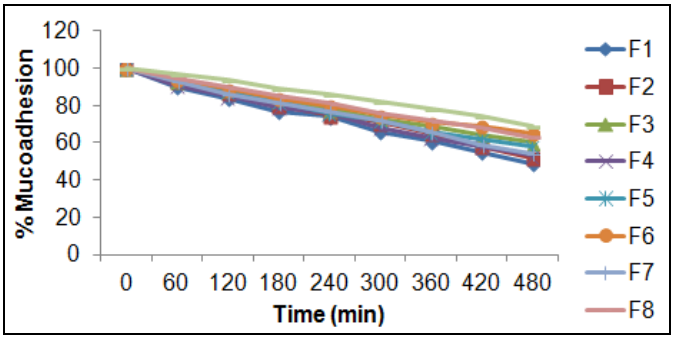


FIG. 5: MUCOADHESION OF METRONIDAZOLE-HPMC K15M MUCOADHESIVE MICROCAPSULES

TABLE 3: PARTICLE SIZE, PERCENTAGE YIELD, DRUG ENTRAPMENT EFFICIENCY, SWELLING INDEX, PERCENTAGE MUCOADHESION, DRUG RELEASE OF METRONIDAZOLE MUCOADHESIVE MICROCAPSULES

Formulation code	Particle size (µm)	Percentage yield (%)	Entrapment efficiency (%)
F1	760.08±0.04	92.48±0.04	85.16±0.01
F2	814.26±0.01	95.25±0.01	87.25±0,04
F3	841.71±0,03	96.04±0,02	90.12±0.05
F4	820.14±0.05	88.51±0.08	88.18±0,02
F5	828.89±0.07	87.67±0.06	86.07±0.04
F6	840.08±0.08	85.860.03	91.10±0.01
F7	838.25±0.02	91.67±0.05	88.23±0.02
F8	835.09±0.02	96.36±0.04	90.15±0.08
F9	852.46±0.04	93.16±0.02	92.07±0.02
Formulation code	**Swelling Index %**	**% Mucoadhesion**	**% Cumulative drug release**
F1	85.88±0.03	49.00±0.01	78.70±0.02
F2	92.70±0.04	52.00±0.03	67.30±0.04
F3	94.52±0.01	60.00±0.04	55.80±0.01
F4	90.15±0.04	53.00±0.02	72.50±0.02
F5	93.70±0.02	58.00±0.06	64.50±0.01
F6	96.06±0.04	65.00±0.02	52.60±0.02
F7	95.23±0.02	54.00±0.02	66.60±0.04
F8	97.15±0.01	63.00±0.03	58.40±0.02
F9	99.25±0.02	69.00±0.04	49.70±0.01

***In-vitro* Dissolution Studies of Metronidazole Mucoadhesive Microcapsules:** The present study showed that metronidazole mucoadhesive microcapsules in most of the formulations were negligible amounts of drug release in simulated gastric fluid (0.1N HCl, pH 1.2); whereas for those formulations were increased amount of drug release in simulated intestinal fluid (pH 7.4), as shown in **Fig. 6**.

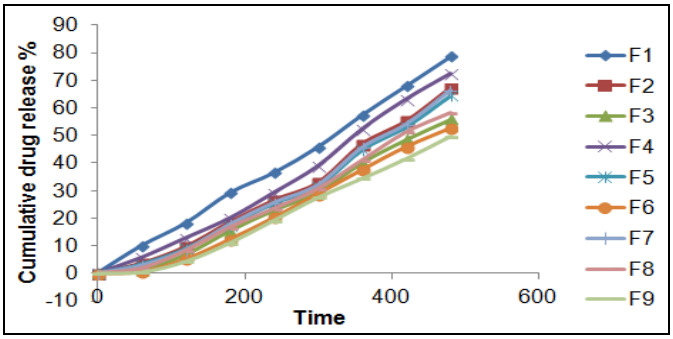


FIG. 6: METRONIDAZOLE - HPMC K15M MUCO-ADHESIVE MICROCAPSULES DRUG RELEASE

It was found that the percentage of cumulative drug release (CDR %) in the range of 78.71 ± 0.02% to 49.70 ± 0.01%, respectively. It was observed that metronidazole- HPMC K15M mucoadhesive microcapsules formulation F9 has slower drug release rates due to higher polymer concentration. The results showed that the drug release was decreased when the polymer concentration was improved attributed to high viscosity of polymer

and drug solution The result was observed that the mucoadhesive microcapsules was slow and spread over an extended period of time when sodium alginate concentration was increased that was similarly reported by previous studies [47, 48, 49].

Drug Release Kinetic Profile: Metronidazole-HPMC K15M mucoadhesive microcapsules F9 was selected as the most potential for its drug release kinetics model like zero order, first order, Higuchi and Korsmeyer-Peppas models. The R^2 of these models were determined and compared. The result of the curve fitting into various mathematical models was shown in **Table 4** and **Fig. 7 - 10**.

The suitability of the model has been observed by best fit to the model using the correlation co-efficient value (R^2).

The data obtained from analysis of drug release kinetics were shown in **Table 4**. The zero order, first order, Higuchi and Korsmeyer-Peppas models were shown in **Fig. 7, 8, 9** and **10**. From the results shown in **Table 4**, it can be observed that the release kinetics of metronidazole mucoadhesive microcapsules from the different formulations showed good fitting with zero order, first order and Higuchi model with R^2 values 0.977, 0.955 and 0.814 respectively. On the other hand, the model with the highest correlation coefficients (R^2) was given by zero order. The n value of Peppas model (0.68) indicates that the mechanism of drug release followed by non-Fickian diffusion. This suggests that drug release occurs mainly by diffusion through polymer matrix from a region of high concentration to lower concentration.

TABLE 4*: IN-VITRO* RELEASE KINETIC MODELS OF METRONIDAZOLE-HPMC K15M MICROCAPSULES

	Zero-order		First order		Higuchi		Korsmeyer-Peppas model		Mechanism of drug release
F9	R^2	K_0	R^2	K_1	R^2	K ($min^{1/2}$)	R^2	n	Non-Fickian
	0.977	0.110	0.955	0.00	0.814	2.388	0.555	0.680	release

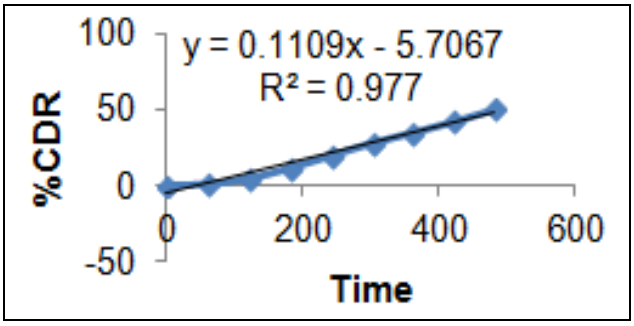


FIG. 7: ZERO ORDER RELEASE OF FORMULATION F9

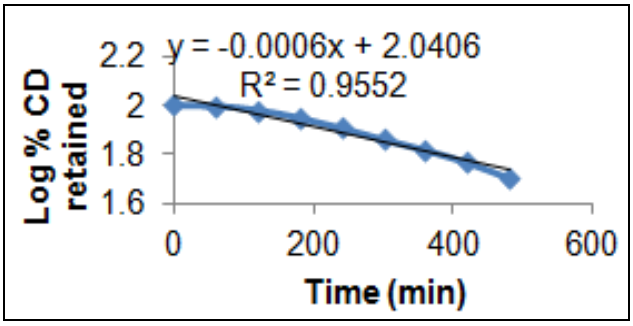


FIG. 8: FIRST ORDER RELEASE OF FORMULATION F9

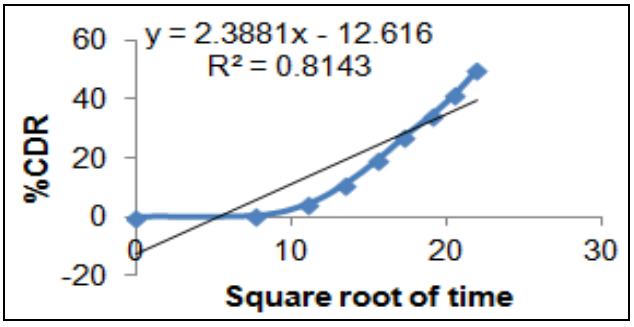


FIG. 9: HIGUCHI MODEL OF FORMULATION

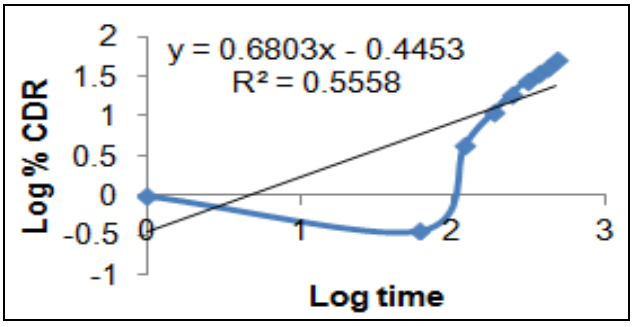


FIG. 10: KORSMEYER-PEPPAS RELEASE OF F9 FORMULATION F9

Statistical Analysis: Metronidazole mucoadhesive microcapsules were used to derive a statistical equation, ANOVA analysis, contour plots, and 3D response surface plots. Statistical analysis was analyzed according to **Table 3**.

Factorial Equation: The result of equation Y that are indicated 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 (Equation 1), drug entrapment efficiency (Equation 2), swelling index (Equation 3), mucoadhesion (Equation 4), and drug release (Equation 5) in metronidazole mucoadhesive microcapsules HPMC K15M were shown in Equation 1, 2, 3, 4 and 5. A positive coefficient represents a synergistic effect, while a negative coefficient indicates an antagonistic effect. Metronidazole mucoadhesive microcapsules regression equation (1, 2, 3, 4 and 5) showed that positive sign X_1 (sodium alginate) and X_2 (HPMC K15M) illustrates synergistic effect, and indicates that if polymer concentration increases; the value of depended variables (particle size, entrapment efficiency, swelling index, mucoadhesion, and drug release) is also increases. Negative effects of X_1^2 and X_2^2 suggest that as the total amount of polymer increases, all depended on variables increases slowly. Positive effects of X_1^2 and X_2^2 suggest that as the total amount of polymer increases, all depended variables increases significantly.

Two independent variables of sodium alginate X_1 had a lower value of co-efficient than HPMC K15M X_2 co-efficient value for metronidazole muco-adhesive microcapsules which indicated that X_2 had a prominent effect on Y. Contrary, sodium alginate X_1 had a higher value of co-efficient than carbopol 934P X_2 coefficient value for metronidazole mucoadhesive microcapsules which implies that X_1 showed much-pronounced effect Y.

$$Y= 830.12+ 18.29X_1 + 19.30X_2 - 6.06X_1^2 - 6.06X_1^2 - 16.85X_1 X_2 \quad \text{…….(1)}$$

$$Y= 87.57+ 1.32X_1+ 1.95X_2 + 0.3800X1^2 + 1.32X_2^2 - 0.2800X_1 X_2 \quad \text{…….. (2)}$$

$$Y= 93.97+ 3.09X_1 + 3.10X_2 + 0.8183X_1^2 -1.00X_2^2 - 1.16X_1 X_2 \quad \text{…… (3)}$$

$$Y= = 58.22 + 4.17X_1 + 6.33X_2 - 0.8333X_1^2 + 0.6667X_2^2 + 1.0000X_1 X_2 \quad \text{…….. (4)}$$

$$Y= 63.76 - 4.50X_1 - 10.03 X_2 - 0.5333X_1^2 - 0.8333X_2^2 + 1.53X_1 X_2 \quad \text{……. (5)}$$

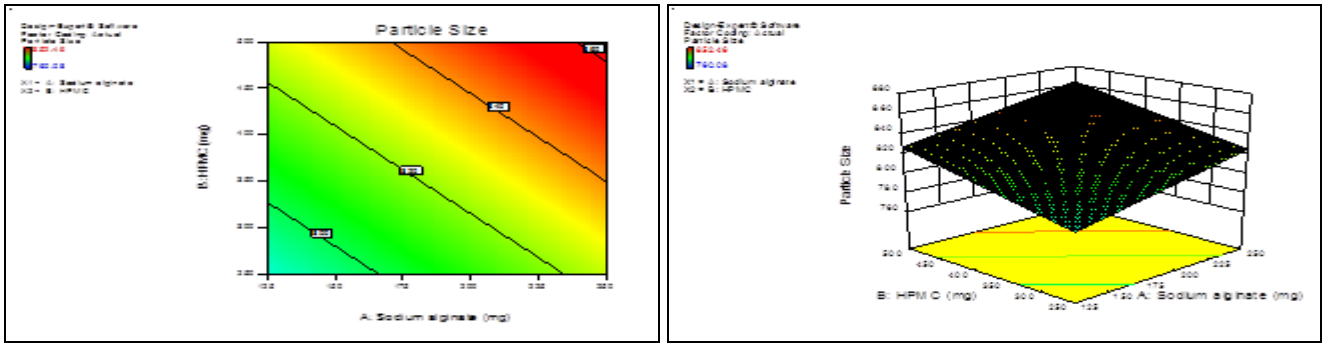
Factorial Design of ANOVA Analysis, 3D Response Surface and Two- Dimensional Contour Plots for Metronidazole Mucoadhesive Microcapsules: ANOVA analysis was used to response combination formulations and it is also used to identify the formulations significant or insignificant. On other hands, three-dimensional 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 is seen that sodium alginate and HPMC K15M value less than 0.0500 which are achieved statistically significant. Metronidazole muco-adhesive microcapsules were prepared using both polymers and models were observed significantly. The contour plot **Fig. 11A - 14A** and response surface plot **Fig. 11B - 14 B** indicates that when the sodium alginate (X_1) and HPMC K15M (X_2) concentration is gradually increased, the all depended variables (without drug release) is gradually improved, as well as sodium alginate (X_1) and HPMC K15M (X_2) concentration is enhances, drug release **Fig. 15A** and **15B** is also decreases.

TABLE 5: ANOVA ANALYSIS VARIANCE FOR PARTICLE SIZE, DRUG ENTRAPMENT EFFICIENCY, SWELLING INDEX, MUCOADHESION AND DRUG RELEASE OF METRONIDAZOLE MUCOADHESIVE MICROCAPSULES

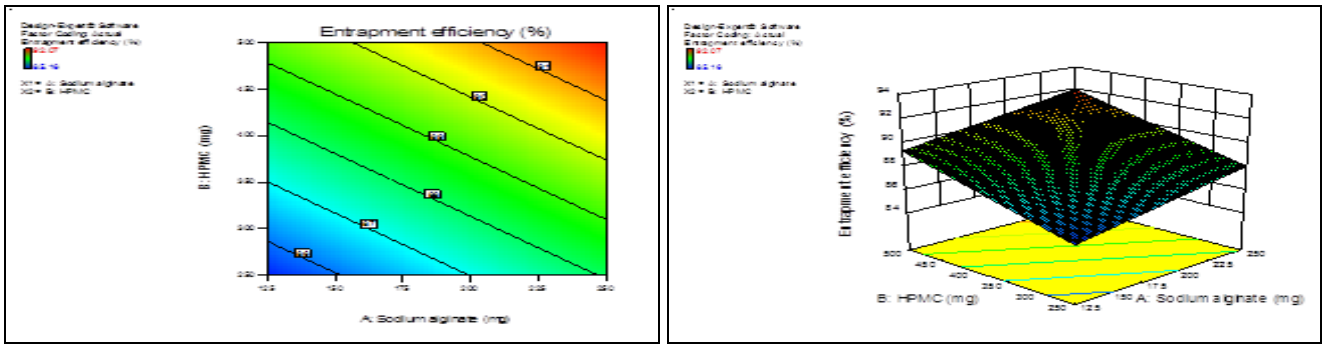
The particle size of metronidazole-HPMC K15M mucoadhesive microcapsules						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	***p-Value* Prob > F**	**Significant/ Insignificant**
Model	4241.68	2	2120.84	7.66	0.0223	Significant
A-Sodium alginate	2007.51	1	2007.51	7.25	0.0359	Significant
B-HPMC K15M	2234.17	1	2234.17	8.07	0.0295	Significant
Entrapment efficiency of metronidazole-HPMC K15M mucoadhesive microcapsules						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	***p-Value* Prob > F**	**Significant/ Insignificant**
Model	33.35	2	16.67	10.07	0.0121	Significant
A-Sodium alginate	10.45	1	10.45	6.32	0.0457	Significant
B- HPMC K15M	22.89	1	22.89	13.83	0.0099	Significant
Swelling index of metronidazole-HPMC K15M mucoadhesive microcapsules						

Source	Sum of squares	df	Mean square	F value	*p-Value* Prob > F	Significant/ Insignificant
Model	104.90	2	52.45	15.03	0.0046	Significant
A-Sodium alginate	47.43	1	47.43	13.59	0.0103	Significant
B-HPMC K15M	57.47	1	57.47	16.47	0.0067	Significant
Mucoadhesion of metronidazole-HPMC K15M mucoadhesive microcapsules						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	***p-Value* Prob > F**	**Significant/ Insignificant**
Model	351.11	5	70.22	36.46	0.0069	Significant
A-Sodium alginate	104.17	1	104.17	54.09	0.0052	Significant
B- HPMC K15M	240.67	1	240.67	124.96	0.0015	Significant
Drug release of metronidazole-HPMC K15M mucoadhesive microcapsules						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	***p-Value* Prob > F**	**Significant/ Insignificant**
Model	2.85	2	1.43	217.00	< 0.0001	Significant
A-Sodium alginate	0.4773	1	0.4773	72.63	0.0001	Significant
B- HPMC K15M	2.37	1	2.37	361.38	< 0.0001	Significant



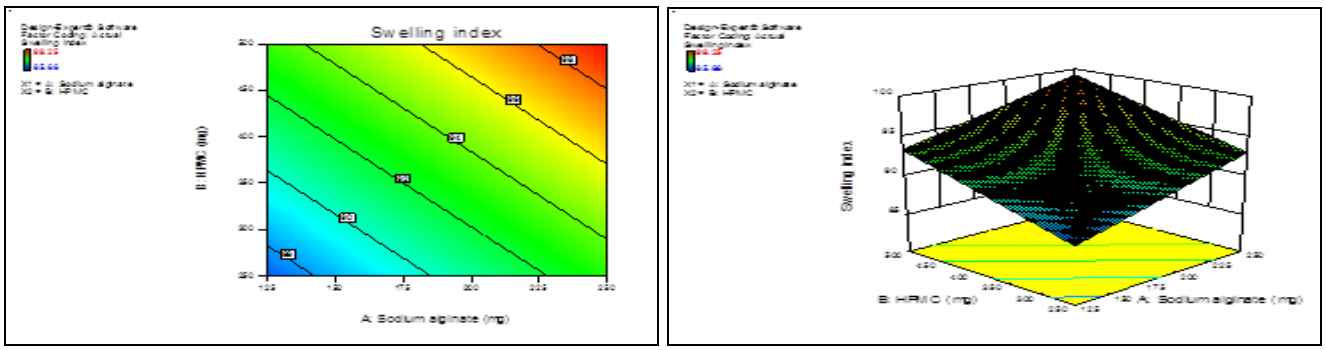
An (HPMC K15M contour plot) **B (HPMC K15M Response plot)**

FIG. 11: METRONIDAZOLE A (CONTOUR PLOT) AND B (RESPONSE PLOT), SHOWING THE EFFECT OF INDEPENDENT VARIABLES ON THE PARTICLE SIZE OF MUCOADHESIVE MICROCAPSULES



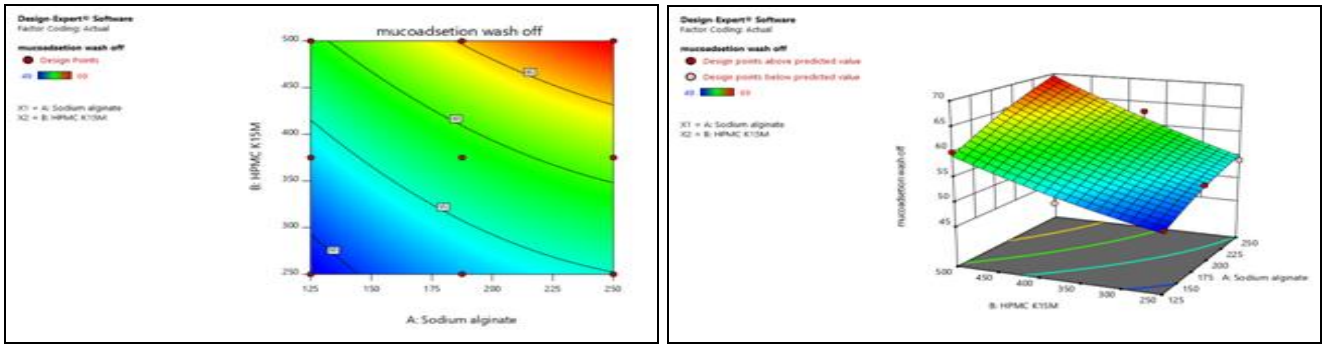
An (HPMC K15M contour plot) **B (HPMC K15M Response plot)**

FIG. 12: METRONIDAZOLE A (CONTOUR PLOT) AND B (RESPONSE PLOT), SHOWING THE EFFECT OF INDEPENDENT VARIABLES ON THE ENTRAPMENT EFFICIENCY OF MUCOADHESIVE MICROCAPSULES



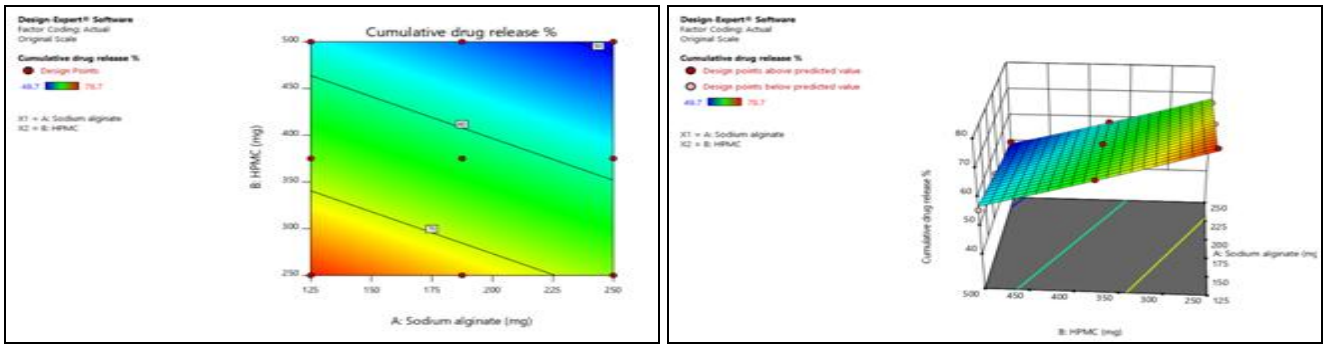
An (HPMC K15M contour plot) **B (HPMC K15M Response plot)**

FIG. 13: METRONIDAZOLE A (CONTOUR PLOT) AND B (RESPONSE PLOT), SHOWING THE EFFECT OF INDEPENDENT VARIABLES ON THE SWELLING INDEX OF MUCOADHESIVE MICROCAPSULES



An (HPMC K15M contour plot) **B (HPMC K15M Response plot)**

FIG. 14: METRONIDAZOLE A (CONTOUR PLOT) AND B (RESPONSE PLOT), SHOWING THE EFFECT OF INDEPENDENT VARIABLES ON THE MUCOADHESION OF MUCOADHESIVE MICROCAPSULES



A (HPMC K15M contour plot) **B (HPMC K15M Response plot)**

FIG. 15: METRONIDAZOLE A (CONTOUR PLOT) AND B (RESPONSE PLOT), SHOWING THE EFFECT OF INDEPENDENT VARIABLES ON THE DRUG RELEASE OF MUCOADHESIVE MICROCAPSULES

CONCLUSION: The observations made during study and results obtained showed the suitability of the investigated polymers for microencapsulation of metronidazole for its sustained release. The ionic gelation method was easy to adopt and also to achieve high drug entrapment efficacy. The result observed that metronidazole mucoadhesive microcapsules of entrapment efficiency, percentage release, particle size, and drug release behavior varies with increased drug-polymer concentration. Additionally, the microencapsulated forms of metronidazole are also anticipated to have enhanced oral bioavailability, minimized harmful side effects and reduced dosing frequency which would be further helpful to improve patient compliance.

The *in-vitro* drug release studies demonstrated that the drug release was sustained about 14 h and non-fickian controlled release mechanism of metronidazole mucoadhesive microcapsules. The results of 3^2 factorial designs revealed that drug and polymer concentration significantly affected dependent variables entrapment efficiency, percentage release, particle size swelling index, mucoadhesion, and drug release.

The metronidazole mucoadhesive microcapsules of the best formulation F9 exhibited high entrapment efficiency, the percentage of mucoadhesion and sustain in gastric mucosa. Therefore, one can assume that metronidazole mucoadhesive micro-capsules are promising pharmaceutical forms by providing controlled-release drug delivery systems.

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