Exploring *Aloe Vera* Leaves Mucilage in Clarithromycin Mucoadhesive Microspheres: Investigating Particle Size and Swelling Index through Design Expert Software

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ABSTRACT

The research aimed to investigate the mucoadhesive properties of Aloe vera leaf mucilage (AVLM) when combined with Clarithromycin (CMN) to create mucoadhesive microspheres. The study involved the preparation of nine batches of CMN mucoadhesive microspheres using carbopol 934P (C-934P) and varying amounts of AVLM. To analyze the effects of AVLM and C-934P levels on particle size (PS) and swelling index (SI) as response variables, a central composite design was employed with design expert software. The results indicated that the PS of the microspheres ranged from 35.2±0.3 to 48.1±0.6µm, with batch B-1 having the smallest PS and B-8 showing the largest size. The PS was determined using the formula: $+49.37+0.3500A+1.73B-0.8750AB-0.2500A^2-8.10B^2$, where A represents the AVLM level and B represents the C-934P level. On the other hand, the SI of the microspheres varied from 56.8 to 61 and increased with higher polymer content. The formula for the SI was: $+59.10+0.2500A+1.90B-0.1500AB+0.2500A^2-0.3000B^2$. The study found that AVLM levels significantly influenced the PS and SI of the microspheres. Moreover, the researchers observed a controlled release of CMN from the microspheres, with satisfactory entrapment efficacy, mucoadhesion, and drug contents, meeting various constraints. Additionally, the microspheres demonstrated potential for targeted drug delivery to the stomach due to C-934P, and the presence of AVLM further enhanced this effect. Scanning electron microscopy images confirmed that the microspheres had a spherical shape with a relatively smooth surface. Overall, the study established the potential of AVLM-based mucoadhesive microspheres for controlled drug delivery, with promising results using CMN as a model drug.

Keywords: Clarithromycin, *Aloe vera* leave *mucilage,* Microspheres, Mucoadhesive, Particle size.

INTRODUCTION

The study's primary focus is to explore innovative methods of enhancing the gastric availability of drugs with patient consent. The researchers aim to develop gastro retentive microspheres, a convenient and easily administered dosage form, to improve the delivery of Clarithromycin (CMN), a broad-spectrum antibiotic. CMN is commonly used in standard eradication treatment for *H. pylori* infection, often combined with other antibiotics and acid-suppressing agents[1].

For effective mucoadhesive systems, the choice of polymer plays a crucial role. Oral drug administration is preferred by many patients due to its convenience, and various polymers have been investigated for mucoadhesive applications, although some of them are rare and expensive. In this study, the researchers have identified Aloe vera leaf mucilage (AVLM) as a new natural polymer with potential mucoadhesive properties. AVLM has also been found to possess antiviral properties, suggesting its potential use in antiviral therapy. By incorporating AVLM into mucoadhesive microspheres of CMN, the researchers aim to achieve a sustained systemic availability of the drug over an extended period[2].

To conduct their experiments, the researchers utilized a Design of Experiment (DOE) approach, specifically employing a Factorial Design (FD) using the Design Expert software version 11.0 trial version from Stat-Ease corporations. This approach allows them to study multiple variables simultaneously and assess their impact on the response (i.e., the properties of the mucoadhesive microspheres). The FD explores all possible combinations of the factors (independent variables) by setting them at two levels: 'high' (+1) and 'low' $(-1)^3$.
Overall, the authors plan to investigate the mucoadhesive properties of AVLM and explore its potential in antiviral therapy. They intend to develop mucoadhesive microspheres of CMN with AVLM to achieve prolonged drug release and steady systemic availability, making it a convenient and effective drug delivery system for short-acting drugs that require continuous medication[4].

MATERIALS AND METHODS

Materials

Clarithromycin was obtained from Cipla Ltd in Bangalore, while Carbopol 934P and dichloromethane were procured from Merck, also in Bangalore. These details are essential for the reproducibility and traceability of the research findings, as the quality and origin of the materials used can influence the experimental outcomes.

Methods

Extraction of mucilage

Aloe vera leaves are known for containing a water storage tissue that mainly consists of viscous mucilage found in the parenchyma cells. The entire *Aloe vera* leaf, including the rind, vascular bundles, and pulp, is ground to obtain a homogenous mixture. The ground mixture is then filtered using cloth filter paper. This step helps remove undissolved and foreign particles, resulting in a clear liquid extract. The filtered extract is then dried in an oven at a temperature of 40°C. This drying process helps remove excess moisture from the extract. The dried extract is ground into a fine powder to facilitate further processing and handling. The powdered extract is passed through a #80 sieve (Remi), which is a fine mesh sieve, to ensure uniform PS and remove any larger particles. The sieved and dried *Aloe vera* whole leaf extract is stored in a desiccator at a controlled temperature of 30°C and a relative humidity of 45% until it is ready for use[5].

Experimental design

The researchers used Stat-Ease Software version 11.0.5.0 from Stat-Ease Inc. to design and analyze their experiments. They employed a quadratic response surface methodology to optimize the AVLM using 9 runs, with the help of a central composite design (CCD). The purpose of this approach was to determine the key, boundary, and quadratic effects of independent variables on the dependent variables. The general form of the quadratic model used is as $Y = B0 + B_1X_1 + B_2X_2 + B_{12}X_1X_2 + B_1X_1^2 + B_2X_2^2$. Y represents the dependent variable (response). X_1 and X_2 represent the independent variables (factors). B_0, B_1, B_2, B_{12}, $B_1X_1^2$, and $B_2X_2^2$ are regression coefficients. The researchers focused on two dependent variables/responses related to AVLM, which were PS (Y_1) – PS and SI (Y_2) - SI. They conducted a total of 9 experimental runs to study the variables and their respective levels used in the screening of AVLM. The table contains information about the ingredients used in various AVLM formulations, which were studied in the experiments. It might provide details about the composition and proportion of different components present in each AVLM sample used in the study[6].

Statistical analysis and experimental design were employed to optimize the properties of AVLM and investigate its potential as a mucoadhesive material for drug delivery applications, as discussed in the earlier provided information.

Table 1. Composition of the CAMM

	Formulations								
Components	**B-1**	**B-2**	**B-3**	**B-4**	**B-5**	**B-6**	**B-7**	**B-8**	**B-9**
Clarithromycin (mg)	500	500	500	500	500	500	500	500	500
Ethyl Cellulose (mg)	40	40	40	40	40	40	40	40	40
AVLM (mg)	50	50	50	75	75	75	100	100	100
Carbopol 934P (mg)	50	75	100	50	75	100	50	75	100
Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
Span 80 (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glutaraldehyde (ml)	1	1	1	1	1	1	1	1	1
Liquid paraffin (ml)	150	150	150	150	150	150	150	150	150

***Preparation of* microspheres**

Dichloromethane was used as a solvent to dissolve Carbopol 934P (C-934P), CMN, ethyl cellulose (EC), and AVLM. These components were mixed together in the solvent. The mixture of dissolved ingredients was continuously stirred using an IKA-R1385 three-bladed propeller stirrer at a speed of 600 rpm. This stirring helped in achieving proper dispersion and uniformity of the components. Liquid paraffin containing span 80 was used as the continuous phase in which the dissolved mixture was dispersed to form microspheres. A concentration of 0.5 ml of glutaraldehyde was added dropwise to the mixture while stirring. The addition of glutaraldehyde likely cross-linked the components, leading to the formation of solid microspheres. After the crosslinking reaction, the CMN-AVLM mucoadhesive microspheres were separated from the liquid paraffin by centrifugation. Then, they were washed with petroleum ether to remove any impurities or unreacted materials. To remove any remaining glutaraldehyde from the AVLM component, the microspheres were suspended in a 5% v/v solution of sodium bisulfite for 15 min. This step likely helped neutralize and eliminate any residual glutaraldehyde. The microspheres were then washed with distilled water to ensure the removal of any remaining impurities or reaction by-products. Afterward, they were dried using vacuum desiccators to preserve their structure and properties[7,8].

Quality by Design (QbD) is a systematic approach to pharmaceutical development that aims to ensure the quality of the final product by understanding and controlling the critical quality attributes (CQAs) throughout the development process. It is a key element described in ICH Q8 (Pharmaceutical Development) and Q9 (Quality Risk Management) guidelines. The Quality Target Product Profile (QTPP) is an essential part of the QbD approach, as it defines the quality attributes of the final product that are critical for its intended use. In the context of the CMN-AVLM mucoadhesive microspheres, the researchers would have established a QTPP based on previous explorations and literature reviews. The QTPP for AVLM would outline the specific attributes and characteristics that the final mucoadhesive microspheres should possess to meet the desired quality standards and performance goals[9].

The QTPP serves as a reference point throughout the development process, guiding decisions on formulation, process design, and analytical methods. The CQAs (Critical Quality Attributes) for AVLM would be identified based on their impact on the final product's quality and performance. These CQAs would be monitored and controlled during the development and manufacturing process to ensure that the final CMN-AVLM mucoadhesive microspheres consistently meet the predefined quality standards and fulfill their intended drug delivery purpose[10].

Evaluation parameters

Microsphere size, shape, and flow properties

An optical microscope to determine the sizes of the mucoadhesive microspheres containing CMN and AVLM. The flow properties of the mucoadhesive microspheres were assessed using the angle of repose (AR) and Hausner's ratio (HR). By evaluating the AR and HR, the researchers can gain insights into the flow properties and compressibility of the mucoadhesive microspheres. These parameters are crucial for understanding the ease of handling and processing the microspheres in manufacturing and administration, as well as for predicting their behavior during drug release and application at the target site. The aim is to achieve desirable flow properties that facilitate proper delivery and administration of the CMN-AVLM mucoadhesive microspheres for efficient drug delivery and therapeutic effectiveness[11].

Particle Size Measurement

The researchers used a stage micrometer scale to assess the PS of CAMM containing CMN and AVLM. A small quantity of the mucoadhesive microspheres was placed on glass slides to create a monolayer of particles for easy visualization and measurement. An eyepiece micrometer, also known as an ocular micrometer, is a calibration tool placed in the eyepiece of the microscope. It consists of a scale with known divisions. By using the eyepiece micrometer, the researchers can establish a reference scale in the microscope's field of view. Using the microscope at an appropriate magnification, the researchers observed the microspheres on the glass slide. They then counted the number of particles present within the field of view. Since the eyepiece micrometer provides a known reference scale, the researchers could measure the size of each microsphere directly in µm or any other suitable unit. To ensure accuracy and statistical significance, the process of counting and measuring the PS was repeated for 100 particles in each batch. By counting and measuring 100 particles from each batch, the researchers obtained a representative sample size to assess the PS distribution of the mucoadhesive microspheres accurately. This method allows them to determine the average PS and understand the uniformity or variability in size among the microspheres in each batch. The results obtained from this analysis contribute to characterizing the microspheres and are essential for evaluating their suitability for drug delivery applications[12].

% yield

The weight yield of microspheres can be calculated using the following equation:

$$\% \text{ Yield} = \frac{\text{Weight of dried CAMM}}{\text{Weight of drug and polymers}} \text{X } 100 \text{--- (1)}$$

Where the weight of dried CAMM is the weight of the final product (microspheres) after the drying process) and the total weight of the drug and polymers used is the combined weight of the CMN and polymers (e.g., AVLM and C-934P) used in the formulation[13].

Entrapment Efficiency

The entrapment efficiency of CMN in the CMN-AVLM mucoadhesive microspheres (CAMM) was determined using spectrophotometric analysis. A known amount of CMN-AVLM mucoadhesive microspheres weighing 100 mg was dispersed overnight in a suitable solvent, in this case, 0.1 M HCl. This step allowed the CMN to be released from the

microspheres into the solvent. After dispersing the microspheres, the mixture was assessed using a spectrophotometer at a specific wavelength, typically 220 nm. At this wavelength, CMN exhibits maximum absorbance, making it suitable for quantification. After the spectrophotometric analysis, the mixture was filtered to separate the microspheres from the solvent. The filtrate, which contains the released CMN, was collected for further analysis. The entrapment efficiency of CMN in the microspheres was calculated by comparing the amount of CMN present in the formulation with the amount initially added during the formulation process. The entrapment efficiency (EE %) can be calculated using the following equation[6]:

$$\%\text{Entrapment efficacy} = \frac{\text{Amount of CMN in CAMM}}{\text{Amount of CMN initially added}} \text{X } 100\text{---- (2)}$$

Where the amount of CMN in microspheres is the quantity of CMN that was released from the microspheres and measured through spectrophotometric analysis. The amount of CMN initially added is the known amount of CMN added during the formulation process. By calculating the entrapment efficiency, the researchers can determine the percentage of CMN that was successfully encapsulated within the mucoadhesive microspheres. A higher entrapment efficiency indicates that a greater proportion of CMN was retained within the microspheres, which is desirable for efficient drug delivery and controlled release.

Swelling Measurement

The SI of the CAMM was determined by conducting a swelling study in 0.1 M HCl. The mucoadhesive microspheres were immersed in a solution of 0.1 M HCl. This step initiated the swelling process. The CAMMs were allowed to swell in the HCl solution for a specific duration, in this case, 3 h. After the swelling time elapsed, the microspheres were removed from the HCl solution and subjected to centrifugation. Centrifugation helped separate the swollen microspheres from the surrounding HCl solution. The weight of the swollen microspheres (Xt) was measured after centrifugation. The SI was calculated using the following equation[14]:

$$\%\ \text{SI} = \frac{\text{Xt}-\text{Xo}}{\text{Xo}} \text{X } 100\text{--- (3)}$$

Where the weight of swollen microspheres (Xt) is the weight of the microspheres after they have swelled in the HCl solution for the specified time (3 h). The initial weight of microspheres (X_0): This is the weight of the CAMM before they were immersed in the HCl solution (i.e., at t=0).

The SI provides valuable information about the swelling behavior of the mucoadhesive microspheres in an acidic environment. It indicates the degree of swelling or expansion of the microspheres due to the uptake of HCl solution. A higher SI suggests greater water uptake and swelling capacity, which can be beneficial for mucoadhesion and controlled drug release in specific applications.

Mucoadhesion Measurement Study

In this study, the researchers employed the wash-off technique to assess the mucoadhesive properties of CMN-AVLM mucoadhesive microspheres (CAMM) in vitro. The experiment involved evaluating the adhesion of the microspheres to freshly cut sections of goat intestinal mucosa. The process began by obtaining freshly cut sections of goat intestinal mucosa, each measuring 5.5 x 1.5 cm. These tissue specimens were mounted onto glass slides of the same

dimensions using cotton thread to hold them securely. The glass slides, along with the tissue specimens, were appropriately connected and supported. For the evaluation, each wetted tissue specimen was coated with approximately 50 microspheres of CAMM. Subsequently, the glass slides, along with the tissue specimens and microspheres, were quickly placed on the arm of a disintegration test machine. As part of the setup, a USP tablet was also included. The tissue specimen with the attached microspheres was then subjected to a moderate up-and-down motion in a test fluid at 37°C. The specific test fluid used in this investigation was 0.1 M HCl. Readings were taken at various time intervals, including 30 min, 1 h, and hourly intervals up to 6 hours. Throughout this time, the test machine operated in a working mode. At each reading interval of up to 6 h, the researchers counted and recorded the number of microspheres that were still adhered to the tissue. This data allowed them to analyze and measure the mucoadhesive properties of the CMN-AVLM microspheres[15]. The formula used to calculate the mucoadhesion of the CAMM microspheres is described in eq. 4.

$$\%mucoadhession = \frac{\text{Number of CAMM (g)}}{\text{Initial CAMM}} \text{X100---} (4)$$

***In Vitro* CMN Release Study**

In this study of the CMN-AVLM mucoadhesive microspheres (CAMM) using the USP-II apparatus, which is commonly employed for dissolution testing of solid dosage forms. The dissolution study aimed to evaluate the release of CMN from the microspheres over time in a simulated physiological environment. The dissolution study was performed using the USP-II apparatus was used with a stirring rate of 50±5 rpm. The temperature was maintained at 37±0.5°C throughout the study. These conditions simulate the physiological environment of the human gastrointestinal tract. The dissolution medium used was 0.1 M HCl with a total volume of 900 ml. This acidic medium mimics the conditions in the stomach, where CMN would be released from the microspheres. During the dissolution study, 5 ml samples were withdrawn at different time intervals, known as breaks. At each break, the volume of the dissolution medium was replenished to maintain a constant volume. The 5 ml samples were subjected to spectrophotometric analysis at a wavelength of 220 nm. At this wavelength, CMN exhibits maximum absorbance, making it suitable for quantification. The spectrophotometric analysis allowed the researchers to determine the concentration of CMN released from the microspheres in each sample. By recording the amount of CMN released at different time points, they could generate a dissolution profile representing the drug release pattern over the 10-hour study period. The dissolution study provided valuable information about the release kinetics and drug release behavior of the CAMM microspheres. It helped assess the controlled drug release properties of the microspheres and their potential for achieving sustained drug delivery[16]. The data obtained from this study contributes to understanding the in vitro performance of the mucoadhesive drug delivery system, which is essential for further optimizing its formulation and predicting its behavior *in vivo*.

Kinetic release study

The drug discharge from CAMM was assessed for its fitting to the zero order, first order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppas models to find out the drug discharge and mechanism[17].

Statistical optimization

Using Design-Expert software, the researchers employed response surface plots and contour plots to analyze the independent influences on responses. The polynomial equations were statistically validated through ANOVA, generating an F value with a 0.05 p-value. This allowed them to assess the model's significance and reliability[18].

RESULTS AND DISCUSSION

Factors screening results

Fit summary

Table 2 presents the fit summary for the responses of PS and SI.

Table 2. Fit summary for the responses

Response 1 (PS)			
Source	**Sequential p-value**	**Adjusted R^2**	**Predicted R^2**
Linear	0.6758	-0.1701	-0.8605
2FI	0.7467	-0.3721	-2.3522
Quadratic	**< 0.0001**	**0.9988**	**0.9963**
Cubic	0.9393	0.9967	0.9257
Response 2: (SI)			
Linear	< 0.0001	0.9748	0.9578
2FI	0.2988	0.9761	0.9671
Quadratic	**0.0268**	**0.9964**	**0.9837**
Cubic		1.0000	

ANOVA results

Table 3 displays the ANOVA results for the responses of PS and SI.

Table 3. Instant of ANOVA of CAMM

Response 1: PS (µm)			
Source	**Sum of Squares**	**F-value**	**p-value**
Model	153.17	1297.43	< 0.0001
A-C-934P	0.7350	31.13	0.0114
B-AVLM	18.03	763.48	0.0001
AB	3.06	129.71	0.0015
A^2	0.1250	5.29	0.1049
B^2	131.22	5557.55	< 0.0001
Residual	0.0708		
Cor Total	153.24		
Response 2: SI (%)			
Model	22.43	448.60	0.0002
A-C-934P	0.3750	37.50	0.0088
B-AVLM	21.66	2166.00	< 0.0001
AB	0.0900	9.00	0.0577
A^2	0.1250	12.50	0.0385
B^2	0.1800	18.00	0.0240
Residual	0.0300		
Cor Total	22.46		

For Particle Size

The obtained F-value of 1297.43 indicates that the model is highly significant in predicting the particle size (PS). The extremely small p-value of 0.01% indicates that the likelihood of such a large F-value occurring by random chance is very low, providing strong evidence for the model's significance.

Among the model terms, A, B, AB, and B^2 are found to be statistically significant (p-values < 0.05), indicating that these factors have a significant impact on the PS response. These terms represent the main effects of factors A and B, the interaction between factors A and B, and the squared effect of factor B, respectively. Model terms with p-values > 0.1 are considered not significant. The Predicted R^2 of 0.99643 and the Adjusted R^2 of 0.9988 are in reasonable agreement, indicating a good fit of the model to the data. The small difference between these two values (< 0.2) suggests that the model can accurately predict the PS. The SD value of 0.1537 indicates the level of accuracy of the predicted values.

For Swelling Index

The model's F-value of 448.60 suggests that the model is statistically significant in predicting the SI. The p-values of the model terms A, B, AB, A^2, and B^2 being less than 0.05 indicate that these factors have a significant influence on the SI response. These terms represent the main effects of factors A and B, the interaction between factors A and B, and the squared effects of factors A and B, respectively. The high Predicted R^2 of 0.9837 and the Adjusted R^2 of 0.9964 indicate that the model fits the data well and is in reasonable agreement. This implies that the model can accurately predict the swelling index based on the factors considered, providing valuable insights into the system's behavior.The small difference (<0.2) between these two values suggests that the model can accurately predict the SI response. The SD value of 0.1 indicates the level of accuracy of the predicted values. Overall, both models (for PS and SI) have high R^2 values, indicating that they are capable of explaining a significant portion of the variance in the responses. The significant model terms provide valuable insights into which factors are most influential in determining the PS and SI of the CAMM microspheres. The statistical analysis and validation of the models help ensure the reliability of the results and the effectiveness of the mucoadhesive drug delivery system.

PS and SI ANOVA particulars

The ANOVA results for PS indicate that the model is significant, as indicated by the F-value. In this case, the independent variables (factors) X_1, X_2, and X_3 (corresponding to AVLM, C-934P, and their interaction) were found to be significant terms in the model, as their p-values were less than 0.05. The final equation for PS, based on the coded factors, is as follows: $PS = +49.37 + 0.3500A + 1.73B - 0.8750AB - 0.2500A^2 - 8.10B^2$

The positive constant term (+49.37) represents the intercept when all factors are at their reference or center levels. The coefficients of the factors (A and B) represent the effect of each factor on PS. The positive coefficient in the equation indicates that an increase in the corresponding factor value leads to an increase in particle size (PS) of the CAMM microspheres. On the other hand, a negative coefficient suggests that an increase in the factor value leads to a decrease in PS. The coefficient of the interaction term (AB) represents the combined effect of factors A (AVLM concentration) and B (C-934P concentration) on PS. The coefficients of the squared terms (A^2 and B^2) indicate the curvature effect of each factor on PS, implying that there might be a non-linear relationship between the factor concentrations and the PS. Based on the equation, the PS of the CAMM microspheres was found to be dependent on the concentration of AVLM and C-934P. When AVLM and C-934P were both at lower levels (50 mg: 50 mg), the mean PS of CAMM was 38 μm. However, at a medium concentration of AVLM and C-934P (75 mg: 100 mg), the mean PS increased to 49.5 μm. This information highlights the importance of the concentrations of AVLM and C-934P in influencing the PS of the microspheres.

Similarly, the equation for the SI in terms of coded factors is as follows:

$SI = +58.70 + 0.2500A + 1.90B - 0.1500AB + 0.2500A^2 - 0.3000B^2$

The positive constant term (+58.70) represents the intercept when all factors are at their reference or center levels. The coefficients of the factors (A and B) represent the effect of each factor on SI. The coefficient of the interaction term (AB) indicates the combined effect of factors A and B on SI. The coefficients of the squared terms (A^2 and B^2) represent the curvature effect of each factor on SI. The elevation of AVLM was found to improve CMN entrapment, possibly due to an increase in the viscosity of AVLM, which stabilizes droplets and prevents the outflow of the drug during the hardening phase.

Diagnostic analysis for PS and SI

The diagnostic plots to assess the goodness of fit of the model for PS and SI of the CMN-AVLM mucoadhesive microspheres (CAMM). These diagnostic plots help evaluate the assumptions and the accuracy of the model predictions. Based on the analysis of these plots, the following observations were made:

For PS, the residuals in the Normality plot (Fig. 1A) indicate a random distribution around zero, suggesting that the hypothesis of normality holds. This is important as normality of residuals is a key assumption in regression analysis. The residuals vs. predicted values plot (Fig. 1B) shows that the residuals are evenly distributed around zero and do not follow any specific pattern, indicating that the assumption of constant variance is met. All the points in the residuals vs. run numbers plot (Fig. 1C) fall within a reasonable range, and there are no distant outliers, suggesting that there were no faraway observations during the experiment. The predicted vs. actual PS plot (Fig. 1D) shows a strong correlation between the predicted and actual PS values, indicating that the model predictions are very similar to the observed data.

Similar to PS, the normality plot for SI residuals (Fig. 1E) also shows a random distribution around zero, supporting the hypothesis of normality. The residuals vs. predicted values plot (Fig. 1F) demonstrates an even distribution of residuals around zero, indicating the assumption of constant variance is met. In the residuals vs. run numbers plot (Fig. 1G) with PS, the residuals in this plot do not show any distant outliers, suggesting no significant deviations during the experiment. The predicted vs. actual SI plot (Fig. 1H) illustrates a close agreement between the predicted and actual SI values, indicating the model fits the data well.

Overall, the diagnostic plots for both PS and SI show excellent model fitting, a lack of significant outliers, and a close match between predicted and observed data. These findings validate the reliability of the statistical model and the accuracy of the predictions, enhancing confidence in the results of the study. The absence of significant residuals and the close agreement between predicted and observed data further support the effectiveness of the ucoadhesive drug delivery system for CAMM microspheres.

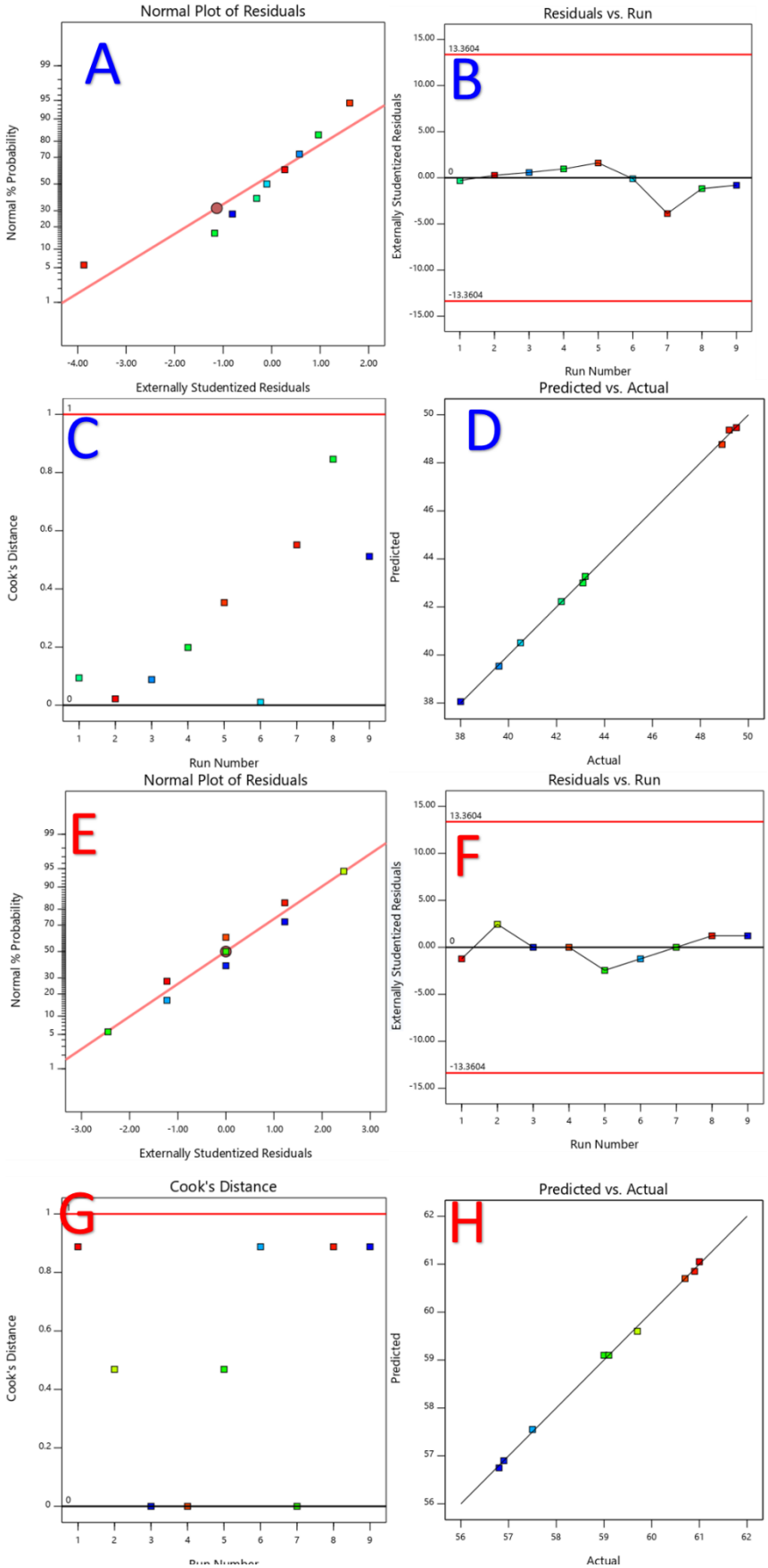

Figure 1. A-H. Plots screening the communication possessions of polymers on PS and SI

In Fig. 2 is the depiction of the PS and SI with contour and 3D plots.

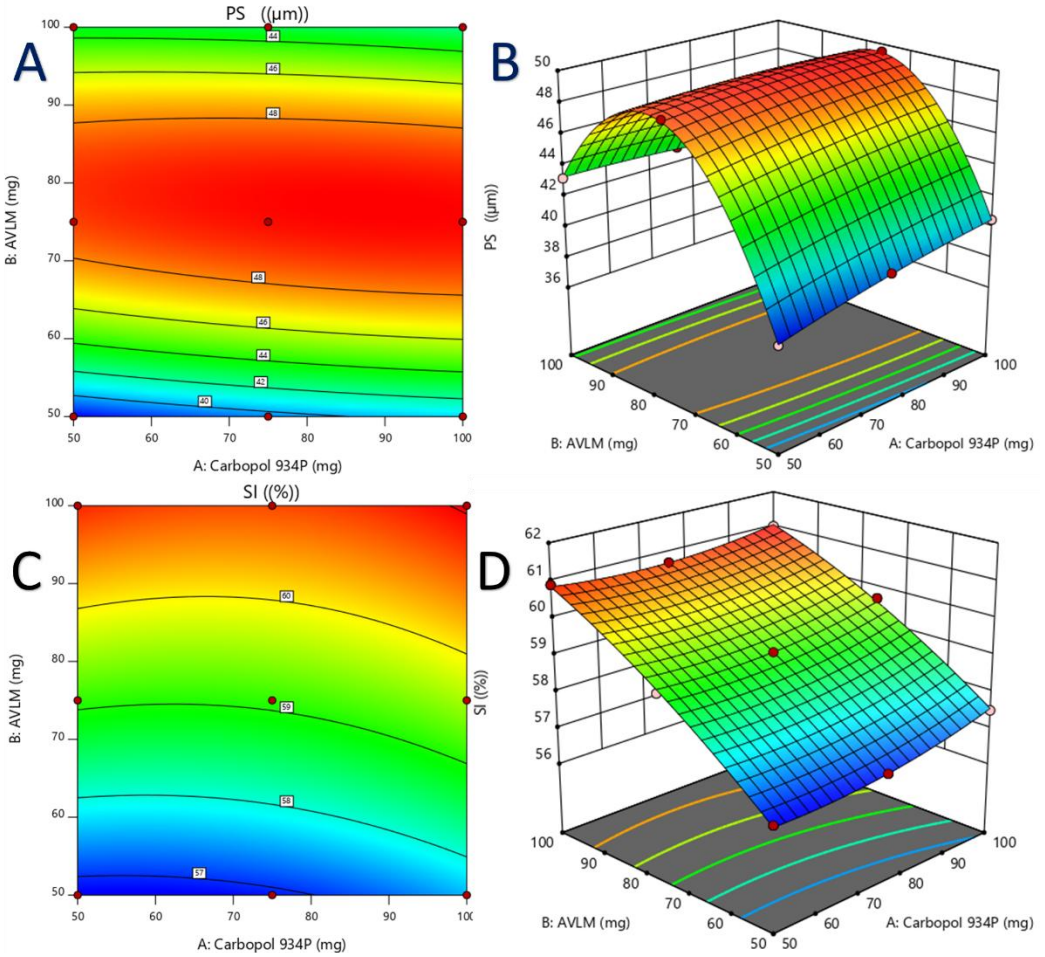

Figure 2. 2D and 3D response plots for PS and SI

Plots like this show the sway of two factors on the response at the same time. The contour plot and response surface plot (Fig. 2) show an equivalent increase with the concentration of the AVLM.

Optimization

Based on the statistical model and the desired PS of 48.82 µm with a SI of 59.70%, the optimal amounts of C-934P and AVLM for the CAMM microspheres can be determined using Fig. 3, which likely represents a response surface plot or contour plot generated by Design-Expert software. From Fig. 3, the researchers can identify the specific combination of C-934P and AVLM levels that would yield the desired PS and SI. Based on the plot, the optimal amounts are found to be approximately 53.74 mg of C-934P and 83.22 mg of AVLM. By preparing the CAMM microspheres with these optimized amounts of C-934P and AVLM, it is expected that the PS will be close to 48.82 µm, and the SI will be around 59.70%, which meets the desired specifications. Using response surface plots or contour plots helps researchers visualize the relationships between the factors and the responses and enables them to identify the optimum conditions for achieving specific target values of the responses. These plots are valuable tools in the optimization and formulation of drug delivery systems to meet specific requirements and ensure desired product performance.

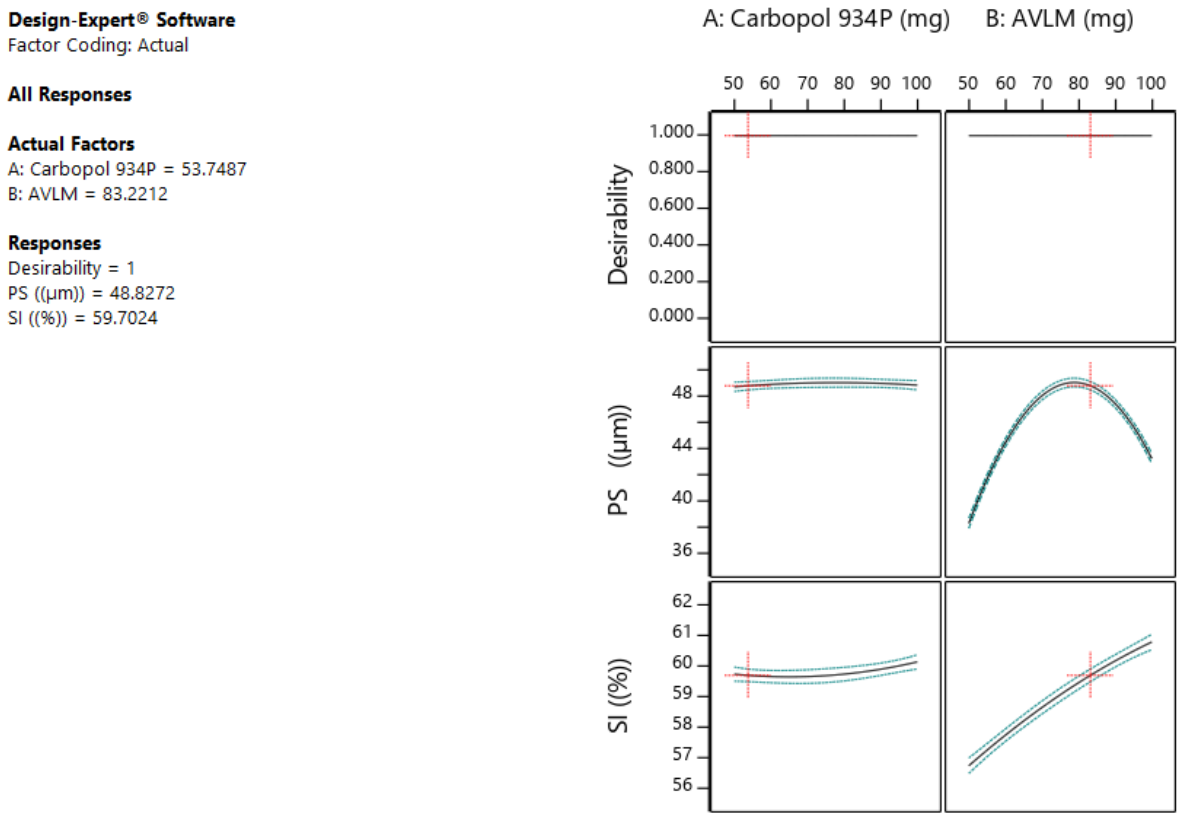

Figure 3. Arithmetical depiction of real factors swaying CAMM response optimization

Depiction of the CAMM

Flow properties

The CAMM microspheres exhibited desirable flow properties, as indicated by various characteristics:

Optimal moisture content is essential for good flowability of microspheres. The presence of the right amount of moisture helps reduce cohesion between particles, promoting better flow and preventing aggregation. It is likely that the CAMM microspheres were prepared with an appropriate level of moisture, contributing to their favorable flow properties.

Reduced cohesiveness between particles is a key factor in achieving good flowability. When particles are less cohesive, they tend to flow freely and do not stick together, resulting in better handling and processing characteristics.

The microspheres' spherical shape is another contributing factor to their excellent flow properties. Spherical particles tend to flow more easily and uniformly compared to irregularly shaped particles.

The angle of repose is a measure of the flowability of granular materials. A lower angle of repose indicates better flowability. The value of 25^{o} for the angle of repose suggests that the CAMM microspheres can flow smoothly and form a stable pile without significant slumping.

Hausner's ratio is a measure of the compressibility and flowability of a powder. It is the ratio of the tapped density to the bulk density of the powder. A Hausner's ratio close to 1 indicates good flow properties, as it suggests minimal changes in volume upon tapping. The range of 1.00 to 1.11 for Hausner's ratio further confirms the excellent flow properties of the CAMM microspheres.

Overall, the combination of optimal moisture presence, decreased cohesiveness, and sphere-shaped morphology contributed to the desirable flow properties of the CAMM microspheres.

These properties are essential for the easy handling, processing, and administration of the microspheres as a drug delivery system. The favorable flow properties ensure uniform dosing and efficient drug release characteristics, enhancing the effectiveness and practicality of the mucoadhesive microspheres for drug delivery applications.

Particle size

The researchers used an optical microscope, specifically a compound microscope with an ocular micrometer that had been calibrated with a stage micrometer, to examine the distribution of dimensions of the CAMM. This allowed them to measure the form and size of the microspheres accurately. To ensure a representative sample, the researchers counted at least 100 microspheres from each batch. The ocular micrometer allowed for precise measurements of the microspheres' dimensions, and by counting a sufficient number of microspheres, they were able to obtain a reliable mean PS for each batch. The results showed that each batch of microspheres had a consistent size, with a mean diameter ranging from 38 µm to 49.5 µm. This suggests that the manufacturing process was robust and reproducible, as the microspheres consistently fell within this size range across different batches. Having a consistent and controlled size distribution is essential for drug delivery systems like microspheres, as it ensures uniform drug release and consistent therapeutic effects. The optical microscope analysis provided valuable insights into the physical characteristics of the CAMM microspheres, confirming their desired size and shape for effective drug delivery applications.

Yield of AVLM

The manufacturing yield of CAMMs was found to be pragmatic, with values ranging between 77.7±4.7% and 92.3±5.6% (as indicated in Table 4). The yield represents the percentage of the final product obtained compared to the theoretical or expected amount. The fact that the yield values varied between different formulations suggests that the production yield was not uniform for all batches. In other words, the process of manufacturing the microspheres resulted in different yields for different formulations. The most likely cause of the low yield observed in some formulations could be the wastage of formulation ingredients during the manufacturing process. Wastage can occur due to various factors, such as loss of material during mixing, handling, or transferring of the ingredients, incomplete encapsulation of the drug, or inefficient drying processes. To improve the yield and reduce wastage, it is essential to identify and address the specific steps in the manufacturing process that are leading to the loss of ingredients. By optimizing the production process and minimizing material loss, it may be possible to achieve more consistent and higher yields across all formulations. A pragmatic yield of around 77.7% to 92.3% is generally considered acceptable for many pharmaceutical formulations. However, efforts to enhance the yield can lead to cost savings, reduced resource utilization, and improved efficiency in the manufacturing process..

% Drug entrapment

The entrapment efficiency of CAMM was determined to range between 70.3±6.6% and 85.3±4.1% (as shown in Table 4). Entrapment efficiency refers to the percentage of the CMN that is successfully encapsulated within the microspheres during the manufacturing process. The researchers observed that the entrapment efficiency of CAMM significantly improved with the use of AVLM during the processing. AVLM was used as a key component in the preparation of the mucoadhesive microspheres. The higher drug extraction into the AVLM during the manufacturing process could be the reason behind the lower entrapment efficiency. When the CMN interacts with AVLM, it may be more effectively incorporated and entrapped within the microspheres. This can be attributed to the mucoadhesive properties

of AVLM, which may lead to stronger interactions with the drug molecules and improve drug retention within the microspheres. The enhanced entrapment efficiency is a positive outcome as it ensures a higher proportion of the drug is successfully incorporated into the microspheres, resulting in more effective drug delivery. However, it is important to strike a balance between entrapment efficiency and drug release characteristics to achieve the desired drug release profile for the intended therapeutic effect. The optimization of the drug extraction process into AVLM and the formulation parameters can further improve the entrapment efficiency and overall performance of the CAMM microspheres as a drug delivery system.

Swelling results

The SI is identified as a crucial factor with a significant impact on the adhesive properties and cohesiveness of mucoadhesive polymers, including the CAMM microspheres. Swelling is a fundamental characteristic of mucoadhesive microspheres, and it plays a vital role in their adhesion to the mucosal tissue and drug release behavior. When the CAMM microspheres were dipped in 0.1 M HCl, all formulations demonstrated significant swelling. This result is expected and desirable because the microspheres are designed to swell upon contact with the acidic environment of the stomach. The absorption and capillary effects of the mucoadhesive microspheres are facilitated by their ability to take up water from beneath the layer of mucosal tissue. This water absorption leads to increased adherence of the microspheres to the mucosa, promoting prolonged contact and sustained drug release at the site of action. Formulations B-8 and B-9, which contained a higher proportion of AVLM, exhibited higher SI values. This can be attributed to the high ionization of AVLM in acidic pH conditions, such as that of 0.1 M HCl. The ionized form of AVLM can absorb a larger amount of water due to its hydrophilic nature, leading to enhanced swelling properties of the microspheres. The increased SI in formulations with higher AVLM content can result in improved mucoadhesive properties, better interaction with the mucosal tissue, and prolonged drug release at the target site. This is advantageous for drug delivery applications, as it ensures prolonged drug exposure and enhances the therapeutic efficacy of the delivered drug. Overall, the significant swelling and higher SI observed in formulations with higher AVLM content contribute to the desired mucoadhesive properties of the CAMM microspheres, making them suitable for effective drug delivery and prolonged drug release in the stomach.

CMN estimation

Obtaining a calibration curve for CMN using a UV-VIS spectrometer at its λ_{max} of 220 nm in a 0.1 M HCl solution is a common method for quantitative analysis of CMN in pharmaceutical formulations. The calibration curve is established by preparing a series of standard solutions with known concentrations of CMN (usually in the range of interest) and measuring their absorbance at 220 nm using the UV-VIS spectrometer. The absorbance values are then plotted against the corresponding concentrations, and a linear regression analysis is performed to obtain the equation of the calibration curve. The calibration curve's linearity in the range of 0-10 μg/ml, as mentioned, indicates that there is a direct relationship between the concentration of CMN and its absorbance at 220 nm within this range. This information is valuable for quantifying the amount of CMN in samples using the spectrophotometric method. In pharmaceutical quality control and content uniformity testing, the calibration curve is used to determine the concentration of CMN in test samples by measuring their absorbance at 220 nm and then applying the equation of the calibration curve. By comparing the absorbance of the test samples to the calibration curve, the content uniformity of CMN in the microspheres can be assessed, ensuring that each batch contains the desired amount of the drug within acceptable limits. Repeated measurements of the

calibration curve (three replicates) help verify its accuracy and precision, ensuring the reliability of the spectrophotometric method for CMN quantification. This data is critical for ensuring the consistency and quality of the drug delivery system and confirming that the formulated microspheres meet the required drug content specifications for effective and safe drug delivery.

Table 4. Flow and physical properties of CAMM

Batch	Angle of repose	BD	TD	HR	CI	Particle size (µm)	%yield	Drug entrapment efficacy (%)	Swelling index (%)	CMN content (%)
B-1	24.98±0.4	0.515	0.546	5.677	1.060	38.0	77.7±4.7	70.3±6.6	56.8	95.8±7.7
B-2	25.75±0.6	0.568	0.597	4.857	1.051	39.6	80.6±3.8	71.5±1.8	56.9	96.8±2.8
B-3	20.22±0.8	0.502	0.538	6.691	1.071	40.5	82.5±5.6	74.7±3.4	57.5	97.2±3.5
B-4	25.65±0.2	0.368	0.389	5.398	1.057	48.9	81.6±2.3	75.9±2.3	59.0	95.6±2.3
B-5	25.47±0.3	0.658	0.678	2.949	1.030	49.2	83.8±5.6	76.5±1.7	59.1	96.9±5.2
B-6	22.87±0.6	0.478	0.502	4.780	1.050	49.5	91.2±2.4	79.8±2.5	59.7	97.4±2.4
B-7	24.98±0.4	0.682	0.704	3.125	1.032	43.2	90.2±3.4	80.3±3.3	60.9	96.9±3.2
B-8	22.54±0.7	0.762	0.789	3.422	1.035	43.1	92.3±5.6	82.8±4.5	60.7	98.6±3.4
B-9	24.99±0.9	0.882	0.899	1.890	1.019	42.2	91.9±3.4	85.3±4.1	61.0	97.9±1.7

Values in mean±SD, N=5

In vitro mucoadhesion efficiency

The % mucoadhesion of the whole CAMM with goat intestine (Fig. 4) demonstrated a strong correlation with the viscosity of the polymer used in the formulation. Viscosity and molecular weight are key factors that influence the mucoadhesive properties of polymers. A higher viscosity and molecular weight of the polymer are generally associated with better mucoadhesion. In the in-vitro wash-off experiment, it was observed that the microspheres containing a high proportion of C-934P in combination with AVLM showed stronger mucoadhesive properties compared to C-934P alone. This indicates that the addition of AVLM enhanced the mucoadhesive assets of the microspheres, leading to better adherence to the mucosal tissue.

The good mucoadhesive activity of the microspheres is a crucial factor for achieving prolonged residence time at the absorption site in the gastrointestinal tract. When microspheres have strong mucoadhesive properties, they can adhere to the mucosal lining and remain at the site of absorption for an extended period. This prolonged residence time enhances drug absorption and oral bioavailability, as the drug is continuously released and absorbed through the mucosa.

The wash-off test results affirm that the formulated microspheres have the desired mucoadhesive activity, which is vital for their drug delivery application. The improved mucoadhesive properties achieved with the combination of C-934P and AVLM make the CAMM microspheres effective for delivering the CMN to the target site, improving drug efficacy, and potentially reducing dosing frequency. Overall, the successful mucoadhesion of the CAMM microspheres indicates their potential as a promising drug delivery system. It offers prolonged drug release and enhanced oral bioavailability, essential factors in developing effective and patient-friendly pharmaceutical formulations.

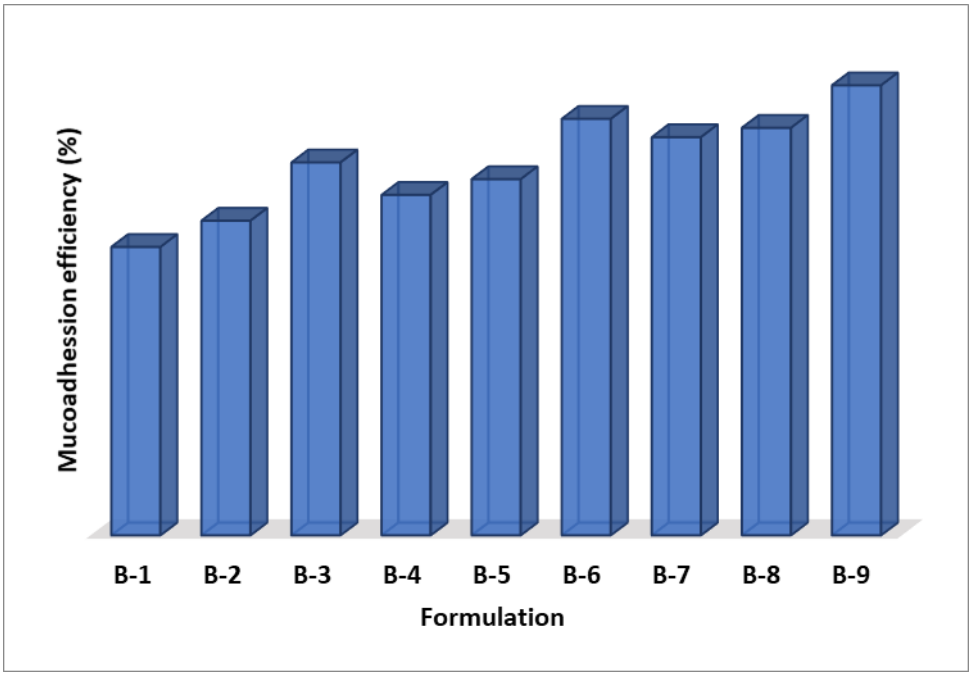

Figure 4. Bar chart presentation *ex vivo* mucoadhesion of CAMM

***In vitro* drug release**

The in vitro release profile of CMN from the CAMMs was assessed in 0.1 M HCl over a period of 10 hours. The results showed that throughout the study, CMN was consistently released by the CAMMs, indicating the effectiveness of the drug delivery system in releasing the drug over an extended period. Fig. 5 illustrates the drug release behavior of the CAMMs in vitro. It can be observed that each formulation of CAMM exhibited drug release for more than 10 hours. This prolonged release of CMN is desirable for controlled drug delivery, as it ensures a sustained and regulated release of the drug over an extended duration, which is beneficial for maintaining therapeutic levels of the drug in the body. Among all the CAMM formulations, B-3, B-6, and B-9 displayed the best-regulated CMN release profile after 10 hours. This enhanced performance can be attributed to the higher percentage of C-934P in these formulations. C-934P is known to transform into a thick gel when in contact with aqueous fluids, which makes it effective in controlling the delivery of highly water-soluble medications like CMN. The effective and rapid drug liberation from hydrophilic matrices, such as the CAMM microspheres, is likely due to the efficient and faster dissolution of water-soluble drugs from the core of the microspheres. As the drug dissolves and spreads out of the microspheres, it creates pores through which solvent molecules can pass. This enables a continuous and controlled release of the drug, contributing to the sustained drug release profile observed in the study. The ability to achieve a regulated and sustained drug release from the CAMM microspheres is a crucial feature for drug delivery applications, as it allows for optimized drug absorption, reduced dosing frequency, and improved patient compliance. The CAMM formulation, with its controlled release characteristics, holds promise for delivering CMN and other water-soluble drugs effectively and efficiently.

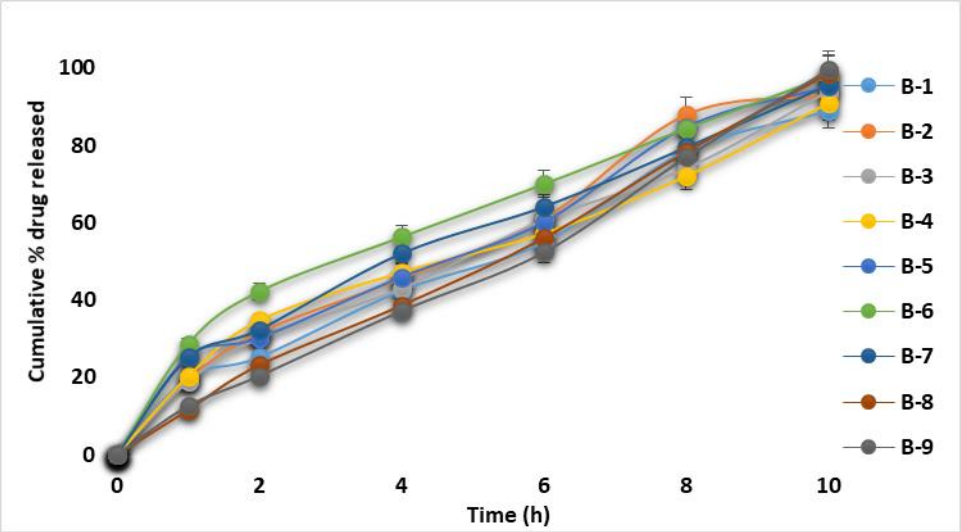

Figure 5. *In vitro* discharge from CAMM

The release kinetics of the CAMMs were examined by fitting the release data to several kinetic models. Among these models, the Korsmeyer-Peppas model was found to be the most suitable for describing the drug release behavior of the formulation. The Korsmeyer-Peppas model is commonly used to analyze drug release from polymeric matrices and can provide valuable insights into the release mechanism. In this model, the drug release exponent 'n' is a crucial parameter that characterizes the release mechanism. The 'n' value < 0.5, indicates Fickian diffusion, where the drug release is controlled by the diffusion of drug molecules through the matrix. This is typical for systems with low polymer concentration or when the drug release is primarily governed by the dissolution of the drug from the surface of the microspheres. The 'n' value= 0.5 I ndicates Case-I or Higuchi diffusion, where the drug release is controlled by a combination of drug dissolution and diffusion. The release profile follows Higuchi's square root of time relationship. The 'n' value > 0.5 indicates non-Fickian diffusion or anomalous transport, where the drug release is a complex interplay of both drug dissolution and diffusion as well as polymer relaxation and swelling. The release profile deviates from traditional Fickian diffusion and follows a more complex mechanism. In Table 5, it was observed that the 'n' value for the CAMM release data was more than 0.5, indicating non-Fickian diffusion as the release mechanism. This suggests that the drug release from the CAMMs is influenced by both drug dissolution and diffusion as well as other factors like polymer relaxation and swelling of the microspheres. The non-Fickian diffusion behavior is typical for polymeric systems that display swelling and relaxation, as observed in mucoadhesive microspheres. The Korsmeyer-Peppas model with non-Fickian diffusion is suitable for describing the drug release pattern from these types of polymeric matrices, providing a comprehensive understanding of the release mechanism. The ability to characterize the release kinetics using the Korsmeyer-Peppas model with a non-Fickian diffusion mechanism is valuable for optimizing the formulation and designing drug delivery systems that achieve the desired release profile for specific therapeutic applications.

Table 5. Kinetic reports of CAMM

	Correlation (r)						
Batch	**Zero-order**	**First order**	**Higuchi**	**Hixson Crowell's**	**Korsmeyer Peppas**	**N**	**Release mechanism**
B-1	0.9678	0.9123	0.9987	0.9629	0.8768	0.6752	Non-fickian
B-2	0.9734	0.9098	0.9576	0.9762	0.8309	0.6654	Non-fickian
B-3	0.9683	0.9053	0.9629	0.9876	0.7903	0.6983	Non-fickian

B-4	0.9788	0.9109	0.9388	0.9448	0.7307	0.7002	Non-fickian
B-5	0.9771	0.9394	0.9529	0.9387	0.6904	0.7003	Non-fickian
B-6	0.9566	0.9306	0.9261	0.9560	0.7129	0.7127	Non-fickian
B-7	0.9902	0.9284	0.9432	0.9328	0.7226	0.7283	Non-fickian
B-8	0.9655	0.8904	0.9842	0.9276	0.7452	0.7481	Non-fickian
B-9	0.9810	0.9121	0.9765	0.9092	0.7193	0.7562	Non-fickian

CONCLUSION

Based on the findings of the study, it can be concluded that the mucoadhesive microspheres of Clarithromycin (CMN) formulated with *Aloe vera* leave mucilage (AVLM) and Carbopol 934 P (C-934P) meet the ideal requirements for mucoadhesive drug delivery systems. The study demonstrated that the mucoadhesive polymers, AVLM, and C-934P, play a crucial role in regulating both the quantity and pace of CMN release in the stomach. The mucoadhesive microspheres effectively released CMN into the stomach when they were broken down. The use of AVLM in the formulation contributed to the enhanced mucoadhesion of the microspheres, resulting in prolonged residence time in the stomach and improved drug delivery to the target site. The scanning electron microscopy revealed that the microspheres had a smooth surface and a spherical shape, indicating good uniformity and stability of the formulation. Furthermore, the study showed that the AVLM content in all batches increased with longer mucoadhesive times, suggesting that higher AVLM content led to stronger mucoadhesive properties of the microspheres. Overall, the mucoadhesive microspheres of CMN with AVLM assisted by C-934P demonstrated excellent mucoadhesive properties, enhanced stomach residence time, and improved oral bioavailability. These characteristics are crucial for the effective delivery of CMN in the stomach and can potentially lead to improved therapeutic outcomes and patient compliance. The study's results support the potential application of these mucoadhesive microspheres as an effective drug delivery system for CMN and possibly other drugs, offering a promising approach to enhance drug delivery and improve patient outcomes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL PERMISSION

Not required as no animal or human subjects were used in the study.

AUTHORS CONTRIBUTION

NSVB, perceiving the offered idea, HAA and ESK developed the theory and made the protocol of the study. NSVB examined the study. HAA supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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