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Original Article

Azadirachta indica Fruit Mucilage Aided Mucoadhesive Microspheres of Acyclovir for Drug Entrapment and Mucoadhesive Time Assets with Design-Expert Software

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ABSTRACT

Introduction: Mucoadhesive microspheres for drug delivery are retained in the stomach for an extended period for localized drug release and effect. **Objectives:** This research aims to explore the mucoadhesive properties of *Azadirachta indica* fruit mucilage when incorporated into mucoadhesive microspheres, utilizing Acyclovir as a model drug. **Materials and Methods:** Employing a Box Behnken design, 13 formulations of microspheres were developed, varying *Azadirachta indica* Mucilage (AIFM) levels, carbomer 934P and stirring speed. Design Expert software was used to assess the impact of these factors on entrapment efficacy and mucoadhesion time. Congeniality studies involved the examination of microspheres for Acyclovir content and discharge. **Results:** Results indicated that Acyclovir entrapment increased with higher AIFM levels and mucoadhesion time was prolonged in formulations with elevated AIFM levels. The optimal stirring speed was determined to be 750 rpm. **Conclusion:** The study concludes that Acyclovir demonstrates effective stomach-specific drug delivery through carbomer 934P, further enhanced by *Azadirachta indica* fruit mucilage, particularly at a stirring speed of 750 rpm in the formulation of mucoadhesive microspheres.

Keywords: Acyclovir, *Azadirachta indica*, Design, Delivery, Microspheres, Mucoadhesion.

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INTRODUCTION

The passage delves into innovative approaches in drug administration, focusing on the development of gastroretentive microspheres, particularly those with mucoadhesive properties.[1] The objective is to improve the obtainability of drugs in the gastric region while ensuring patient acceptance. The drug of interest in this context is Acyclovir (AVR), a purine nucleoside analog utilized for treating viral infections like herpes simplex virus, herpes zoster and chickenpox.[2]

The discussion begins by highlighting the challenges associated with AVR, including its relatively low oral bioavailability (15-30%) and a short half-life of approximately 2 hr. Despite being well absorbed into the stomach after oral administration, there is a need to enhance its therapeutic efficacy. Gastro-retentive microspheres are presented as a promising solution due to their ease of preparation and administration.[3]

The choice of polymer in mucoadhesive systems is emphasized as a critical factor influencing their effectiveness. In the search for an effective and economical polymer, the study explores the use of *Azadirachta indica* Fruit Mucilage (AIFM).[4] The combination of AIFM with carbopol 934 P is examined and the impact of stirring speed during the formulation process is considered. Notably, AIFM is highlighted for its antiviral properties, suggesting a potential dual role in both drug delivery and antiviral therapy.[5]

The study's overarching goal is to attain steady-state systemic obtainability over an extended period, aligning with the design of precision liberation systems. These systems are particularly valuable for short-acting drugs and those requiring incessant medication.

The passage critiques traditional research methods that manipulate only one variable at a time, pointing out the limitations of such an approach in capturing the dealings between factors. It introduces the concept of Design of Experiments (DOE) as an essential method for systematically exploring a partial number of factors, screening for responses and optimizing outcomes.[6] The levels in a full Factorial Design (FD) are labeled 'high' (+1) and 'low' (-1) and the study employs design expert software to evaluate the impact of independent variables on the response, specifically in the context of screening mucoadhesive microspheres of AVR.[7]

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The passage not only highlights the challenges in drug administration but also presents an innovative approach using mucoadhesive microspheres. The study explores the use of AIFM and Carbopol 934 P, incorporating the principles of DOE to systematically optimize the formulation for enhanced drug delivery, with a focus on AVR.[8]

The BBD is a type of Response Surface Methodology (RSM) used in experimental design. It is particularly useful for optimizing processes and studying the response of a system to different factors or variables. Here are some key features of the BBD including the following:[9]

The design is based on three levels of each factor: low, medium and high. The design is orthogonal, meaning that the impact of each factor is independent of each other. This allows for an efficient estimation of the main effects and interactions. BBD requires fewer experimental runs compared to a full factorial design. This is especially beneficial when experiments are time-consuming or expensive. The design includes center points to estimate the pure error or experimental error. This is useful for assessing the response variability that the factors do not explain.[10] The BBD is particularly well-suited for fitting a second-order polynomial model to the data. This allows for the examination of curvature in the response surface. The goal of using the BBD is to control the optimal conditions for a process or system by identifying the levels of the factors that maximize or minimize the response of interest.

This study focuses on the development of mucoadhesive microspheres for AVR by combining synthetic (Carbopol 934 P) and natural (*Azadirachta indica* fruit mucilage) polymers, with an examination of the impact of stirring rate on mucoadhesion and AVR entrapment. The experimental design follows a BBD, systematically varying Carbopol 934 P concentration, AIFM concentration and stirring rate. The objective is to assess the individual and interactive effects of these factors on Mucoadhesion Time (MT) and Entrapment Efficiency (EE). The study aims to understand the synergistic or antagonistic influences of combining synthetic and natural polymers in mucoadhesive microspheres with AVR and it seeks to identify optimal conditions for maximizing the effectiveness of the formulation. The findings have potential implications for enhancing the therapeutic efficacy of AVR in treating viral infections, contributing to the development of an optimized mucoadhesive Drug Delivery System (DDS).

MATERIALS AND METHODS

Materials

Acyclovir was gifted from Ranbaxy Labs, Hyderabad, Telangana. Carbomer 934P, ethyl cellulose, dichloromethane, span 80, glutaraldehyde and liquid paraffin were from Merck, Hyderabad.

Methods

Extraction and cleansing of AIFM

As described by Ahad *et al.*, the expression was conducted using *Azadirachta indica* fruits.[11] The process involved washing the fruits, removing the outer layer, soaking them in water, boiling them for 1 hr and allowing them to cool. After removing the seeds, fractionation was sequentially carried out with petroleum ether (50%), ethyl acetate and butanol. The mucilage obtained was then separated by mining it with a multi-layer muslin cloth bag to eliminate the marc. Subsequently, the mucilage underwent a series of steps, including drying in an oven (Stericox-India) at 40°C, being poised, ground, passed through a # 80 sieve (Remi) and finally being stored in a desiccator (B-3082045 Foxx Life Sciences) at 30°C and 45% RH. This comprehensive procedure outlines the extraction and preparation of AIFM for further utilization in the study.[12,13]

In the experimental procedure described by Hindustan *et al.*, the substance AIFM underwent a series of meticulous steps to attain purification.[14] Initially, the AIFM was homogenized using Biologics-150VT and treated with 5% trichloroacetic acid. The resulting mixture underwent centrifugation in a Remi R-303 centrifuge, followed by neutralization with sodium hydroxide. The neutralized substance then underwent dialysis using a SURDIAL-X apparatus with distilled water, a process designed to separate molecules based on their size and charge. The subsequent addition of ethanol (95%) induced the precipitation of certain components. To further refine the purification, the precipitate was carefully treated with acetone and diethyl ether in a progressive scrubbing process. These intricate steps represent a comprehensive approach to purifying AIFM, potentially for use in subsequent analytical or experimental endeavors. The use of various solvents and precise techniques underscores the attention to detail in the pursuit of obtaining a highly purified form of the substance.

Experimental design

In the optimization process of Acyclovir Mucoadhesive Microspheres (AMM), the Design-Expert software (version 11.0.5.0, Stat-Ease Inc.) played a pivotal role. A quadratic response surface methodology was employed using a 13-run Box-Behnken Design (BBD) to create and assess the response surfaces. The independent variables considered were carbopol 934P (X_1), AIFM (X_2) and stirring speed (X_3), each having three levels. The study focused on two dependent variables: EE and Mucoadhesive Time (MT), both crucial parameters in the performance of the AMMs. A total of 13 experimental designs, as outlined in Table 1, were systematically utilized to define the variables and their respective levels for the optimization of AMMs. The BBD is particularly advantageous in efficiently exploring the response surface with fewer experimental runs associated with a full factorial design.[15] This systematic and structured approach, facilitated by the

Design-Expert software, allowed for a comprehensive assessment of the influence of the independent variables on the response variables. This methodology is integral to attaining an optimized formulation of AMMs, contributing to the enhancement of both EE and MT, essential factors for the efficacy and functionality of the AMMs.[16]

Preparation of AMM

The preparation of AMMs involved a carefully orchestrated series of steps. Carbomer 934P, AVR, EC and AIFM were dissolved in a solution of acetic acid (2% v/v) and dichloromethane. This mixture was then introduced into liquid paraffin, which contained span 80, using a three-bladed propeller stirrer (IKA-R1385) operating at 200 rpm. Throughout this process, Glutaraldehyde (GAD) was added dropwise at intervals of 1 hr. The stirring was unremitting for a total duration of 3 hr.[17]

Following the synthesis, the resulting AMMs were subjected to further processing. They were centrifuged and washed with petroleum ether to eliminate residual liquid paraffin. Subsequently, the AMMs underwent suspension in a 5% w/v sodium bisulfite solution for 15 min, aimed at removing any remaining GAD. The final purification step involved washing the AMMs with distilled water. The washed AMMs were then dried and stored in vacuum desiccators.

This comprehensive procedure reflects a methodical approach to the fabrication of AMMs, ensuring the removal of impurities and the preservation of the AMMs for subsequent use or analysis. The incorporation of GAD and the subsequent removal steps contribute to the quality and features of the final product.[18]

Evaluation

Identification of AVR

The comparison of the sample spectrum of AVR with a combination spectrum is a common practice to assess changes in functional groups. Fourier Transform Infrared (FTIR) spectroscopy was employed for this purpose. The reference spectrum serves as a baseline or standard, representing the expected spectrum for the pure substance without any alterations. Comparing the AVR spectrum to the AVR with the combination of excipients helps identify changes in the vibrational or magnetic frequencies of the atoms in the molecule. These changes can indicate modifications in the chemical structure or functional groups of the substance. In FTIR spectroscopy, shifts or intensity changes in distinctive absorption bands corresponding to specific functional groups (like C-H, O-H, or C=O bonds) can signify chemical changes or impurities in the sample. This helps to identify if any modifications or impurities are present in the sample, allowing for a more comprehensive understanding of the chemical assets of the substance under investigation.

Finding the melting point of AVR

The finding of the melting point of the AVR delivers valuable information about the purity and identity of the compound. In this case, the open capillary method was employed to ascertain its melting point. The open capillary method involves placing a small amount of the substance into a capillary tube, which is then carefully heated. The temperature range at which the substance transitions from a solid to a liquid state is observed and recorded as the melting point. The open capillary method is relatively straightforward and is suitable for a wide range of substances.

Table 1: The ingredients in various AMM.

Formulation	AVR (mg)	C-934P (mg)	AIFM (mg)	EC (mg)	DCM (mL)	Span 80 (minims)	GAD (minims)	LP (mL)	rpm
AMM-1	200	50	50	40	25	2	3	150	750
AMM-2	200	100	50	40	25	2	3	150	750
AMM-3	200	50	100	40	25	2	3	150	750
AMM-4	200	100	100	40	25	2	3	150	750
AMM-5	200	50	75	40	25	2	3	150	500
AMM-6	200	100	75	40	25	2	3	150	500
AMM-7	200	50	75	40	25	2	3	150	1000
AMM-8	200	100	75	40	25	2	3	150	1000
AMM-9	200	75	50	40	25	2	3	150	500
AMM-10	200	75	100	40	25	2	3	150	500
AMM-11	200	75	50	40	25	2	3	150	1000
AMM-12	200	75	100	40	25	2	3	150	1000
AMM-13	200	75	75	40	25	2	3	150	750

AVR: Acyclovir; C-934P: Carbopol 934P; AIFM: *Azadirachta indica* fruit mucilage; EC: Ethyl cellulose; DCM: Dichloromethane; GAD: Glutaraldehyde; LP: Liquid paraffin; rpm: rotations per minute.

The melting point is a characteristic property of a pure substance and any deviation from the expected melting point can indicate impurities or a mixture of substances. Therefore, this method is valuable for quality control purposes, ensuring that the AVR being analyzed is in its pure form.

By determining the melting point using the open capillary method, researchers can gain insights into the thermal behavior of AVR and use this information for identification and quality assessment. It's worth noting that the accuracy of the melting point assessment depends on the precision of the experimental setup and the purity of the sample being tested.

Drug Excipient compatibility studies

DSC

A mixture of AVR and AIFM in a 1:1 ratio, totaling 10 mg, was meticulously prepared and subjected to analysis using a Differential Scanning Calorimeter (DSC) (Venchal Scientific-412105-USA). The DSC scan encompassed a temperature range of 50°C to 300°C. This analytical technique allows for the observation of thermal transitions within the sample. As the DSC measured the heat flow in and out of the sample, any associated peaks or changes in heat flow were indicative of phase transitions, such as melting or crystallization. The resulting thermogram provided valuable insights into the temperature-dependent behavior of the AVR and AIFM mixture, aiding in the characterization of its thermal properties and offering a deeper understanding of the stability and compatibility of these substances in the specified 1:1 ratio.

FTIR

The synergistic interaction between AVR and the excipients used was investigated using FTIR spectroscopy (Bruker instrument). The FTIR analysis involved scanning the sample in the wavenumber range of 4000 to 400 cm^{-1}. By examining the FTIR spectrum over this range, researchers can identify characteristic peaks associated with specific molecular vibrations, offering valuable insights into the chemical composition and bonding within the AVR and with the excipient mixture. This method is particularly useful for assessing the potential synergy between these components, as changes or shifts in the FTIR spectrum can indicate molecular interactions or modifications in the chemical structure, contributing to a more comprehensive understanding of their combined behavior.

Initial risk assessment

Following the principles outlined in ICH Q8 and Q9, the Quality Target Product Profile (QTPP) is viewed as dynamic within the framework of Quality by Design (QbD). This approach acknowledges the evolution of quality attributes throughout the product development process. It is recognized that the QTPP is not static and can be refined and adjusted as new insights emerge during the development stages. The initial stages of product development may not always provide a completely unbiased perspective, but QbD principles enable an iterative and adaptive approach.

For the specific case of AMM, the QTPP plays a pivotal role in guiding the development process. It leverages product attributes to ensure that the final product aligns with the essential quality standards. Through a thorough analysis of past explorations and literature decisions, the QTPP and Critical Quality Attributes (CQAs) for AMM have been scrutinized. This involves a comprehensive evaluation of the desired characteristics and performance criteria of the product, providing a foundation for the systematic design and optimization of the manufacturing process. The QbD framework, as per ICH guidelines, emphasizes a proactive and science-based approach to development, ensuring that the QTPP and CQAs are repeatedly assessed and refined as the understanding of the product evolves. This dynamic and iterative process contributes to the production of a high-quality pharmaceutical product with consistent performance and attributes.

Assessment of physical constraints

Particle Size

The determination of AMMs particle size was resoluted using a stage micrometer scale. In this method, dry AMMs samples were carefully placed on a clean glass slide. The examination was conducted using an eyepiece micrometer and the size of the AMMs particles was measured. The counting process involved a minimum of 200 individual AMMs per batch, ensuring a representative sample size for accurate particle size assessment.[19]

This approach allows for a systematic and statistically relevant evaluation of the particle size distribution within each batch of AMMs. By employing a stage micrometer scale and eyepiece micrometer, researchers can ensure precision in the measurements, contributing to the reliability of the particle size data. Monitoring and controlling particle size are crucial aspects of pharmaceutical development, as they directly impact the performance and characteristics of the product.

Production yield

The production yield was calculated using a straightforward ratio involving the average weight of the dried AMMs collected from each of the three trials and the total initial dry weight (e.q.-1).[20]

$$\%\text{Percentage Yield} = \frac{\text{Average Weight of Dried AMM (W1)}}{\text{Total Initial Dry Weight (W2)}} \text{X } 100 \text{--- (1)}$$

Here,

W_1 represents the average weight of dried AMMs collected from each of the three trials and,

W_2 is the total initial dry weight.

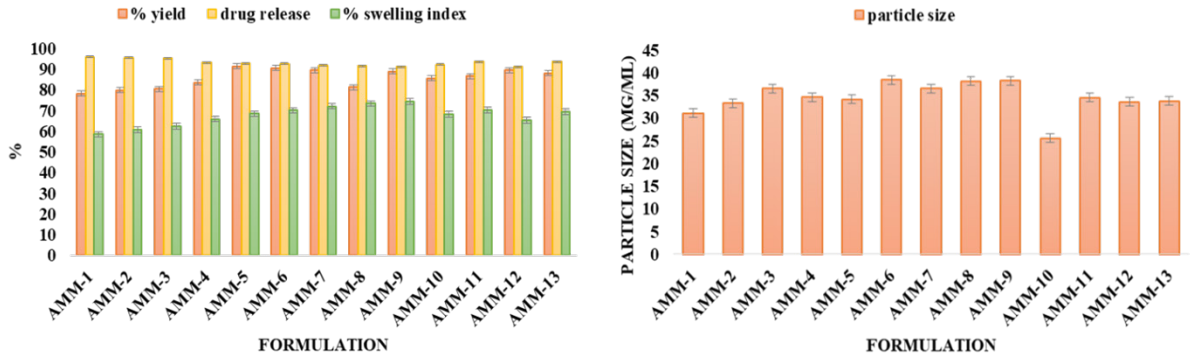

Figure 1: Various *in vitro* assessments for AMMs.

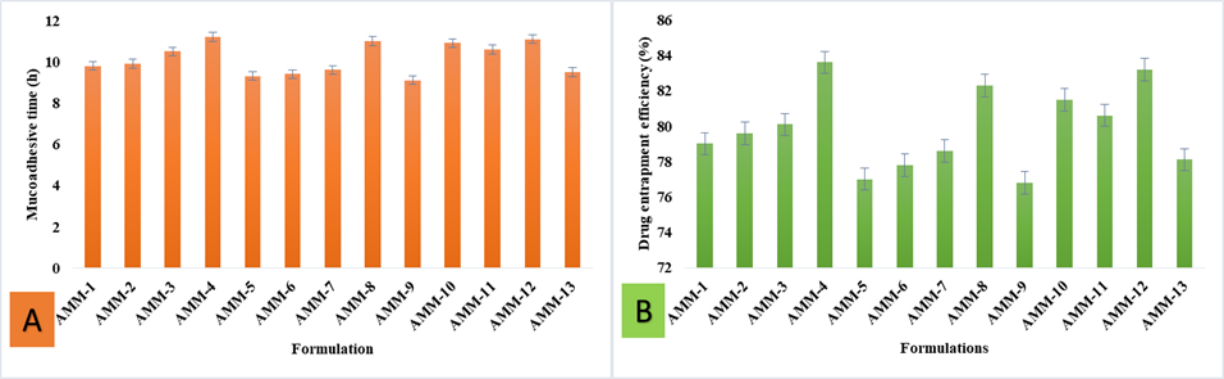

Figure 2: EE and MT of AMMs.

This calculation delivers a percentage that reflects the efficiency of the production process, indicating the proportion of the initial dry weight that was positively recovered as dried AMMs. A higher production yield is generally desirable, as it signifies a more efficient and effective manufacturing process for the AMMs.

Entrapment Efficiency

In the EE determination for the AMMs, 100 mg of the microspheres were dispersed in 0.1 M HCl overnight, accompanied by intermittent shaking. Following this dispersion, the mixture was filtered and the resulting filtrate was subjected to spectrophotometric analysis using a UV Spectrophotometer (Elico SL-174). The analysis was performed at a wavelength of 254 nm, which is likely the specific wavelength associated with the absorbance peak of the AVR.[19]

The EE was then calculated by considering the ratio between the actual amount of antibiotic present in the formulation, as determined through spectrophotometric analysis and the initially added amount. The EE is a critical parameter in DDS, reflecting the proportion of the AVR that is magnificently encapsulated within the AMMs during the manufacturing process. The spectrophotometric analysis at 254 nm delivers a quantitative measure of the AVR content in the formulation, allowing for an accurate assessment of the EE. This information is valuable in evaluating the effectiveness of the AMMs in retaining the AVR (e.q.2).[21]

$$EE = \text{yield theoretical} X\ 100 \text{---} (2)$$

Swelling index determination

The swelling behavior of AMMs were investigated by immersing them in 0.1M HCl. Following a 3 hr incubation, the samples were extracted and centrifuged and the gained weight was determined by calculating the difference between the weight at time t (Xt) and the initial weight at time t=0 (X0)(e.q.3).[22]

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

Where Xt-weight of the AMMs after time t; Xo- Initial weight of the AMM.

Determination of mucoadhesion

A 5 cm section of freshly cut sheep stomach, procured from a local slaughterhouse within 60 min of the animal's demise and subsequently eviscerated through washing with isotonic saline, was utilized to determine MT. Accurately weighed AMMs were applied to the mucosal surface and affixed to a polyethylene plate positioned at a fixed angle of 40° relative to a straight line. A solution of HCl (0.1M), maintained at 37±1°C, was administered to the tissue at a controlled rate of 5 mL/min. The time required

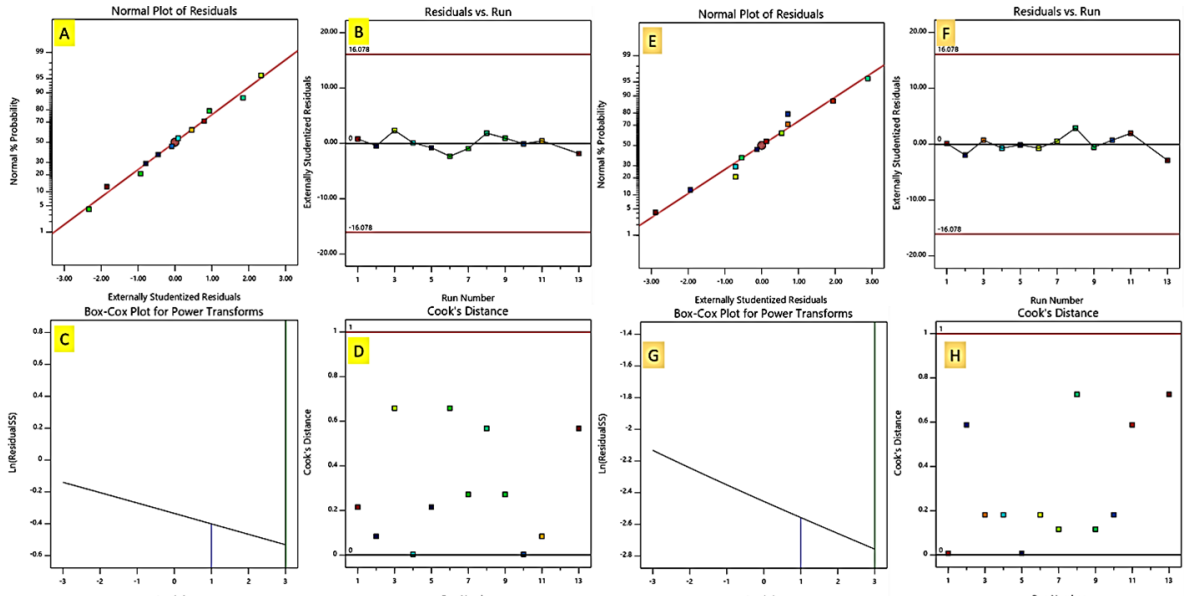

Figure 3A-H: Plots showing the interaction effect of polymers on EE and the MT of AMMs.

for complete detachment of all AMMs from the mucosal surface of the sheep's stomach was then measured through visual inspection (e.q.4).[23]

$$\text{Force of adhesion (N)} = \frac{\text{Mucoadhesive strength (g)} \times 9.81}{1000} \text{--- (4)}$$

In vitro AVR Release Study

The dissolution characteristics of AMMs were examined using the USP-II apparatus. The dissolution study involved stirring the AMMs samples at a rate of 50±5 rpm in a dissolution medium consisting of 0.1 N HCl at 37±0.5°C. The dissolution medium had a volume of 900 mL. At various time intervals, a 5 mL sample was withdrawn from the dissolution medium and the volume of the dissolution medium was replenished to maintain a consistent dissolution environment. The withdrawn samples were then subjected to spectrophotometric analysis at a wavelength of 254 nm. Spectrophotometric analysis at this specific wavelength is likely utilized to quantify the concentration of AVR, as it corresponds to a characteristic absorbance peak associated with the compound. This dissolution study, performed under controlled conditions mimicking the physiological environment, allows for the assessment of how effectively the AMM discharges AVR over time. Monitoring the dissolution profile is crucial for understanding the AVR release kinetics from the AMMs and is valuable information for AVR formulation and delivery optimization. The frequent sampling and subsequent spectrophotometric analysis offer a dynamic view of the dissolution process and help in generating dissolution profiles for further analysis.[24,25]

Statistical optimization

In the experimental design and analysis, Design-Expert software was employed to estimate the independent impacts on the retorts. The assessment of these effects was visualized through contour plots in two Dimensions (2D) and response surface plots in three Dimensions (3D). These plots deliver a graphical depiction of the association between the independent variables and the response. The statistical validation of polynomial equations derived from these plots was conducted by evaluating Analysis of Variance (ANOVA) tables. The ANOVA tables assessed the significance of the model terms and determined whether the observed variations in the response were statistically significant. The model's adequacy and predictive capability were established through statistical indicators derived from the ANOVA tables. Specifically, the F value, with a significance level (p-value)>0.05, was utilized to determine whether the model was statistically significant. A p-value below 0.05 indicates that at least one of the model terms has a significant effect on the response variable.

RESULTS

Identification of AVR

The AVR was yellowish-white in color. The observation of color and the congeniality assessment collectively underscore the meticulous selection and understanding of AVR's properties in the formulation process, emphasizing the importance of attaining a harmonious blend for the optimal development and efficacy of the final pharmaceutical product.

The comparison of the sample spectrum of AVR with the reference spectrum revealed a noteworthy observation: no significant changes were observed in the functional groups. This analysis suggests that the chemical structure and composition of AVR in the sample remained consistent with the reference standard, indicating the stability and integrity of the compound under the studied conditions. The absence of significant changes in functional groups is a crucial finding, as alterations in these

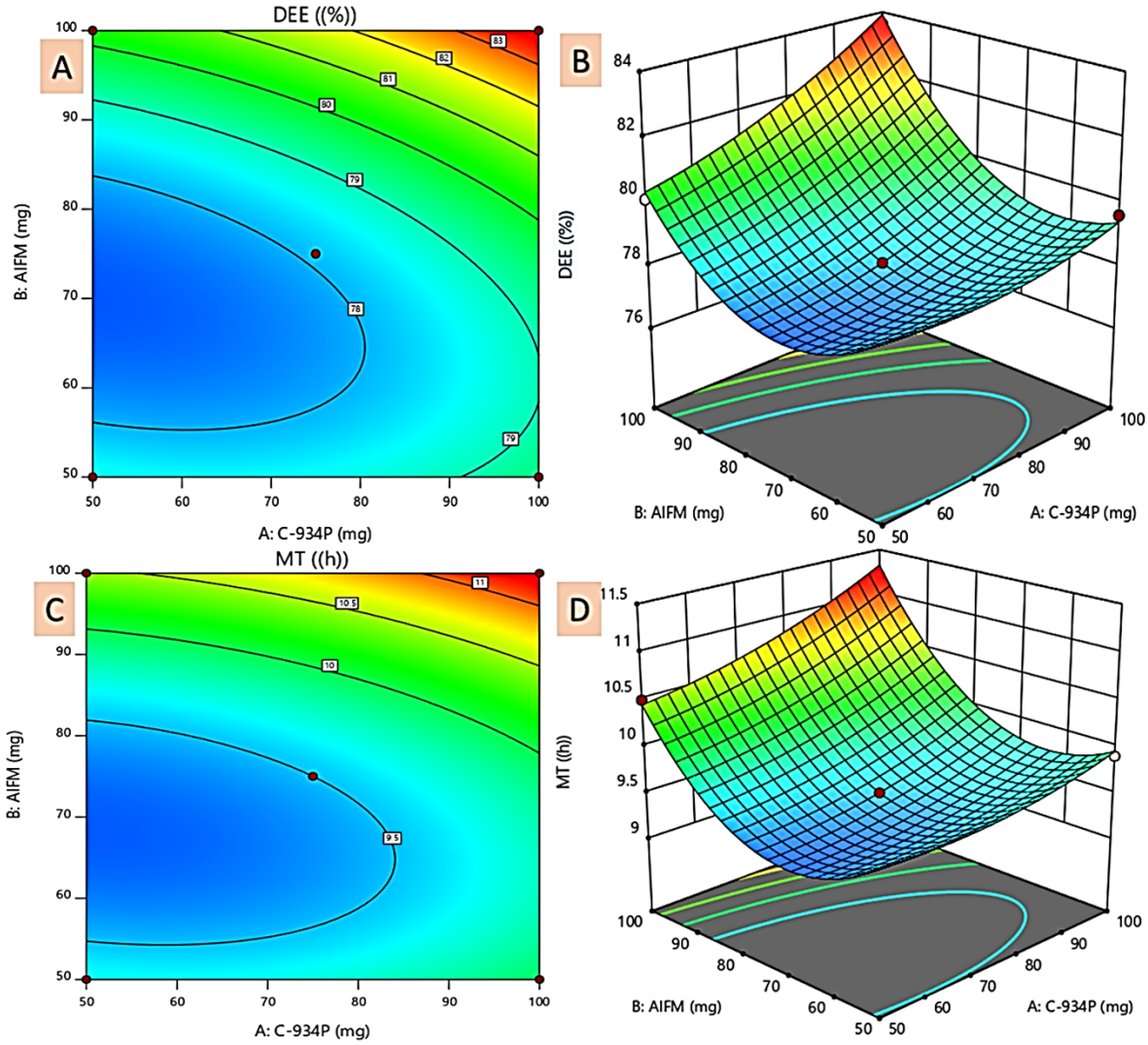

Figure 4: Contour plot and 3D response plot for EE and MT.

groups could potentially impact the drug's efficacy, safety and overall performance. The comparison reaffirms the reliability and quality of the AVR sample, aligning closely with the established reference standard. This analytical approach delivers essential insights into the molecular characteristics of the drug, contributing to the assurance of its consistency and suitability for use in pharmaceutical formulations or research endeavors.

Melting Point

The determination of the standard melting point of AVR yielded a specific value within a defined range: 254.9±2.4°C. This information is crucial for understanding the thermal behavior of the compound. The melting point is a fundamental characteristic of a substance, representing the temperature at which it transitions from a solid to a liquid state under standard atmospheric pressure. The specified range, along with the precision indicated by the ±2.4°C, delivers a degree of confidence in the accuracy of the measurement. Consistency in the melting point is indicative of the purity and crystalline nature of the AVR sample. Any deviations from this standard range could raise concerns about the compound's identity or quality, making the reported melting point a valuable parameter in assessing the stability and suitability of AVR for various applications, particularly in pharmaceutical formulations.

Compatibility studies

The results of the Differential Scanning Calorimetry (DSC) analysis revealed that pure AVR exhibited a distinct and sharp endothermic peak, indicative of its purity. When combined with excipients, this peak shifted left and became broader. DSC observations suggest that AVR does not interact significantly with the excipients.

In the FTIR analysis, bands corresponding to secondary amines, phenyl esters and carboxylic groups were evident in the pure AVR spectrum. Interestingly, in the blend (AMM-9), peaks and stretches similar to those in the pure AVR spectrum were observed. Additionally, the spectrum of AVR with excipients displayed unobstructed peaks and stretches, further indicating

Table 2: **Fit summary for the responses.**

Fit summary for EE (%)			
Source	**Sequential *p*-value**	**Adjusted R^2**	**Predicted R^2**
Linear	0.0059	0.6457	0.4655
2FI	0.4707	0.6407	0.2185
Quadratic	0.0248	0.9565	
Fit summary for MT (h)			
Linear	0.0162	0.5525	0.3216
2FI	0.3440	0.5993	0.1473
Quadratic	0.0221	0.9552	

Table 3: **ANOVA for the responses.**

EE (%)						
Source	**Sum of Squares**	**d_f**	**Mean Square**	**F-value**	***p*-value**	
Model	61.00	9	6.78	30.35	0.0086	Significant
A-C-934P	9.24	1	9.24	41.40	0.0076	
B-AIFM	19.22	1	19.22	86.06	0.0027	
C-rpm	16.82	1	16.82	75.31	0.0032	
AB	2.10	1	2.10	9.41	0.0546	
AC	2.10	1	2.10	9.41	0.0546	
BC	1.10	1	1.10	4.94	0.1128	
A^2	0.4375	1	0.4375	1.96	0.2561	
B^2	9.49	1	9.49	42.49	0.0073	
C^2	0.3432	1	0.3432	1.54	0.3032	
MT (h)						
Model	6.83	9	0.7594	29.40	0.0090	Significant
A-C-934P	0.6612	1	0.6612	25.60	0.0149	
B-AIFM	2.31	1	2.31	89.47	0.0025	
C-rpm	1.62	1	1.62	62.71	0.0042	
AB	0.0900	1	0.0900	3.48	0.1588	
AC	0.4225	1	0.4225	16.35	0.0272	
BC	0.4225	1	0.4225	16.35	0.0272	
A^2	0.0357	1	0.0357	1.38	0.3245	
B^2	1.20	1	1.20	46.51	0.0064	
C^2	0.0914	1	0.0914	3.54	0.1565	

that the presence of excipients did not interfere with the characteristic features of AVR in the FTIR analysis.

Quality Target Product Profile

The QTPP was employed to document CQAs and anticipated dosage forms. The method employed in the production of AMM demonstrated strength and reproducibility, ensuring alignment with drug product CQAs. Leveraging QbD principles, we explored the inspiration of CQAs, specifically EE and MT, on AMM responses. A careful examination of AMM identified AIFM and carbomer 934P, elucidating their impact on AVR discharge and MT and contributing to a comprehensive understanding of the formulation's behavior.

Physical properties

Particle size

Particle size analysis was conducted for all formulations utilizing optical microscopy. The AMMs exhibited a range in particle size, spanning from 31.9±0.7 to 39.9±0.3 µm (Figure 1).

Notably, formulation AMM-5 stood out with a larger particle size associated with the other formulations. This data delivers valuable insights into the physical characteristics of the AMMs, offering a quantitative perspective on their sizes and highlighting the potential variation among different formulations. Particle size is a critical parameter inducing AVR release kinetics and overall performance, making this analysis an essential aspect of the comprehensive characterization of the AMMs.

Yield of AMM

The AMMs demonstrated varying yields across formulations, ranging from 81.9±0.8 to 97.0±1.5%. Notably, formulations AMM-4 and AMM-6 exhibited the highest yields, as depicted in Figure 1. The percentage yield is a critical parameter in pharmaceutical manufacturing, reflecting the efficiency of the process in producing the desired AMMs. Higher yields, as observed in AMM-4 and AMM-6, suggest optimal conditions and reproducibility in the manufacturing process, indicating a positive and efficient synthesis of these particular formulations.

AVR entrapment

The EE of AMMs was observed to be 90.8±0.77 to 96.1±2.48, AMMs with lesser AIFM gave good AVR entrapment (Figure 2).

Swelling assessment

The study suggests that AIFM can serve as a valuable indicator for assessing mucoadhesion by analyzing the discharge pattern of AVR. This consideration is crucial when formulating mucoadhesive preparations. Subsequently, the impact of various concentrations of AIFM on the swelling indices of AMMs was investigated. The results indicated a steady decrease in the swelling index as the concentration of AIFM decreased. Among the batches tested, AMM-9 exhibited the highest swelling, followed by AMM-1, AMM-2 and AMM-3. It is suggested that lower concentrations of AIFM may not provide sufficient content to induce significant swelling, while AMM-7, AMM-8 and AMM-9, with potentially higher concentrations of AIFM, demonstrated increased swelling.

The presence of several polar compounds in AIFM contributes to its ability to absorb and retain water, resulting in notable swelling properties. These findings underscore the importance of AIFM concentration in influencing the swelling behavior of AMMs, which is a critical factor to consider in the formulation of mucoadhesive systems.

Mucoadhesion time

The mucoadhesive properties of all batches of microspheres were found to range from 17.3±0.12 g (AMM-1) to 19.3±0.05 g (AMM-9) (Figure 2). The data suggests that an increase in AIFM content, coupled with varying levels of Carbopol 934P, is associated with an anticipated increase in MT. This observation implies that the combination of higher AIFM content and Carbopol 934P levels enhances the ability of the mucoadhesive microspheres to adhere to the mucosal surface, resulting in prolonged MT.

In vitro AVR release

In the dissolution study of AMMs, a comparison between formulations AMM-6 and AMM-4 revealed favorable results. Specifically, the data indicated that at the end of 10 hr, there was a substantial AVR discharge of 92.2±2.3% for AMM-6 and 91.5±2.2% for AMM-4, as illustrated in Figure 2. This suggests that both formulations exhibit high and comparable rates of AVR release over the specified period, emphasizing their effectiveness in achieving the desired dissolution profile.

AVR estimation

Using a UV-vis spectrometer, a calibration curve for AVR estimation in a 0.1M HCl solution at a wavelength of 254 nm was successfully obtained. Beer's law was applied to construct the calibration curve and the curve was found to be within the range of 0-10 µg/mL. This calibration process was repeated three times, ensuring the reliability and reproducibility of the results. The generated calibration curve is valuable for determining the content uniformity of Acyclovir in the studied formulations, providing a basis for accurate and consistent estimation of AVR concentrations in the 0.1M HCl solution.

Fit summary

It appears that there is a mention of a "Fit Summary" for the responses of EE and MT in Table 2. This summary likely provides an overview of how well the experimental data fits the model used to analyze these responses. The fit summary in Table 2 might include statistical measures such as R^2 values, *p*-values, or other relevant metrics that assess the goodness of fit for the models employed in the study. These values are crucial in evaluating the reliability and accuracy of the models in predicting or explaining the variability in the responses (EE and MT).

ANOVA for Quadratic model

The mention of ANOVA for the responses, specifically EE and MT, in Table 3 suggests that statistical analysis has been performed to assess the variability and significance of these responses. ANOVA is commonly used to determine whether there are any statistically significant differences between the means of three or more groups.

Table 3 likely presents the results of the ANOVA analysis and it might include various statistical parameters such as F-values, *p*-values and degrees of freedom. These values help in understanding the significance of the factors or variables considered in the responses (EE and MT). The outcomes of the ANOVA are crucial in concluding the impact of different factors on the observed variations in EE and MT in the study.

The results of the ANOVA for two models are presented, indicating the significance of the model and specific terms within each. In the first model, the substantial model F-value of 30.35 underscores its overall significance, with only a 0.86% probability that such a large F-value could result from random noise. Model terms X_1, X_2, X_3 and X_2^2 are identified as statistically significant, as reflected in their p-values being less than 0.05. Similarly, the second model demonstrates significance, as evidenced by a model F-value of 29.40 and a mere 0.90% probability of such an F-value arising due to noise. The p-values less than 0.05 for specific model terms in the second model further emphasize their statistical significance. These findings collectively affirm the importance of the considered factors and their respective contributions to the variability observed in the responses under investigation.

ANOVA details for the responses

The F-value analysis indicates the overall significance of the model and in this case, the model terms X_1 and X_2 are found to be significant. Model terms with a value greater than 0.1 are considered not significant. Based on the coding factors, the final equation for EE is provided as EE (%)=+78.10+1.07X_{1+}1.55X_2+1.45X_3+0.725X_1X_2+0.725X_1X_3-0.525X_2X3+0.4375X_1^2+2.04X_2^2+0.3875X_3^2. This equation, with coded factors, allows for the prediction of response levels based on the values of the factors. The equation helps identify the relative impact of the factors, where factors with high levels are coded as +1 and those with low levels as -1.

Similarly, the final equation for MT in terms of coded factors is expressed as MT=+9.50+0.2875X_1+0.5375X_2+0.45X_3+0.15X_1X_2 +0.325X_1X_3 - 0.325X_2X_3+0.125X_1^2+0.725X_2^2+0.2X_3^2. This coded equation can be employed to predict responses based on the specified levels of each factor, where high levels are coded as +1 and low levels as -1. The coded equation facilitates the comparison of factor coefficients, aiding in the identification of the relative impact of the factors on MT.

Diagnostic analysis for EE

The goodness of fit for the EE was assessed using diagnostic plots (Figure 3A-D). In the outwardly studentized residuals plot, the colored points representing EE cluster around the normal probability line, affirming the normality of the residuals and validating the appropriate analysis of the response data. This conformity is further supported by the stratified arrangement of residuals around a straight line, meeting the normality hypothesis (Figure 3A). The residuals versus predicted values plot demonstrates that the colored points of EE fall within acceptable limits, indicating the validity of the assumption of constant variance (Figure 3B), as supported by the random distribution of studentized residuals. The residuals versus run number plot identify variables predictive of EE and all points suggest that the data closely approximates a normal distribution (Figure 3C). Additionally, Cook's distance plots for EE<1 have indicated acceptable effects, suggesting that data points are less likely to significantly impact predictions (Figure 3D). These diagnostic plots collectively support the adequacy of the model for EE, validating its reliability and appropriateness for further analysis.

Diagnostic analysis for MT

Diagnostic plots were employed to assess the goodness of fit for MT (Figure 3E-H). In the normal likelihood plot of superficially studentized residuals, the majority of colored points representing MT align closely with the normal probability line, confirming the normality of residuals and endorsing the appropriate analysis of response data. This alignment indicates that the residuals are distributed normally, as evidenced by the straight line (Figure 3E). The plot of externally studentized residuals versus predicted values reveals that the colored points of MT fall within the predetermined limits. The random distribution of studentized residuals in this plot supports the correctness of the assumption of constant variance (Figure 3F). These diagnostic plots collectively suggest that the model for MT adequately fits the data, confirming the normality of residuals and supporting the validity of assumptions made during the analysis. This plot reveals variables that could have prejudiced MT based on residuals versus the run numbers. A consistent pattern of no outlying reflections and steady running was evident at all the points (Figure 3G). The cook's distance plots for the MT below 1 is an acceptable effect and the data point is less likely to significantly impact the predictions (Figure 3H).

To navigate the design space effectively, the model utilizes 2D contour plots and 3D response surface plots, as depicted in Figures 4A and 4B. These plots serve as valuable tools for studying the interaction effects of the factors on the responses for EE.

The contour plot and response surface plot, illustrated in Figures 4C and 4D, respectively, reveal a consistent increase in the MT of AMMs with a concurrent escalation in the levels of carbomer 934P and AIFM. These plots provide visual insights into the relationships between the factors and the response, aiding in the interpretation of the complex interactions that influence the MT. The design space exploration facilitated by these plots enhances the understanding of the formulation parameters and their impact on the desired responses.

DISCUSSION

The study embarked on a comprehensive exploration of the development and characterization of AMMs. Multiple facets of the investigation were meticulously discussed, encompassing the physical and chemical attributes of the constituents, the intricacies of the manufacturing process, the optimization of the formulation through the application of QbD principles and the scrutiny of CQAs.[26]

The initial observation highlighted the yellowish-green color of the fresh AIFM, a crucial component serving as an aid in the formulation of AMMs. The compatibility of AVR was assessed

using spectral analysis, revealing that there were no significant alterations in the functional groups. Additionally, the standard melting point of AVR was determined, providing essential information about its thermal behavior.[27]

DSC played a pivotal role in further unraveling the characteristics of AVR. The analysis indicated that pure AVR exhibited a sharp endothermic peak, signifying its purity. Notably, the study suggested that the interaction between AVR and excipients did not substantially impact AVR's purity, providing important insights into the formulation's stability.

The adoption of QTPP principles was crucial in systematically documenting CQAs and anticipated dosage forms. This approach ensured a robust and reproducible method for the production of AMM. The study, guided by QbD principles, delved into understanding the impact of critical quality attributes (EE and MT) on AMM responses.

The study conducted an in-depth analysis of particle sizes within AMM formulations, uncovering a diverse range of sizes. Notably, a specific formulation, AMM-5, stood out with a larger particle size compared to the others. This detailed examination of particle sizes is crucial as it offers insights into the physical characteristics of the AMMs, persuading aspects such as AVR release kinetics and overall performance. In addition to particle size analysis, the study delved into other key parameters to comprehensively evaluate the performance of different formulations. The percentage yield, a measure of the efficiency of the manufacturing process, was assessed. Formulations exhibiting higher yields suggest more effective and reproducible production methods, contributing to the economic viability of the manufacturing process.[28]

The study also examined AVR entrapment within the AMMs. The EE is a critical factor in DDS as it indicates the proportion of the AVR positively encapsulated within the AMMs. Formulations with high AVR entrapment values are indicative of effective AVR loading, which is essential for the therapeutic efficacy of the AMMs.[29]

Furthermore, the assessment of mucoadhesion properties is pivotal for understanding the interaction between the AMMs and mucosal surfaces. Mucoadhesive properties play a significant role in prolonging AVR residence time at the target site, enhancing therapeutic effectiveness. The study demonstrated overall favorable performance across different formulations concerning mucoadhesion properties, suggesting that the AMMs exhibit the desired adhesive characteristics. The in-depth particle size analysis, along with the evaluation of percentage yield, AVR entrapment and mucoadhesion properties, delivers a comprehensive understanding of the physical attributes and performance characteristics of AMM formulations. This multifaceted analysis is crucial for optimizing formulation parameters, ensuring efficient AVR delivery and advancing the development of effective AMMs.[30]

The dissolution studies conducted in this investigation play a pivotal role in understanding the discharge pattern of AVR from AMMs. The results of these studies offer valuable insights into the performance of different formulations, specifically highlighting the excellent discharge percentages observed in formulations AMM-6 and AMM-4.

Dissolution studies are crucial in pharmaceutical research as they simulate the conditions under which AVR is expected to be discharged into the human body. In this case, the dissolution studies focused on AMM formulations containing AVR. The fruitful discharge of AVR from the AMMs is a critical aspect of the formulation's efficacy. Formulations AMM-6 and AMM-4 demonstrated exceptional discharges, indicating that these specific formulations are highly effective in delivering AVR into the dissolution medium. The factors contributing to the superior discharge from these formulations could include optimized particle size, EE, or specific characteristics of the excipients used in the formulation. This information is crucial for further development and optimization of the AMM formulations.[31]

Additionally, the establishment of a calibration curve for AVR estimation in a 0.1 M HCl solution is a noteworthy aspect of the study. This calibration curve serves as a quantitative tool to determine the concentration of AVR in solution at 254 nm. By utilizing a calibration curve, researchers can accurately assess the content uniformity of AVR in the dissolution medium, ensuring that the AVR is consistently present at the desired concentration.

The determination of content uniformity is a critical quality attribute in pharmaceutical formulations, ensuring that each dosage unit contains the intended amount of the active pharmaceutical ingredient. The calibration curve in 0.1M HCl solution delivers a reliable method for estimating AVR concentration during dissolution studies, enhancing the precision of the content uniformity assessment.

In this study, rigorous statistical analyses, including ANOVA and diagnostic plots, were systematically employed to validate mathematical models concerning EE and MT. The application of ANOVA allowed for an in-depth assessment of the significance of differences among group means, specifically determining whether the formulated models for EE and MT were statistically significant. This analysis involved comparing variances within and between groups, with a significant F-value indicating the substantial contribution of at least one model term to the observed variation in the response variables.[32] Complementing ANOVA, diagnostic plots, such as Normal Probability Plots, Residuals vs. Predicted Values Plots and Residuals vs. Run Number Plots, were instrumental in scrutinizing the assumptions and adequacy of the statistical models. These plots provided insights into the normality of residuals, the constancy of variance and the presence of patterns or outliers. Furthermore, the assessment of the significance of various model terms elucidated the individual impact of factors

and their interactions on EE and MT. A careful examination of *p*-values allowed for the identification of statistically significant terms, ensuring the accuracy and reliability of the mathematical models.[33] Collectively, these statistical approaches facilitated a nuanced understanding of the interactions between different factors and the observed responses, enhancing the credibility and robustness of the study's findings.

CONCLUSION

In the context of mucoadhesive drug delivery systems, control over the rate and quantity of Acyclovir (AVR) release is influenced by the mucoadhesive polymers utilized. In this study, AVR was formulated as mucoadhesive microspheres in conjunction with *Azadirachta indica* Fruit Mucilage (AIFM) and carbomer 934 P. Notably, formulations AMM-1 to AMM-3 exhibited favorable entrapment, particularly as the AIFM content decreased. Interestingly, higher concentrations of AIFM were associated with increased mucoadhesive time across all batches. This observation suggests that mucoadhesive microspheres of AVR, incorporating AIFM with the assistance of carbomer 934 P, meet the desired criteria for effective mucoadhesive microspheres. Such formulations demonstrate the potential to be retained in the stomach, offering enhanced bioavailability and thereby presenting a promising avenue for mucoadhesive drug delivery systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AVR: Acyclovir; **hr:** Hour; **AIFM:** *Azadirachta indica* fruit mucilage; **DOE:** Design of experiments; **RSM:** Response surface methodology; **BBD:** Box Behnken Design; **MT:** mucoadhesion time; **EE:** Entrapment efficiency; **DDS:** Drug delivery system; **%:** Percent; **RH:** Relative humidity; **AMM:** Acyclovir mucoadhesive microspheres; **C-934P:** Carbopol 934P; **EC:** Ethyl cellulose; **DCM:** Dichloromethane; **GAD:** Glutaraldehyde; **LP:** Liquid paraffin; **rpm:** Rotations per minute; **FTIR:** Fourier Transform infrared; **DSC:** Differential Scanning Calorimeter; **QTTP:** Quality Target Product Profile; **QbD:** Quality by Design; **CQA:** Critical Quality Attributes; **HCl:** Hydrochloric acid; **2D:** Two dimensions; **3D:** Three dimensions; **ANOVA:** Analysis of Variance.

SUMMARY

- Mucoadhesive microspheres are required for localized drug delivery in the stomach.
- The mucoadhesive microspheres were made with *Azadirachta indica* fruit mucilage with the use of Acyclovir.
- Box Behnken Design was adopted using Design expert software with *Azadirachta indica* Fruit Mucilage (AIFM), carbomer 934P and stirring speed as inputs and drug entrapment and mucoadhesion time as dependent variables.
- Higher concentrations of AIFM showed good Acyclovir entrapment with extended mucoadhesion time.

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