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A Novel Delivery Of Ketoprofen Loaded Gastroretentive Mucoadhesive Nanoparticles Fabricated By Gelatin/Pluronic F68 For The Management Of Rheumatoid Arthritis

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ABSTRACT

A promising medication delivery method that can target specific medications at the absorption site is the use of mucoadhesive nanoparticles. This experiment was conducted to develop mucoadhesive gastroretentive ketoprofen nanoparticles, a non-steroidal anti-inflammatory medicine used to treat rheumatoid arthritis (RA).

To carry out the research, a new experimental design was fabricated. This new design made use of a three-factor, two-level central composite. The desolvation procedure was applied to generate nanoparticles that are mucoadhesive and gastroretentive. The formulations were evaluated by various preliminary parameters and transmission electron microscopy(TEM), Fourier-transform infrared spectroscopy (FTIR), zeta potential, particle size, and entrapment efficiency.

To ascertain the adhesion characteristics and release kinetics of the improved batch, in vitro drug release and ex-vivo mucoadhesion tests evaluation were conducted. The idea was to develop a formulation that would enable the stomach to deliver drugs continuously. Keeping therapeutic drug levels in the stomach for a longer period may be used to treat RA symptoms effectively.

Keywords: *Ketoprofen, Nanoparticles, Mucoadhesion, Gastroretentive, Desolvation.*

INTRODUCTION

The administration of a medicine by the oral route has the highest rate of patient compliance. However, drugs having short half-lives and rapid absorption from the gastrointestinal tract (GIT) require frequent dosing to maintain therapeutic drug levels. Sustained-release oral formulations aim to gradually release medication into the GIT and sustain therapeutic concentrations over an extended period.[1-3] Various pharmacological formulations have been developed for gastric retention, including floating, swellable, high-density, and mucoadhesive systems.[4-5] Mucoadhesive drug delivery systems have received significant

attention as gastroretentive formulations. These systems can adhere to the epithelial surface and prolong drug retention at the absorption site, resulting in controlled release over an extended duration and reducing dose requirements.[6-7] Nanoparticles that remain attached to the gastric mucosa i.e. Stomach-specific mucoadhesive nanoparticles (SSMN) continuously release the drug before the absorption site to provide regulated delivery while maximizing bioavailability.[7-8] The development of mucoadhesive particulate formulations serves two key purposes for oral administration in treating conditions like rheumatoid arthritis (RA).

Firstly, mucosal adhesion prolongs the transit time of drug carriers in the GI tract, extending the release window. Secondly, increased drug absorption can occur as mucoadhesion swells and fills mucosal fissures, there by increasing the effective surface area that is in contact with the intestinal mucosa and producing high local drug concentrations as a result.[6, 8] This study aimed to develop ketoprofen-loaded mucoadhesive nanoparticles using desolvation with polymers gelatin and Pluronic F68. The suitability of the produced nanoparticles for drug delivery was evaluated.

MATERIAL AND METHOD

Materials

The Shanghai Huirui Chemical Technology Co. in China was the source of the ketoprofen that was used. In India, Sigma Aldrich Chemicals Pvt Ltd was the vendor for the acquisition of pluronic F68. Sisco Research Laboratories Pvt. Ltd., located in India, was the supplier of the acetone. A local vendor was consulted for the procurement of gelatin as well as other reactants. Each one of the compounds was of an analytical grade.

Methodology

Assessment of Drug-Excipient Compatibility

Through the use of Fourier-transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) analysis, it was possible to determine whether or not ketoprofen and excipients in physical combinations were compatible with one another. FTIR was performed to detect any interactions between materials based on shifts or changes in functional group vibrations. DSC analyzed thermal behaviour and checked for the presence of any new thermal events indicative of interactions.[9]

Differential scanning calorimetry

An apparatus with model number DSC-4000 was used to carry out the differential scanning calorimetry (Perkin Elmer, USA). An indium standard was used in the calibration process of the instrument. A nitrogen environment with a flow rate of 60 mL/min was used, and the samples, each weighing 2 mg, were placed in aluminium pans, sealed, and heated from 60 degrees Celsius to 240 degrees Celsius at a rate of 10 degrees Celsius per minute. When recording the sample thermograms, a pan that was otherwise empty was used as the reference.[10-11]

Fourier-Transform Infrared Spectroscopy

Equipment designated as an FT-IR Alpha Bruker 1206 0280 was utilized in order to carry out Fourier-transform infrared (FTIR) spectroscopy (Germany). By combining 1 mg of each sample with 1 mg of potassium bromide, we were able to create thin pellets. The spectra were recorded at a temperature of room temperature between 4000-400 cm-1.

A mucoadhesive polymeric nanoparticle formulation of ketoprofen is presented here.

Using an approach that involved modification of the desolvation process, ketoprofen-loaded polymeric nanoparticles were created.[12] Gelatin was dissolved in water that had been distilled at a temperature of 40 degrees Celsius, give or take one degree, and magnetic stirring was used throughout the process. A different amount of ketoprofen was mixed with organic solvent and then disseminated. Along with the Pluronic F-68, this medication dispersion was put into the polymeric solution. HCl or NaOH was utilized to bring the pH of the mixture down to 2.0-0.05 while it was magnetically agitated at a predetermined speed for thirty minutes.

Next, dropwise acetone was added at a controlled rate. After 10 minutes, glutaraldehyde was introduced to crosslink the nanoparticles. The reaction continued with stirring for a set time and speed. The nanoparticles were purified via centrifugation at 16,000g for 20 minutes at 4°C, then redispersed in an acetone: water mixture. The supernatant was removed, and nanoparticles were resuspended in distilled water before storage in vials for further analysis.[9,12]

The goal was to develop a modified desolvation technique for producing mucoadhesive ketoprofen nanoparticles with optimized properties for drug delivery applications. Various processing and formulation parameters were evaluated.

Experimental design

Utilizing the response surface methodology, systematic optimization was carried out with the goal of determining the components that were the most important and gaining an understanding of the connection between responses and factors (RSM). The amounts of Pluronic F-68 and gelatin that were selected for optimization were determined based on screening investigations. A core composite design was utilized within the Design-Expert program for planning each of the seventeen experiments. The design called for five center-point replicates to be carried out in a randomized sequence.[13]

The data were analyzed using a technique called response surface regression. In order to choose the most appropriate polynomial model, we looked at terms that had a significance level of p 0.05, a lack of significance in terms of lack of fit, a low coefficient of variance, and correlation coefficients. In order to facilitate curvature evaluation and rotatability, a face-centred central composite design was adopted.[14] We defined the upper and lower limits for the variables that were independent.

The RSM was examined using a design with two factors and three levels. The coded and actual values of the independent variables and the limits placed on the dependent variables are presented in tables 1 and 2. The results of the dependent variables are listed in Table 2 for a variety of stirring speeds and amounts of polymer that were utilised to construct nanoformulations. The significance of the effect that independent variables had on response regression coefficients was investigated using ANOVA. In addition, F-tests and p-values were computed by the software. Through the utilisation of 3D response surfaces and contour plots, we were able to analyze the relationship that exists between the dependent and the independent variables. These plots are useful for investigating the influences of numerous factors at the same time as well as anticipating the responses of the dependent variables at intermediate levels of the independent variables.[15]

Table 1: Conversion of encoded levels into real values

Coded Level	Gelatin (X1)	Pluronic F 68 (X2)	Stirring Speed (X3)
-1	200 mg	100 mg	200 rpm
0	500 mg	275 mg	600 rpm
+1	800 mg	450 mg	1000 rpm

Table 2. Observed responses of formulations and the degree of independent variables.

Formulation	Independent Variables			Dependant Variables	
	Gelatin (X1)	Pluronic F 68 (X2)	Stirring Speed (X3)	Particle size (nm)	Entrapment efficiency (%)
F1	500	275	-72.717132202972	543 ±1.3	54.47 ±0.1
F2	200	450	1000	550 ±0.3	61.79 ±0.4
F3	500	-19.3137453388	600	680 ±0.3	59.36 ±0.9
F4	800	450	1000	567 ±0.9	76.45 ±0.1
F5	800	450	200	850 ±1.4	65.19 ±0.2
F6	200	100	200	578 ±0.1	50.98 ±1.0
F7	800	100	200	798 ±1.0	54.37 ±0.1
F8	800	100	1000	650 ±3.2	63.29 ±0.6
F9	200	100	1000	449 ±2.0	55.46 ±0.1
F10	500	569.3137453388	600	844 ±1.4	69.34 ±0.9
F11	500	275	600	316 ±1.3	92.93 ±1.0
F12	500	275	600	325 ±1.5	91.19 ±1.1
F13	500	275	600	314 ±0.9	91.46 ±0.1
F14	500	275	1272.717132203	170 ±0.5	67.34 ±1.0
F15	200	450	200	860 ±0.1	59.87 ±0.1
F16	1004.5378491522	275	600	880 ±1.0	70.1 ±0.3
F17	-4.5378491522288	275	600	750 ±0.1	58.93 ±0.2

An investigation of the mucoadhesive and gastroretentive properties of nanoparticles

Analysis of the Size of Particles

Using a Nano-ZS Zetasizer, the particle size and polydispersity index (PI) were determined at 25°C (Malvern Instruments, Malvern, UK). Using disposable cells, the data were obtained by averaging three runs at a 173° scattering angle.[16]

Effectiveness of Entrapping

Using UV–visible spectrophotometry with a wavelength of 258.3 nm, we were able to estimate the amount of ketoprofen that was present in the supernatant.[17] For the purpose of determining the entrapment efficiency, the following equation was utilized:

Entrapment efficiency (%) = (Amount of ketoprofen initial - Amount of ketoprofen in supernatant) / Amount of ketoprofen initial x 100

Morphological Assessment

In order to investigate the specimens transmission electron microscope was utilised (H-7100, Hitachi Ltd., Tokyo, Japan). After being diluted 1:10 in deionized water, the samples were inspected under a microscope. One drop of the diluted sample was placed on a copper grid that was three millimetres in thickness and covered with carbon. The samples were given a negative stain using a uranyl acetate solution that contained 2 percent (w/v) uranyl acetate for two minutes. The filter paper was then used to remove any excess stain that had been applied. After allowing the samples to dry at room temperature for a period of time, images were taken of them using the integrated camera system.[18]

Drug Release Studies in Vitro

The dialysis sack method with Sigma's DO405 Dialysis tubing 2315mm (Frankfort, Germany) was used for the aim of researching the kinetics of drug release. A dialyzing membrane (10-12 KD) that had previously been prepared with 5 mL of the optimal Formulation was given an initial addition of 0.1 N HCL in a volume of 100 mL. This step was performed immediately after the initial step. Aliquots were taken out at regular intervals over 8 hours, with the old medium being replaced with the new medium. These samples were then examined with a spectrophotometer set at 258 nm after being appropriately diluted.[19-20]

Measurement of Mucoadhesion

The technique assesses the polymer's adherence to the mucosa. Mucoadhesive nanoparticles were meticulously diluted and applied to a glass slide containing goat intestinal mucosa. In order to facilitate interaction with the membrane, the slide was incubated for 15 minutes at 90% relative humidity. After that, it was positioned in a 45° angle cell that was attached to an assembly, and 1 ml/min of 0.1 N HCl was poured over the membrane and nanoparticles. Following the collection, washing, separation, and weighing of the nanoparticles at various intervals, the percentage of mucoadhesion was computed as follows: % mucoadhesion = Wa/W1x100, where Wa represents the initial weight of the nanoparticles, and W1 represents the weight of the nanoparticles collected.[21-23]

RESULT AND DISCUSSION

Compatibility of the Excipients

The FT-IR spectrum of pure ketoprofen had characteristic peaks that were compatible with the research that had been done in the past.[24] There were peaks found at 3294 cm-1 for the C=C aromatic stretching, 1658 cm-1 for the ketone C=O stretching, 1691 cm-1 and 1228 cm-1 for the carboxylic acid C=O and CO stretching, respectively, and 2733-3290 cm-1 for the carbonyl group stretching (O-H broad band stretching). The spectra of physical mixtures and optimized formulations showed these same characteristic peaks, indicating no interactions between ketoprofen and excipients. Figure 1 shows no apparent changes or loss of peaks, suggesting a lack of drug-ingredient or ingredient-ingredient interactions.

Thermal analysis was performed using differential scanning calorimetry (DSC), as shown in Figure 2. Pure ketoprofen exhibited an endothermic peak at 97.68°C consistent with its reported melting point.[25] Similarly, the physical mixture and optimized batch showed no new peaks or shifts in peak position, indicating no drug-polymer or polymer-polymer interactions. These FTIR and DSC studies confirmed the compatibility of ketoprofen within the optimized Formulation.

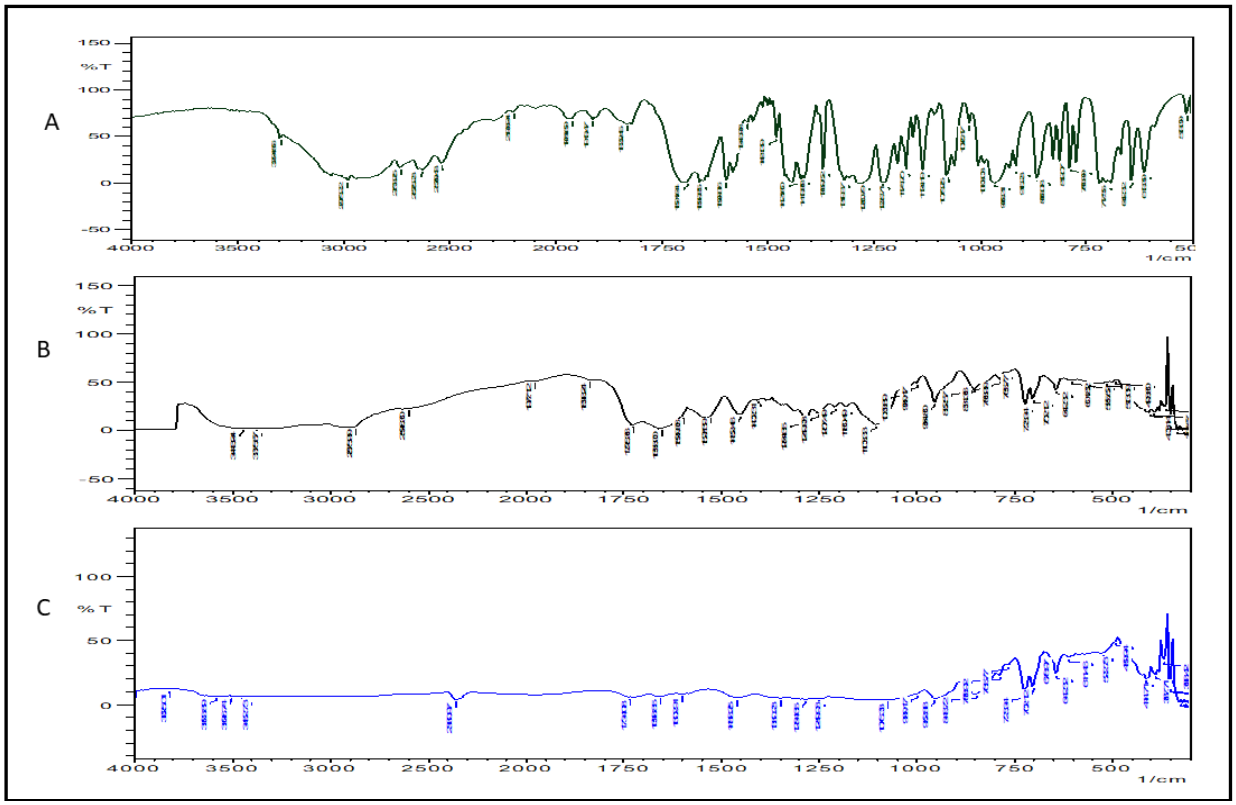

Figure 1. Spectrum of FTIR

A. Pure drug ketoprofen B. Physical Mixture with polymers C. Optimized Formulation

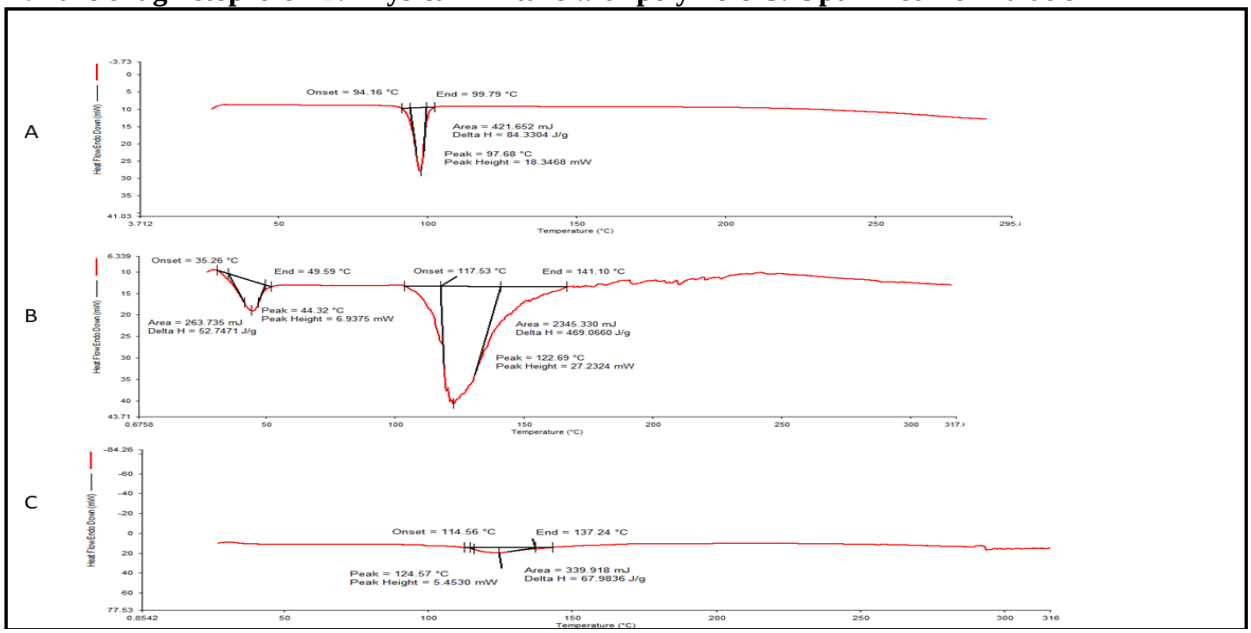

Figure 2. DSC thermogram of Pure drug(A), Physical mixture(B) and Optimized batch (C)

Variables in the gastroretentive mucoadhesive nanoparticle optimization approach

The encapsulation effectiveness and particle size of ketoprofen nanoparticles were investigated using a three-factor, two-level central composite design.[26] The three independent variables were polymer concentration (X1), solvent type (X2), and stirring rate (X3). Table 2 displays the results of the desolvation technique used in the preparation of seventeen different formulations (F1-F17).

The encapsulation efficiencies ranged from 50.99% to 92.93%, according to Table 2. It was discovered that the polymer concentration, the kind of solvent, and the stirring rate all had a substantial impact on the amount of drug loading and the efficiency of encapsulation.[27-28] The average particle sizes of the formulations ranged from 170-870 nm. Figure 3 shows that the optimized batch F11 had a mean particle size of approximately 300 nm.

The zeta potential of Formulation F11 was -41.4 mV, as shown in Figure 3. Higher zeta potential leads to greater electrostatic repulsion between particles and thus improved stability. Transmission electron microscopy (Figure 4) revealed that the nanoparticles were spherical and porous in structure. Overall, formulation F11 demonstrated desirable encapsulation, particle size, zeta potential, and morphology for oral delivery applications.

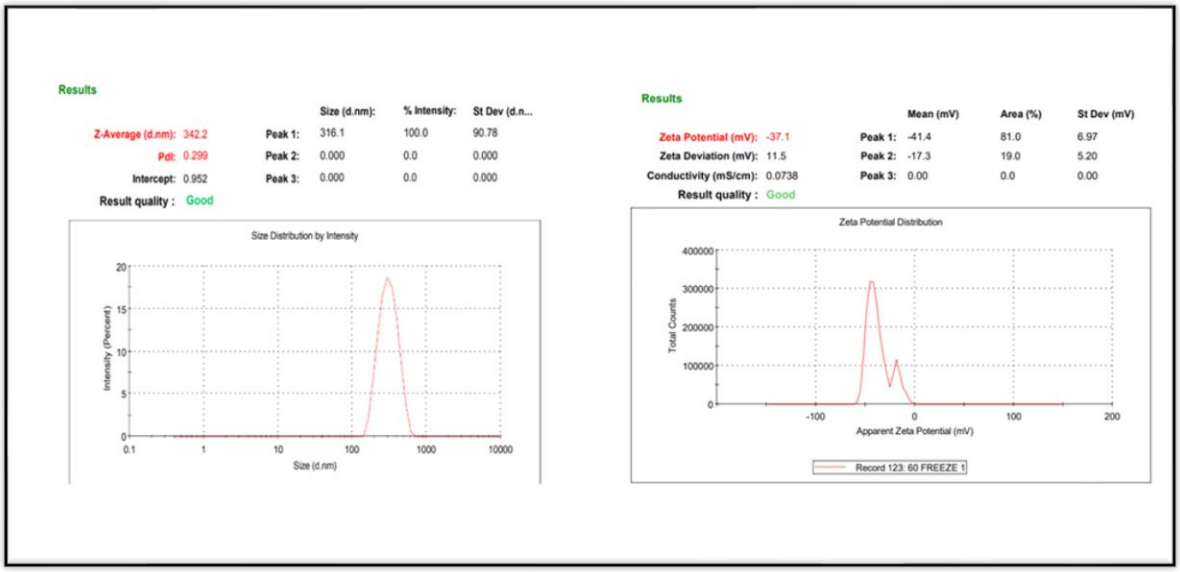

Figure 3: Optimized batch's zeta potential, as well as its mean particle size after optimization

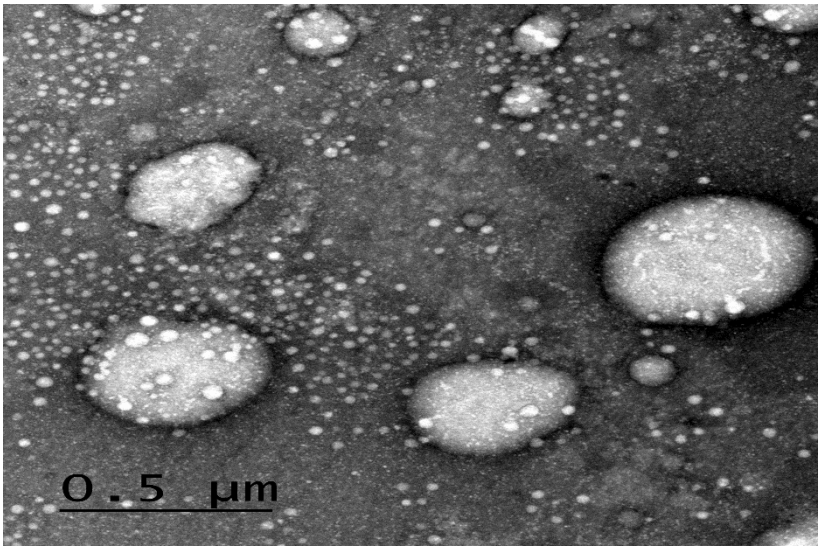

Figure 4. TEM Images of the optimized batch

Statistical Analysis

The coded equations can be used to predict responses based on factor levels and understand their relative impacts by comparing coefficients.[28-29]

Table 3. The coded equation for the responses Y1 and Y2

For encapsulation efficiency (Y1)
Y1=318.43+47.35A+45.97B-10964C-51.75-51.75AB+1.00AC-39.50BC+175.26A2+156.52B2+13.15C2
For particle size (Y2)
Y2 = 91.88 + 3.68A + 4.09B + 3.54C + 1.06AB + 1.77AC - 0.0588BC - 9.79A2 - 9.85B2 - 11.06C2

These equations show the effect of independent variables (A: polymer concentration, B: solvent type, C: stirring rate) on the dependent variables (Y1: encapsulation efficiency, Y2: particle size). Coefficients were compared to identify factors with the greatest influence.

Table 3: ANOVA findings for R1 and R2

Responses	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Adequate Precision	
R1	Quadratic	< 0.0001	0.1413	0.9966	0.9888	73.125	Suggested
R2	Quadratic	< 0.0001	0.2191	0.9848	0.9514	30.9388	Suggested

The predictive power of the models can be evaluated based on the coefficient of determination (R2) value. For encapsulation efficiency (Y1), the R2 value was close to 0.98, indicating that the model can explain around 98% of the total variability in the response. For particle size (Y2), R2 was approximately 0.99, suggesting the model accounts for 99% of the variation in Y2. The remaining variability may be due to noise or other factors not included in the models. Overall, the high R2 values demonstrate that the developed models fit the experimental data very well and can provide an accurate description of the influence that independent factors have on the replies.

Table 4: Results of an ANOVA on the relationship between particle size and entrapment efficiency

	Response R1		Response R2	
Source	Model	Lack of fit	Model	Lack of fit
Sum of Squares	7.902 E+05	1093.06	28.6081	17.66
df	9	5	9	5
Mean Square	87804.28	218.61	317.87	3.53
F-value	529.06	6.37	114.34	3.91
P-value	<0.0001		<0.0001	0.2159
	Significant	Not significant	Significant	Not significant

The fact that the model F-values for responses R1 and R2, which came in at 529.06 and 114.34, respectively, were extremely significant (p less than 0.0001) demonstrates that the models were accurate in their prediction of the answers. P-values for the model terms that were less than 0.05 indicated that they played a significant role in influencing the answers. The lack of fit F-values for R1 and R2 were not significant ($p > 0.05$), demonstrating that the models were viable for navigating the design space. The lack of fit F-values were 6.37 for R1 and 3.91 for R2. Therefore, the fact that the lack of fit was not statistically significant and the high F-values suggest that the models that were built were appropriate for describing the real process within the test ranges.

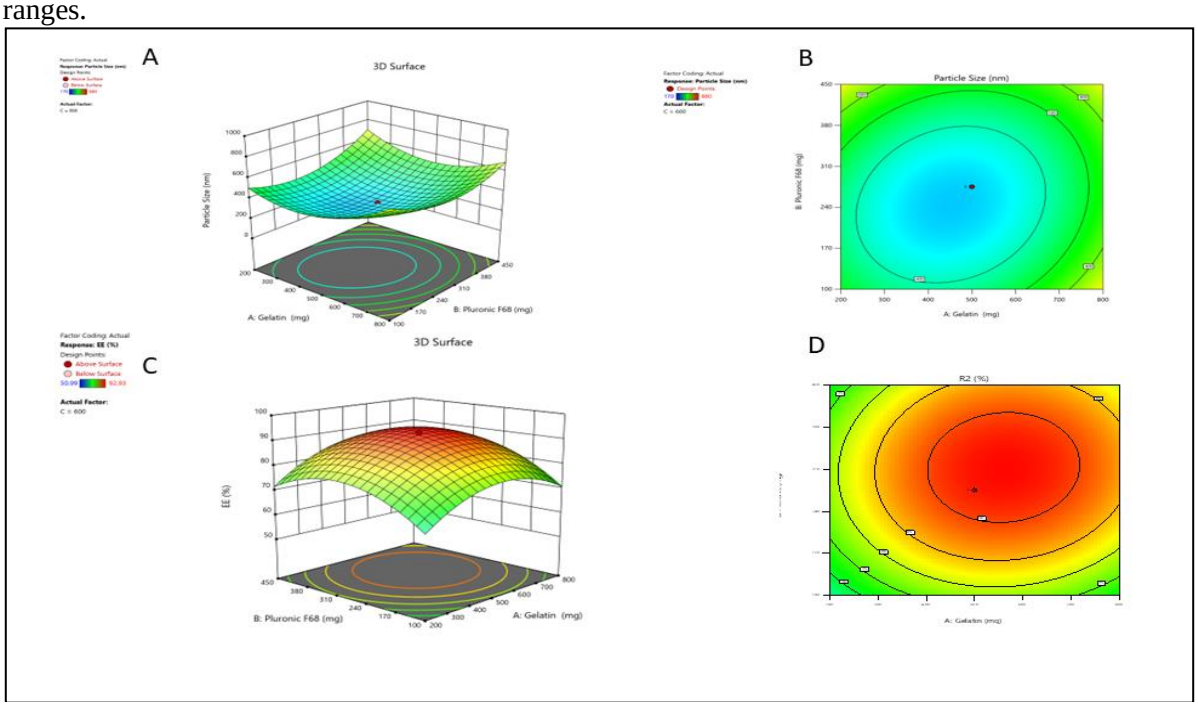

Figure 5: Plots of 3D response surfaces show how stirring speed and polymer concentration affect particle size and entrapment at various drug-to-polymer ratios.

A visual depiction of the relationship between the independent variables and the answers is provided by the 3D surface plots and the 2D contour plots that are generated by the Design-Expert software. As seen in Figure 5, particle size (Y2) increased with rising polymer concentration (A) but was less influenced by changes in stirring speed (C). An optimal polymer ratio is necessary to form stable nanoparticles during processing. Within the design space, a minimum particle size could be achieved by maintaining the polymer amount while allowing flexibility in agitation. These plots aid in identifying critical factor levels for obtaining an optimized formulation.

Mucoadhesion Study

The mucoadhesion of the nanoparticles was evaluated using an ex vivo wash-off method to simulate gastrointestinal conditions. This test measures the ability of the polymer coating to adhere to gastrointestinal mucosa upon continuous washing with simulated fluids. A higher mucoadhesion percentage indicates stronger adhesion to mucin surfaces and better localization at the absorption site. The optimized Formulation (F11) exhibited 74.65% mucoadhesion, demonstrating good adhesion properties of the polymer coating. This would facilitate increased residence time and drug absorption from the delivery system.

Drug Release

Examining the in vitro drug release from the nanoparticles is the goal of this study; a dialysis bag diffusion method was utilized. Figure 6 is a scatter plot depicting the cumulative proportion of medication that has been released from formulation F11 over time. To gain an understanding of the process underlying the release, the data were evaluated using several different kinetic models, including zero-order, first-order, Higuchi, Korsmeyer-Peppas model and Hison-crowell cube root plots. As can be shown in Table 5, Formulation F11 had the highest correlation coefficient (R2 = 0.9922), which indicated that it follows the Korsmeyer-Peppas model release kinetics the most accurately compare to the r^2 values. This suggests that the release of the drug was super case-II transport existed between the dissolving liquid and the delivery device.

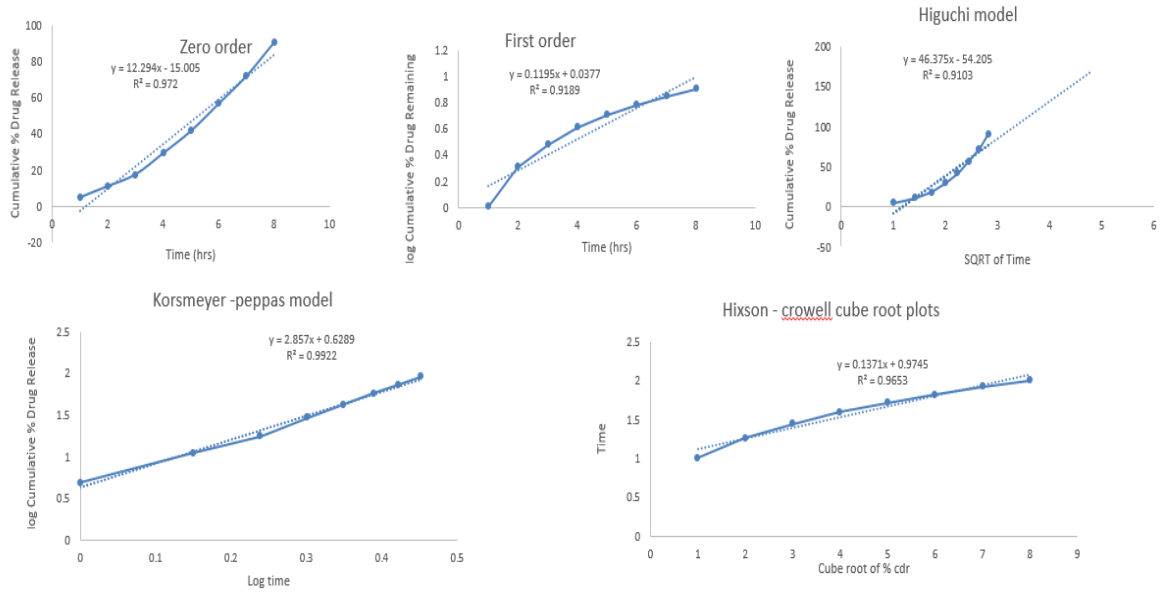

Figure 6. Kinetic release profile of drug- Zero order, First order, Higuchi, Korsmeyer-Peppas model and Hixson-Crowell cube root plots

Table 5: Optimal batch kinetic release model

Optimized Batch	Zero – Order (r^2)	First-order (r^2)	Korsmeyer–Peppas (r^2)	Higuchi(r^2)	Hixon-Crowell(r^2)
F11	**0.972**	**0.9189**	**0.9922**	**0.9103**	**0.9553**

4. CONCLUSION

Ketoprofen mucoadhesive nanoparticles were successfully optimised by the central composite design. This ensured the greatest possible medication release and stability at the location that was being targeted. After

conducting compatibility tests with DSC and FTIR, the researchers found that ketoprofen and the polymers did not interact with one another. Because of their diminutive size, nanoparticles have a huge surface area, which contributes to increased solubility and dissolution. The strong zeta potential helps to improve the repulsion of particles and the stability of dispersion. It was confirmed that Formulation F11 is suitable for use as a gastroretentive delivery system by the fact that it displayed a high entrapment efficiency (92.93 percent) and mucoadhesion (74.65 percent). The results of the statistical study confirmed that F11 was the best formulation. However, in vivo investigations are still necessary to establish the in vitro efficacy of these polymeric mucoadhesive nanoparticles for oral ketoprofen administration. These nanoparticles were tested in a laboratory setting.

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