"Optimizing Pharmaceutical Formulations: Advancements in Nanosuspension Pre-Formulation to Enhance Solubility and Bioavailability of Active Agents"

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Abstract: This study aimed to enhance the bioavailability of a poorly water soluble drug by developing a nanosuspension through an innovative formulation method. The nanosuspension was produced with meticulous attention to the materials reliability and excellent quality. Nepafenac was characterised in the pre-formulation study by establishing its melting point, solubility in different solvents, organoleptic properties, and creating a calibration curve. We utilised UV spectroscopy to determine the maximum wavelength (λmax) of Nepafenac and construct a standard calibration curve in ethanol. To ensure the compatibility of the medicine with the polymers (Pluronic F127 and HPMC E 5), Fourier Transform Infrared Spectroscopy (FTIR) was utilised for drug interaction analysis. The nanosuspension was produced using the solvent diffusion approach. The effect of different concentrations of Poloxamer 407 and HPMC E 5 on the formulation's effectiveness was investigated experimentally using a 32 complete factorial design. We used Response Surface Methodology (RSM) and statistical analysis to enhance the formulation concerning in vitro release, particle size, and viscosity. The pre-formulation characteristics of Nepafenac met the standards outlined in the Pharmacopoeial regulations. The maximum absorption wavelength (λmax) of Nepafenac was confirmed by UV spectroscopy, and a reliable calibration curve was created. The FTIR analysis showed no chemical interaction between the medicine and polymers. RSM and experimental design indicated that Poloxamer 407 and HPMC E 5 had a substantial impact on the performance of the nanosuspension. Analysis of variance (ANOVA) confirmed the model and parameters that influence controlled drug release percentage, particle size, and viscosity. Optimal conditions for enhancing drug delivery and reducing particle size were determined by the analysis of contour and response surface plots. The optimal parameter values, as indicated in the desirability plots, closely matched the anticipated values. The experimentation confirmed the viability and effectiveness of the nanosuspension formulation generated based on the RSM findings.

Keywords: Nanosuspension, bioavailability, poorly soluble drug, formulation, pre-formulation study, optimization, experimental design, drug delivery.

INTRODUCTION

The pursuit of enhancing drug bioavailability, particularly for medicines with low solubility, continues to be a significant focus in pharmaceutical research. The restricted bioavailability of these medications is frequently attributed to their low solubility, which presents considerable obstacles in attaining effective therapeutic results. Throughout time, researchers have investigated many methods of drug delivery to improve the solubility and absorption rates of drugs.[1-2]

Out of these options, nanosuspensions have become a favourable approach because they may effectively address problems related to solubility and enhance the absorption of drugs. Nanosuspensions are composed of medication particles that are only a few nanometres in size and are stabilized in a dispersion medium.[3-4] These suspensions provide faster dissolving rates and better absorption into the body, resulting in higher bioavailability. Although there have been significant breakthroughs in medication delivery using nanotechnology, there is still a lack of study in comprehending the important connection between pre-formulation studies and the effective creation of nanosuspensions. [5-6]

The research of pre-formulation factors, such as drug characterization, solubility assessments, compatibility studies, and their impact on the formulation and development of nanosuspensions, is an area that has not been well investigated. This work seeks to address this disparity by conducting an in-depth analysis of the pre-formulation studies related to nanosuspensions.[7-8] The objective is to uncover the crucial factors that influence the development, stability, and improvement of the bioavailability of medications with low solubility through the formulation of nanosuspensions.[8-9]

This research aims to use careful analysis and experimentation to get useful knowledge on how to improve nanosuspension formulations. The ultimate goal is to enhance the delivery and effectiveness of medications that have low solubility.

The instruments and methods:

The study utilised various critical components to formulate and produce a nanosuspension with the goal of improving the bioavailability of a poorly soluble medication. Nepafenac, the active pharmaceutical ingredient (API), was obtained from Maya Biotech in Chandigarh. Pluronic F 127, an amphiphilic triblock copolymer, was obtained from Sigma-Aldrich in Bangalore to improve the stability and dispersion of the formulation. HPMC E 5, a hydrophilic polymer serving as a stabiliser and viscosity enhancer, was purchased from Loba chemie pvt. Ltd. Mumbai. Additionally, ethanol, sourced from the same supplier, was used as a solvent to help dissolve and solubilise the components of the formulation. Benzalkonium chloride, obtained from Loba chemie pvt. Ltd. in Mumbai, was used as a surfactant to stabilise the nanosuspension.

Pre-formulation analysis:

In the pre-formulation analysis of nepafenac, a calibration curve was established to ensure the highest quality of the drug and accurate assessment of its characteristics [10-14]. This involved rigorous testing, including sensory analysis to evaluate hue, scent, and visual attributes. The determination of the drug's melting point was carried out using the capillary approach, cross-referenced against a reference standard for accuracy. Furthermore, the solubility of nepafenac in various solutions, such as pure water, methanol, ethanol, DMSO, and DMF, was investigated. Solubility tests involved subjecting devices containing different solvents to an excess of the active pharmaceutical ingredient (API), stirring at intervals, and conducting spectrophotometric analysis on filtered solutions at a wavelength of 235 nm. These comprehensive analyses form a critical foundation for understanding nepafenac's physical and chemical properties, ensuring its suitability for pharmaceutical formulation.[15-17]

UV Spectroscopic Analysis:

Determining the maximum wavelength (λ max) of Nepafenac:
The UV spectrophotometer played a crucial role in the analysis of Nepafenac, with a focus on determining its maximum absorbance (λ max) through careful dilution. A meticulous calibration curve was established for Nepafenac by dissolving it in ethanol, with reagents prepared according to predefined standards to ensure accuracy. The process involved dissolving 5 mg of Nepafenac in a 10 ml volumetric flask using methanol, and subsequent extraction of 0.1 ml to 0.5 ml from this sub-stock buffer, employing methanol as the reagent. This systematic approach generated dilutions ranging from 1 µg/ml to 10 µg/ml, laying the groundwork for in-depth analysis. Simultaneously, a drug interaction study was conducted to investigate potential interactions, adding another layer of insight into the compound's behaviour and suitability for pharmaceutical applications. [18-19]

Fourier Transform Infrared Spectroscopy (FTIR):
The exploration of drug polymer interactions involved the analysis of Fourier transform infrared (FTIR) spectra using a Shimadzu FTIR 8400S optical bench from Japan. A physical amalgamation of the drug alongside two polymers, Pluronic F-127 and HPMC E 5, each weighing 10 mg, was meticulously prepared. This combination was then blended with 400 mg of potassium bromide. Through the application of a hydraulic press at a pressure of 15 tons, approximately 100 mg of this amalgam was compressed, resulting in the formation of a translucent pellet. The scanning of the spectra encompassed a range from 4,700 to 340 cm using the Shimadzu FTIR 84008 spectrophotometer. Subsequently, a comparative analysis was conducted between the IR spectra of the physical amalgamation and those of the individual pure medication and polymers, aiming to discern any potential incompatibilities or interactions among the components.[20]

Nanosuspension formulation using the solvent diffusion method:
The solvent diffusion method was employed for the formulation of Nepafenac, involving a step-by-step process. A predetermined quantity of Nepafenac was dissolved in 5 ml of the organic solvent, Ethanol. This solution was then slowly injected into a 25 ml aqueous phase composed of HPMC E 5 and Poloxamer 407, utilizing a syringe equipped with a 24-gauge needle. Subsequently, the mixture underwent homogenization for 10–20 minutes at a high speed of 12,000–18,000 rpm, facilitated by a Digital Ultra Turrax homogenizer from Germany. To eliminate any residual solvent, the formulation was subjected to magnetic stirring for 2 to 3 hours. This meticulous process ensures the uniform distribution of Nepafenac in the formulation and the removal of any traces of the organic solvent, contributing to the overall quality and stability of the final product.[21]

Table 1: Composition of Nanosuspension

Materials	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9
Nepafenac	25	25	25	25	25	25	25	25	25
Ethanol	7	7	7	7	7	7	7	7	7
Pluronic	1.0	1.0	1.0	0.15	0.15	0.15	0.2	0.2	0.2
HPMC E 5	0.3	0.4	0.5	0.3	0.4	0.5	0.3	0.4	0.5
Benzalkonium chloride	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Experimental Design (3^2 full factorial Design):

In the experimental design phase, a robust 32 full factorial design was employed to systematically investigate the impact of key variables on the formulation process of Nepafenac emulgel. The two critical factors under scrutiny were the concentration of Poloxamer 407 (X1) and the concentration of HPMC (X2). These variables were varied at three levels: -1, 0, and +1, resulting in nine distinct experiments or batches. Design Expert 13 software was utilized for efficient statistical analysis and response surface modeling.

The viscosity values for both Poloxamer 407 and HPMC were selected as the dependent variables. Multiple linear regression analysis was conducted, leading to the development of quadratic models. These models, expressed through quadratic equations encompassing coefficients for individual factors and their interactions, provided a comprehensive representation of the factors' impact on viscosity.

The resulting polynomial equations allowed for the evaluation of how changes in each factor, both independently and in combination, influenced the specific responses. The examination of the coefficients magnitudes and mathematical signs within the equations enabled the identification of positive or negative influences of each factor on viscosity outcomes. [22-25]

Table 2: Factor levels and formulation specific responses in a 32-way complete factorial study.

Elements (unrelated variables)	Factors level used		
	Low (-1)	Medium (0)	High (+1)
Amount of Poloxamer 407	0.1	0.15	0.2
HPMC E 5	0.3	0.4	0.5
Responses (dependant variables)			
Y1=% In Vitro Release **Y2=**Particle size (nm) **Y3=**viscosity (cps)			

RESULTS AND DISCUSSION:

The pre-formulation analysis of Nepafenac reveals its distinctive organoleptic properties as a yellow, odourless, crystalline, and amorphous powder. The determined melting point of 177±3°C aligns with the specifications in the 2011 US Pharmacopoeia, signifying the temperature range for its phase transition. Solubility assessments in various solvents highlight its insolubility in water but solubility in ethanol and DMSO, crucial information for formulation considerations. This comprehensive examination ensures the quality and characteristics of Nepafenac are well-established, forming a critical foundation for subsequent pharmaceutical formulation endeavors.

Analysis using UV spectroscopy:

Measurement of the maximum wavelength (λmax):

The UV spectroscopy analysis of Nepafenac revealed a peak absorbance at 237.2 nm (λmax), which aligns with the specified requirement. The Nepafenac Indian Pharmacopoeia exhibited an absorption maximum at a wavelength of 237.2 nm.

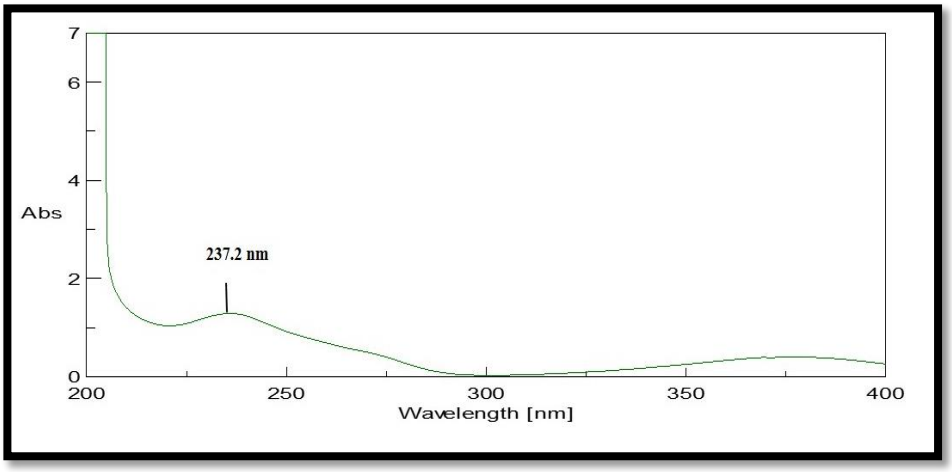

Figure 1: Aspects of Nepafenac UV absorption spectrum

Nepafenac Conventional Calibration Relationship:

A calibration curve for nepafenac was generated using concentrations ranging from 4 µg/ml to 20 µg/ml in ethanol, with measurements taken at a wavelength of 237.2 nm. A plot was created to show the relationship between absorbance and concentration, and the data was analysed using linear regression. A graphical representation of the drug's standard calibration curve in ethanol was presented.

Table 3: Calibration curve for Nepafenac in ethanol

Sr. No	Concentration (µgm/ml)	Absorption
1	0	0
2	4	0.2872
3	8	0.5256
4	12	0.7757
5	16	1.0627
6	20	1.3319

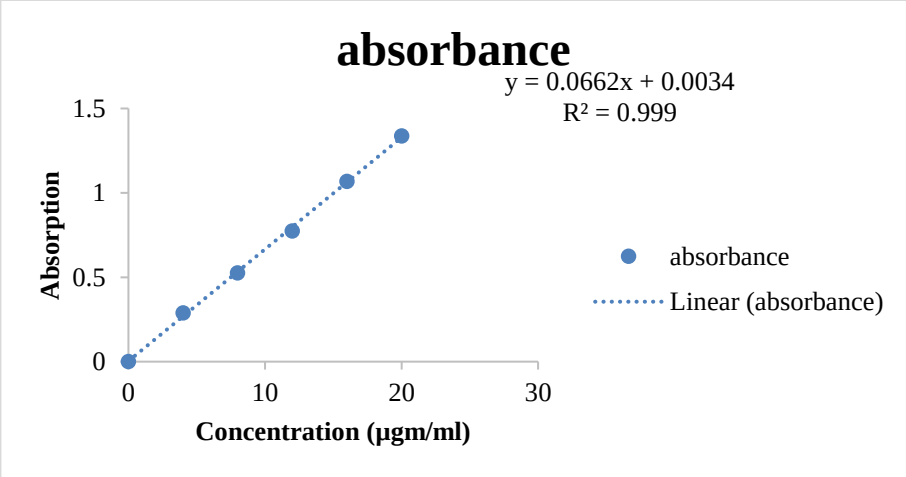

Figure 2: Nepafenac calibrating curve in ethanol

Infrared Spectroscopy using a Fourier Transform (FTIR):

The study employed Fourier Transform Infra-Red Spectroscopy (FTIR) to investigate the interaction between Nepafenac, excipients (Poloxamer 407 and HPMC E 5), and their physical mixture. The FTIR spectra analysis revealed no new peaks in the physical combination, indicating no significant interactions or elimination of the drug and polymers. Specific frequencies in Nepafenac's FTIR spectra aligned with the reference spectrum, confirming its identity. Similarly, the FTIR spectra of Poloxamer 407 and HPMC E 5 matched reported frequencies, affirming their composition. The physical mixture's FTIR analysis showcased the presence of all major peaks, consistent with the

reference spectrum. This comprehensive FTIR study ensures the compatibility of components, supporting the formulation's integrity and potential pharmaceutical applications.

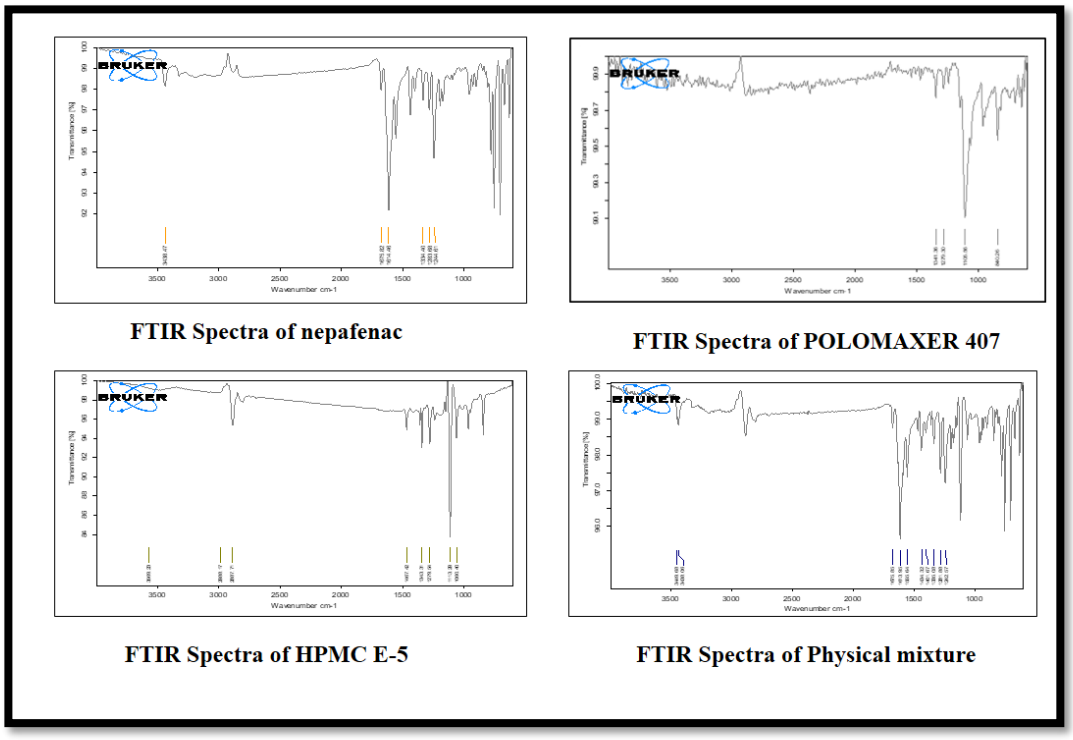

Figure 3: Fourier Transform Infra-Red Spectroscopy (FTIR) study

Design of the Experiment (3^2-Fall Factorial Design):

The nanosuspension formulation was optimized using a two-level (32) factorial design, with nine batches prepared based on specified formulation variables. The study focused on assessing the impact of Poloxamer 407 and HPMC E 5 on in vitro drug release (DR), particle size (PS), and viscosity (CP) using Response Surface Methodology (RSM). Three quadratic equations (Equations 1-3) were formulated to express the relationships between the independent variables and the responses. The coefficients in these equations were analyzed to determine the measurable effects of Poloxomer 407 (A) and HPMC E 5 (B) on each response. The negative or positive signs of the coefficients in the equations provided insights into the direction and strength of the variables' influence. The statistical significance of the correlation coefficient (r2) was highlighted, confirming the reliability of the quadratic model in describing the relationships among in vitro drug release, particle size, and viscosity. This approach facilitated the identification of significant factors and their optimization for enhanced nanosuspension formulation performance.

Table 4: Factorial experiment design with three variables (Poloxamer 407, HPMC E 5, and responses:

Batch	Variable Level (Coded Form)	Variable Level (Actual Form)	Poloxamer 407	HPMC E 5	% In-vitro Release	Particle Size (nm)	% Viscosity
F1	0.5	0.5	1.0	0.3	88	739	65
F2	0.5	0.75	1.0	0.4	82	835	58
F3	0.5	1	1.0	0.5	81	721	72
F4	0.75	0.5	0.15	0.3	90	449	68
F5	0.75	0.75	0.15	0.4	85	600	70
F6	0.75	1	0.15	0.5	82	428	75
F7	1	1	0.2	0.5	95	400	95
F8	1	0.75	0.2	0.4	73	450	85
F9	1	1	0.2	0.5	72	510	76

This table outlines the factorial experiment design with three variables (Poloxamer 407, HPMC E 5, and responses: % In-vitro Release, Particle Size, % Viscosity) at different levels, both in coded and actual forms. The design follows a 32 factorial design, and the responses are measured for each batch in the fall season.

DR Response I (% in-vitro Dung Release *):
The model is found to be significant (P = 0.0053), indicating that at least one of the factors has a significant effect on the response. The individual factors (A-Poloxamer, B-HPMC E 5) and their interactions (AB) are also significant, with low P values. The residual, representing unexplained variation is relatively small. The F values and P values help determine the significance of each factor and interaction

Table 5: Analysis of variance (ANOVA) for the percentage of drug release (% DR) in the nanosuspension formulation.

Parameter	Value
Std. Deviation	1.22
R^2	0.9865
Mean	80.89
Adjusted R^2	0.9640
C.V%	1.50
Predicted R^2	0.8369
PRESS	-
Adeq. Precision	19.4413

The corrected R^2 of 0.9640 is in good agreement with the expected R^2 of 0.8369, suggesting a discrepancy of less than 0.2. It seems that the model provides a satisfactory explanation for the observed data variability. The signal-to-noise ratio is a measure of precision. With a ratio greater than 4, it is ideal to have adequate signals, and a result of 15, 19, and 441 is considered excellent.

It is evident that A and B have a significant impact on the visual perception. The variation in percentage of drug released in vitro, as influenced by factors A and B, was shown using counter and response surface plots using a complete factorial design (32). It was determined that a moderate amount of A and a low level of B provide favourable conditions for achieving a larger percentage of in-vitro drug release.

Response 2 (Particle size):
The analysis of Particle Size in the Nanosuspension batches indicates that an increase in both Poloxamer 407 and HPMC E 5 results in an increase in particle size. The Particle Size for various Nanosuspension batches ranged from 298 nm to 835 nm. Notably, Batch P2 exhibited a larger particle size, with a measured composition of Poloxamer 127: HPMC at a ratio of 0.1:0.4.

The statistical analysis shows that the model is significant, indicating that the factors under consideration (Poloxamer 407 and HPMC E5) have a substantial impact on Particle Size. The individual factors, A (Poloxamer 407) and A2, also exhibit significant effects on Particle Size. However, the interaction term (AB) and the squared term of HPMC E5 (B2) do not show statistical significance in influencing Particle Size.

Fit Statistics:
The signal is considered sufficient based on the ratio of 17.038. This paradigm is applicable for navigating the de.

Response 3 (% Viscosity) (%CP):
Regression analysis of equation (3) shows that the coefficient of G is positive and B is positive. This indicates that when the amount of Poloxamer 407 (A) increases, the viscosity percentage also increases. The viscosity of various Nanosuspensions ranges from 65% to 95%. The batch fg exhibited the greatest maximum viscosity among all the batches tested, with a composition of Poloxamer 407 to HPMC in a ratio of 0.2:0.5.
Model F-value of 34.74 shows the model is significant at the statistical level. Only 0.74% of the time can an F-value of this size be explained by random chance.

Statistical significance of the model terms is indicated by p-values lower than 0.0500. Important model terms in this case are A, B, and A^2. Any value above 0.1000 indicates that the terms in the model are not significant. Model reduction may improve your model if it has many unnecessary words (apart from those required for hierarchy).

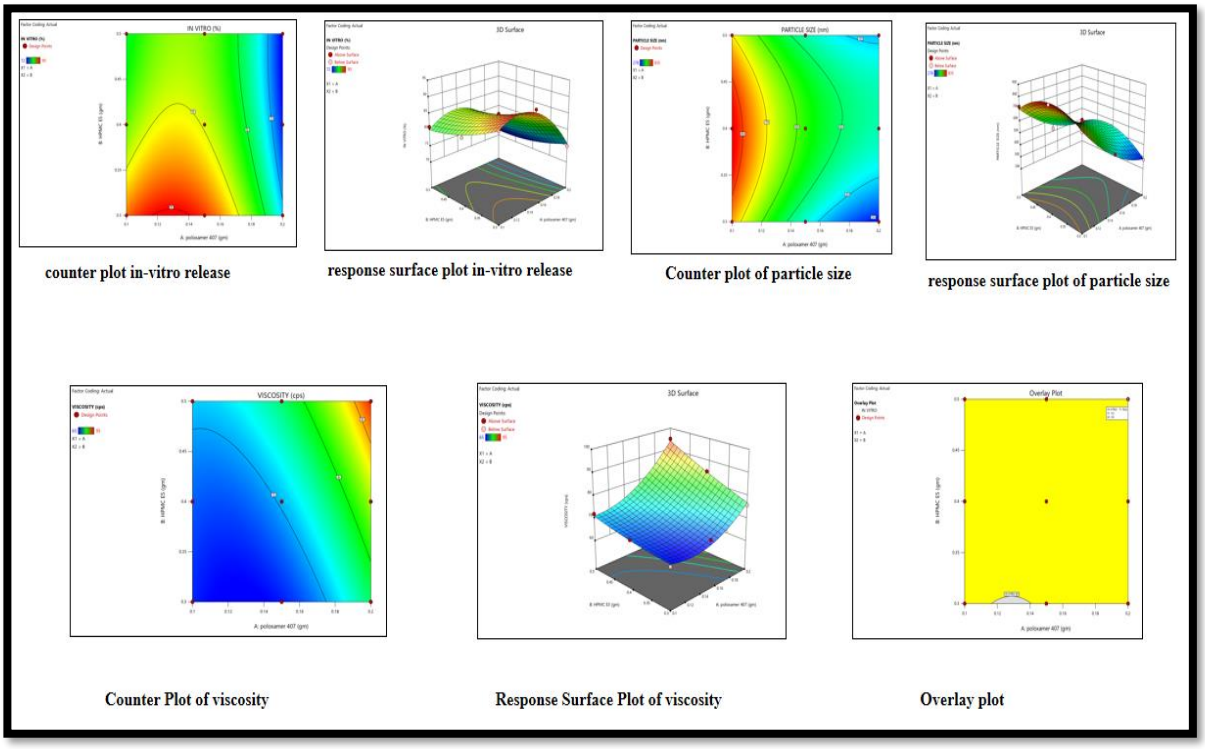

Figure 4: Design of the Experiment (3^2-Fall Factorial Design)

Desirability and Overlay Plot:

All relevant measurable responses that may impact the product's quality were considered throughout the optimization phase. The percentages of drug release, particle size (in nanometers), and viscosity of 9% were established as the maximum criterion in the in vitro setting. The optimal value was obtained by combining each response criteria using overlay plotting. The study findings' optimization outcomes are shown in the Table 6.

Table 6: Characteristic of optimum formula

Object	Poloxamer 407 (X1)	HPMC E5	% Invitro release	Particle size (nm)	Viscosity (Cpc)	Desirability	
Predicted	0.2	0.5	71.77	382.88	94.11	1.00	Selected
Actual	0.2	0.5	72	400	95		

The desirability value varied from 0 to P, where P represents the ideal value. The desirability parameter for the nanosuspension formulation was 1.00. Since the predicted parameter values were near to the ideal values, we can say that everything went according to plan.

Validation of RSM results:

The results of the in-vitro drug release (%), particle size (nm) (Y2), and viscosity (%) (N3) derived from the experimental model are presented in the table. The percentage of drug release (Y1), particle size in nanometers (Y2), and viscosity percentage (Y3) were obtained using both anticipated and experimental models. The table displays the outcomes. For the Y1, Y2, and Y3 response variables, the calculated percentages of drug release in vitro, particle size in nanometers, and viscosity percentage were -0.32, -4.41, and -0.94, respectively. The highest PRE value recorded was (-4.47). Nevertheless, the values were determined to be 55%, therefore affirming the appropriateness of the experimental design. The theoretical features of the preparation were well demonstrated.

Conclusion:

In present study, it was observed that, given formulation shows increased bioavailability of poorly soluble drug Nepafenac. Careful pre-formulation research verified that appropriate materials were chosen and that drug-polymer interactions were compatible. By using experimental design and response surface modelling (RSM), the formulation parameters were optimized which leads to remarkable effects on drug release, particle size, and viscosity. Future

perspectives encompass the exploration of in vivo studies to validate the enhanced bioavailability and therapeutic efficacy of the developed Nanosuspension along with long-term stability and scale-up feasibility.

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