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Original Article

Formulation Development and Evaluation of Bicalutamide Nano Suspension with Enhanced Solubility

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ABSTRACT

Background: Bicalutamide, a non-steroidal anti-androgen, is commonly prescribed for the treatment of prostate cancer. A drug categorized under BCS Class II due to its low solubility and higher permeability. The inherent poor aqueous solubility of the drug leads to delays in its onset of action. Therefore, the primary objective of this research is to leverage nanosuspension technology to formulate a solution that enhances the dissolution rate of the drug, consequently improving its bioavailability. **Materials and Methods:** Nanosuspensions were synthesized using the solvent diffusion method, incorporating selected stabilizers at varying concentrations. **Results:** The nanosuspensions underwent characterization for Polydispersity Index, mean particle size, zeta potential, entrapment efficiency and *in vitro* drug release. *In vitro* dissolution testing validated that the drug's dissolution rate was notably higher compared to the pure drug. the % drug release was found to be 95.4% which is the highest drug release as compared to other batches in 6 hr. A 2^3 factorial design was employed to examine the effects of independent variables, specifically, Stabilizer Concentration [X1], Surfactant concentration [X2] and sonication time [X3], on the dependent variables: mean particle size (Y1) and *in vitro* release study (Y2). The obtained data underwent statistical analysis and were further analyzed using three-dimensional response surface methodology to discern the impact of variables on responses. Subsequently the optimized batch underwent evaluation of *in vitro* parameters including particle size and *in vitro* drug release. **Conclusion:** The nanosuspension formulation presents a promising solution for enhancing the bioavailability of poorly water-soluble drugs when administered orally, offering improved efficacy and cost-effectiveness compared to traditional methods. The investigation of Bicalutamide-loaded nanosuspension serves as a novel delivery platform aimed at enhancing the oral bioavailability of the drug.

Keywords: Bicalutamide, Nanosuspension, Solvent Diffusion method, Solubility Enhancement, Bioavailability.

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INTRODUCTION

Because of insufficient drug solubility and low bioavailability, the oral delivery of pharmaceutical substances is often restricted. Water solubility is a problem for more than 40% of newly discovered medications.[1] Limited drug solubility has hampered the commercialization of numerous medications because solubility is so important in drug formulation. Traditional approaches like micronization,[2] usage of surfactants,[3] solubilize,[4] solvency,[5] crystallization,[6] solid dispersions,[7] Self-Micro Emulsifying Drug Delivery System (SMEDDS),[8] Complexation[9] and Polymorphism[9] have been employed to enhance drug solubility. The formulation of nanocrystals or nanosuspension is one of the most common techniques currently being studied (NS). A drug is reformed during the formulation of NS and it is classified as such by the Food and Drug Administration. There are two fundamental methods for Nanostructured (NS) formulation: Top down and Bottom up technologies. In the top-down technique, large crystalline particles are reduced to the desired size range. The bottom-up approach entails dissolving the pharmaceutical compound in a suitable solvent, followed by its introduction into a nonsolvent to precipitate nanocrystals. This process is conducted under controlled conditions, incorporating a stabilizing agent to facilitate particle formation. Nitrendipine, simvastatin, carvedilol, efavirenz, risperidone, and other drugs have had their solubility and bioavailability improved using these methods.[10]

Nanosuspensions represent colloidal dispersions containing drug particles at the nanometer scale, which are stabilized by surfactants. Alternatively, they can be characterized as a biphasic system comprising pure drug particles dispersed within an aqueous medium, wherein the suspended particle diameter is less than 1 µm. Nanosuspensions can enhance the solubility

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of drugs that exhibit poor solubility in both aqueous and lipid environments. This is one of its distinct advantages over other methods for increasing Solubility. It proves beneficial for substances characterized by low solubility, permeability or reactivity all of which present significant challenges for modelers. Due to their reduced particle size, poorly soluble medications can be administered intravenously without causing blockages in blood capillaries.[11]

Bicalutamide has been used for non-steroidal antiandrogen prescribed for prostate cancer. Bicalutamide falls under BCS class II (log P of 2.92), characterized by poor solubility and high permeability, leading to restricted oral bioavailability. The Bicalutamide competes with androgens for binding affinity to androgen receptors, it inhibits the stimulation of both normal and malignant prostatic tissue growth by androgens derived from the adrenal glands and testes. It exhibits insolubility in water and limited solubility in methanol. The dose of the drug is 50 1mg tablet once daily and the half-life is 5.9 days.

Nanosized suspensions are being studied as a promising approach to improve the solubility and, consequently, the bioavailability of poorly water-soluble active compounds categorized within BCS classes II and IV. A pharmaceutical nanosuspension comprises submicron-sized solid drug particles uniformly suspended within an aqueous medium, intended for delivery via topical, pulmonary, oral or parenteral routes. Solid particles present in nanosuspensions typically exhibit the particle size distribution of less than 1 micron, with a mean particle size ranging from 50 to 600 nm.[12]

MATERIALS AND METHODS

Bicalutamide drug was obtained from Emcure Pharmaceuticals (Pune, India) as a gift sample. Sodium Lauryl Sulphate, Pluronic f127 (Research grade) and Eudragit L100 were purchased from Sisco Research Laboratories Pvt. Ltd., All the solvents and other chemicals used in the study were of analytical grade.

Methods of Preparation of Nanosuspension

Bicalutamide nanosuspension was prepared by solvent diffusion method. Bicalutamide (50 mg) was accurately weighed dissolved in 10 mL of methanol and sonicated for 10 min. This solution is considered an organic phase hence it contains organic solvent methanol. The surfactant Sodium Lauryl sulphate (100 mg) and stabilizer as a Pluronic f127 were added separately in 20 mL of water and kept in an ice water bath for 10 min. This solution is considered an Aqueous solution. The organic solution was added dropwise to the aqueous solution under continuous stirring by a magnetic stirrer at room temperature and then the formulated solution was stirred at 10,000 to 25,000 rpm for 1 hr. Then the Bicalutamide Nanosuspension was formed.

Preformulation Studies

Determination of Melting Point

The melting point of drug was determined by using Melting Point Apparatus.

Determination of absorption maxima

The conc of 100 ug/mL was prepared in 1% SLS. The UV spectrum of Bicalutamide solution was taken in range of 200-400 nm against reference as blank. The peaks observed were compared with official compendia.

Calibration curve of drug in 1% SLS

Bicalutamide equivalent to 10 mg was carefully weighed and dissolved in 10 mL of 1% SLS in 10 mL of volumetric flask, yielding conc of 1000mg/mL solution. Then take 1 mL from this and dilute it to 10 mL yielding conc 100 mg/mL then take conc of 5 to 25 ug/mL in 10 mL. The absorbance was measured at 270 nm against blank.

Solubility study

10 mg of drug dissolved in 10 mL of each solvent like 1% SLS, 1% SLS plus 6.8 PBS, water and methanol in vortex mixer for 15 min. Then this mixture kept it for 24 hr. Centrifuge this at 5000 rpm for 15 min. From the Supernatant layer take 0.1 mL and dilute it with 10 mL of solvents. A UV-visible spectrophotometer was used to detect OLP in 254 nm using PBS as a blank.[13,14]

FTIR Study

FTIR spectrum measurements of Drug and physical mixers of Bicalutamide with Pluronic f127 and Sodium lauryl Sulphate were taken at the proper temperature.[15]

Differential Scanning Calorimetry

A differential scanning calorimeter was used to record the DSC thermograms. In punctured aluminium pan (AL- Crucibles 40 rate of 50 mL/min) approximately 2-5 mg of each sample was heated. The STAR e programme (version 1210) was used to analyze the thermal data of DSC thermograms from 300 to 3000°C at heating rate of 100 C/min under a stream of nitrogen at a flow rate of 50 mL/min.[16]

Experimental Optimization of Prepared Nanosuspension Design of Experiment

A prepared Bicalutamide Nanosuspension was optimized by a 2^3-factorial design. In this study Design, Expert Version13 was used for the experimental design and statistical analysis (Stat-Ease, USA). Three factors and two responses were used in the trials (Table 1). Based on the results of the trial batches, the amounts of stabilizer (X1 mg), surfactant (X2 mg) and sonication time (X3 Min) were determined to be in the ranges of 50-200

for Pluronics f127, 50-100 for SLS and 30-60min for sonication time.[10]

Evaluation of Prepared Nanosuspension

Particle size, Polydispersity Index Zeta Potential

1 mL of nanosuspension was diluted in 10 mL of distilled water and the resulting formulation was subjected to be Particle size, Polydispersity index and Zeta potential by using Nanoparticle Analyzer manufactured by HORIBA SCIENTIFIC SZ 100, Japan.[17]

Entrapment Efficiency

Using a cool ultracentrifuge, the freshly optimized nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature. The amount of subtracting the amount of free drug in the supernatant from the total amount of drug consumed, DEE was computed.[18]

In vitro drug release study

The dialysis bag was soaked overnight in dissolving media to ensure that the membrane was thoroughly soaked. 5 mL of NS were placed in dialysis bag and connected to the paddle then bag was put into dissolution medium, which contained 900 mL of 1% SLS at 37±0.5°C and dissolution was carried out for 250 min at a stirring speed of 50 rpm. An aliquot of 1 mL was taken every 30 min, diluted, filtered and tested by using UV Spectrophotometer (Shimadzu 1700, Japan) at 270 nm.[19]

Stability Study

Stability tests for optimized formulation were carried out according to ICH Q1A (R^2) criteria. Samples were subjected to storage conditions at 40±2°C temperature and 75%±5% relative humidity, corresponding to Climatic Zone IV for three months to evaluate their physical changes and stability.[20]

RESULTS

Preformulation Study

Melting point

Bicalutamide has a melting point of 195°C. The melting point of Bicalutamide lies between 191 and 193°C, indicating that it is solid. The medication obtained is 100% pure.

Table 1: Factors and responses for 2^3 factorial designs for Bicalutamide Nanosuspension.

Factors	Lower Limit	Higher Limit
X1=Stabilizer Concentration (mg)	50	20
X2=Surfactant Concentration (mg)	50	100
X3=Sonication Time	30	60
Responses	Limit	
Y1=Particle Size	Goal Minimize	
Y2=Percentage *in vitro* Drug Release	Maximize	

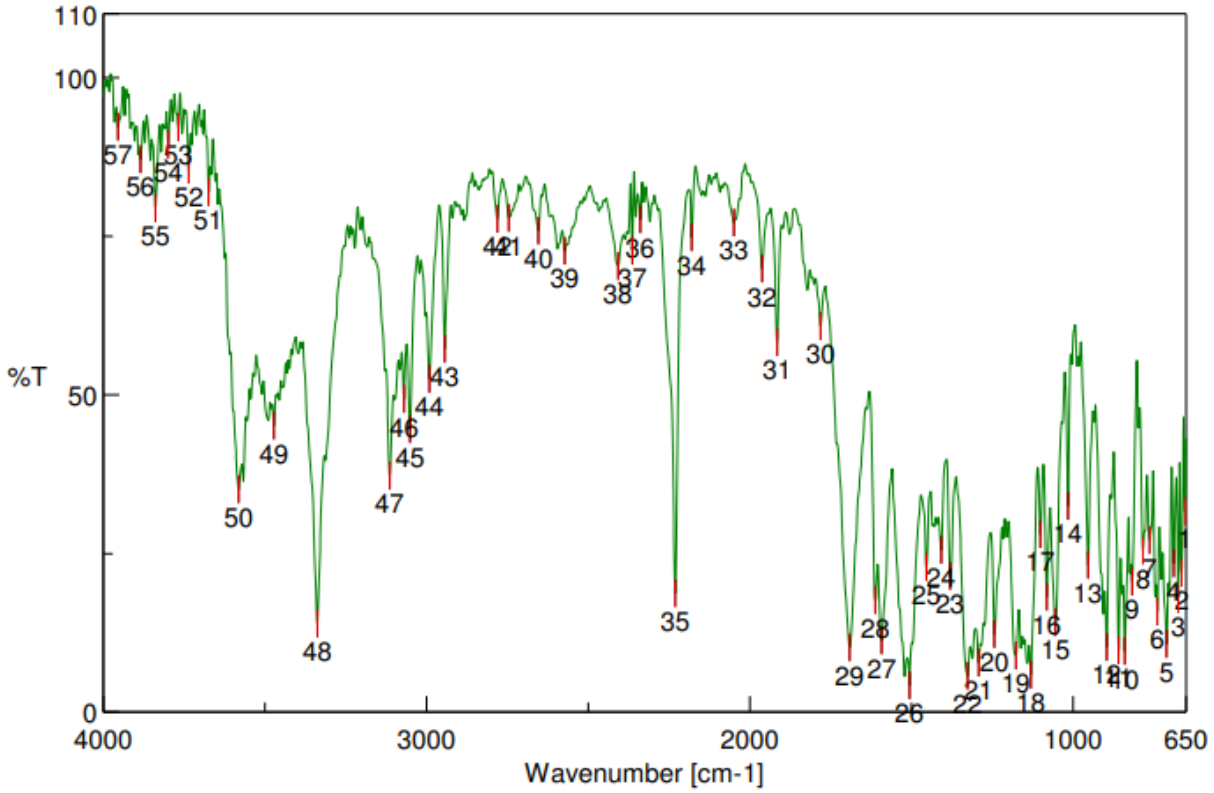

Figure 1a: FTIR spectra of Bicalutamide.

Absorbance maximum (λ_{max})

The λ_{max} of Bicalutamide was found to be 287nm indicating drug purity.

Calibration curve of Bicalutamide in 1% SLS

The bicalutamide calibration curve was obtained in a 1% SLS at 270 nm. Within the concentration range of 5 to 25 µg/mL, Beer's Law was followed. According to the Beers-Lambert Law, the absorption maximum of Bicalutamide was measured at 270 nm, demonstrating greater linearity (R^2=0.9965) from the concentration range of 5-25 µg/mL, moving from origin. The linearity equation for the Bicalutamide Standard Calibration Curve at 270 nm in 1% SLS was reported to be y=0.0044x-0.0059. These measurements were utilized to construct the standard calibration curve for Bicalutamide in the presence of 1% SLS at 270 nm.

Solubility study

The solubility study revealed that the drug exhibited improved Solubility in 1% Sodium Lauryl Sulfate (SLS) solution. The incorporation of Methanol in the drug was assessed by estimating the absorbance of the blank/control nanosuspension and 5 mL diluted supernatant solution at 270 nm using a UV spectrophotometer. Which is shown in Table 2.

FTIR Study

The physical mixture containing the excipients, as well as the FTIR spectra of Bicalutamide were discovered. The spectra show that the pharmacological functional groups have similar peaks, this indicates that the drug and polymers had not undergone any chemical change. FTIR spectral data is shown in Table 3 and Figures 1a-1c.

Differential Scanning Colorimetry (DSC)

The DSC thermogram of Bicalutamide exhibits an endothermic peak corresponding to its melting point 194.45°C (Enthalpy=119.42 J/g). There was negligible variation observed in the peak position. It can be concluded that polymers and drugs do not interact with each other. Also, Bicalutamide didn't form a complex with the polymer as the endothermic peaks remained unchanged in position (Figure 2).

Experimental Design

The study includes, a 2^3 factorial design was employed, which involved three factors studied at two levels each. All possible combinations of these factors were tested, resulting in a total of eight experimental trials. In this investigation, the Mean particle size [Y1], Entrapment Efficiency [Y2] and Cumulative drug release [Y3] over the specified period for Bicalutamide-loaded nanosuspension were assessed and are all affected by factors such as poloxamer 407 (X1), SLS (X2) and Sonication time (X3). As a result, they were used for further investigation. The two dependent variables (Y1, Y2) revealed a variation in particle

Table 2: Drug solubility profile across different media.

Solvents	Solubility (mg/mL)
Water	5
1% SLS	12
Methanol	10
1%SLS+6.8 PBS	8

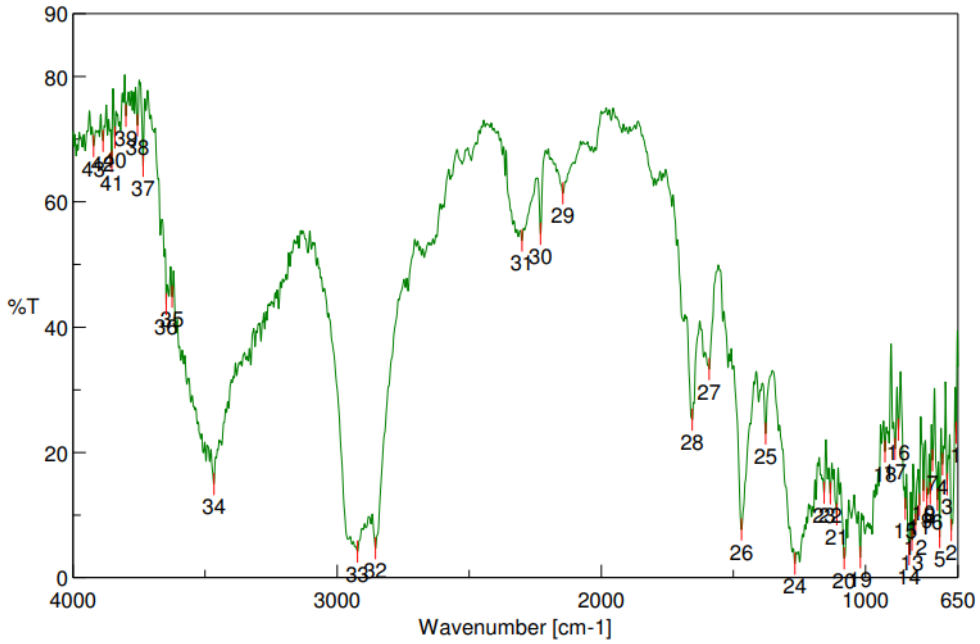

Figure 1b: FTIR spectra of Drug with SLS.

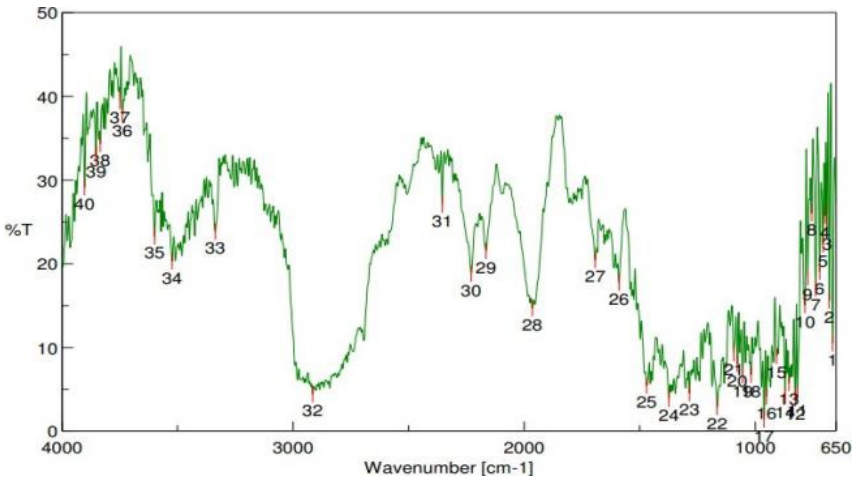

Figure 1c: FTIR spectra of Drug with Pluronic f127.

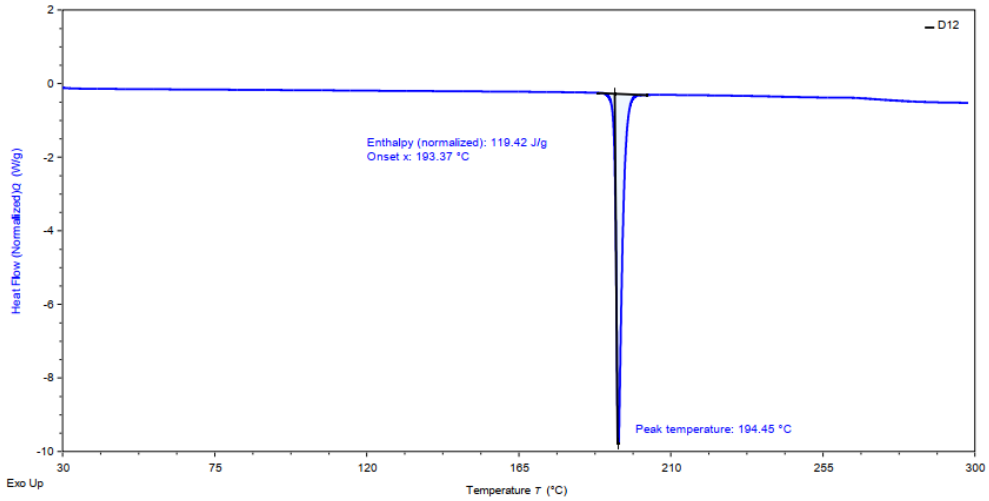

Figure 2: Differential Scanning Calorimetry of Bicalutamide drug.

Table 3: Spectral data for FTIR Spectroscopy.

Sl. No.	Functional group	Standard value	Pure Drug	Drug with SLS	Drug with Pluronics f127
1.	C=O	1780-1650	1780	1655	1693
2.	C=-N	2260-2220	2230	2230	2229
3.	C-OH	3300-2500	2572	2855	2915
4.	N-H	3500-3300	3338	3466	3337
5.	C-F	800-400	652	656	665
6.	C=C	1680-1600	1612	1655	1590
7.	C-H	3300-2700	2745	2855	2915

size and CPR for all 8 batches. The responses of all dependent variables of 2^3 factorial designs are shown in Table 4.

Effect of Formulation composition on particle size

The principle aim of this study is to enhance the dissolution rate and solubility profile of bicalutamide, a poorly soluble medication. This is visually represented in Figure 3. This is depicted in a three-dimensional plot. The particle size of optimized batch F1 of Nanosuspension was observed to be 201.0 nm and a Polydispersivity Index was found to be -13.5. In this optimized batch, the lowest particle size is observed as the concentration of stabilizer is 200 mg, surfactant concentration is 100 mg and sonication time is 30 min. This results in the concentration of the stabilizer being increased and particle size decreases.

Impact of formulation composition on cumulative drug release

The second most important dependent variable is % cumulative drug release. The % cumulative drug release of the optimized batch (F1) is 95.4% in 6 hr. This results that as the concentration of stabilizer increases and surfactant concentration decreases then the Particle size decreases and % the cumulative drug release increases and solubility will also increase. The 3D plot of % cumulative drug release is shown in (Figure 4).

Characterization of Optimized batch (F1)

Particle size and polydispersity index

For the Optimized batch (F1) the particle size was found to be 201.0 nm and PDI was found to be 0.261. In the formulation using 200 mg as a stabilizer, the particle diameter was shown to decrease. The average particle size of the optimized formulation was determined (F1) containing 200 mg of Pluronic f127 as a stabilizer and 50 mg of SLS as a surfactant and the sonication time is 30 min. The average PDI value of the nanosuspensions F1 to F8 ranged from 0.26 to 0.49 pointing to good results. The particle size distribution curve can be given in the following Figure 5.

Zeta Potential

The Optimized batch exhibited a zeta potential of -13.5 mV, suggesting enhanced stability of the prepared nanosuspension. The zeta potential curve as shown in Figure 6.

Entrapment Efficiency

The Bicalutamide Nanosuspension exhibited Drug Entrapment Efficiency within the range of 79 to 94%. The percentage of Entrapment Efficiency for the Optimized formulation was determined to be 94%, This suggests that there is a correlation between the concentration of stabilizer and the increase in entrapment efficiency.

In vitro drug release evaluation

The Bicalutamide nanosuspension exhibited a percentage of drug release within the range of 84.5 to 95.4. For the optimized batch (F1) the % drug release was found to be 95.4% (Figure 7) which is the highest drug release as compared to other batches in 6 hr, resulting as a particle size decreases and % drug release increases with an increase in stabilizer concentration and also increases the dissolution rate of nanosuspension.

Stability Study

The stability assessment was conducted on the optimized formulation. At intervals of 1, 2, and 3 months, samples were retrieved and reanalyzed for % *in vitro* drug release and particle size. No notable alterations were observed in either parameter, indicating that the formulation maintained its stability over the entire three-month period as shown in Table 5.

Table 4: Composition of all optimized batches and their response.

		Factor-1	Factor-2	Factor-3	Response-1	Response-2
Sl. No.	Batch Code	Stabilizer Concentration (mg)	Surfactant Concentration (mg)	Sonication Time (min)	Particle size (nm)	% drug release
1.	F1	200	100	30	201.0	95.4
2.	F2	50	50	30	344.1	86.24
3.	F3	200	50	60	307.9	90.5
4.	F4	200	50	30	267.8	91.48
5.	F5	50	100	60	420.1	85
6.	F6	50	50	60	426.7	84.5
7.	F7	50	100	30	346.7	85.80
8.	F8	200	100	60	235.5	94.5

Nc=no change.

Table 5: Assessment of the stability of the optimized batch [F1].

Time (days)	Physical change		Chemical change	
	Appearance	Colour	Particle Size (nm)	% Drug release
0	NC	White	201.0	95.4
30	NC	White	204.1	93.5
60	NC	White	207.4	92
90	NC	White	208.6	90

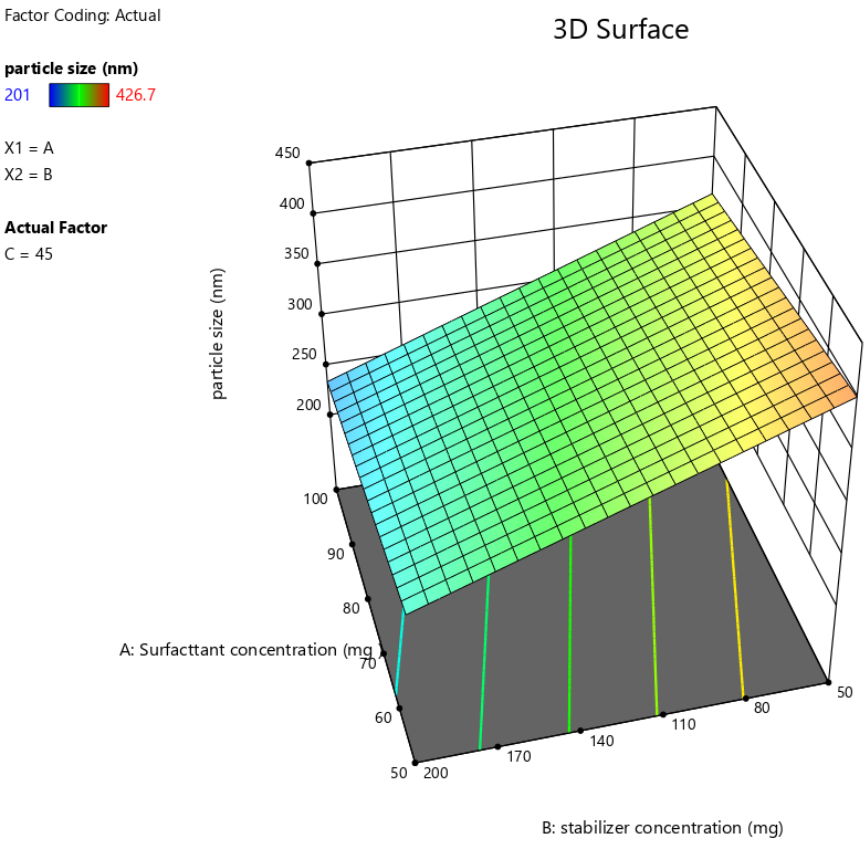

Figure 3: 3D Response surface Plot of Particle Size.

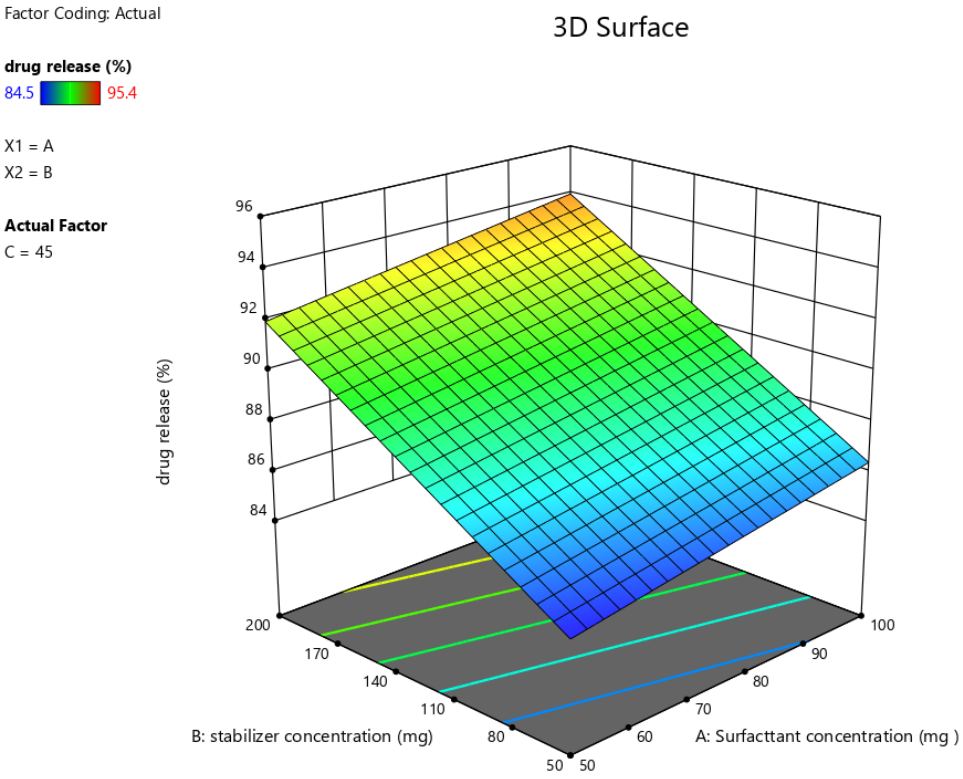

Figure 4: 3D Response surface plot of Percentage Drug release.

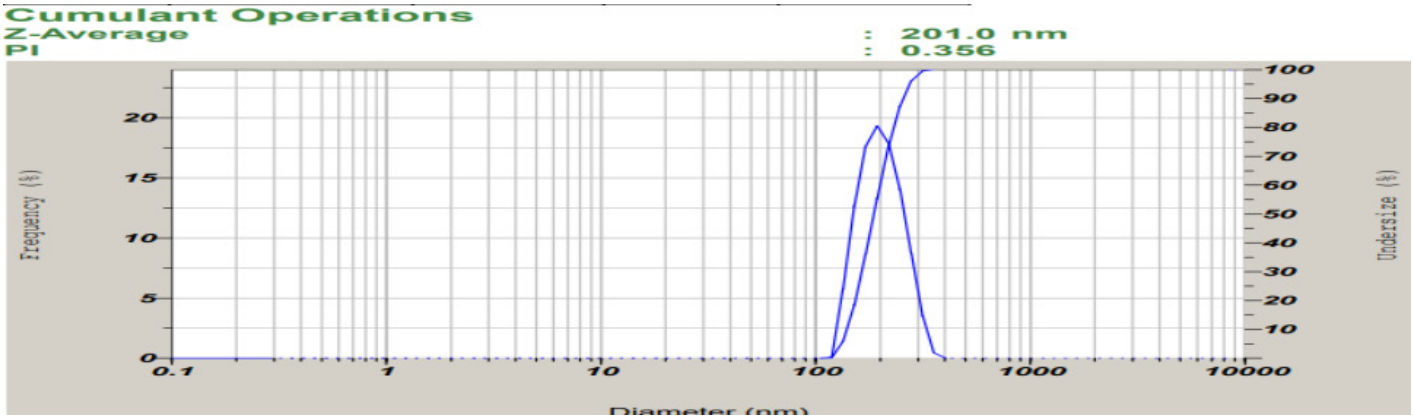

Figure 5: The particle size dispersion of the optimized batch (F1).

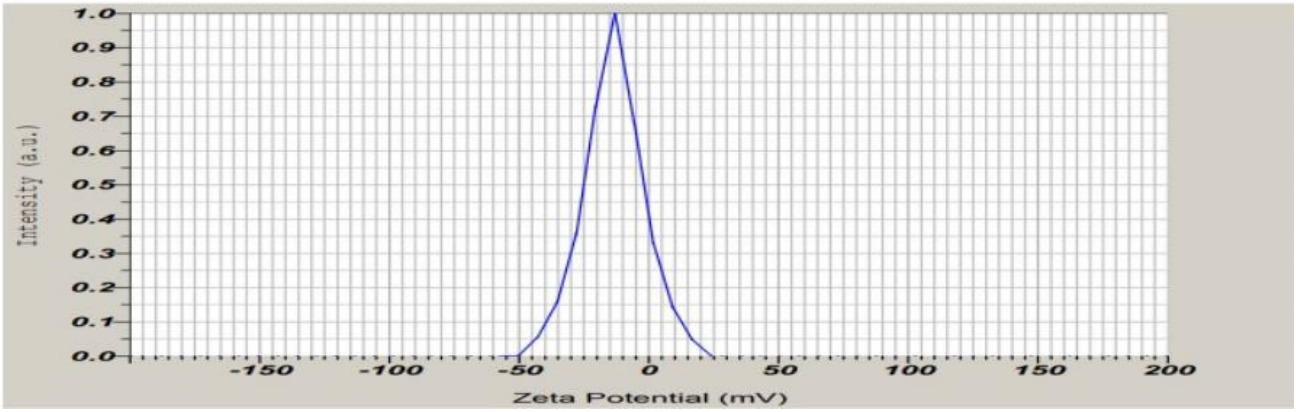

Figure 6: The Zeta Potential of the Optimized Batch (F1).

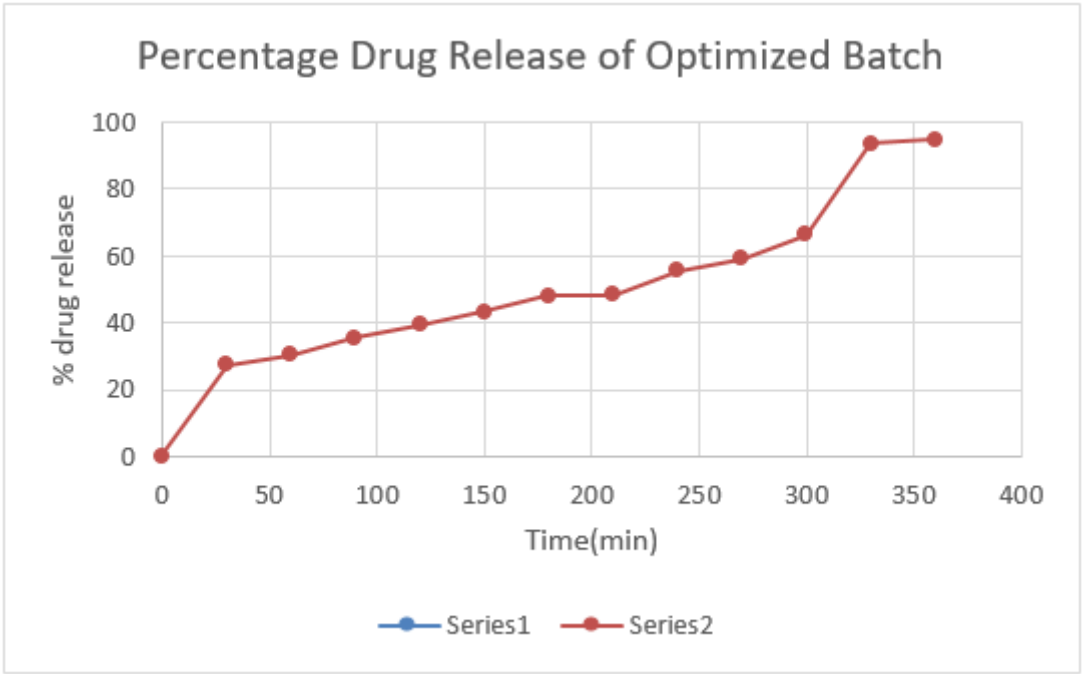

Figure 7: *In vitro* release study of optimized batch.

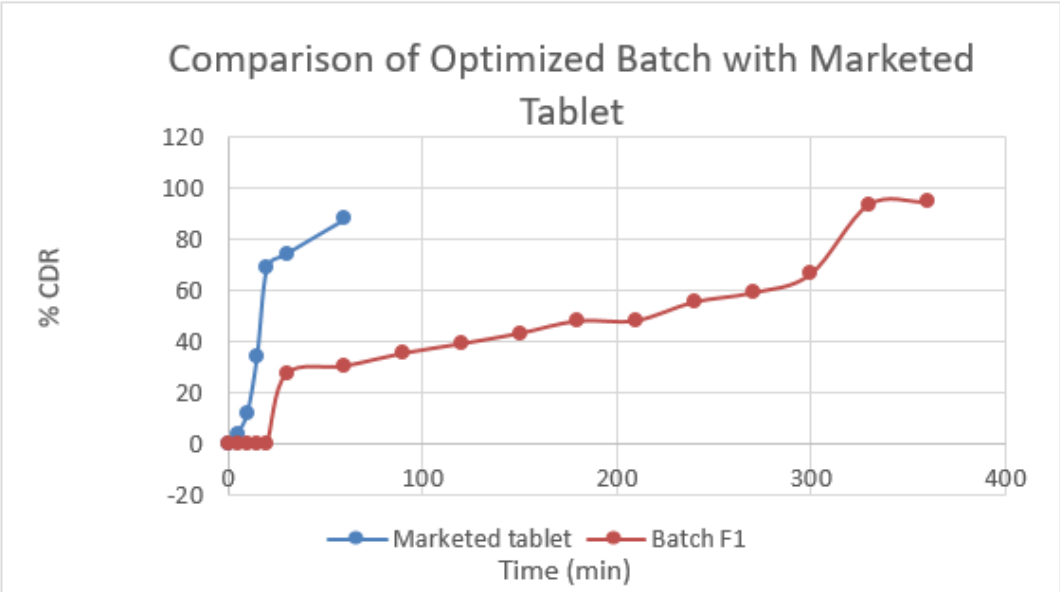

Figure 8: Comparisons of the optimized batch with the marketed tablet.

Comparison between optimized formulation and the marketed formulation

The results of dissolution trials for the F1 formulation of Bicalutamide nanosuspension and the marketed tablet were compared. When compared to ideal nanosuspension formulations and the marketed tablet, the F1 batch of Bicalutamide nanosuspension shows a maximum drug release of 95.4% within 6 hr as compared to the marketed tablet drug release of 87.80% within 1 hr shown in Figure 8. Bicalutamide Nanosuspension were prepared successfully by applying a 2^3-factorial design using the Solvent Diffusion method. The Preformulation study was carried out to check the compatibility of API with Excipients by FTIR spectroscopic method. From 8 batches F1 batch was optimized by applying the ANOVA technique, the reason that the F1 batch showed better results as compared to other batches. F1 formulation contains a high concentration of stabilizer as compared to others and a low concentration of surfactant and has a particle size of 201.0 nm and also has a dissolution rate of 95.4% which is more than the marketed tablet of Bicalutamide. It has been concluded that the prepared formulations showed enhanced dissolution characteristics hence Bicalutamide Nanosuspension proved to be the most potent formulation for oral drug release with enhanced Bioavailability and Dissolution rate.

DISCUSSION

The nanosuspension formulation exhibits a mean particle size of 191 nanometers with a Polydispersity Index (PDI) of 0.28.[17] For the Optimized batch (F1) the particle size was found to be 201.0 nm and PDI was found to be 0.261. The nanosuspension formulation exhibits a zeta potential of -12 millivolts.[17] The optimized batch exhibited a zeta potential of -13.5mV, suggesting enhanced stability of the prepared nanosuspension. The Entrapment Efficiency (EE%) of the drug was notably impacted by the inclusion of non-ionic surfactants, specifically a combination of Tween 80 with Tween 20, at a ratio of drug: polymer of 1:4:4. This formulation exhibited a substantially increased EE% of 98.74%.[18] The percentage of Entrapment Efficiency for the Optimized formulation was determined to be 94%, This suggests that there is a correlation between the concentration of stabilizer and the increase in entrapment efficiency. In the dissolution study, it was found that Lactose Bicalutamide achieved a dissolution rate of 94% within a 10-min interval.[19] while for the optimized batch (F1) the % drug release was found to be 95.4% which is the highest drug release as compared to other batches in 6 hr.

CONCLUSION

The nanosuspension formulation presents a promising solution for enhancing the bioavailability of poorly water-soluble drugs when administered orally, offering improved efficacy and cost-effectiveness compared to traditional methods. The investigation of Bicalutamide-loaded nanosuspension serves as a novel delivery platform aimed at enhancing the oral bioavailability of the drug. The method of preparation of Bicalutamide nanosuspension was found to be simple. The formulation were optimized by using design of experiment by employing a 2^3 factorial design.

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ABBREVIATIONS

BCS: Biopharmaceutical Classification System; **NS:** Nano Suspension; **FDA:** Food and Drug Administration; **SLS:** Sodium Lauryl Sulphate; **UV:** Ultra Violet Spectrophotometer **FTIR:** Fourier Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **EE:** Entrapment Efficiency; **RPM:** Revolution Per Minute; **DEE:** drug Entrapment Efficiency; **API:** Active Pharmaceutical Ingredients; **ANOVA:** Analysis of Variance; **PDI:** Polydispersity Index.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

In this research Paper, the investigation of Bicalutamide-loaded nanosuspension serves as a novel delivery platform aimed at enhancing the oral bioavailability of the drug. The formulations were optimized by using design of experiments by employing a 2^3 factorial statistical design and design expert software. For the optimized batch (F1) the % drug release was found to be 95.4%.

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