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Original Article

Formulation Development and Evaluation of Iloperidone Loaded Nanosuspension for Solubility Enhancement

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ABSTRACT

Introduction: Iloperidone is categorised as an antipsychotic drug and is used to treat schizophrenia. Iloperidone belongs to the Biopharmaceutics Classification System class II due to its low water solubility. **Objectives:** The primary aim of this work is to develop a nanosuspension of iloperidone to address issues such as low solubility and insufficient bioavailability. A 3^2 complete factorial design was employed to explore the influence of stabiliser concentration (A) and probe ultrasonication time (B) at three different levels as independent factors on the size of particles (Y1) and saturation solubility (Y2) as dependent variables. **Materials and Methods:** Solvent-antisolvent method with probe ultrasonication was used for the formulation of nanosuspension. Optimized nanosuspension was then assessed for several parameters like saturation solubility, particle size, SEM, % of the drug, zeta potential, thermal analysis, FTIR, *in vitro* and *in vivo* analysis. **Results:** The optimized formulation has shown 286.67 nm particle size and 164.33 µg/mL saturation solubility. Zeta potential measurement of optimized formulation has indicated -25.8 mV potential which was enough to keep suspension in nano size. SEM images of lyophilized optimized nanosuspension formulation revealed particle size less than 1 µm. Optimized formulation has shown near about 100% drug release in 20 min. *In vivo* studies have indicated that the optimized nanosuspension exhibited a 2.88-times increase in C_{max}, a 2-fold increase in AUC and less time to reach T_{max}. Stability studies indicated acceptable stability over three months. **Conclusion:** In conclusion, the results of the present investigation indicate that nanosuspension technology can enhance the solubility as well as the bioavailability of iloperidone.

Keywords: Solubility, Nanosuspension, Pharmacokinetic, Schizophrenia, Factorial design.

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INTRODUCTION

Continual advancements in pharmaceutical research are leading to the constant development of novel medications to address a wide range of diseases and ailments. Approximately 40% of newly created active compounds encounter the issue of aqueous solubility.[1] The solubility of a drug is an important aspect in determining how quickly it dissolves and gets absorbed into the body.[2] The poor rates of dissolution of less water-soluble pharmaceuticals can cause problems in developing formulations, leading to low oral bioavailability. Several methods have been investigated to tackle this problem, such as salt creation, surfactant utilisation, prodrug development and micronization.

Micronization is the process of reducing the size of particles to the micron scale, usually between 2-10 microns. This can enhance the dissolution and solubility of medications. However, for drugs with extremely low solubility, micronization alone may not be enough. As a result of this constraint, nanoscale systems have been created.

A nanosuspension is an example of a nanoscale system. Nanosuspension is defined as colloids consisting of particles of drug suspended in suitable medium in their pure form, with a size in the nanometer range. Degrading the size of particles from micron scale to nanometer scale leads to a significant enhancement in surface area as well as dissolution rate.[2]

The field of nanoparticle engineering enables the preparation of less soluble drugs either as nanosuspensions alone or in conjunction with pharmaceutical excipients. This approach offers promising potential for enhancing drug solubility and oral bioavailability. Various methods are employed for the formulation of active pharmaceutical ingredients in nanosuspension form. One such method is the solvent-antisolvent method followed by the use of probe-ultrasonic homogenisation.[3] In this method drug whose nanosuspension is to be prepared is 1st dissolved in a liquid in which the drug is soluble while other ingredients

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are dissolved in a liquid in which the drug is insoluble, hence called solvent-anti-solvent. The drug solution is then added with high-speed stirring in an anti-solvent to cause precipitation. This method can be followed by probe ultrasonication for further reduction of particle size in the nanoscale.[4,5]

Iloperidone is used in the treatment of schizophrenia.[6] Schizophrenia is a chronic condition affecting the brain, characterized by disturbances in thinking, perception, emotions and behaviour.[7] Iloperidone functions as a blocker at dopamine D_2 receptors and serotonin 5-HT_{2A} receptors within the brain. It also has antagonistic activity at other receptors such as serotonin 5-HT_6 and 5-HT_7 receptors. This multifactorial mechanism of action is believed to contribute to its therapeutic effects in managing the symptoms of schizophrenia. The Biopharmaceutical Classification System (BCS) class of iloperidone is II which means it has less solubility and high permeability. Increasing the water solubility of pharmaceuticals is a desirable objective to enhance their therapeutic efficacy. The pace at which a drug dissolves is crucial for how much of it can be absorbed by the body and is influenced by both its capacity to dissolve in a liquid and the amount of surface area it has. As a drug's solubility rises, so does its dissolution rate. Likewise, an expansion in the drug's surface area results in an elevated dissolution rate.

The novelty of this study lies in the development of an optimized nanosuspension of iloperidone, which will enhance bioavailability and therapeutic efficacy in treating schizophrenia. The rationale is grounded in addressing the drug's poor solubility and bioavailability, offering a potentially more effective and patient-friendly treatment approach compared to conventional formulations.

The goal of the current investigation is to prepare and optimize a formulation of iloperidone in nanosuspension form by using suitable stabilisers and the solvent-anti-solvent approach, before probe ultrasonication. A 3^2 factorial model was utilised to investigate the impacts of two formulation factors at three distinct levels: the concentration of stabiliser (A) and the duration of probe ultrasonication (B). The saturation solubility (Y1) and particle size (Y2) were considered as responses. Formulated nanosuspension was evaluated for responses and then optimized formulation was lyophilized and evaluated for other parameters.

MATERIALS AND METHODS

Materials

The drug Iloperidone was gifted by Aripolis Biotech Pvt. Ltd., Ludhiana, Punjab, India. Poly-Vinyl-Pyrrolidone K30 (PVPK30), Sodium Lauryal Sulphate (SLS) and Methanol were purchased from Sudharshan Scientific Ltd., Nandgoaon, Nashik, Maharashtra. For the study, double-distilled water was utilised.

Methods

Preformulation study

Melting point

By the application of the capillary technique, melting point of iloperidone was determined. A minute quantity of drug was placed into the capillary from the open end while the other end was closed by use of flame. Subsequently, the capillary was affixed to a thermometer and submerged within liquid paraffin in Theil's tube. Theil's tube was then heated on a flame till the drug started to melt. The temperature at which the iloperidone sample underwent melting was recorded as a melting point.[7]

Solubility

The solubility of the selected Active Pharmaceutical Ingredient (API) was determined by the use of the flask shake method. An excessive amount of Iloperidone was introduced into a 50 mL volume of water and methanol as solvents contained in glass vials. These vials were sealed placed on a shaker (Remi Rs-12 R, India) and subjected to agitation at room temperature for 48 hr. Saturated solutions then were filtered by use of Whatman filter paper, appropriately diluted and analysed using a UV spectrophotometer (Shimadzu UV-1800).[8]

Preparation of Calibration curve in distilled water, methanol and phosphate buffer, 6.8 pH

A carefully measured quantity of 10 mg of iloperidone was added into a 10 mL volumetric flask. Iloperidone was completely dissolved with 10 mL of methanol to get a solution of 1000 µg/mL. The prepared stock solution was then suitably converted into serial dilutions by using water, methanol and phosphate buffer, 6.8 pH. The UV spectrophotometer was utilised to measure the absorbance of prepared solutions at 227 nm. A calibration curve was constructed by plotting absorbance values versus corresponding concentrations.[9]

Drug Excipient Compatibility Study

To determine possible drug-excipient interaction, iloperidone and excipients were subjected to compatibility testing by use of Fourier Transfer InfraRed (FTIR) (Jasco-4600, Japan). Iloperidone was mixed with excipients thoroughly in a 1:1 ratio and spectra were recorded.

Selection of solvent and antisolvent

The preparation of the nanosuspension was achieved using the solvent-antisolvent precipitation approach followed by treatment with an ultrasonic probe. Solvent and anti-solvent for the formulation of nanosuspension were decided depending on the solubility of the API, stabilizer and polymer. Also, the anti-solvent should be completely miscible with the solvent used. From the solubility study, it was observed that iloperidone was freely soluble in methanol and hence methanol was used as a solvent

for this method. Since selected excipients were soluble in water and iloperidone was insoluble, water was used as an anti-solvent for nanosuspension formulation.[10-14]

Selection of independent and dependent variables

In the formulation of stable nanosuspensions, various factors contribute to their stability. The independent variable is the factor intentionally controlled or altered by the investigator in a study and is assumed to represent the reason or effect. In contrast, the dependent variable is the factor that is seen or quantified in response to variations in the independent variable. It represents an effect or result that investigators are interested in.[14,15]

The stabiliser amount and the duration of the probe ultrasonication were determined as independent variables in the present investigation and the particle size and saturation solubility of the resultant nanosuspension were examined as dependent variables. Stabilizers and surfactants perform a very vital role in the formulation of nanosuspension. Stabilizers help to keep the nanosuspension in nano form. Without stabilizers, particles of nanosuspension will undergo rapid Ostwald's ripening increasing in particle size. Surfactants improve the dispersibility of nanoparticles in the liquid medium by reducing interfacial tension, leading to the formation of a stable colloidal system. Surfactants prevent the coalescence of nanoparticles by forming a protective monolayer or bilayer around them, inhibiting their fusion. In pharmaceutical nanosuspensions, surfactants can augment the absorption of weakly aqueous-soluble drugs by facilitating their absorption. Probe ultrasonication causes a reduction in particle size due to ultrasonic waves. Therefore, it was essential to decide the proper concentration of stabilizer as well as probe ultrasonication time for the formulation of nanosuspension.

Optimization

Optimization was performed by 3^2 full factorial design. The concentration of stabilizer (A), as well as probe ultrasonication time (B), were two independent variables at three different levels that were decided for the formulation of nanosuspension. A total of 9 formulations will be considered for formulation of nanosuspension. These 9 formulations, as indicated in Table 1, will be screened for particle size (Y1) and saturation solubility (Y2) as dependent variables. The nanosuspension which will yield optimum results will be considered as optimized and further studies will be carried out by using optimized nanosuspension.

Formulation of nanosuspension

Using the anti-solvent precipitation process followed by probe ultrasonication (Aczet Pvt. Ltd., Mumbai), the nanosuspension was developed. The drug 10 mg iloperidone was solubilized in 10 mL methanol as the drug was soluble in methanol. The mixture was then subjected to sonication for 5 min. Selected amounts of PVPK30 and SLS (5 mg) were dissolved in 50 mL distilled water which was previously cooled up to 5°C with the help of an ice-water bath (anti-solvent). The solution of surfactant and stabilizer was kept in a beaker with high-speed stirring (1000 rpm). A syringe containing a solution of the drug in methanol was positioned over the beaker and added dropwise. After the addition of all the drug solution stirring was continued for 2 hr. After 2 hr, the sample was immediately transferred into the beaker and was treated with the ultrasonic probe of 6 number 3 mm deep for different time intervals resulting in the travelling of waves in downward and upward direction.[16-18]

Lyophilization of nanosuspension

Optimized formulated nanosuspensions of iloperidone were converted into dry powder using mannitol as a cryoprotectant by using Lyophilizer (FreeZone, Triad, Labcono, USA). In the lyophilization process sample was kept in the chamber and the temperature was kept at -80°C for 8 hr. After 8 hr nanosuspension was converted into the dry powder which was then removed from the chamber and placed in a hermetic container for further work.

Evaluation of Nanosuspension

Initially, formulated nanosuspensions were evaluated for saturation solubility and particle size. Based upon these parameters optimized formulation was selected which was then evaluated for various parameters.

Saturation Solubility Study

Conical flasks with stoppers were filled with 10 mL of distilled water and an excessive amount of iloperidone nanosuspension. The flasks were then put in a rotary shaker at room temperature for 24 hr. Immediately after the 24 hr, samples were collected, filtered and examined using a UV spectrophotometer.[19]

Particle size analysis

The Anton Paar Litesizer 500 particle size analyser was used to calculate the particle size analysis of several batches of iloperidone nanosuspension. The particle size distributions obtained were used to verify the existence of particles at the nanoscale, hence confirming the development of the nanosuspension.[20]

Determination of drug content

A 10 mg lyophilized optimized batch of iloperidone was accurately measured and solubilised in methanol (10 mL) followed by filtration. The filtrate was then suitably diluted and analysed using UV spectroscopy. The following formula was used to determine the concentration of the drug in formulation.[21]

$$\text{Drug content} = \frac{\text{Drug content determined}}{Total\ Drug\ added} \times 100$$

Differential Scanning Calorimetry (DSC)

The DSC (Mettler, DSC 8000, Switzerland) evaluation of Iloperidone API and nanosuspension was done. 5 mg of API as well as lyophilised nanosuspension was precisely balanced and hermetically packed in an aluminium pan. The sample undergoes heating from ambient temperature to 300°C at a rate of 10°C/min. To preserve a stable environment, nitrogen gas with a purity of 100% was introduced into the system at an average rate of flow of 100 mL/min.[22]

Fourier Transform Infrared (FTIR)

FTIR analysis was performed to analyse lyophilized iloperidone nanosuspension and API. The FT-IR spectra of the lyophilized nanosuspension were recorded using an FT-IR spectrophotometer (Jasco 4600, Japan). Approximately 5 mg of samples were mixed with an equal weight of dried KBr and compressed to form a disc. Subsequently, the pellets were inserted into the spectrophotometer and spectral data was collected in a frequency range spanning from 4000 to 400 cm^{-1}.[23]

Scanning Electron Microscopy (SEM)

To confirm that the produced nanosuspension indeed included nanoparticles and to study its surface shape, a SEM analysis (FEI Quanta 200) was performed.

X-ray Diffraction (XRD)

The XRD analysis of the Iloperidone API and lyophilised nanosuspensions was conducted using the PANalytical X' Pert Pro, Netherlands, instrument. The X-ray tube utilised in this study features a Copper (Cu) target. A wavelength of 1.54184 Å was employed in the X-ray Diffraction (XRD) analysis. The instrumental parameters utilised in this study included a 2θ angle, a range spanning from 10 to 90°, with a counting time of 3 seconds for each step. The counting step for the 2θ angle was set at 0.04°.[24]

Zeta Potential (ZP)

The ZP of the optimised batch was determined using the AntonParLitesizer 500 instrument with the Omega cuvette Mat. No. 225288. Henry factor 1.5 (Smoluchowski) was applied. A relative permittivity of 78.37 was applied with a target temperature of 25°C.

In vitro dissolution study

The *in vitro* dissolution study of the optimised batch was conducted testing using the USP type II (Paddle type) (VDS-6, VBTech automation, Gujarat, India) instrument. The optimized nanosuspension formulation was put in an overnight pre-soaked dialysis bag (Himedia, Mumbai) in a dissolution medium. The experiment was carried out at a temperature of 37±0.5°C, using a medium as a 900 mL pH 6.8 phosphate buffers at 50 rpm. 5 mL nanosuspensions were put inside the dialysis bag which was then tied to the shaft. Samples of 5 mL were taken out of the basket's centre at the time interval of 2, 5, 10, 20, 30, 40, 50 and 60 min. Samples were then filtered by use of a 0.2 µm Polytetrafluoroethylene (PTFE) filter. The same amount of preheated fresh media was then placed in dissolution media. Withdrawn samples were appropriately diluted and then analysed by use of a UV instrument. From the standard calibration curve, the quantity of API present in each aliquot was estimated.[25,26] The *in vitro* drug release of the optimised nanosuspension formulation of iloperidone was then compared with the marketed tablet formulation.

In vivo pharmacokinetic studies

The *in vivo* pharmacokinetic studies were performed according to the rules set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India. The Institutional Animal Ethics Committee of KBHSs Trust's Institute of Pharmacy, Malegaon, India (1566/PO/a/11/CPCSEA) approved the conduction of animal studies. A total of 18 male rats weighing between 200 and 250 g were chosen for *in vivo* pharmacokinetic research. The selected animals

Table 1: 3^2 Optimization of Iloperidone nanosuspension.

Formulation code	Iloperidone (mg)	PVP K30 (Stabilizer) (A) (mg)	Ultrasonication time (B) (min)
IF1	10	10	5
IF2	10	10	10
IF3	10	10	15
IF4	10	20	5
IF5	10	20	10
IF6	10	20	15
IF7	10	30	5
IF8	10	30	10
IF9	10	30	15

were split into three groups the control group, another group was administered a pure drug coarse suspension and a third group was given an optimized formulation. A nanosuspension formulation, containing 3 mg of the pure medication suspended in water using tragacanth as a suspending agent, was prepared. 1st group received coarse nanosuspension, 2nd group received an oral dose of the nanosuspension formulation equivalent to 3 mg/kg of the drug. 0.5 mL blood samples were obtained from the retroorbital vein of rats at stated time intervals of 0, 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 hr. The blood samples were placed into containers containing heparin and promptly separated in a centrifuge at a speed of 3000 revolutions per minute for 10 min. Afterwards, the plasma samples were reserved at -20°C till the next examination. During the analysis, the plasma samples were appropriately diluted and analysed using a previously established Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method.[27-29]

Stability study

The optimized iloperidone nanosuspension batch was subjected to a 3-month stability assessment, as per the rules set by the International Council for Harmonisation (ICH). The stability research was performed at a temperature of 40°C with a tolerance of±2°C and a Relative Humidity (RH) of 75% with a tolerance of±5%. The nanosuspension underwent evaluation for particle size and saturation solubility at specific time intervals: 0, 15 days and 1, 2 and 3 months.[30,31]

RESULTS

Preformulation study

Melting point

The melting point was determined by the use of Theil's tube by use of the capillary method. The observed melting point of pure iloperidone was 120 to 122°C which corresponds to the standard melting point of 118 to 122°C.[32]

Solubility

The solubility of pure iloperidone was determined by the use of the shake flask method in water as well as in methanol. In water drug was found to be very less soluble with a solubility of

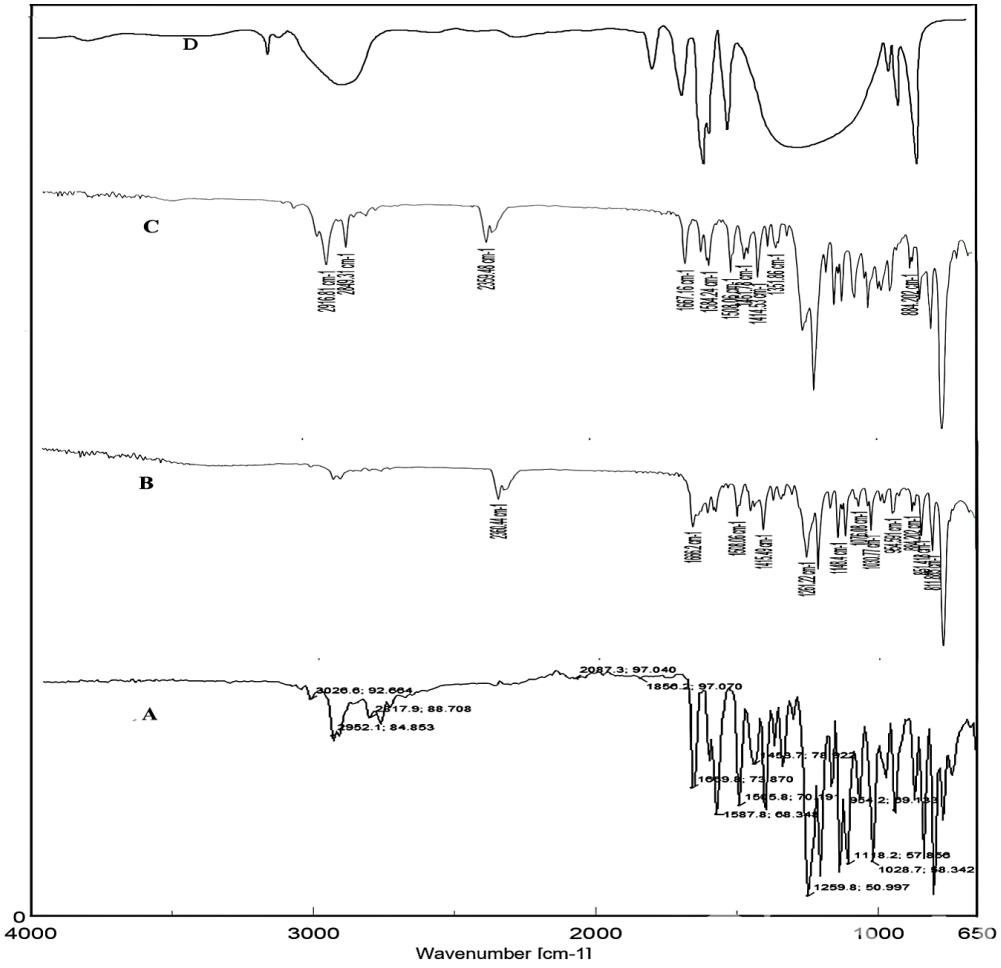

Figure 1: FTIR results of compatibility study: Iloperidone (A), Iloperidone+SLS physical mixture (B), Iloperidone+PVPK30 physical mixture (C), lyophilized Iloperidone nanosuspension (D).

Table 2: Layout of observed responses of 3^2 factorial designs for iloperidone nanosuspension.

Formulation code	Stabilizer Concentration (mg)	Probe Ultrasonication time (min)	Particle size (nm)	Saturation Solubility (µg/mL)
IF1	10	5	440.3±1.54	159.87±3.08
IF2	10	10	378.4±3.27	159.45±3.83
IF3	10	15	343.6±1.65	162.34±1.77
IF4	20	5	356.4±1.57	158.40±3.07
IF5	20	10	324.1±6.74	160.35±3.80
IF6	20	15	286.7±5.65	164.33±2.37
IF7	30	5	334.5±1.95	159.74±3.24
IF8	30	10	298.9±2.11	163.21±2.93
IF9	30	15	305.4±2.46	164.56±4.27

*(Mean±SEM, *n*=3).

0.026 mg/mL while in methanol drug was highly soluble with a solubility of 4.974 mg/mL.

Calibration curve

The calibration curve of iloperidone was plotted in water, methanol and 6.8 pH phosphate buffer. All the plotted calibration curve showed R^2 values more than 0.99 indicating good linearity.

Compatibility study

FTIR results of the compatibility study of iloperidone with SLS (Figure 1B) and PVP K30 (Figure 1C) indicated no major changes in the FTIR spectrum of iloperidone (Figure 1A) which showed compatibility between the drug and excipients. FTIR spectra of iloperidone showed distinct peaks corresponding to the functional groups like C-F stretching at 1259 cm^{-1}, N-O stretching at 1350 cm^{-1}, C=O stretching at 1669 cm^{-1} and 2952 cm^{-1} due to C-H stretching vibration.

Optimization of nanosuspension

Optimization of iloperidone nanosuspension was done by 3^2 factorial designs. This resulted in the formulation of a total of 9 formulations. The results obtained from the optimization study are indicated in Table 2.

Surface response plots were plotted by use of Design Expert Software (Version 13) to observe the interaction between the independent variable, concentration of surfactant and probe ultrasonication time and dependent variable, particle size and saturation solubility. From the surface response graph, as indicated in Figure 2A, the saturation solubility of iloperidone in the nanosuspension form increases as probe ultrasonication time goes on increasing. The surface response curve also indicates how the concentration of the stabiliser affects saturation solubility. The concentration of stabilizer also causes increased saturation solubility of iloperidone nanosuspension. When both the parameters were combined i.e. probe ultrasonication time and concentration of stabilizer saturation solubility was found to be increased. The surface response graph from Figure 2B depicts the impact of two independent factors, the amount of stabiliser and the duration of probe ultrasonication, on the size of particles. From the obtained data all the prepared formulations show particle size in nanoscale. Also, as evident in the surface response curve, an increase in probe ultrasonication after the formation of suspension by solvent-antisolvent method causes a further decrease in particle size. Probe ultrasonication time of 15 min has yielded the least particle size i.e. 286.7±5.65 nm at the polymer concentration of 20 mg. From the obtained data as well as statistical analysis batch number IF6 was decided as optimized where particle size was 286.7±5.65 nm and saturation solubility of 164.33±2.37 µg/mL at the probe ultrasonication time of 15 min and 20 mg concentration of stabilizer i.e. PVP k30. This iloperidone nanosuspension-optimized batch was then utilised for further studies. Formulation and process parameters for the optimised batch are indicated in Table 3.

Design expert software (Version 13) predicted the following polynomial equations for dependent variables over the range of independent variables.

$$\text{Particle size}=315.312-37.255A-32.6067B+16.88AB+27.8117A2+10.6667B2$$

$$\text{Saturation Solubility}=160.669+0.975A+2.2033B+0.5875AB+0.5016A2+0.5366B2$$

Particle size analysis of optimized batch

The study of the particle size of the optimised batch revealed a particle size of 286.7±5.65 nm. This finding demonstrates that the suspension was successfully formed in the nano-size range using the solvent-antisolvent approach.

Saturation solubility study

Saturation solubility of optimized nanosuspension batch was performed to check enhancement in solubility of iloperidone. From the study, it was observed that the optimized iloperidone batch has shown 164.33±2.37 µg/mL solubility which 6.32-fold enhancement in the solubility of iloperidone.

Determination of drug content

The surplus of optimised batch IF6 was diluted with methanol and filtered. The drug amount was quantified using a UV-visible spectrophotometer at a wavelength of 227 nm. The study revealed that the optimised formulation consists of 91.48±3.20% of the active pharmaceutical ingredient.

DSC

Figure 3 depicts the DSC spectra for pure iloperidone (Figure 3C) and the optimised lyophilized nanosuspension (Figure 3D). Pure iloperidone showed a strong endothermic peak at 123.59°C, confirming a crystalline structure as well as the melting point of API. In contrast, the DSC spectra of the lyophilized nanosuspension showed a broad peak near about 120°C, indicating a change from crystalline to amorphous state as the peak is not sharp. This might be due to inadequate time for iloperidone to crystallise.

FTIR

The FTIR spectra of pure iloperidone exhibited numerous valleys conforming to the various groups present in the pure drug. Conversely, the FTIR spectra of the nanosuspension displayed reduced and broad valleys as shown in Figure 1D. This reduction in peak intensity and broadening could be credited to the decreased crystallinity of the iloperidone in the nanosuspension, as well as the presence of excipients in the formulation.

SEM

Figure 4 shows the SEM results of the optimised batch, indicating a nanoformulation with particles smaller than 1 µm in size. Lyophilization may also increase particle size due to aggregation of particles.

XRD

The XRD spectra of iloperidone (Figure 3A) displayed numerous sharp peaks at 17, 20, 21, 23 and 25. 2-theta values demonstrating the crystallinity of iloperidone. However, the XRD spectrum of the optimized lyophilized nanosuspension (Figure 3B) exhibited fewer peaks.

Zeta potential

Optimized nanosuspension formulation has indicated a mean zeta potential value of -25.8 mV. This indicates the formation of stable nanosuspension as to stabilize nanosuspension, ±30 mV value of zeta potential is required.[33] SLS causes stabilization of nanosuspension through electrostatic as well as stearic stabilization, while PVP K30 causes stabilization of nanosuspension through stearic stabilization.

Table 3: Formulation and process parameters for an optimized batch of iloperidone nanosuspension.

Formulation/Process Parameter	Quantity / Condition
Quantity of Iloperidone	10 mg
Quantity of PVP K30	20 mg
Quantity of SLS	5 mg
Amount of Solvent (methanol)	10 mL
Amount of Antisolvent (Water)	50 mL
Stirring speed	1000 rpm
Stirring time	2 hr
Probe Ultrasonication time	15 min

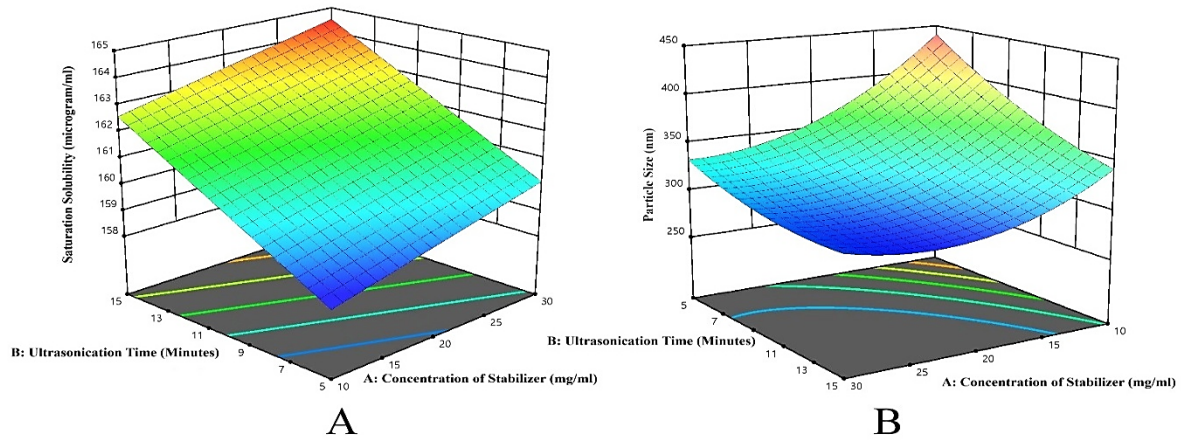

Figure 2: 3D surface response plot showing the effect of ultrasonication time and concentration of stabilizer on saturation solubility (A) and on particle size (B) of iloperidone nanosuspension.

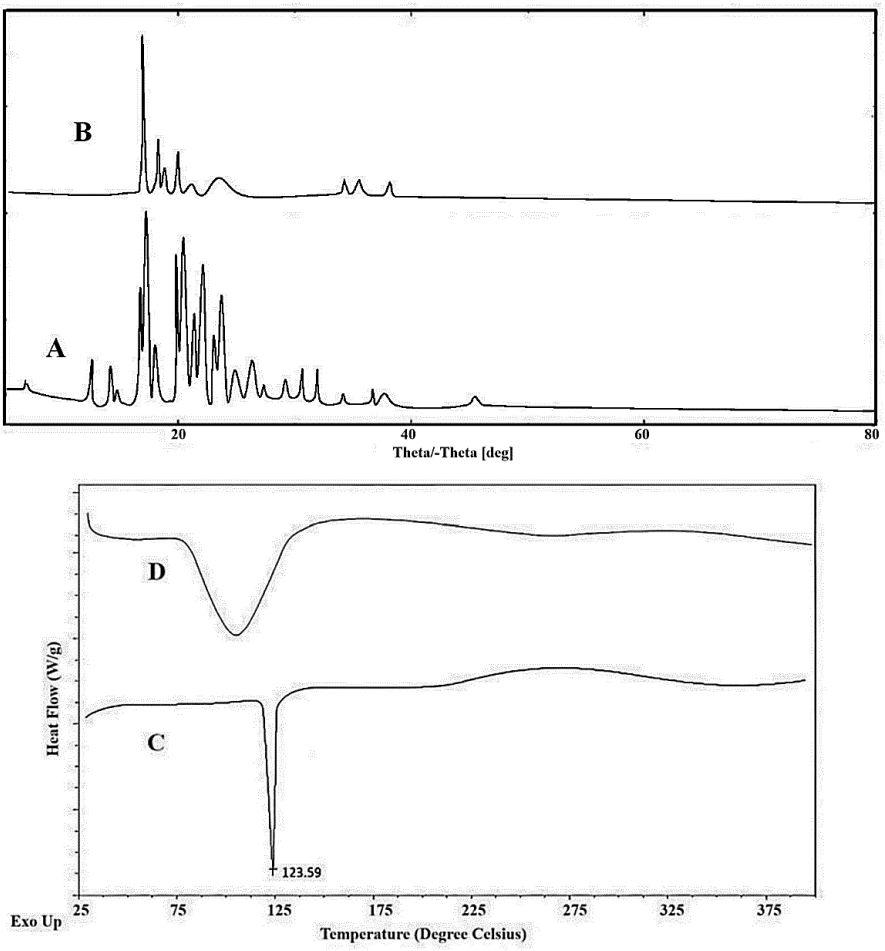

Figure 3: Comparative XRD of pure iloperidone (A) and optimized iloperidone nanosuspension (B) and DSC spectra of pure iloperidone (C) and optimized iloperidone nanosuspension (D).

In vitro dissolution study

An investigation on *in vitro* dissolution was conducted using phosphate buffer with a pH of 6.8. Figure 5 shows the dissolution profiles of the optimised batch of iloperidone and the marketed formulation. During the *in vitro* drug dissolution trial, almost 80% of the drug was released after 5 min from the optimised nanosuspension, while nearly 100% of drug release was reached within 20 min. It was observed that the formulated optimized nanosuspension exhibited superior drug release compared to the marketed tablet formulation.

In vivo pharmacokinetic study

To conclude if an optimized formulation of nanosuspension can enhance the Pharmacokinetic (PK) parameters of iloperidone, *in vivo* pharmacokinetic studies were performed in wrister rats. In the PK study drug coarse suspension was compared for various parameters like AUC, C_{max} and T_{max} with optimized nanosuspension. Results obtained from the study are indicated in Table 4.

From the obtained result it can be concluded that iloperidone nanosuspension has shown enhanced pharmacokinetic parameters than coarse suspension. Iloperidone nanosuspension has shown about a 2.88-fold increase in C_{max}. Also, the time required to reach maximum concentration was nearly halved. AUC has also increased by about 2 times. T_{max} of nanosuspension was also achieved in less time as compared to coarse suspension because of the smaller particle size in nanosuspension and larger surface area available for dissolution and absorption, leading to faster dissolution and more quick absorption of the drug. As an outcome, the drug reaches peak plasma concentration more quickly, leading to a shorter T_{max}. Also, this increase in overall PK parameters can be attributed to reduced first-pass metabolism. These overall results indicate the usefulness of particle size reduction in the form of nanosuspension for the improvement of pharmacokinetic parameters.

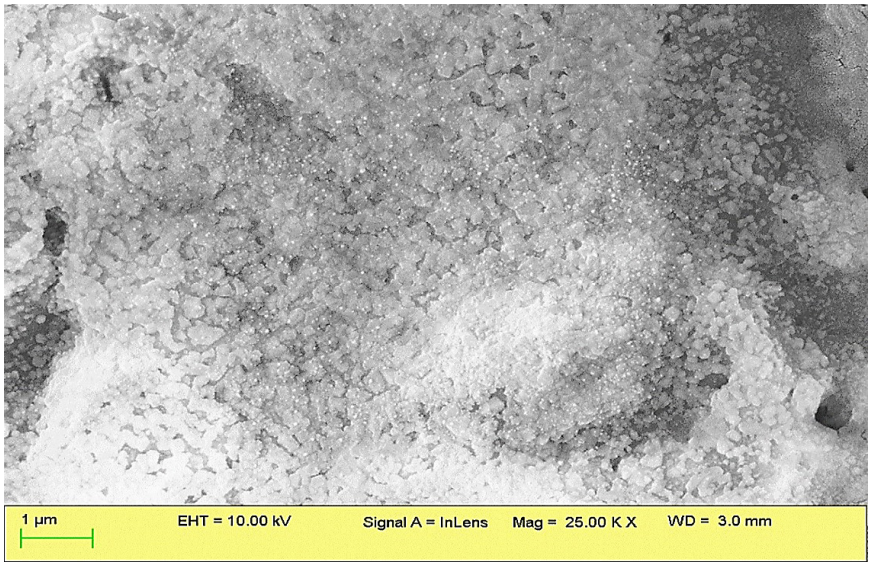

Figure 4: SEM analysis of optimized iloperidone lyophilized nanosuspension batch.

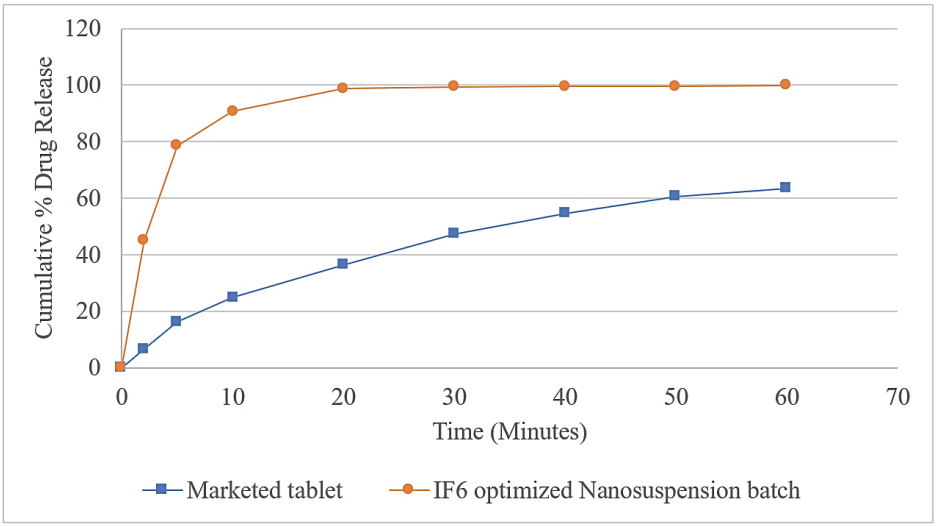

Figure 5: Graph indicating drug *in vitro* drug release pattern of optimized nanosuspension formulation and marketed tablet formulation.

Stability study

Selected formulations were retained for a 3-month stability analysis under a temperature situation of 40°C±2°C and relative humidity of 75%±5%. The particle size and saturation solubility of materials were assessed at 0, 15 days, 1, 2 and 3 months. The study findings are presented in Table 5. Upon completion of the stability studies, it was noted that there was a slight growth in the particle size of the nanosuspension. Furthermore, it has been observed that the saturation solubility decreases over time.

DISCUSSION

The preformulation studies provided a comprehensive understanding of iloperidone's physicochemical properties, which were crucial for developing an optimized nanosuspension. The melting point determination confirmed the purity of iloperidone, with observed values aligning with the standard. Solubility studies revealed iloperidone's poor aqueous solubility and high solubility in methanol, emphasizing the need for a formulation approach to enhance its solubility.

The compatibility studies using FTIR spectroscopy showed no significant interaction between iloperidone and the excipients (SLS and PVP K30), ensuring that the excipients chosen would not adversely affect the drug's stability or efficacy. The successful formulation and optimization of iloperidone nanosuspension using a 3^2 factorial design demonstrated the impact of stabilizer concentration and probe ultrasonication time on particle size and saturation solubility. The optimized batch (IF6) had a particle size of 286.7±5.65 nm and saturation solubility of 164.33±2.18 µg/mL, indicating a significant enhancement compared to the pure drug.

Table 4: Comparison of PK parameters of optimized nanosuspension and coarse suspension.

PK parameter	Coarse suspension	Optimized Nanosuspension
C_{max} (µg/mL)	2.80±0.17	8.08±0.42
T_{max} (hr)	3±0.45	1.8±0.17
AUC_{0-24} (µg/mL·h)	85.90±0.79	158.60±1.38

(Mean±SEM, *n*=6).

Table 5: Stability study parameters of optimized iloperidone nanosuspension.

Time	Parameter	
	Particle size (nm)	Saturation solubility (µg/mL)
0 days	286.7±5.65	164.33±2.18
15 days	295.4±3.04	162.45±3.38
1 month	320.6±5.07	158.56±3.21
2 months	346.7±6.40	156.74±4.19
3 months	380.4±4.15	153.80±3.27

(Mean±SEM, *n*=3).

Surface response plots illustrated that both increased stabilizer concentration and prolonged ultrasonication time contributed to reduced particle size and enhanced saturation solubility. This finding aligns with previous studies indicating that nanoscale particle size and stabilizer presence improves drug solubility and stability. Oktay and others (2020) formulated flurbiprofen nanosuspensions using an experimental design where they found that stabiliser was an important parameter in the formulation of nanosuspension.[34]

The particle size analysis of the optimized batch confirmed the nanoscale range, essential for improving solubility and bioavailability. The DSC analysis indicated a transition from crystalline to amorphous state in the nanosuspension, contributing to the solubility enhancement. FTIR and XRD analyses further supported this transition, showing reduced crystallinity in the optimized nanosuspension. Jog and Burgess (2017) reviewed that the precipitation of drug particles in nanosize in amorphous form has increased the rate of dissolution.[35] SEM analysis confirmed the nanoscale morphology and zeta potential measurements (-25.8 mV) indicated good stability of the nanosuspension.

In vitro dissolution studies revealed that the optimized nanosuspension exhibited significantly faster and higher drug release compared to the marketed tablet formulation, with nearly 100% release within 20 min. This rapid dissolution was likely due to the increased surface area of the nanoparticles, facilitating faster drug dissolution and absorption.

In vivo pharmacokinetic studies in Wistar rats demonstrated that the optimized nanosuspension had a significantly higher C_{max} (8.08±0.42 µg/mL) and AUC (158.60±1.38 µg/mL·hr) compared to the coarse suspension. The reduced T_{max} (1.8±0.17 hr) further indicated faster absorption, likely due to the enhanced dissolution and reduced particle size of the nanosuspension. These findings suggest that the nanosuspension formulation can improve the bioavailability of iloperidone, potentially leading to better therapeutic outcomes. Sahu and Das (2014) also proved that nanosuspension enhanced the oral bioavailability of felodipine in rats.[36]

Stability studies over three months showed slight increases in particle size and decreases in saturation solubility, attributed to Ostwald ripening. However, the changes were not substantial enough to compromise the formulation's overall stability and efficacy.

The development of iloperidone nanosuspension using a solvent-antisolvent method followed by probe ultrasonication significantly improved the drug's solubility, dissolution rate and bioavailability. These enhancements can potentially lead to better therapeutic efficacy and patient compliance in treating schizophrenia.

CONCLUSION

The formulation and optimization of iloperidone nanosuspension using a solvent-antisolvent method followed by probe ultrasonication significantly improved the drug's solubility, dissolution rate and bioavailability. The optimized batch (IF6) achieved a nanoscale particle size of 286.7±5.65 nm and saturation solubility of 164.33±2.18 µg/mL. *In vitro* studies demonstrated rapid and complete drug release within 20 min, surpassing the marketed tablet formulation. *In vivo* pharmacokinetic studies confirmed enhanced bioavailability, with a 2.88-fold increase in C_{max} and a 2-fold increase in AUC compared to the coarse suspension. Stability studies showed the nanosuspension remained relatively stable over three months. In summary, the iloperidone nanosuspension presents a promising strategy to enhance solubility and bioavailability, potentially improving therapeutic outcomes and patient compliance in schizophrenia treatment. Future research should focus on further optimization and long-term stability to fully realize its clinical potential.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BCS: Biopharmaceutical Classification System; **PVPK30:** Poly-vinyl-pyrrolidone; **SLS:** Sodium Lauryal Sulphate; **API:** Active Pharmaceutical Ingredient; **UV:** Ultraviolet-Visible; **FTIR:** Fourier Transfer InfraRed; **rpm:** Rotation Per Minute; **DSC:** Differential Scanning Calorimetry; **SEM:** Scanning Electron Microscopy; **XRD:** X-ray diffraction; **ZP:** Zeta Potential; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **RP-HPLC:** Reversed-Phase High-Performance Liquid Chromatography; **ICH:** International Council for Harmonisation; **SEM:** Standard Error of the Mean; **AUC:** Area Under Curve.

SUMMARY

Iloperidone, a BCS Class II antipsychotic, has low water solubility, limiting its bioavailability. This study aimed to develop a nanosuspension using a 3^2 factorial design to optimize stabilizer concentration and ultrasonication time. The solvent-antisolvent method with probe ultrasonication was used for formulation. The optimized nanosuspension had a particle size of 286.67 nm, saturation solubility of 164.33 µg/mL, and zeta potential of -25.8 mV, ensuring stability. *In vivo* studies showed a 2.88-fold increase in C_{max} and 2-fold increase in AUC, confirming enhanced bioavailability. Stability studies indicated three-month stability. Overall, nanosuspension technology effectively improves iloperidone's solubility and bioavailability, enhancing its therapeutic potential.

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