

Effect of Lyophilization on Characteristics of Iloperidone Nanosuspension

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ABSTRACT

Objective: The primary objective of this study is to develop the nanosuspension formulation of iloperidone and to check the effect of lyophilization on different characteristics of iloperidone nanosuspension.

Methods: Solvent-antisolvent method with probe ultrasonication was used for the formulation of nanosuspension. The nanosuspension was then converted into lyophilized form by using mannitol as a cryoprotectant and then evaluated for various parameters, including particle size, saturation solubility, scanning electron microscopy (SEM), thermal analysis, fourier-transform infrared (FTIR), *in-vitro* drug release study.

Result: From the study it was observed the increase in concentration of cryoprotectant while lyophilization has increased the particle size of nanosuspension. As there was an increase in particle size after lyophilization, there was a decrease in saturation solubility. *In-vitro* drug release also indicates a slight decrease in drug release after lyophilization. Overall, lyophilization might be used in the improvement of stability of nanosuspension, parameters must be chosen carefully for the process of lyophilization in order to obtain nanosuspension with good characteristics.

Keywords: Lyophilization, Iloperidone, Nanosuspension, Schizophrenia, Particle size.

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INTRODUCTION

A nanosuspension is a colloidal dispersion consisting of nanosized particles of a drug stabilized by surfactants. These suspensions are used to improve the solubility and bioavailability of poorly water-soluble drugs. The particles in a nanosuspension typically range in size from 10 to 1,000 nm, and their small size allows for enhanced dissolution rates and, consequently, better absorption in the body.^{1,2}

Post-processing of nanosuspensions is a critical step to ensure the stability, efficacy, and usability of the final product. Some common post-processing techniques used in the preparation of nanosuspensions are freeze-drying, milling, sterilization, and coating.

Freeze drying, or lyophilization, is a critical post-processing technique for nanosuspensions that converts a liquid nanosuspension into a dry, stable powder by sublimating the solvent (usually water) from the frozen state under reduced pressure. This method is particularly valuable for enhancing the stability, shelf-life, and ease of handling of nanosuspensions.³

Freeze drying, or lyophilization, can significantly affect the characteristics of nanosuspensions. Understanding these effects is crucial to optimizing the process for maintaining the quality and efficacy of the nanosuspension.

Freeze drying of nanosuspensions can cause possible aggregation of particles of nanosuspension which can cause a possible decrease in effective surface area and hence decrease can decrease the rate of dissolution.⁴

The current study mainly deals with the effect of lyophilization on different characteristics of iloperidone nanosuspension.

Iloperidone is an atypical antipsychotic medication primarily used for the treatment of schizophrenia in adults. It works by modulating neurotransmitter activity in the brain, particularly targeting dopamine and serotonin receptors, which are believed to play a crucial role in schizophrenia.^{5,6}

MATERIALS AND METHODS

The drug iloperidone was obtained as a gift sample from Aripolis Biotech Pvt. Ltd., Ludhiana, Punjab, India. Poly-vinyl-pyrrolidone K30 (PVPK30), sodium lauryl sulphate (SLS), mannitol and methanol were purchased from Sudharshan Scientific Ltd., Nandgoan, Nashik, Maharashtra. Double distilled water is used for the study. All the chemicals were used without further purification.

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Preparation of Iloperidone Nanosuspensions

The nanosuspension was prepared using the solvent-anti-solvent precipitation method. The drug 10 mg iloperidone was dissolved in 10 mL methanol as the drug is soluble in methanol. The mixture is then subjected to sonication for 5 minutes. About 20 mg of PVPK30 and SLS (5 mg) are 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 is kept in a beaker with high-speed stirring (1000 rpm). A syringe containing a solution of the drug in methanol is positioned over the beaker and added dropwise. After the addition of all the drug solution stirring is continued for 2 hours. After 2 hours, the sample was immediately transferred into the beaker and was treated with the ultrasonic probe of 6 number 3 mm deep for 15 minutes resulting in the travelling of waves in downward and upward direction.^{7,8}

Lyophilization of Nanosuspension

Formulated iloperidone nanosuspension was lyophilized by use of mannitol at 5 and 10% levels to study the effect of change in concentration of cryoprotectant by using lyophilizer (FreeZone, Triad, Labcono, USA). For lyophilization, the formulation was kept at -80°C at 0.02 mBar for 8 hours. Lyophilized nanosuspension of iloperidone was then kept in an airtight container in a desiccator at room temperature (25 ± 5°C) for further examination.

Characterization

Saturation solubility study

About 10 mL of distilled water was placed in stoppered conical flasks and excess iloperidone, iloperidone nanosuspension formulation in liquid form as well as lyophilized form was added. The flasks were placed in a rotary shaker at room temperature and sealed hermetically for a whole day. Samples were collected right after 24 hours, filtered, and then analyzed with a UV spectrophotometer.⁹

Particle size analysis

The Anton Paar Litesizer 500 particle size analyzer was used to calculate the particle size analysis of iloperidone nanosuspension in liquid form as well as lyophilized form.¹⁰

Differential scanning calorimetry

The differential scanning calorimetry (DSC) instrument (Mettler) was used to finish the DSC evaluation of Iloperidone API and nanosuspension in lyophilized form. About 5 mg of lyophilized nanosuspension was precisely balanced and hermetically packed in an aluminum pan. The lyophilized nanosuspension is heated from ambient temperature to 300°C at a rate of 10°C per minute. 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 per minute.¹¹

Fourier transform infrared

Fourier transforms infrared (FTIR) analysis was conducted to analyze lyophilized iloperidone nanosuspension and API. The FTIR spectra of the lyophilized nanosuspension were recorded using an FTIR spectrophotometer (Jasco FT/

IR-4600 FTIR Spectrometer, Japan). Approximately 5 mg of samples were mixed with an equal weight of dried KBr and compressed to form a disc. The pellets were then placed in the spectrophotometer, and spectra were recorded over a frequency range of 4000 to 400 cm⁻¹.¹²

Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was performed to study the surface morphology of lyophilized nanosuspension and to confirm the nanoparticle nature of lyophilized nanosuspension.

X-ray diffraction

The X-ray diffraction (XRD) analysis of the Iloperidone API and lyophilized nanosuspension was conducted using the PANalytical X' Pert Pro instrument. The X-ray tube utilized in this study features a copper (Cu) target. A wavelength of 1.54184 Å was employed in the XRD analysis. The instrumental parameters utilized 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°.¹²

In-vitro dissolution study

In-vitro dissolution studies of prepared nanosuspension of iloperidone in liquid form as well lyophilised form were performed by use of USP type II (Paddle type) apparatus. The nanosuspension formulation was put in an overnight pre-soaked dialysis bag (Himedia, Mumbai) in a dissolution medium. The study was performed at 37 ± 0.5° using 900 mL 6.8 pH phosphate buffer as a medium at 50 rpm. About 5 mL nanosuspensions were put inside the dialysis bag and the bag 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 minutes. Samples were then filtered by use of a 0.2 µm PTFE filter. The same amount of preheated fresh media is then placed in dissolution media. Withdrawn samples were appropriately diluted and then analyzed by using a UV spectrophotometer. The amount of drug present in each aliquot was determined from the standard calibration curve. The *in-vitro* release was then compared with the marketed tablet formulation.^{13,14}

RESULT AND DISCUSSION

Lyophilization

After lyophilization of nanosuspension of iloperidone with 5% and 10% mannitol, a fluffy white porous powder was obtained. Table 1 indicates the result of the amount of cryoprotectant on the particle size of nanosuspension.

From the results, it was concluded that a 5% concentration of mannitol yielded the least particle size in comparison to the 10% mannitol. Hence, further studies were carried out by the use of lyophilized nanosuspension, which has having 5% concentration of cryoprotectant.

Saturation Solubility Study

Saturation solubility of iloperidone API, Iloperidone nanosuspension in liquid form as well as the lyophilised form

Table 1: Effect of concentration of cryoprotectant on the particle size of lyophilized nanosuspension.

Cryoprotectant concentration	IF6 batch particle size trial (nm)			
	1	2	3	*Mean
5%	340.3	367.4	355.3	354.3 ± 6.40
10%	423.3	403.4	418.5	415.1 ± 4.89

*(Mean ± SEM, n = 3)

Table 2: Saturation solubility study

API and nanosuspension	Saturation solubility (µg/mL)
Iloperidone	0.026 ± 0.02
Iloperidone Nanosuspension	164.33 ± 2.37
Lyophilised nanosuspension	154.28 ± 3.23

Table 3: Particle size analysis result

Formulation	Particle size (nm)
Iloperidone nanosuspension	286.7 ± 5.65
Lyophilized nanosuspension	354.3 ± 6.40

*(Mean ± SEM, n = 3)

was performed to check the possible effect of lyophilization on iloperidone nanosuspension. The results of the study are indicated in Table 2.

From the saturation solubility data, it can be observed that iloperidone in pure form shows very little solubility in the water, while in the nanosuspension form, there is a drastic increase in the solubility of iloperidone. This might be due to a decrease in particle size and an increase in effective surface area. In the case of lyophilized nanosuspension, there is a slight decrease in saturation solubility. This can be attributed to the aggregation of particles due to lyophilization which leads to a decrease in surface area.

Particle Size Analysis

Particle size analysis of iloperidone nanosuspension in liquid, as well as the lyophilized form, was performed to check the possible effect of lyophilization on particle size. The results of the study are indicated in Table 3. From the results, it was observed that lyophilization has caused increased particle size of nanosuspension. The particle size of nanosuspension in liquid form is less than the particle size of nanosuspension in lyophilized form.

Differential Scanning Calorimetry

DSC thermogram of pure iloperidone indicated a sharp peak at 123.59, corresponding to the melting point of pure API. Also, the sharp peak obtained can be indicative of the crystalline state of API. In the case of nanosuspension of iloperidone, a broad peak is obtained near 120. Broad peaks may result due to changes in the physical state of API from crystalline to amorphous. The reduction in particle size of iloperidone can be a possible reason behind this change. Results are shown in Figure 1.

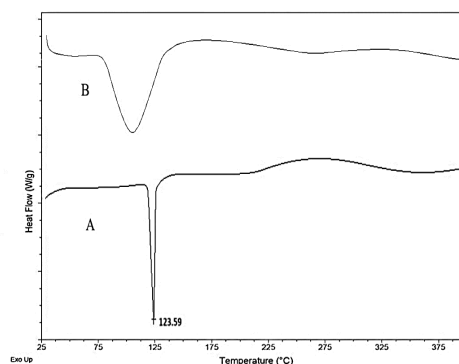


Figure 1: DSC DSC thermogram of (A) Pure iloperidone, (B) lyophilized iloperidone Nanosuspension

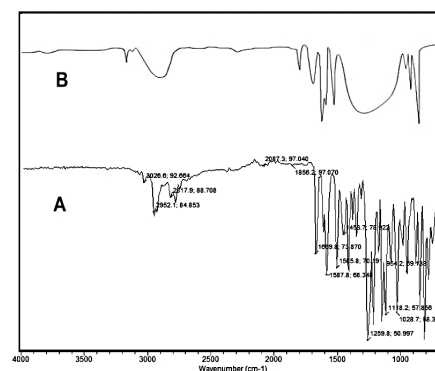


Figure 2: FTIR spectra of (A) Pure iloperidone, (B) lyophilized iloperidone nanosuspension

Fourier Transform Infrared

Lyophilized nanosuspension of iloperidone showed most of the characteristic peaks of API iloperidone, as shown in Figure 2, which indicated the suitability of the process for the formation of nanosuspension; however, the peak heights are reduced. The drug's transition from a crystalline to an amorphous state could be the underlying cause. Also, the presence of excipients has shown some additional peaks.

Scanning Electron Microscopy

The surface topology of particles in SEM was found to be thin, flat particles with a diameter of less than 1-µm as indicated in Figure 3. Also, particle size was observed to be slightly increased, which might be due to the aggregation of particles that occurred during lyophilization in the presence of cryoprotectant.

XRD

The XRD graphs of API have shown numerous peaks which was indicative of the crystalline nature of the API. However, the XRD graphs of the lyophilized nanosuspension have shown a significant reduction in peaks as well as peak height. This might be due to the change of state of the drug from a crystalline state to an amorphous state. This change in state from crystalline to amorphous can be due to the rapid addition of the drug in anti-solvent at low temperatures, which exhibited occurring

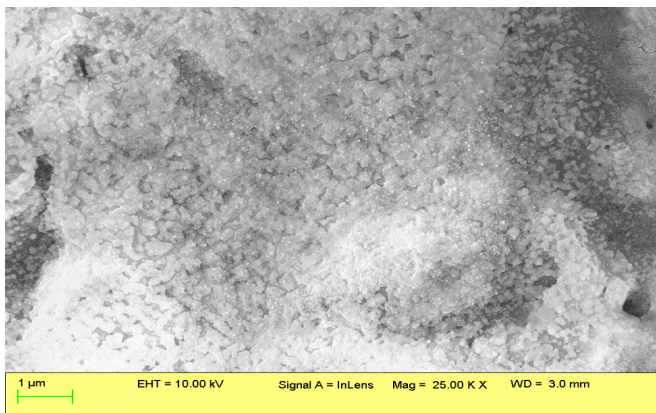


Figure 3: SEM of lyophilized iloperidone nanosuspension

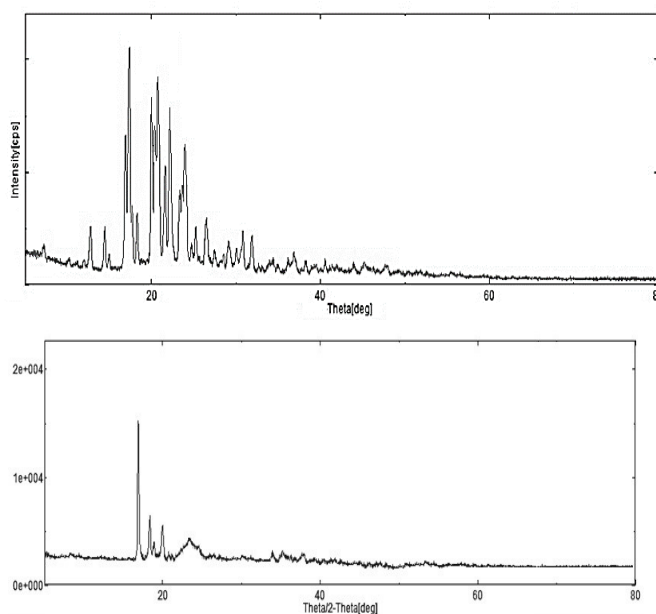


Figure 4: XRD of iloperidone nanosuspension and lyophilized nanosuspension

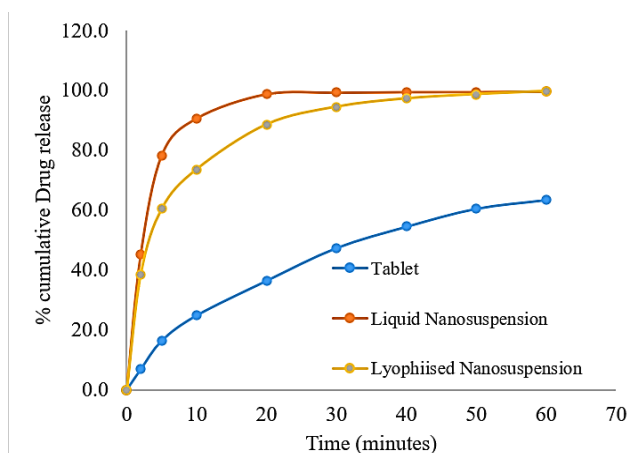


Figure 5: Cumulative drug release of tablet, liquid nanosuspension and lyophilized nanosuspension

of nucleation required for crystal growth. The results of XRD are indicated in Figure 4.

***In-vitro* Dissolution Study**

In-vitro dissolution studies of prepared nanosuspension in liquid form and lyophilized form were performed by use of USP type II (Paddle type) apparatus. The nanosuspension formulation was put in an overnight pre-soaked dialysis bag in a dissolution medium and drug release was checked.

The *in-vitro* dissolution profiles of iloperidone nanosuspension, lyophilized form and tablet formulation are presented in Figure 5.

From the dissolution study, it was observed that iloperidone nanosuspension in lyophilized form had shown less drug release as compared to liquid form. This decrease in drug release can be attributed to the solid nature of the lyophilized form as well as the increase in particle size.¹⁵

CONCLUSION

From the study, it can be observed that lyophilization has a significant effect on various characteristics of nanosuspension formulation. While lyophilization can be used for the improvement of stability it can decrease the saturation solubility of the drug. It can increase the particle size due to aggregation and can affect the dissolution profile of the formulation. It is important to determine the optimum concentration of cryoprotectant while carrying out the process of lyophilization to ensure maximum stability while maintaining the other characteristics of nanosuspension.

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